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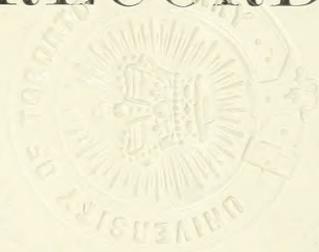


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# THE ANATOMICAL RECORD



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# THE GROWTH OF THE BODY AND ORGANS IN ALBINO RATS FED WITH A LIPOID-FREE RATION

SHINKISHI HATAI

*The Wistar Institute of Anatomy and Biology*

Nearly seven years ago the writer attempted to raise stunted albino rats with the hope that a forced retardation of growth would induce some disturbance in the firm relation which normally exists between the weight of the body and of the central nervous system. The stunted rats were produced by feeding them with a minimum amount of nitrogenous food. It was found, however, that in this instance the artificial stunting did not modify the weight relation between the body and the central nervous system (Hatai '08). Although it was highly desirable to pursue this investigation further, yet on account of inconstancy and uncertainty of the outcome in raising stunted rats by the method employed, the investigation was postponed.

In 1911 Professors Osborne and Mendel published a series of remarkable papers in which the results of maintenance experiments by means of various isolated proteins were fully described. According to these investigators, albino rats about one-third grown can maintain their body weight for a considerable period without revealing any sign of nutritional or physical deterioration. This satisfactory and constant procedure for producing undersized rats renewed my interest in the problem mentioned.

During the past two years I have been so fortunate as to receive a number of stunted rats with their controls for examination. These came through the courtesy of Dr. McCollum, who raised the rats by feeding them with a 'lipoid-free ration.' These rats fall into two series: the series of 1913 and the series of 1914. The present paper contains the results of the anatomical examination of these interesting rats, and I take this

opportunity to thank Dr. McCollum for his courtesy in putting these animals at my disposal.

The rats used were from those bred in the colony at The Wistar Institute in Philadelphia and sent to the University of Wisconsin. In each case rats belonging to the same litter were divided by Dr. McCollum into two lots with nearly identical body weights. The one lot was used for control and received the normal mixed ration, while the other lot, which was used for the experiment, received a specially prepared diet. As to the dietary formula, the following statements were kindly furnished by Dr. McCollum: The ration of the experimented rats which received the lipid-free food was as follows:

Casein.....	18 per cent	Agar-agar. ....	.2 per cent
Lactose.....	20 per cent	Dextrin.....	.56 per cent

The salts were as stated below:

<i>Salt mixture</i>	<i>Per 100 grams of ration</i>	
	<i>No. 174</i> <i>gm.</i>	<i>No. 185</i> <i>gm.</i>
NaCl.....	0.808	0.168
MgSO <sub>4</sub> (anhydrous).....	0.264	0.264
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O.....	0.336	0.336
K <sub>2</sub> HPO <sub>4</sub> .....	0.936	0.964
CaH <sub>4</sub> (PO) <sub>2</sub> ·H <sub>2</sub> O.....	0.528	0.528
Fe citrate.....	0.096	0.096
Ca lactate.....	2.000	1.300

The salt mixtures no. 174 and no. 185 were given at different periods in the case of both series.

At the end of the experiment these rats were shipped back to The Wistar Institute for the anatomical examination, where the writer determined the weights of the following organs: Brain and spinal cord, heart, lungs, kidneys, liver, spleen, alimentary tract, testes and ovaries, suprarenals, thymus, thyroid, hypophysis and eyeballs. Some of these organs were preserved for further histological examination. Besides the organs mentioned, the bones also were examined.

Although the methods employed in determining the relative amount of alteration in the various organs of the experimented rats, and also the technique for the preparation of the bones and separation of the encephalon into the four parts can be found

in my papers recently published (Hatai '13 and '14), I shall briefly restate the essential points.

The encephalon was divided into four parts in the following way:

1. *Olfactory bulbs.* The protruding portions of the olfactory tract with bulbs were cut from the rest of the encephalon by section of the tract just caudad to the bulb.

2. *Cerebrum.* The cerebrum is separated from the stem by a cut passing just in front of the dorsal edge of the anterior colliculi and just caudad to the corpus mammillare on the ventral surface.

3. *Cerebellum.* The cerebellum is separated by severing the peduncles.

4. *Stem.* The structure which is left after removal of these three parts mentioned above, is called the stem.

The bones were prepared as follows: The bones are freed from the main bulk of muscles and placed in a hot aqueous solution of 2 per cent 'gold dust washing powder.' After maceration for several hours at nearly  $90^{\circ}\text{C}$ ., the remaining soft parts are removed. The bones thus prepared are gently wiped with blotting paper and are weighed. This gives the 'fresh weight.' These weighed bones are then dried at  $95^{\circ}\text{C}$ . for one week and the amount of moisture determined from the weight of the dried residue.

In order to determine the amount of modification following the experimental ration, we have employed our usual method of comparing the observed values with those found in a series of reference tables that have been compiled in this laboratory. These tables present for normal rats adequate data on all the organs and characters under consideration and in each case the graph representing the table can be expressed by a mathematical formula (Hatai '13; Hatai '14).

In making the comparison between the observed values and those in the tables—the body length is always used as the basal measurement and the weight of the body or organs as observed compared with the corresponding values given in the reference tables. In this manner comparison is made not only for the

experimented rats but also for those used as controls. The departures of the observed values from those in the tables having been observed in each case—the difference between that found for the experimented animals and that for the controls is obtained and this figure is used to indicate the amount by which the experimented animals have been modified.

Two examples will serve to illustrate this procedure. They are taken from table 3—C, normal males, 1914 series: (1) On the 'mixed ration' the average tail length for the three rats is 172 mm., for the given body length, 196 mm. We expect from the reference tables a tail length of 165 mm. The observed value is therefore plus 4.2 per cent. The two rats on the "lipoid-free diet and egg fat" give a tail length of 151 mm. for a body length of 168 mm. From the reference tables we should expect a tail length of 139 mm. The observed value for the tail length of the experimented group is therefore plus 8.6 per cent. The difference between these two percentage shows the tail length in the experimented group to be  $8.6 - 4.2$ , or 4.4 per cent greater than that of the controls. This is the value given in table 3. (2) Taking the brain weights for the groups just used we find by following the method employed above for the tail length, that the group on the "mixed ration" has a brain weight 4.8 per cent below the reference table value, while the group on the "lipoid-free ration plus egg-fat" has a brain weight which is 6.4 per cent deficient. Thus the brain weight in the experimented group is  $- 6.4$  less  $- 4.8$  or 1.6 per cent lower than in the controls. This is the value entered in table 2. All the percentage differences in the accompanying tables have been obtained in a manner similar to that illustrated by the two examples just given.

The only modification in procedure to which attention need be drawn is in the cases where the data from two series, 1913 and 1914, have been combined. In those cases the percentage deviation which is given in the table is the mean of the deviations for each series computed separately.

## GROWTH OF BODY IN WEIGHT

The modifications of the growth of the body in weight due to the lipid-free ration are shown in tables 1 and 2. Table 1 refers to the growth of the albino rats belonging to the 1913 series, while table 2 refers to the growth of the 1914 series. We note in both tables that the rats fed with the mixed ration made nearly normal growth in respect to their ages (see Donaldson '06). The spring

TABLE 1

*Showing the weight of the body as modified by the lipid-free ration compared with that of the rats raised on the mixed ration (1913 series)*

DATE		MALES		FEMALES	
		Mixed ration (3)	Lipoid-free ration (4)	Mixed ration (4)	Lipoid-free ration (5)
1913					
April	16.....	94.7	93.8	85.0	76.2
	30.....	129.7	122.7	112.0	92.4
May	7.....	137.7	127.8	125.7	98.6
	14.....	153.7	134.8	146.7	108.4
	21.....	149.3	136.5	litters	107.4
	28.....	159.7	139.0	" "	108.4
June	4.....	166.3	140.7	" "	108.4
	11.....	173.3	131.5	129.0	103.8
	23.....	185.0	124.8	140.7	107.8
	30.....	196.7	134.2	143.3	109.0
July	7.....	209.7	139.7	151.0	111.6
	14.....	223.3	143.2	156.7	111.0
	21.....	222.7	149.2	161.0	107.2
	28.....	229.0	151.0	166.0	111.8
August	15.....	243.3	155.0	167.7	118.6
September	1.....	249.7	153.5	172.7	125.2

rats in 1913 made much better growth than the autumn rats in 1914. On the other hand, the experimented rats in both series made a noticeably poor growth when contrasted with the controls. In the 1913 series we notice that the experimented rats made continuous and steady growth throughout the period of experimentation, although the total amount of growth in weight was very slight. Curiously enough the experimented rats belonging to 1914 made a still smaller growth, and indeed in some cases the final body weight is no higher than the body weight at the begin-

TABLE 2  
*Showing the weight of the body as modified by the lipid-free ration compared with that of the rats raised on the mixed ration (1914 series)*

DATE	INTACT MALES			INTACT FEMALES			CASTRATED	
	Mixed ration (3)	Lipoid-free (2)	Lipoid-free and egg fat (2)	Mixed ration (3)	Lipoid-free (2)	Lipoid-free and egg fat (4)	Mixed ration (5)	Lipoid-free and egg fat (6)
November 26.....	96.3	79.0	90.0	82.3	95.0	80.0	84.2	94.8
December 3.....	118.0	100.5	105.5	101.7	102.0	82.0	100.8	103.2
10.....	133.3	104.0	105.0	113.7	98.0	84.5	115.4	105.7
17.....	147.0	106.5	107.0	121.7	103.5	85.2	122.4	108.2
26.....		106.5	118.0		107.0	90.2		108.5
January 1.....	171.3	104.0	119.5	136.3	111.0	91.5	150.4	111.2
7.....	181.0	103.0	121.0	143.0	103.5	92.8	162.2	110.0
14.....	182.3	102.0	111.0	144.3	107.0	91.2	167.6	106.5
February 3.....	198.7	96.5	113.0	141.0	111.5	95.2	169.6	108.2
16.....		87.0	108.0		105.5	93.3		104.8
24.....	207.3	84.0	107.0	166.3	108.0	93.2	179.0	111.5
March 5.....		84.5	110.5	litters	108.5	99.5		119.2
12.....	209.0	82.5	105.5	" "	105.0	96.5	182.6	117.7
19.....	212.0	82.5	105.5	" "	104.0	96.8	182.2	116.8
26.....	207.3	81.0	104.5	" "	103.0	93.0	184.6	115.2
31.....	210.3	79.0	102.5	155.7	105.0	94.5	187.6	119.3

ning of the experiment. This difference in growth in the two series may probably be due to the different physiological condition of the rats in these two series, combined with slight differences in the preparation of the ration. One point is clear, however: that the rats cannot continue the normal rate of growth on the lipid-free ration in combination with the salt mixture which was used.

In table 2 we have also the data on the growth of the body of the albino rats which were fed first with the lipid-free ration and later with the same ration to which a minute quantity of the egg-fat had been added. For convenience, these last mentioned rats will be designated simply as 'egg-fat series.'

It was found by McCollum and Davis ('13) that the rats whose body growth had ceased for a long period as the result of the lipid-free ration, could be made to grow by the addition of a minute quantity of the extract of egg to the experimental ration. In order to see whether or not the rats thus treated would show any modifications other than those shown by the rats fed with the simple experimental ration, a small series was carried on. As will be seen from table 2, the 1914 rats given the extract of egg did not show the improvement in the growth of the body which was to be anticipated.<sup>1</sup> Thus the growth of the body is nearly identical in both the lipid-free series and in the egg-fat series. Why in the present experiment the egg-fat series did not show a noticeable improvement in the growth of the body is not clear. However, from the fact that the control rats belonging to the 1914 series did not make satisfactory growth when contrasted with the 1913 series, we conclude that the failure to grow was

<sup>1</sup> "Our experience in feeding synthetic rations in this laboratory has convinced us that there exists a great variation in the vitality of individual rats as indicated by their ability to grow on such rations. It is unfortunate that practically all of the animals employed in the work here reported were not sufficiently vigorous to grow for a time in a nearly normal manner on the experimental ration, or to respond by a period of active growth when the ration was supplemented with egg yolk fats. We have individual rats in our colony at the present time which have been on the diet employed in the lipid-free period with egg yolk fats added, during more than six hundred days, and which compare favorably with our stock rats in size and well-being."—E. V. MCCOLLUM.

probably due to a peculiarity of the rats rather than a peculiarity of the experimental ration.

Osborne and Mendel ('12) obtained normal growth of the rats with the ration from which the lipid had been almost entirely removed. They carried the experiment for a considerable length of time by beginning with albino rats slightly over 30 days in age. In one series the experiment lasted for nearly 160 days. In every instance, so far as one can judge from the graphs, the body weight of the experimented rats was nearly identical with that of the control rats, while McCollum and Davis' rats, fed with the lipid-free ration, did not grow at any period to the size of the controls (McCollum and Davis '13; see also present series).

This difference in growth between the rats of Osborne and Mendel, on the one hand, and those of McCollum and Davis on the other, was undoubtedly due to the nature of the inorganic salts and some extracts still contained in the food. The Osborne and Mendel rats received the inorganic salts from protein-free milk, while those of McCollum and Davis received the salts which were a laboratory mixture of pure chemicals. In reference to the varying effects of different salt mixtures McCollum and Davis state ('13) that

"Young rats have been found to be very sensitive to variations in the character of the salt mixtures supplied, but with certain mixtures we have been able to obtain practically normal growth for periods varying from 70 to 120 days. Beyond that time little or no increase in body weight can be induced with such rations. The rats may remain in an apparently good nutritional condition on those rations for many weeks after growth ceases."

#### ANATOMICAL ANALYSIS

We now wish to present the results of the anatomical examination of these interesting rats reared by McCollum at the University of Wisconsin.

Although the growth rate was dissimilar in the two consecutive years, nevertheless it was found that the alterations shown by the various characters are nearly identical in the two series of experiments, and on account of this uniformity in the results, as well as to avoid unnecessary complication by presenting the

two series of data separately, I have combined the results. Consequently, unless otherwise stated, the figures given in the tables represent the averages of the two sets of data belonging to the 1913 and the 1914 series combined.

#### CENTRAL NERVOUS SYSTEM

If the lipid-free ration is able to produce any alterations in the lipid content of the organs, the central nervous system would naturally be expected to indicate such effects, since the central nervous system of the albino rat at about 200 days of age normally contains some 60 per cent of lipid in the dried residue (Koch '13). This lipid content is certainly greater in the nervous system than in any other organ (Koch '11). The weights of the central nervous systems of the experimented and of the control rats are shown in table 3 (see also page 16). As will be seen from this table, the weight of the brain with respect to the body length is generally slightly smaller in both the lipid-free and egg-fat series. Only one exception is found in the female rats (B) fed with the lipid-free ration in which the experimented rats show a slight over weight of 0.7 per cent. This slight increase is probably due to the abnormally small brain weight of the control rats, thus raising the relative weight of the central nervous system of the experimented rats. Indeed the normal brain weight of the female rats corresponding to the body length of 189 mm. should be 1.80 grams as against the observed weight of 1.73 grams, i.e., the observed weight of the control is nearly 4 per cent less than the normal brain weight. Without making any correction, however, we find on the average that the experimented rats show about 2 per cent less brain weight than the controls.

Similarly we find a reduction of 2.1 per cent in the weight of the spinal cord when compared with that of the control rats. This reduction of 2 per cent in weight in both the brain and spinal cord is somewhat greater than what we might expect from the normal fluctuation, nevertheless it is certainly far smaller than one might anticipate from the nature of the experiment. It seems reasonable, therefore, to conclude that the central nervous

TABLE 3

Showing the various measurements of the rats fed with the lipoid-free ration compared with the control rats

	LENGTH (MM.) OF		BODY WEIGHT (GRAMS)		WEIGHT OF		PER CENT		SEX GLANDS		AGE Days	NO. OF RATS	
	Body	Tail	Initial	Maxim.	Final	Brain	Sp. cord	Brain	Sp. cord	Testes			Ovaries
<i>A. Normal males, 1913 and 1914 series combined</i>													
Mixed ration.....	204	175.0	96	249	216	1.833	0.571	78.26	71.36	2.1820		193	6
Lipoid-free ration.....	179	158.0	84	133	118	1.678	0.466	78.04	70.78	0.8830		193	6
Per cent: Exp.-Control.....		4.2				-3.9	-1.4	-0.22	-0.58	-44.2			
<i>B. Normal females, 1913 and 1914 series combined</i>													
Mixed ration.....	189	164.0	84	165	151	1.730	0.528	78.28	71.79	0.0407		196	6
Lipoid-free ration.....	179	153.0	81	140	131	1.709	0.491	78.13	71.13	0.0303		195	8
Per cent: Exp.-Control.....		-0.7				0.70	0.30	-0.15	-0.66	-21.6			
<i>C. Normal males, 1914 series</i>													
Mixed ration.....	196	172.0	96	218	191	1.751	0.555	78.36	71.60	2.0320		186	3
Lipoid-free and egg fat.....	168	151.0	90	139	96	1.599	0.435	78.19	71.32	0.7830		186	2
Per cent: Exp.-Control.....		4.4				-1.6	-2.4	-0.17	-0.28	-44.2			
<i>D. Normal females, 1914 series</i>													
Mixed ration.....	185	161.0	84	157	137	1.729	0.529	78.21	71.82	0.0404		192	3
Lipoid-free and egg fat.....	162	147.0	79	128	100	1.569	0.416	77.98	71.23	0.0248		191	4
Per cent: Exp.-Control.....		5.1				-3.1	-4.9	-0.23	-0.59	-13.1			
<i>E. Castrated, 1914 series</i>													
Mixed ration (1).....	193	168.0	84	188	182	1.780	0.548	78.25	71.50			189	5
Lipoid-free and egg fat (2).....	175	158.0	95	119	131	1.709	0.480	77.98	71.13			188	6
Per cent: (2)-(1).....		4.5				1.2	0.70	-0.27	-0.37				

system is adequately supplied with the necessary amount of the lipoids from the body and this fact in turn leads us to assume that the body has the ability to synthesize the lipoids from the non-lipoid materials. McCollum ('12) has demonstrated that the phosphorus needed by the animal for phosphatid formation can be drawn from inorganic phosphates, and that phosphatids can be synthesized anew in the animal body. Further investigation of McCollum ('12) indicates that certain complex lipoids of the lecithin type can be synthesized in large amounts by birds.

The percentage of water found in the central nervous system indicates also that the chemical composition has not been noticeably altered since the difference between the control and experimented rats is only 0.2 per cent in the brain and 0.5 per cent in the spinal cord; both in favor of the controls. It is to be noted, however, that a small reduction appears in all the experimented series, thus indicating a strong tendency to a slight modification.

To determine whether or not the reduction of 2 per cent in the weight of the central nervous system was mainly caused by the alteration in the white substance, in which the lipoids are predominant, the brain was divided into four parts and the weights and water content of those parts were found separately. The results of the investigation are shown in table 4. We notice from the table that although the percentage of water tends to be smaller in the experimented rats in all four parts, nevertheless the greatest relative reduction appears in the olfactory bulbs, the cerebellum comes next and the cerebrum and stem come in the order named. Thus the stem where the lipid constituent is greatest is least modified and the olfactory bulbs and cerebellum, where the lipid constituent is least, are most affected. From this we infer that the gray substance is most affected, and the white substance, in which one might anticipate the largest alteration, is least modified.

This fact of greater change of the gray matter is interesting, since it is found that during partial starvation with non-nitrogenous food for three weeks, the total brain weight shows a reduction of 5 per cent (Hatai '04) but the amount of myelin, as can be seen from the normality in the amount of alcohol and

TABLE 4

Showing the relative weights of the parts of encephalon and percentage of water found in them, of the rats fed with the lipoid-free ration, compared with the control rats

	INTACT MALE RATS			INTACT FEMALE RATS			CASTRATED RATS		
	Mixed ration	Lipoid-free ration	Lipoid-free and egg fat	Mixed ration	Lipoid-free ration	Lipoid-free and egg fat	Mixed ration	Lipoid-free and egg fat	Mixed ration
Body length (mm.).....	204	179	168	189	179	162	193	175	175
Weight of brain (grams).....	1.833	1.678	1.654	1.730	1.707	1.570	1.780	1.709	1.709
Cerebrum.....	1.128	1.026	1.064	1.098	1.086	1.006	1.135	1.092	1.092
Stem.....	0.354	0.324	0.310	0.335	0.333	0.306	0.347	0.332	0.332
Cerebellum.....	0.260	0.226	0.230	0.244	0.239	0.215	0.250	0.239	0.239
Olfactory bulbs.....	0.053	0.041	0.051	0.052	0.040	0.042	0.048	0.046	0.046
Proportional weight of:									
Brain.....	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Cerebrum.....	62.850	63.450	64.320	63.520	64.010	64.210	63.800	63.870	63.870
Stem.....	19.750	20.060	18.750	19.360	19.640	19.550	19.530	19.460	19.460
Cerebellum.....	14.440	13.980	13.890	14.130	13.990	13.650	14.040	14.000	14.000
Olfactory bulbs.....	2.970	2.510	3.050	2.990	2.350	2.600	2.630	2.670	2.670
Per cent of water:									
Brain.....	78.260	78.040	78.190	78.280	78.130	77.980	78.250	77.980	77.980
Cerebrum.....	78.950	78.750	79.040	78.950	78.940	78.720	78.980	78.870	78.870
Stem.....	74.500	74.340	74.390	74.480	74.530	74.510	74.560	74.410	74.410
Cerebellum.....	79.570	78.630	78.610	79.460	78.910	78.680	79.330	78.810	78.810
Olfactory bulbs.....	82.370	81.630	81.820	83.160	81.910	81.930	82.100	81.120	81.120
Age, days.....	193	193	186	196	195	191	189	188	188
Number of rats.....	6	6	2	6	8	4	5	6	6

ether extract as well as from the Weigert preparations, is not altered (Donaldson '11). We conclude therefore that the absence of lipoids from the ration does not affect the amount of the lipoids in the central nervous system, but on the contrary, the gray substance is affected. This alteration of the gray substance is similar to the effect of partial starvation on the brain of the albino rat.

#### SKELETAL SYSTEM

The skeletal system is naturally looked on as another structure which might show some alteration owing to the use of the artificial mixture of the pure chemicals contained in the ration in place of the salts normally present. For this purpose the following bones were examined: femur, tibia and fibula, humerus, radius and ulna. The results of the investigation are given in table 5. We note from this table that the ratio between body length and bone length and the ratio between body weight and bone weight are not altered. However, the water content found in these bones shows a distinct alteration in the experimented rats. The difference amounts to as much as 5.5 per cent in the case of the lipid-free ration and 5.3 per cent in the case of the egg fat series; both in favor of the experimented rats. This difference of over 5 per cent is far greater than the usual incidental fluctuations. Furthermore, its constancy in direction in all these cases indicates that the chemical composition of the bones must be affected as the result of the experimental ration.

#### SEX GLANDS

The alterations thus far recorded are all of small magnitude, but we now come to the consideration of the one very obvious alteration. This is manifested by the testes and ovaries.

#### TESTES

We notice from table 3 that the experimented male has considerably smaller testes than the control. The difference amounts to as much as 44 per cent against the experimented. We notice also that the initial body weight of the experimented male rat

TABLE 5  
*Showing the weights and lengths of various bones of the rats fed with the lipid-free ration compared with the control rats*

	INTACT MALE RATS			INTACT FEMALE RATS			CASTRATED RATS		
	Mixed ration	Lipoid-free	Lipoid-free and egg fat	Mixed ration	Lipoid-free	Lipoid-free and egg fat	Mixed ration	Lipoid-free and egg fat	Lipoid-free and egg fat
Body length, mm. ....	196.000	169.000	168.000	185.000	178.000	162.000	193.000	175.000	175.000
Body weight, grams { Obs. ....	191.000	95.000	96.000	137.000	128.000	100.000	182.000	141.000	141.000
{ Calc. ....	181.000	113.000	111.000	161.000	142.000	106.000	172.000	125.000	125.000
Bone length. ....	116.000	101.000	102.000	112.000	103.000	96.000	114.000	102.000	102.000
Bone weight { Moist. ....	2.688	1.660	1.905	2.340	1.868	1.569	2.564	2.007	2.007
{ Dried. ....	1.813	1.001	1.191	1.459	1.188	0.969	1.733	1.098	1.098
Per cent water, bones. ....	32.560	39.730	37.500	34.870	36.420	38.220	32.440	45.290	45.290
Body length: Bone length. ....	59.34%	59.70%	59.51%	60.27%	57.81%	59.26%	58.96%	58.46%	58.46%
Body weight, calc.: Bone weight. ....	1.48%	1.47%	1.72%	1.39%	1.32%	1.48%	1.49%	1.60%	1.60%
Age, days. ....	186	190	186	192	190	191	189	188	188
Number of rats. ....	3	2	2	3	2	4	5	6	6

was 87 grams; this body weight calls for nearly 1.09 grams of testes, while the observed final weight is but 0.83 grams, thus showing a difference of nearly 23 per cent. We conclude therefore that the testes not only failed to grow during nearly six months of the special diet, but that there is a clear indication of an actual loss in weight.

#### OVARIES

In the case of the ovaries the difference between the controls and experimented is less than one-half of that found in the case of testes. The difference amounts to 17.4 per cent against the experimented. The initial body weight of the female rat was 80 grams; this body weight calls for nearly 0.015 grams of ovaries, while the observed final weight is 0.028 grams, thus showing an increment of more than 80 per cent during the experimental period of nearly six months. Thus it is clear that although the weight of the ovaries was 17 per cent smaller than that of the controls, nevertheless the ovaries of the experimented rats made steady growth, and indeed the final weight of the ovaries was nearly double the initial weight. The functional normality of the ovaries in the lipoid-free series is demonstrated by the fact that some of the females raised on the lipoid-free ration produced litters (McCullum and Davis '13).

#### EFFECT OF LIPOID-FREE RATION ON CASTRATED MALE RATS

The reduction of the testes in weight as the result of the experimental ration (1913 series) suggested that the same experimental ration when given to castrated male rats might produce a different alteration. To determine this point a series of castrated rats was sent to Dr. McCullum. Five of these castrated rats were fed with a mixed ration and six others were fed with the lipoid-free ration, to which latter a small amount of the egg-fat was added occasionally. The results of the investigation are given in tables 2, 3, 4 and 5. As is seen from table 2, the growth of the body in weight in castrates fed with the lipoid-free ration is similar to that of the intact rats fed in the same way. Thus castration, plus the lipoid-free ration, does not produce any other alterations, the testis excepted, than those shown by the intact rats fed in the same way.

We further note from tables 3 (E) and 4 that the central nervous system of the castrates fed with the experimental ration is not different from that of the intact rats fed in the same way. Thus it is clear that the effect of the ration is not modified by castration. This conclusion applies also to the ratio between body length and bone length and the ratio between body weight and bone weight. The only difference is found in the percentage of water in bones of those castrates fed with lipoid-free ration.

We note that the difference in the water content of the bones between the castrates fed with the mixed ration and the castrates fed with the experimental ration, amounts to 13 per cent, which is much more than the difference between the intact rats on a mixed ration and those on the experimental ration. This greater difference of water content is found also in all individual cases. We conclude, therefore, that castration followed by the lipoid-free ration produces no further alteration than is found in the non-castrated rats fed with the same experimental ration, except in the water content in the bones. No explanation is possible for this singular result until further experiments have been made.

#### THE VISCERAL ORGANS AND DUCTLESS GLANDS

As has already been stated, the visceral organs and ductless glands, together with the eyeballs of these rats, were also examined. However, in view of the greater variability of these organs as well as the relatively small number of rats examined, I have decided not to attempt at this moment to interpret the alterations recorded by these organs. Nevertheless, for the reader who may wish to know the weight relations between the controls and the experimented rats shown by these organs, table 6 is given, where the results of the investigation are presented.

It may be important to add one word concerning the weight of the lungs. As will be seen from table 6, the weights of the lungs belonging to the experimented rats are always considerably smaller than those of the controls. This means that the lungs of the experimented rats were in a healthy condition and that the greater weights found in the controls were due not to sound lungs of larger size, but to a diseased condition. This fact must

be considered when interpreting the weight relations given by various other organs whose weights vary with the condition of the lungs (Hatai '13).

#### CONCLUSIONS

1. The lipoid-free ration diminishes the normal rate of the growth of the body.

2. The weight of the central nervous system shows a reduction of about 2 per cent as the result of the experimental ration. The percentage of water found in the central nervous system shows a very slight diminution.

3. The different parts of the encephalon are differently altered. In general, the parts where the gray substance is predominant are more affected than the parts where the white substance is predominant.

4. The weight and length of the longer bones with respect to body weight and body length are not modified. The percentage of water found in these bones, however, is constantly greater (5 per cent) in the experimented rats. This indicates that the chemical composition of the skeletal system has been somewhat altered.

5. The testes of the experimented rats showed not only a deficiency of 44 per cent as a result of six months of the lipoid-free diet, but there is a clear indication of actual loss in weight (23 per cent).

6. The ovaries of the experimented rats were smaller in weight by 17.4 per cent but no loss of the gland has occurred and growth was continuous.

7. The reactions shown by the lipoid-free ration and egg fat series are similar to those produced by partial starvation, especially with respect to the responses by the central nervous system and by the sex glands.

8. Although the lipoid-free ration causes a marked atrophy of the testes, yet in castrates on the lipoid-free ration no special alteration occurs which can be referred to castration, save the diminution of the solids in the bones.

9. Two incidental observations call for comment: (a) The loss of solids in the bones of the rats receiving a lipoid-free diet is of interest owing to the possible use of the phosphorus of the

TABLE 6  
*Relative weights of visceral organs, ductless glands and eyeballs of the rats fed with the lipid-free ration compared with the control rats*

	HYPOTHY- MUS	THYROID	THYMUS	HYPOPHYSIS	HEART	KIDNEYS	LIVER	SPLEEN	ALIMEN- TARY	LUNGS	EXTRALIM- ENARY
<i>A. Normal males, 1913 and 1914 series combined</i>											
	<i>mm.</i>										
Mixed ration. . . . .	204	0.0332	0.0237	0.0934	0.0075	1.813	10.896	0.442	8.001	1.994	0.292
Lipoid-free ration. . . . .	179	0.0271	0.0156	0.0483	0.0051	1.196	6.939	0.427	6.438	1.106	0.274
Per cent: Exp- Control . . . . .	8.0	5.9	-26.7	-5.9	-12.9	-3.5	-10.4	32.0	-7.1	-38.4	-6.0
<i>B. Normal females, 1913 and 1914 series combined</i>											
	<i>mm.</i>										
Mixed ration. . . . .	189	0.0489	0.0189	0.0700	0.0092	1.307	8.364	0.472	6.193	1.916	0.269
Lipoid-free ration. . . . .	179	0.0338	0.0219	0.0761	0.0068	1.198	7.446	0.493	6.624	1.286	0.270
Per cent: Exp- Control . . . . .	-20.2	21.8	3.7	-6.3	10.8	5.9	2.1	24.1	14.2	-42.7	0.6
<i>C. Normal males, 1914 series</i>											
Mixed ration. . . . .	196	0.0311	0.0217	0.0364	0.0069	1.727	8.786	0.425	7.160	2.181	0.283
Lipoid-free and egg fat. . . . .	168	0.0261	0.0158	0.0610	0.0053	1.233	6.961	0.450	7.589	0.900	0.275
Per cent: Exp- Control . . . . .	4.4	4.5	-3.1	-10.4	4.1	9.3	12.9	58.8	36.8	-77.5	-2.7

TABLE 6—Continued

BODY LENGTH	SUPRA-RENALS	THYROID	THYMUS	HYPOPHYSIS	HEART	KIDNEYS	LIVER	SPLEEN	ADRENAL TRACT	LUNGS	EYE-BALLS
<i>D. Normal females, 1914 series</i>											
<i>mm.</i>	<i>gms.</i>										
Mixed ration. . . . . 185	0.0468	0.0178	0.0485	0.0075	0.657	1.307	6.870	0.441	5.957	2.151	0.267
Lipoid-free and egg fat. . . . . 162	0.0263	0.0131	0.0612	0.0054	0.478	1.242	5.454	0.352	5.804	0.726	0.253
Per cent: Exp.-Control. . . . .	-5.3	2.1	9.0	11.2	-0.9	32.3	6.9	17.9	20.3	-115.6	-5.1
<i>E. Castrated rats, 1914 series</i>											
Mixed ration (1). . . . . 193	0.0357	0.0198	0.1108	0.0103	0.733	1.470	7.250	0.490	7.071	2.287	0.291
Lipoid-free and egg fat (2). . . . . 175	0.0287	0.0157	0.0598	0.0073	0.557	1.327	7.072	0.441	7.020	1.116	0.282
Per cent: (2)-(1). . . . .	-2.6	-0.6	-23.8	-16.0	2.3	17.3	19.0	19.3	7.0	-81.2	-3.0

bone in the formation of lipoids. (b) On the lipid-free diet, as well as in various forms of underfeeding, and after long-continued exercise, the rats become remarkably resistant to the lung infection which appears in the controls.

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## THE SOURCE OF THE HYDROCHLORIC ACID FOUND IN THE STOMACH

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At the present time two opinions exist as to the source of the hydrochloric acid found in the stomach. The work of Miss Fitzgerald (1910) tends to show that the parietal cells of the gastric glands are the seat of the direct formation of the acid. Harvey and Bensley (1912), on the contrary, report that their experiments demonstrate that while these cells may form precursors of the acid, they do not produce the acid itself. Previous to these publications, Fränkel (1891) as well as Edinger (1880), by using dyes, had demonstrated an acid reaction below the surface epithelium of the gastric mucosa; but they could not accurately localize the source of the acid.

The disagreement between the conclusions of Miss Fitzgerald and of Harvey and Bensley appears to call for a critical review of their work, such as is here undertaken. In preparing it, I must thank both Dr. Frederic T. Lewis and Dr. Otto Folin for generous assistance.

Miss Fitzgerald found direct proof of the presence of acid in the lumina of the gastric glands, in the canaliculi of the parietal cells, and even in the parietal cells themselves. She found in these places a deposit of Prussian blue after injecting a ferrocyanide and a ferric salt into the ears of the animals experimented upon. This precipitate is formed in the presence of hydrochloric acid and the two salts mentioned. She found the precipitate in no other place than the immediate vicinity of the parietal cells. From her findings she draws the conclusion that the parietal cells of the gastric glands produce the hydrochloric acid found in the stomach.

Harvey and Bensley consider that Miss Fitzgerald's conclusions are unjustified, for reasons which are discussed seriatim in the following paragraphs.

1. *The reaction is not constant.* In some of Miss Fitzgerald's experiments the Prussian blue reaction was not obtained, and Harvey and Bensley, who repeated her work, likewise report unsuccessful experiments. No explanation, other than mere conjecture, is offered to account for these failures.

2. *When present, the reaction is restricted to limited areas.* Regional activity of the glands, decreased blood supply, and inhibition of secretory function due to the toxic effects of the injected salts, are all sufficient to explain this effect; the actual cause is unknown.

3. *Within the areas which respond, the reaction occurs in only a few cells.* The non-reacting cells can be considered either as resting cells, or as those which have already discharged their acid and are prevented from further activity by the toxic effect of the injected salts. It is to be noted that the parietal cells "of the deeper third of the gland tubules, that is, the third farthest from the free surface, never contained the Prussian blue." This may be correlated with the fact that the upper end or neck of the tubules is the source of new cells, as indicated by the presence of mitotic figures. And further, as stated by Kölliker (1902, p. 158), "the parietal cells are infrequent at the bottom of the glands, where they may be entirely absent; they increase in the body of the gland and are most frequent in the region of its neck." Thus the Prussian blue reaction appears to occur where the parietal cells are most active and most numerous.

4. *The reaction occurs in other places than the gastric glands.* Miss Fitzgerald did not find the Prussian blue precipitate in any tissues but those of the stomach wall. As a result of the toxic action of the injected salts a marked inflammation occurred. This would signify an increased permeability of the cell walls, and the secreted acid of the parietal cells could escape into the neighboring tissues as well as into the natural pathway. That

only traces of the precipitate were found outside of the glands proper is fairly convincing evidence of its intensive localization.

Although Harvey and Bensley found a precipitate in the liver, spleen, and blood vessels of the cardiac muscle, yet this precipitate may indeed not have been typical Prussian blue. We find, for example, that lactic acid, when added to a mixture of the salts in question, causes an atypical precipitate. This may explain Harvey and Bensley's apparently contradictory results of finding no precipitate in the heart's blood but finding it in the vessels of the heart muscle. In all probability it was precipitated there by the liberation of lactic acid from the dead muscle. Moreover we find that blood serum, blood plasma, and various salts which occur in the blood (sodium bicarbonate, sodium carbonate, sodium chloride, di-sodium phosphate, and mono-sodium phosphate, each in 0.1 per cent solution) may be added to a mixture of potassium ferrocyanide and iron and ammonium citrate without causing a precipitate. This is contrary to Harvey and Bensley's conclusion that "the Prussian blue is precipitated in the blood stream when solutions of these salts (sodium ferrocyanide and iron and ammonium citrate) are injected into it." At least there is no precipitate of Prussian blue when the salts are added to fresh normal blood *in vitro*.

Some of Harvey and Bensley's results with the Prussian blue reaction afford interesting evidence in support of Miss Fitzgerald's ideas. For instance, they find the Prussian blue precipitate on the mucous surface of the stomach, and prove that there is no backing up of the precipitate into the lumina of the glands; but occasionally they find the Prussian blue precipitate in these lumina. Therefore the Prussian blue must have been formed in the lumina of the glands. This necessitates acid, yet Harvey and Bensley deny the presence of acid in this situation. It seems as if they had furnished evidence of the presence of acid in the lumina of the glands of the gastric mucosa.

Furthermore, as might be expected on physiological grounds, they find that poisons and a decreased or restricted blood supply do not increase the formation of the Prussian blue precipitate. These influences would be expected to decrease the liability of

precipitate formation, so that only the favored locations would be able to respond. Both by their criticisms and their own work with the Prussian blue reaction, Harvey and Bensley appear to have strengthened greatly the conclusions of Miss Fitzgerald. After summarizing her experimental findings they state—"Our own results have confirmed these facts entirely." To prove that the cells of the gastric glands do not produce hydrochloric acid as such, Harvey and Bensley attempted injection experiments with various dyes; but this line of work did not yield satisfactory results.

The next form of attack was to immerse portions of the gastric mucous membrane from a freshly killed animal in a solution of the dye *cyanamin* in normal sodium chloride solution. This dye yields distinctive colors for acid, alkaline, and neutral solutions. With this method they found the contents of the gland cells to be alkaline. The acid reaction occurred on the surface of the mucosa and extended inward as far as the bottom of the gastric pits or foveolae. There it changed rapidly through neutral to alkaline, and so it extended through the lumina of the glands and into the secretory canaliculi of the parietal cells, which were thus strikingly demonstrated.

We have prepared cyanamin according to the directions given by Witt (1890) and have repeated Harvey and Bensley's experiment, obtaining similar results; but we draw different conclusions from these results.

Harvey and Bensley found that the concentration of the gland secretion is quite different from that of normal saline solutions. This being so, the laws of osmosis and diffusion come into play and we can not go on the supposition that the addition of the dye to the normal saline solution renders it isosmotic with the cell contents. Apparently no attempt was made to determine the relative concentrations of the reacting substances, e.g., dye and hydrochloric acid.

In order to determine for ourselves whether the dye or the hydrochloric acid diffused the more rapidly, experiments were conducted to that end. As might be expected, it was found that the acid diffused with by far the greater rapidity under conditions

approximating those in which the tissue was used. Having established this fact we have the following mechanical process as an explanation of Harvey and Bensley's results.

The hydrochloric acid secreted by the gland cells diffuses out of the cells, through the canaliculi and into the lumina towards the free surface, faster than the dye diffuses inward along the same path. Consequently the mucous surface of the tissue and the foveolar contents show acidity. The tissue, after removal from the organ, does not continue to perform its secretory function, nor does it excrete save by diffusion. This then leaves the cell contents alkaline, as is shown by the fact that the slowly moving dye stains the cells with the alkaline reaction. Supposing we have hydrogen weakly bound to protein and ionizing in the cell to  $(H)^+$  and protein. We know we have sodium ions and chlorine ions present.  $NaCl = (Na)^+ + (Cl)^-$ . Removing the hydrogen ions and the chlorine ions we have an excess of sodium ions, thus making the cell contents alkaline.

The localization of the reaction between the dye and the acid is dependent upon the relative velocity of the participating constituents. Inasmuch as the acid has the higher velocity, we get the recorded results and a stable confirmation of Miss Fitzgerald's experiments and conclusions.

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## STROPPING MACHINE FOR MICROTOME KNIVES

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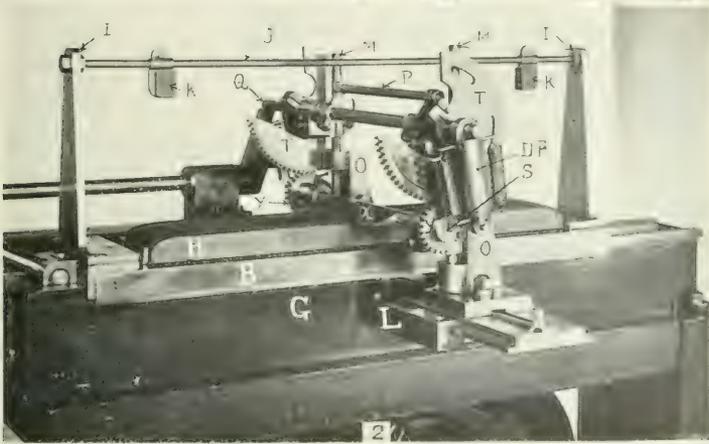
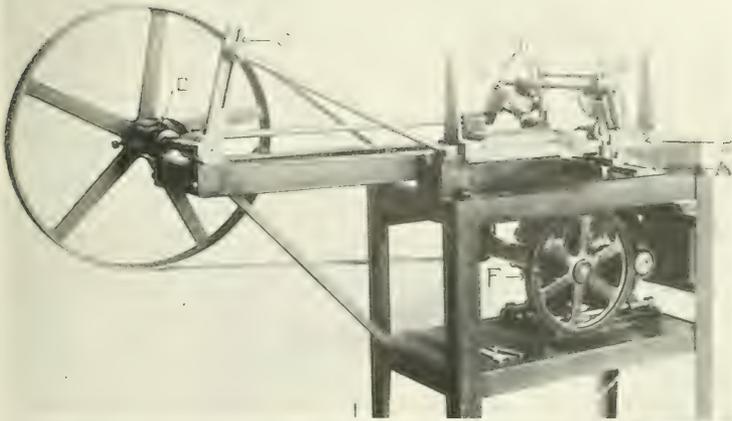
*The Wistar Institute of Anatomy and Biology*

This machine consists of two reciprocating carriers moving at right angles to each other, one (*A*) bearing the knife with the reversing mechanism and the other (*B*) carrying the strop. These carriers are actuated by cranks of unlike speeds (*C*, *D*) geared together (at *E*) and driven by a belt from a motor (*F*). The entire apparatus is constructed on an angle iron (2 inches  $\times$  2 inches) frame. Carrier *B* (fig. 2) is a heavy brass plate 19 inches long,  $3\frac{3}{4}$  inches wide, and designed to carry a strop (*H*) 15 inches long and  $2\frac{1}{4}$  inches wide. This carrier is grooved and accurately fitted to a base casting (*G*) upon which it travels. At each end of the carrier is a pillar (*I*). A  $\frac{3}{8}$  inch steel rod (*J*) connects the tops of these pillars and carries a lug (*K*) at each end.

Carrier *A* is 12 inches long and is designed to carry a knife of any length from  $3\frac{1}{2}$  inches to 7 inches. This carrier is mounted and moves in a base *L* secured at right angles to the base *G*.

*O* and *O* are vertical pillars carrying  $\frac{1}{2}$  inch steel rods *M*, *M*, which move freely in a direction vertical to the plane of the strop. To the rods (*M*, *M*,) are attached by universal joints, the axis *Q* carrying the reversing mechanism *R* and the gear wheels *T*, *T*. Axis *S* (in which the knife forms the central portion) is also attached by universal joints to the vertical rods, *M*, *M*. It has also a gear wheel at each end meshing with the gear wheel *T* of the corresponding end of axis *Q*. The supporting pillar *O* may be adjusted upon carrier *A* to accommodate a longer or shorter knife. Axis *S* is made up of the knife *X* and the gear wheels *Y*, *Y*, with their hollow spindles as shown in figure 3. By means of pin *Z* the shank of the knife is prevented from turning in the hollow spindle of the gear wheel *Y*.

The gear wheel *Y* carries a wrist pin *XX* (fig. 3), to which is attached the lower end of the dash-pot *DP* (fig. 2); the upper member of the dash-pot is attached to axis *Q*. The dash-pot consists of an outer steel cylinder having an air outlet at the top (the size of which outlet may be changed by a screw) and an inner brass plunger with an air inlet at the bottom controlled by a valve. The springs on each side of the dash-pot tend to force the plunger into the cylinder by driving the air through the outlet at the top. The dash-pot springs, acting upon the wrist pin of the axis *S*, tend to tilt the knife edge downwards. The



3

dash-pot prevents the knife from striking too hard upon the strop when reversed.

The machine operates as follows: Carrier *B* moves to the left (fig. 2) while carrier *A* moves at right angles to carrier *B*. The knife is thus moved from end to end of the strop, and at the same time it moves part way across the strop. The gear ratio is such that the knife traverses the same path only once in every 137 strokes, thus bringing every portion of the knife edge in contact with every portion of strop surface. As carrier *B* passes to the end of its stroke to the left, the rod *R* comes in contact with lug *K*, and axis *Q*, with gear wheels *T*, *T*, is turned slightly anti-clock-wise. This movement gives the axis *S* a half turn clock-wise, thus throwing the knife edge to the right, at which instant the carrier *B* begins its stroke to the right. This process is repeated at each end of the stroke. The dash-pot springs pull the knife over firmly but slowly into its new position at each stroke. The knives used with this machine have a short shank at each end (fig. 3). The vertical rods *M*, *M*, move freely upward and downward carrying with them the knife and all the reversing mechanism. This permits the knife to follow over the surface of the strop and to conform to any irregularities which the strop may present.

The strop consists of a piece of Russia leather stretched over a piece of wood and secured at the ends. The strop is secured to the carrier by dowel pins and may be easily removed; the flesh side of the leather comes in contact with the knife. Any abrasive material may be smeared upon the strop, but experience proves that the best results are obtained by using castor oil in small quantities. The resulting edge is one free from the saw-toothed appearance and may be described as a polished line.

This machine was designed and constructed to do both honing and stropping. In actual use, however, it is found in most cases that a few moments on the honing stone is all that is necessary to prepare the knife for stropping. The practice followed at The Wistar Institute, where the machine has been in constant use for two years, is to give a knife about 10 or 15 minutes hand treatment on the honing stone, and then place it in the stropping machine for 10 to 30 hours.

Where any considerable amount of section cutting is done the time saved in sharpening microtome knives is a very considerable item. The machine was built at The Wistar Institute and may be duplicated by any good machinist. A quarter horse power motor running at 800 r.p.m. is used to drive the machine. The strop makes 25 complete strokes per minute.

## A SIMPLE APPARATUS FOR MICROSCOPIC AND MACROSCOPIC PHOTOGRAPHY

DANIEL DAVIS

*From the Physiological Laboratory, Johns Hopkins University*

### THREE FIGURES

One of the pioneers in photomicrography was Leon Foucault,<sup>1</sup> who in 1844 was the first to make daguerreotypes with the microscope by means of electric light. Since that time many forms of photomicrographic instruments have been designed. All of these cameras, however, can be divided into three types, the horizontal, the vertical, and the convertible, each type possessing advantages peculiar to itself.

Of these three classes of apparatus the horizontal is certainly the oldest and perhaps still the most popular. Its chief advantage is that it allows unlimited bellows extension. This was at first of primary consideration, for in the early days of photomicrography the water and the oil immersion objectives, to say nothing of the apochromat, were unknown. Consequently, the only way of obtaining high magnifications was by means of a comparatively low-power objective used without any ocular, the image being projected a considerable distance onto the plate. This method is still of value when great depth of field is desired. Furthermore, it is somewhat easier to obtain critical illumination with the horizontal camera, since the beam of light can be projected directly into the microscope, obviating the use of the mirror. A simple and efficient camera of this type has been constructed by Parker,<sup>2</sup> while very complete and ingenious machines have been described by Barnard<sup>3</sup> and Buxton.<sup>4</sup>

Owing to its greater compactness the vertical camera is often preferred. With the modern highly perfected lenses great bellows extension is no longer essential for high-power work. This is due to the fact that the image formed by an apochromatic objective can be very greatly enlarged by means of a compensating or projection ocular without losing any of its original sharpness. The vertical camera is of course the only type to be used in photographing specimens in liquid. Perhaps the best camera of this type is the Van Heurck, but a very simple

<sup>1</sup> E. J. Spitta: *Jour. Quekett Micr. Club*, London, 1907-08, n. s. x, 51.

<sup>2</sup> H. B. Parker: *Bulletin No. 7*, of the Hygienic Laboratory, Washington, 1902, p. 7.

<sup>3</sup> J. E. Barnard: *Tr. Jenner Inst. Prevent. Med.*, London, 1899, 2 s, p. 248.

<sup>4</sup> B. H. Buxton: *Jour. Applied Micr.*, Rochester, 1901, vol. 4, p. 1366.

outfit has been described by Borden,<sup>5</sup> while Terras<sup>6</sup> has designed a vertical camera resting on the floor in which the microscope is carried on a very low shelf, thus making possible the convenient use of long bellows extensions. The simplest upright camera is a light box fitting directly on the tube of the microscope. The description of an aluminum camera of this character has recently been given by Wilson.<sup>7</sup>

It is to possess the advantages peculiar to each of the above types that the numerous forms of convertible cameras have been designed, and it is to this type that the machine which I wish to describe belongs. It is obvious, however, that such an instrument can not also possess the many little refinements peculiar to either the horizontal or the vertical form. This machine is the result of three years' experimentation. If it merits a description it is because it is so easily and so cheaply built, and because it is so simple and accurate of manipulation.

The stand consists of two parts, the optical bench, and the camera rest. Each is of the same width, length, and thickness. The optical bench is formed from two pieces of  $\frac{7}{8}$  inch poplar 3 feet long and 3 inches wide. These strips are screwed and glued to two cross-pieces, one at each end, each piece measuring  $\frac{7}{8} \times 3 \times 8\frac{1}{4}$  inches. There are two other cross-pieces only 2 inches wide, equally spaced across the bottom. In order to make the bench still more rigid, a batten measuring 3 feet  $\times \frac{7}{8} \times 1\frac{3}{4}$  inches is fastened along either side. These prevent the bench from sagging. On the top of this bench is a track on which the various auxiliary condensers slide. This track is formed of two similar brass tubes, 3 feet long,  $\frac{5}{8}$  inch outside diameter, and  $\frac{1}{16}$  inch thick. The distance between the centers of these tubes is  $5\frac{1}{4}$  inches.

The camera rest is formed of four poplar strips, all of which are 3 feet long and  $\frac{7}{8}$  inch thick. Two of the strips are 1 inch while the others are 2 inches in width. Only two cross-pieces are used, one at each end. These supports are of exactly the same dimensions as the end cross-pieces fitted to the optical bench. Battens are similarly fastened to the sides, the 1 inch strips coming next to them, the 2 inch strips then being fastened in place with a space of 1 inch between them and the narrower pieces. This will leave just enough room between the center pieces for the bolts holding the camera on the rest, while the tubes on the optical bench fit into the spaces on either side when the stand is folded up. The stand complete is shown in figure 3. It will be seen that the parts are fastened together with bracket hinges, while the camera rest can be clamped in a vertical position by means of oak side braces held in place by small bolts fitted with thumb nuts. The exact dimensions and position of these braces are immaterial, depending somewhat on the size and type of camera used, but the pieces should be slotted at one end as shown, so that to lower the rest to the horizontal position it will be necessary only to loosen the thumb nuts.

<sup>5</sup> W. C. Borden: *Am. Month. Micr. Jour.*, Washington, 1896, vol. 17, p. 193.

<sup>6</sup> J. A. Terras: *Proc. Scot. Micr. Soc.*, London and Edinburgh, 1899-1903, vol. 3, p. 210.

<sup>7</sup> L. B. Wilson: *American photography*, Boston, 1914, vol. 8, p. 204.

Furthermore, it is important to arrange the braces so that the camera may be clamped to either side of the rest. This rest will accommodate nearly any style of plate camera not larger than the  $6\frac{1}{2} \times 8\frac{1}{2}$  inch size with a bellows extension of not more than 3 feet.

The lamp and the various auxiliary condensers are supported on the track of the optical bench by means of geometric slides, as described by Barnard.<sup>8</sup> The slide is made from a piece of  $\frac{7}{8}$  inch poplar  $3\frac{1}{2}$  inches wide and  $10\frac{1}{2}$  inches long. Four inches from one end across the bottom of this piece is cut a V-shaped groove  $\frac{1}{2}$  inch deep. The sides of this groove are at  $90^\circ$  to each other; one of the tubes of the track fits into this groove, and it is by this means that the slide is kept in alignment.

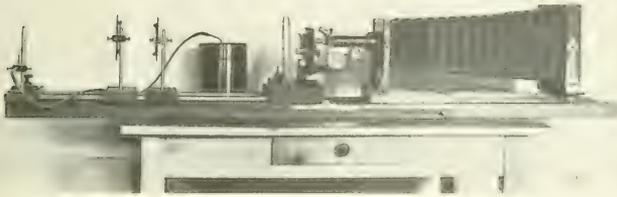


Fig. 1 Camera in horizontal position. The U-shaped frame on the microscope table is used to support the ray filter. The condensing lenses, a plano-convex and the meniscus, are arranged for high-power work.

The other end of the support resting on the other tube is cut away till the slide sits level upon the track. There is, of course, one such support for each lens that is to be carried on the optical bench, the lens being attached to an upright rod of convenient length fastened to the slide, as shown in figure 2. I have found it convenient to use three lenses, all 4 inches in diameter. Two are plano-convex of 12 inches focal length, while the other is a double convex lens of 18 inches focal length, thus forming a simple Kohler condensing system, as suggested by Barnard.<sup>8</sup> These lenses are mounted on  $\frac{1}{8}$  inch sheet iron rings  $5\frac{1}{2}$  inches in diameter with a  $3\frac{3}{4}$  inch hole in the center, the lens being supported by three equally spaced machine screws fitted with washers and short spiral spring sleeves. A slotted 6 inch iron rod of the same diameter as the upright on the slide (in this case  $\frac{3}{8}$  inch) is riveted radially to the ring, this rod being fastened to the upright by means of an adjustable clamp.

<sup>8</sup> J. E. Barnard: Practical photomicrography, Edward Arnold, London, 1911.

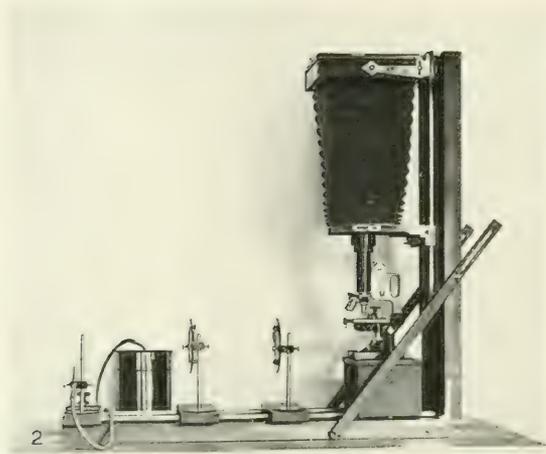


Fig. 2 Camera in vertical position. The two plano-convex condensing lenses arranged for medium-power work. For low-power work the meniscus lens is substituted for the plano-convex lens nearer the light.

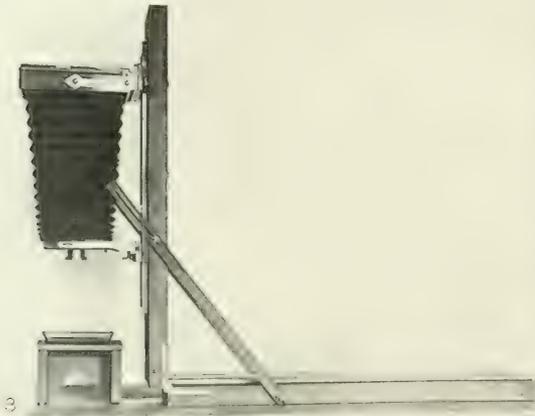


Fig. 3 Camera fitted with a wide angle lens and fastened to the back of the rest for the photographing of embryos.

Acetylene has proved an excellent illuminant. The light is very actinic, and perfectly steady. A  $\frac{1}{2}$  foot burner is ample, and the gas can readily be made in any simple generator. The gas should be passed through a fairly large bottle before being fed to the burner. This serves to maintain a steady pressure as well as cooling the acetylene and allowing the condensation of water. Such a light used with the condensers just described has proved ample for magnifications of over 1000 diameters.

The microscope is supported on a table, the legs of which just straddle the track on the optical bench to which this table is clamped. Having been properly centered, the microscope is fastened in position by means of an oak strip extending across the horseshoe base. This strip is bolted to the microscope table. Two small blocks are attached to the table, as shown in figure 1. These fit snugly against the side of the horseshoe base, serving as guides to the correct position of the microscope should it be removed from the stand. All fastenings used on this table should of course be fitted with thumb nuts. The height of the table must be such as to cause the optical axes of the microscope and of the camera to coincide. The condensing lenses and burner are then adjusted. When the camera is placed vertically another table is used of such a height as to make the optical axis of the condensing system center upon the microscope mirror. The condensers thus need no readjustment when changing the camera from the horizontal to the vertical position.

In use this outfit has proved very satisfactory. When in the horizontal position the field can be examined by sliding back the camera front, or perhaps more conveniently by removing the microscope from the stand. By means of the guide blocks the microscope can readily be replaced in correct alignment. When using the greatest bellows extension, focusing is accomplished by means of a waxed silk cord passing around a little pulley fitted to the knob of the fine adjustment. When used vertically the microscope, once having been adjusted, is not moved. If it is desired to examine the field it is but necessary to raise the camera front a few inches and then lower the camera rest out of the way into the horizontal position. When ready to photograph the camera can quickly be swung up over the microscope. Finally, by clamping the camera to the back of the stand, as shown in figure 3, and using a photographic lens of short focal length, a most convenient arrangement is obtained for copying drawings, or photographing embryos or other macroscopic specimens.

## INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with Vol. 18, price per volume, \$7.50; foreign, \$8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price per volume, \$5.00; foreign, \$5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, beginning with Vol. 25, price per volume, \$7.50; foreign, \$8.00.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

36TH STREET AND WOODLAND AVENUE

PHILADELPHIA, PA.

# PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

## THIRTY-FIRST SESSION

*At the Washington University Medical School, St. Louis, Mo.,  
December 28, 29 and 30, 1914*

MONDAY, DECEMBER 28, 9.30 A.M.

The thirty-first session of the American Association of Anatomists was called to order by President G. Carl Huber, who appointed the following committees:

*Committee on Nominations:* G. S. Huntington, chairman; Irving Hardesty, Florence R. Sabin.

*Auditing Committee:* T. G. Lee, chairman; A. T. Kerr.

President G. Carl Huber, in recognition of the great loss the Association had sustained in the death of Professor Minot, suggested that some arrangement be made for expression from the Association. Prof. F. T. Lewis moved that the following committee be appointed to draw up appropriate resolutions: Chairman, Prof. George S. Huntington; Members, Professors G. Carl Huber and R. J. Terry, the resolutions to be presented at a future meeting of the Society.

TUESDAY, DECEMBER 29, 12.00 M. ASSOCIATION BUSINESS MEETING, PRESIDENT G. CARL HUBER, PRESIDING.

The Secretary reported that the minutes of the Thirtieth Session were printed in full in *The Anatomical Record*, volume 8, number 2, pages 69 to 145, and asked whether the Association desired to have the minutes read as printed. On motion, seconded and carried, the minutes of the Thirtieth Session were approved by the Association as printed in *The Anatomical Record*.

Prof. T. G. Lee reported for the Auditing Committee as follows: The undersigned Auditing Committee has examined the accounts

of Dr. Charles R. Stockard, Secretary-Treasurer of the American Association of Anatomists, and finds the same to be correct, with proper vouchers for expenditures, and bank balance on December 23 of \$285.85. (Signed) T. G. LEE, A. T. KERR; St. Louis, Mo., December 29, 1914.

The Treasurer made the following report for the year 1914:

<i>Account of G. Carl Huber, former Secretary-Treasurer, closed January, 10, 1914</i>			
Balance on hand December 26, 1913, when accounts were last audited.....	\$213.03		
Collections made from December 26 to January 10, 1914.....	55.00		
Total deposit.....	\$268.03	\$268.03	
Expenses of Secretary-Treasurer attending Philadelphia Meeting, December 29-31, 1913.....	\$49.00		49.00
Balance sent by draft to Charles R. Stockard, Secretary-Treasurer.....			\$219.03
Amount of draft.....	\$219.03		
Receipts for dues, 1914.....	1520.20		
Total deposits for 1914.....	\$1739.23	\$1739.23	
Expenditures for 1914:			
Postage (\$45.42), printing (\$34.00).....	\$79.42		
To 305 subscriptions to 1 volume of the American Journal Anatomy and 1 volume of the Anatomical Record @ \$4.50	1372.50		
To collections and exchange on foreign and domestic drafts	1.46		
Total expenditures.....	\$1453.38	\$1453.38	
Balance.....			\$285.85
Balance on hand, deposited in the name of the American Association of Anatomists in the Corn Exchange Bank, New York City, December 23, 1914.			

On motion the reports of the Auditing Committee and the Treasurer were accepted and adopted.

The Committee on Nominations, through its Chairman, Prof. George S. Huntington placed before the Association the following names for members on the Executive Committee for terms expiring in 1918: J. L. Bremer and H. von W. Schulte.

On motion the Secretary was instructed to cast a ballot for the election of the above named officers.

The Secretary presented the following names recommended by the Executive Committee for election to membership in the American Association of Anatomists:

WAYNE JASON ATWELL, A.B., Instructor in Histology, 1335 Geddes Avenue, Ann Arbor, Michigan.

- HENRY K. DAVIS, A.B., A.M., Instructor in Anatomy, *Cornell University Medical College, Ithaca, New York.*
- ARNOLD HENRY EGGERTH, Assistant in Histology, *University of Michigan, Ann Arbor, Michigan.*
- J. F. GUDERNATSCH, Ph.D., Instructor in Anatomy, *Cornell University Medical College, New York City.*
- ELMER R. HOSKINS, A.B., A.M., Instructor in Anatomy, *University of Minnesota, Minneapolis, Minnesota.*
- CHARLES EUGENE JOHNSON, Ph.D., Instructor in Comparative Anatomy, *Department of Animal Biology, University of Minnesota, Minneapolis, Minnesota.*
- BEVERLY WAUGH KUNKEL, Ph.B., Ph.D., Professor of Zoology, *Beloit College, Beloit, Wisconsin.*
- PAUL EUGENE LINEBACK, A.B., M.D., Teaching Fellow in Histology and Embryology, *Harvard Medical School, Boston, Massachusetts.*
- C. C. MACKLIN, M.D., Assistant in Anatomy, *Johns Hopkins Medical School, Baltimore, Maryland.*
- WILLIAM ELI McCORMACK, M.D., Instructor in Embryology and Histology, *University of Louisville, Louisville, Kentucky.*
- D. GREGG METHENY, M.D., *Jefferson Medical College, Philadelphia, Pa.*
- ROY L. MOODIE, A.B., Ph.D., Assistant Professor of Anatomy, *University of Illinois, Chicago, Illinois.*
- HENRY R. MULLER, M.D., Assistant in Anatomy, *Johns Hopkins Medical School, Baltimore, Maryland.*
- JAY A. MYERS, A.B., Ph.D., Instructor in Anatomy, *University of Minnesota, Minneapolis, Minnesota.*
- H. W. NORRIS, B.S., A.M., Professor of Zoology, *Grinnell College, Grinnell, Iowa.*
- JAMES WENCESLAS PAPEZ, B.A., M.D., Professor of Anatomy, *Atlanta Medical College, Atlanta, Georgia.*
- FRANKLIN P. REAGAN, A.B., *Princeton University, Princeton, New Jersey.*
- RANDOLPH TUCKER SHIELDS, A.B., M.D., Dean, *University of Nanking, Medical School, Nanking, China.*
- P. A. WEST, B.A., *Johns Hopkins Medical School, Baltimore, Maryland.*
- HARRY OSCAR WHITE, M.D., Professor of Anatomy, Histology and Embryology, *Medical Department, University of Southern California, Los Angeles, California.*
- JOHN LOCKE WORCESTER, M.D., Instructor in Anatomy, *University of Michigan, 1214 Willard, Ann Arbor, Michigan.*

On motion the Secretary was instructed to cast a ballot for the election of all the candidates proposed by the Executive Committee. Carried.

The following proposed amendment, having been a matter of record at the last meeting, was presented for action by the Association. "These officers shall be elected by a ballot at the annual meeting of the Association and their official term shall commence with the close of the annual meeting."

“At the annual meeting next preceding an election, the President shall name a nominating committee of three members. This Committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual meeting. Additional names for any office may be made in writing to the Secretary by any five members at any time previous to balloting.”

The Association voted the adoption of this amendment.

The following proposed amendment, also recorded at the last meeting, was presented for action by the Association: Amendment of Article VI of the Constitution. The first sentence of the article “the annual dues shall be \$5.00,” it is proposed to amend to read “the annual dues shall be \$7.00.”

The Association voted the adoption of this amendment.

It was pointed out by Dr. M. J. Greenman, Director of The Wistar Institute, that since the dues of the Anatomists were now advanced to \$7.00 per year, the members of the Association would receive all numbers of The American Journal of Anatomy and The Anatomical Record, six numbers of the Journal of Anatomy and twelve numbers of the Record yearly.

The special Committee on Pre-Medical Work in Biology presented through its chairman, Dr. H. McE. Knowler, the following report:

Your Committee was appointed to confer with the Zoölogists to ascertain what coöperation may be expected toward standardizing work in Biology required of students looking forward to the study of medicine; and to formulate the considerations which would seem practical to incorporate in plans for such courses.

The Zoölogical Society promptly appointed a committee for this conference, and the following questions were discussed, not only with this committee, but with a number of other representative members of the Zoölogical Society. Besides this, published statements of courses and of discussions on this subject were examined.

The following questions seemed to be most important:

Question 1. Is the work given in different colleges in the elementary, general course in Biology adapted to satisfy the requirements of pre-medical training in this subject?

Question 2. Is it possible to so select and standardize the work of the first year in Biology in different colleges as to make it uniform, and to include, here, all needed to make it *an adequate course*?

Question 3. If an ideal course, including sufficient preliminary work can not be secured within the one year period advocated, what principles should be urged to govern the planning of the biological work of students looking forward to the study of medicine, so that they will profit most by the training of the first year, and be best prepared to follow this up in special departments of Biology more directly related to medicine.

Question 4. What additional work is to be advised, which is not to be obtained in the first year's general course?

Both committees agree that it is of the first importance to urge the selection of only thoroughly trained scientific men as teachers for this work. Such men can be trusted to insist on real scientific methods and to select the best material and treatment to give the beginner a practical introduction and basis for further work.

Beyond this point, however, the committees were unable to proceed. The Zoölogists suggested that the Anatomists should draw up a statement of what they desire the Zoölogists to do, in preparing students for anatomy. After this has been done, the Zoölogists are ready to consider how far it is practical to meet these needs. Several attempts have been made in this direction, and your committee submits the following statement to the Association for its approval and transmission to the Zoölogists.

At the present time a one-year's course in biology is generally required as a preparation for the work of the medical school. This study of biology must serve as a preparation for medical work in physiology, pathology, bacteriology and parasitology, as well as anatomy, and it may fairly be questioned whether a single college course is adequate for this purpose. The study of botany alone is obviously insufficient, and the domain of zoölogy is so vast that much care should be exercised in the choice of the phases of the science to be presented to young students. Courses which are primarily experimental and deal with the functions and reactions of animals, although excellent in preparation for the physiological work of the medical school, are not the proper basis for the study of human anatomy. It is the purpose of this report to point out only those features of the college preparation which experience has shown to be desirable, and in fact essential, for the successful study of gross and microscopic anatomy.

No uniform or stereotyped preparatory course is recommended, for it is recognized that every teacher should give special attention to those subjects and groups in which he is particularly interested, and to the knowledge of which he has contributed by his own researches. Success depends in large part upon the ability of the teacher, but the following purposes of instruction should not be forgotten if the preparatory work is to satisfy the requirements of anatomy.

1. Students frequently begin the study of human anatomy with an insufficient knowledge of the lower forms of animal life. The broad knowledge of the various classes of animals and of invertebrate and lower-vertebrate morphology, which was the inspiration of the great anatomists of the past, is now too often replaced by vague considerations of the method of science and ideals of observation. A return to the study of animals, as objects of interest in themselves, apart from theoretical considerations and possible relations to human society, is therefore recommended. The student should obtain a synoptic knowledge of the animal kingdom, and should be able to classify in a general way, and to describe the life histories of the common forms of animals, aquatic and terrestrial, which may be collected in his locality. A beginning in such work may well be made by the student independently, or perhaps in high-school courses, but such fragmentary and elementary studies should be supplemented by a thorough college course. The first-hand familiarity with animals thus obtained should serve as the basis for all further work.

2. As a result of the knowledge of genera and species which the student should have obtained directly for himself, by studying some group of animals or plants; questions of the origin of species and of the relation of the great classes of animals to one another are inevitably before him as philosophical problems. Collateral reading then becomes as necessary for the biologist as for the man of learning in any other branch of knowledge. Selected works of Lamarck, Darwin, Huxley, Mendel, and others should be freely consulted. This literature, which in its influence upon human thought has far outspread the bounds of biology, should not be neglected by the student of zoölogy, whose particular heritage it is. Since the idea that science cannot be read, and that there is no knowledge in books, is often taught as a cardinal principle, it has come about that students of zoölogy have little knowledge of, or respect for, the writings of the makers of their science.

3. Before beginning the study of human histology, every student may reasonably be expected to be familiar with the use of the microscope and with the simpler methods of preparing specimens for microscopic examination. This technique can be learned in connection with various courses, perhaps the most useful of which is a general study of the cell with a comparative study of the elementary tissues. The maturation of the germ cells and the processes of fertilization and segmentation cannot be properly presented in the medical curriculum, and these fundamental biological phenomena should therefore be observed in college courses. The development of the chick, which was studied primarily by physicians to explain the growth of the human embryo, can likewise receive little attention in the medical school. These subjects are all very desirable in themselves, and if studied by laboratory methods, will supply the requisite skill in the use of the microscope.

4. In preparing for human dissection, comparative anatomy should be studied with the same standards of thoroughness which obtain in



animals and their life histories. While the Anatomists in adopting this statement as given in the report, undoubtedly, expect the physiological aspects of these mechanisms to be considered as necessary accompaniments of such first hand familiarity with animals; it is urged in the report that the introductory college course shall not be "primarily physiological." It is earnestly desired that the work should involve a rigorous grounding in comparative morphology, especially of lower forms, which furnishes not only the best basis for human anatomy, but is a very essential preliminary for comparative and human physiology. (b). It is urged that the theoretical and philosophical considerations which accompany the course shall follow a practical acquaintance with animals; rather than that special animal structures should serve chiefly as illustrative material for lectures on general biological theories, with a neglect of a thorough study of a series of animal forms. (c). The additional principles which should govern the planning of the introductory course, beyond those just stated, are: The selection of suitable teachers; The undesirability of attempting to establish a uniform preparatory course, or courses especially limited to applications to medicine; The acquirement of skill in the use of the microscope, and of correct scientific method of work in connection with the work of the course; The beginnings of embryology and cytology.

4. As to the last question, number 4, the report does not attempt to decide what proportion of the recommended preparation for anatomy can be obtained by a student in the first year's course. This must be indicated by the zoölogists. It seems evident to a student of present conditions, however, that most of the work desired in cytology and comparative, general histology; comparative anatomy of vertebrates; or systematic zoölogy will have to be elected by students looking forward to medicine, after they have taken the introductory course. It is to be hoped that the elements of vertebrate embryology will be included in that course. Some of this work may well be done in one of the excellent Summer Laboratories.

5. Finally, the importance of collateral reading in the masterpieces of biological literature is strongly emphasized.

H. McE. KNOWER, *Chairman*

On motion, the report as presented was adopted and the Committee was continued and instructed to confer with the Committee of the Zoölogists on the basis of the report.

At the final session the Committee for Resolutions on the death of Professor Minot presented through its chairman, Prof. George S. Huntington, the following:

The American Association of Anatomists, assembled in the Thirty-First Session at St. Louis, record their profound sense of sorrow and their deep feeling of loss sustained by the death of Prof. Charles Sedg-

wick Minot of Harvard University: For many years Doctor Minot has stood for the best development of science and medical education both in this country and abroad. He has been particularly identified with the progress of the American Anatomical Association. In his official connections as President and Member of the Executive Committee his brilliant and constructive mind guided the affairs of the Society with marked success. Much of the progress of The Wistar Institute of Anatomy originated in his keen executive ability as Chairman of its Advisory Board. He was one of the founders of The American Journal of Anatomy and of its offspring, The Anatomical Record. His colleagues realize that these first American anatomical publications owed their success during their early and experimental years largely to his judgment and wisdom. Later he became most active in establishing and maintaining the eminently valuable relations now existing between the journals and the Publication Department of The Wistar Institute. These are mere outlines of a few of the more formal points of contact between Doctor Minot and the Anatomical Association. Important and far-reaching as these have been, and lasting as their impress will prove in the future development of the Society, they are fully equalled in value by the influence of his marked personality on the individual life and work of the members with whom he came in closer contact. Keen and yet considerate judgment, kindly help, both with advice and material, were freely extended by him. The Association, in mourning the loss of a stimulating leader, of a wise counsellor and of a personal force which has always directed forward the lone advance of national science, directs the following resolutions:

1. That the foregoing minute be published in the Proceedings of the Thirty-First Session and that the Secretary be requested to forward a copy to Doctor Minot's family.

2. That Prof. Frederick T. Lewis of Harvard University be requested to prepare, on behalf of the Association, a memorial of Doctor Minot's personal and academic life, with full consideration of his educational and scientific achievements, for publication in The Anatomical Record.

Before adjournment it was voted: That this Association extend a vote of thanks to Washington University, Professor Terry and his Staff, our hosts at this session, and of congratulations to them on the completion of their carefully planned and admirably equipped Institute of Anatomy.

CHARLES R. STOCKARD,

Secretary of the Thirty-First Session, of  
the American Association of Anatomists



## ABSTRACTS OF PAPERS

1. *The rhinencephalon of the dolphin* [Delphinus delphis] WILLIAM H. F. ADDISON, University of Pennsylvania.

In the adult dolphin, the olfactory bulbs and tracts are lacking, and that portion of the mesethmoid, which corresponds to the cribriform plate of the ethmoid of the ordinary mammal is imperforate. Thus the dolphin is entirely anasmotie, and it has been interesting to study the more centrally placed parts of the rhinencephalon, to see in them the extent of the regression which has accompanied the disappearance of the olfactory tracts and bulbs.

During the past summer, I had the opportunity of examining thin sections of the brain of an adult dolphin in the Frankfurt Neurological Institute, under the direction of Professor Edinger, to whom I am greatly indebted.

In addition to the lack of olfactory bulbs and tracts, the olfactory cortex of the basal surface of the frontal lobe is also wanting. At this region the corpus striatum of each side comes to the surface and protrudes as a convex oval area. This area, smooth and free from fissures, was named *lobe désert* by Broca. The parolfactory cortex is also much reduced, but at least some definite remains of it are seen. This is interesting in the light of Edinger's view, that the tuberculum olfactorium is not a part of the olfactory system, but is the end-station of tracts conveying impulses by way of the fifth nerve from specialized sensory structures in the snout region. To the sense, which this mechanism serves, he has given the name of 'oral sense.'

Of the several connections of the olfactory and parolfactory cortical cells with the hippocampus, only Zuckerkandl's bundle is definitely present. The fimbria is a slender band of fibers arising from the hippocampus. True fornix fibers are not seen, and in the usual region of the corpora mammillaria there are no well-developed rounded protuberances, and in the gray tissue of this region are seen no medullated nerve fibers. This would indicate that the tractus cortico-mammillaris is very small or perhaps lacking. There is the usual arrangement of a psalterium or crossing of fibers between the two hippocampi. Indeed, the psalterium is so well developed that the possibility is suggested that it may contain other fibers in addition to the commissural hippocampal fibers. The anterior commissure is much reduced, evidently the olfactory portion being entirely lacking.

Of the other possible connections of the olfactory and parolfactory cortical cells, both the taenia thalami and the taenia semicircularis are seen, as are their respective end-stations, the ganglion habenulae and the nucleus amygdalae. The hippocampi are very degenerate small

structures, and it is with difficulty that one sees the analogy with even the microsmatic type of hippocampus.

Thus, in the brain of the dolphin, accompanying the loss of the olfactory bulbs and tracts, there is found a recession of the frontal cortex, exposing the corpus striatum over a considerable area; loss of the olfactory portion of the anterior commissure; great diminution of the hippocampus, and reduction or loss of the uncrossed fibers from it to the corpora mammillaria (tractus cortico-mammillaris or true fornix); also, the usual connections between the olfactory cortex and the hippocampi are lacking except Zuckerkandl's bundle, and the corpora mammillaria are greatly reduced.

2. *The artificial production of spina bifida by means of ultra-violet rays.*

W. M. BALDWIN, Department of Anatomy, Cornell Medical College, New York City.

The purpose in presenting this paper is two-fold: first, by reason of the method employed the condition of spina bifida in tadpoles may be produced at will and the level of the bifurcation of the neural tube predetermined; second, the method gives considerable insight into the developmental potentials of the various portions of the ovum, and, in this instance, of the nature and location of the neural tube formative substances. This method consists in the illumination of small surface areas of the fertilized ovum of the frog by means of ultra-violet light of intensity sufficient to kill the area exposed in from 10 to 30 seconds.

It was found that in the undivided ovum the chemical organ-building substances, 'ferments,' or proanlagen, of the neural tube are neither located in any portion of the yolk hemisphere, nor along the equator. These proanlagen occupy a superficial position well up on the surface of the pigmented hemisphere of the egg and attain their definitive extent and position by a process of backward migration or differentiation, keeping pace in this migration with the corresponding shifting of the dorsal lip of the blastopore. These two processes occur synchronously, so that when the rate of the latter is interfered with (as from the presence of an area of dead yolk), the neural tube proanlagen differentiate into half anlagen and then half tubes at approximately their former rate of backward progression, but now along a line corresponding to the equator of the egg and not, as is usual, along the median plane of the egg. The yolk mass is finally completely drawn into the body of the embryo, and as a result the two neural-tube halves are approximated to a greater or less degree. Subsequent fusion of the tube-halves does not, however, occur. Each half-tube by a later shifting of its cell becomes a whole tube.

The experiments add one more fact towards the establishment of the conception of the egg as a composite structure containing, from the first, the organ-building substances of the various body systems, restricted to more or less definitely localized areas in the egg substance (mosaic theory of Roux). Furthermore, the conclusion seems justifiable, tentatively, at least, that the various chemicals such as salts of sodium and of lithium, which have been used by Morgan, Hertwig,

Herbst, Jenkinson and others in the production of this malformation, have, at least, produced their effect by acting upon portions of the yolk hemisphere and not necessarily upon the proanlagen restricted to the pigmented hemisphere.

C. R. BARDEEN, see abstract 57, page 137.

3. *Some effects of mammalian thyroid and thymus-glands upon the development of Amphibian larvae.* G. W. BARTELMIEZ, Department of Anatomy, The University of Chicago.

The following data are taken from some attempts made with a view to analyzing the reactions reported in the feeding experiments of Guder-natsch and others. They are based on experiments upon larvae of *Amblystoma tigrinum* and *Rana catesbiana* treated with sheep's thyroid and thymus in three different ways, appropriately controlled. (1) Using the glands as food. (2) Feeding normally but adding saline extracts of the glands to the water of the culture dishes. (3) Injecting strong extracts into the coelom or dorsal musculature.

*Experiments with thyroid gland. Amblystoma: Effects of feeding (spring, 1913).* *Amblystoma* is a favorable form in that the larvae can be hatched and reared in the laboratory, that they are fairly hardy, more especially that they are carnivorous and the effects of feeding can be studied separately from those resulting from suspensions of the glands in the culture water. When exclusive thyroid feeding was begun soon after hatching (12 to 15 mm. larvae) there was a high mortality but the survivors showed only the effects of a meagre diet such as was obtained by feeding egg albumen or by starving. They grew little or not at all and the limbs did not differentiate as rapidly as in the controls. No clear cut results were obtained unless the larvae were at least 30 mm. long, had three or four fingers and leg buds. In these cases a few individuals survived to undergo partial metamorphosis. Still older larvae, if they were well nourished, after two or three feedings of thyroid underwent metamorphosis in the course of eleven to sixteen days. Fairly normally constituted adults were thus obtained from 35 to 80 mm., long whereas the normal in this vicinity at the time of metamorphosis is from 130 to 150 mm. long. My conclusions from these observations are that the thyroid feeding does not stimulate differentiation in *Amblystoma* since the differentiation of the limbs is not accelerated but it does bring about certain changes in the gut which in turn induce other changes characteristic of metamorphosis.

*Amblystoma: Effects of thyroid extracts (spring and summer, 1914).* In these experiments the evil effects of an unnatural food were eliminated by giving the larvae first entomostraca and then anuran tadpoles as food and between the feedings adding the extract to the water in which they were living. Starting with larvae 15 to 17 mm. long, after six treatments in the course of six weeks no differences were noted as compared with the controls. During this time they grew and differentiated normally, reaching a length of from 20 to 40 mm. Continuing the treatment for two and a half months, small adults were obtained. Begin-

ning with older larvae the process was somewhat more rapid. The chief differences between these and the feeding experiments lie in the lowered mortality and the more complete metamorphoses in the former set. Both agree in that the thyroid has no specific effect until the larva has reached a definite minimal stage in development and in that the metamorphosis is not wholly normal.

*Amblystoma: Effects of hypodermic injections (summer, 1914).* The results of injecting small doses of strong extracts of thyroid were somewhat variable, largely no doubt because in different cases different proportions of the injection oozed from the wound. Animals under 50 mm. in length gave no signs of metamorphosis before death. In older ones two doses were sometimes necessary to start the process and then it began within four to seven days and was completed in fourteen to thirty days: a period somewhat longer than normal.

*Rana catesbiana: Experiments with thyroid gland.* The results with bull frog tadpoles were complicated by various factors and only a few experiments can be summarized here. The reaction varied according to the stage of development of the larva, its age (1st, 2nd or 3rd season), the length of previous confinement in the laboratory and the season of the year in which the treatment was begun. The individual resistance was also variable and this was a factor as the supply of material was limited and each tadpole was measured and observed until its death.

*Effects of feeding.* Larvae fed only twice with thyroid and the rest of the time with lymph node reacted like those fed exclusively on thyroid and as the death rate was lower, the former treatment was used in most cases. In larvae of all ages five or six days after the first feeding the body became more slim than in the protein fed controls. This was due to the marked reduction in the length and in the position of the spiral gut. After this differences were noted which depended upon the stage of development reached by the animal at the time thyroid feeding was begun. If the legs had developed so far that the toes were differentiated at time of first feeding, the legs grew as they do shortly before metamorphosis, but true metamorphosis did not set in until after six or seven weeks. Some of this group, however, developed a marked resistance to the thyroid. To cite a single case: An individual which had been accustomed to a lymph node diet and then to thyroid by six feedings between January 19 and April 7, was fed weekly with thyroid until May 21 (seven times) then on alternate days twelve times, died half way through metamorphosis on June 16. In this and the cases mentioned above the gut reduced at a faster rate than it does normally. This fact is accentuated by the following class of cases. When thyroid feeding was begun before the larva had gone beyond the stage of leg buds a peculiar kind of metamorphosis was brought about. The gut shortened and assumed practically the adult condition, the head showed some signs of metamorphosis but there was no reduction of gills, little reduction of the tail and no more development of the leg buds than in the control.

The results of treatment with thyroid extract and the injection of thyroid suspensions in general confirmed these results with feeding.

Upon the assumption that lymph node tissue is similar as a food to thymus, but that it has no internal secretion, some series of experiments were made with these two tissues using them both as foods and as extracts in the culture dishes. In *Amblystoma* they gave practically identical results in all feeding experiments—and there was no proof of a retardation of development. Larvae treated with thymus extract metamorphosed normally but sooner and when the animals were smaller than the controls. Furthermore, the hypodermic injection of thymus extract did not retard or inhibit metamorphosis in the larger larvae. In *Rana* the feeding of both thymus and lymph node produced more rapid development than was observed in the plant fed control.

4. *The development of the sympathetic nervous system in Elasmobranchs.*

GEO. A. BATES, Tufts College Medical School, Boston, Mass.

The first appearance of the sympathetic in *Squalus* is in the form of a series of ganglia lateral to the aorta in 15 mm. embryos. At the time of its formation the dorsal and ventral roots of the somatic spinal nerves, in their ventral growth, have reached this level. The ganglion of the sympathetic is formed in immediate connection with the dorsal root, from cells that arise from this source. It appears as a cluster of cells attached to the dorsal root and embedded in a protoplasmic mass quite distinct from the surrounding mesenchymal cells.

In embryos of from 7 to 8 mm. cells of the ventro-lateral wall of the neural tube have begun to migrate into the space between the tube and myotome. At the same time mesenchymatous cells from the disintegrating sclerotome migrate dorsally into the same region. The medullary cells are easily distinguishable from the sclerotomic cells in sections prepared by the vom Rath method. These cells arrange themselves along the margin of the myotome and are distinctly marked off from the surrounding cells.

There are no medullary cells among the cells of the mesenchyma. In the latter, however, two varieties of cells are present; one reacting to the basic stain quite intensely, the other less so. These facts are demonstrated in sublimate fixed, hematoxylin stained sections, and particularly in sections stained with iron hematoxylin.

Such cells are present throughout the mesenchyma and at points where neither the ventral root cells nor cells from the dorsal crest have begun their migration.

The claim that the deeply staining cells in the mesenchyma are medullary cells which subsequently will become incorporated into the sympathetic ganglion, seems unwarranted for the following reasons:

At this stage, 7 to 8 mm. there is no sign of the formation of the sympathetic ganglion. At the level of the aorta and lateral to it a mass of sclerotomic cells may be seen and it is here that the two sorts of cells, above mentioned, are found.

The difference in staining property seems to be the result of the presence or absence of chromatin due, probably, to the state of activity of the cell. Such conditions have frequently been observed in mesodermic cells in the formation of the liver, and also by various observers in other regions. In other words, the difference in staining properties of cells in the region of the dorsal aorta affords no foundation for the inference that the more deeply staining cells are destined to become sympathetic cells. On the contrary, they are mesodermal in their origin and are not genetically related to the sympathetic.

As above stated, the sympathetic ganglion is developed directly from the dorsal root of the somatic spinal nerve at a stage of about 35 to 17 mm. At the time of development there are relatively few cells present in the motor root, and the question of contribution to the ganglion of cells from that source, while not improbable, is doubtful.

5. *The growth of the head and face in American (white), German-American, and Filipino children (lantern and photos).* ROBERT BENNETT BEAN, The Tulane University of Louisiana.

Materials:

146 Filipino girls	}	Manila, Philippine Islands
579 Filipino boys		
309 German girls	}	Ann Arbor, Michigan
324 German boys		
412 American girls		
415 American boys	}	

2185, total

*The growth of the head diameters (length, breadth and height).* Between the ages of 6 and 16 the head grows in length least, 0.9 cm., in the American girls, and most, 1.6 cm., in the Filipino boys; in breadth least, 0.5 cm., in the American girls, and most, 1.1 cm., in the German boys; and in height least, 0.5 cm. in the German and American girls, and most 1.1 cm. in the Filipino boys. The heads of the Filipinos grow more rapidly in length between 6 and 11 years of age than between 11 and 16 years of age, whereas the heads of the Germans and Americans grow more rapidly in the latter than in the former period. What is true of the Germans and Americans in relation to the Filipinos is also true of the boys in relation to the girls.

The head size as represented by the module (length plus breadth plus height) increases least, 19 points, in the American girls and most 35 points in the Filipino boys.

At 6 years of age the heads of the Americans of both sexes are the largest, the heads of the Filipinos are the smallest, and the heads of the Germans are nearly as large as those of the Americans. At 16 years of age the heads of the Filipinos are the smallest, and the heads of the Americans are nearly as large as those of the Germans.

The cephalic index decreases with age for the length-breadth index least, 0.0, for the Filipino girls, and most, 3.3, for the Filipino boys;

and for the length-height index least, 0.4. for the Filipino boys, and most, 2.7, for the German girls.

*Growth of head circumferences* (frontal, forehead, parietal and occipital.) The forehead and occipital regions are large in the boys and in the Americans, the frontal and parietal regions are large in the girls and in the Germans and Filipinos. The forehead and frontal regions together are large in the girls and in the older children, and the occipital and parietal regions together are large in the boys and in the younger children.

From 6 to 16 years of age, the forehead, frontal, and parietal regions grow most in the Filipinos, less in the Germans, and least in the Americans, but the reverse is true of the occipital region. The forehead, frontal and occipital regions grow more in the boys than in the girls, and this is especially true of the occipital region, whereas the parietal region grows more in the girls than in the boys.

It is notable that, in relation to each of the other regions, the forehead increases in size and the parietal region decreases with age.

The large size and greater growth of the parietal region are characteristic of the girls and of the young children, and the large size and greater growth of the occipital region are characteristic of the boys and of the older children. The Filipinos resemble the girls in this respect, and the Americans resemble the boys, whereas the Germans are more or less intermediate.

The Hypo-types are like the Filipinos, the Hyper-types are like the Americans and the Meso-types are like the Germans.

*The growth of the face* (length, breadth and facial angle): The growth of the face as a whole may be considered by taking the product of the length and breadth. From this standpoint the growth from 6 to 16 years is least in the Filipino girls, greatest in the American boys, with the others in between, the boys greater than the girls. The face increases about 33 per cent in the girls and about 50 per cent in the boys during the ten year period.

The face length increases with age about 2 cm. in 10 years. The girl's face grows more from 6 to 11 years and the boy's from 11 to 16 years. The face of the Filipino is shorter than that of the German and American, about 1 cm. at 16 years and about 0.3 cm. at 6 years. The face of the Filipino grows less in length than that of the German and American from 6 to 16 years.

The face breadth increases with age from 11.3 cm. at 6 years to 13.1 at 16 years. The face breadth of the girls grows more rapidly from 6 to 11 and that of the boys from 11 to 16. The face of the Filipino is as broad as that of the German and broader than that of the American, and the growth of face is about the same in breadth for the three peoples.

The face index increases with age, the face becomes longer relative to its width, and this increase is greatest in the Americans, less in the Germans, and least in the Filipinos. The increase in the Germans is greatest from 6 to 11 years and in the Americans it is greatest from 11 to 16 years.

The facial angle represents the projection of the maxilla, and with

increase of age this is greater in the American boys and less in the Filipino boys than is apparent in the German and American girls and the German boys. The Filipino girls have no records made of the facial angle.

*Cephalo-facial index* (originated by the author). This represents the size of the face in terms of the head, the latter always 100. The face grows relatively more than the head from 6 to 16 years, relatively more from 6 to 11 in the Germans and Americans and relatively more from 11 to 16 in the Filipinos. At 6 years the Filipinos have relatively the largest faces, and the Americans relatively the smallest, with the Germans in between, at 11 this is reversed, and at 16 all are about the same.

The cephalic index decreases with age, and it decreases from the Filipinos through the Germans to the Americans. The face index increases with age, and it increases from the Filipinos through the Germans to the Americans. If the process of development recapitulates the progress of evolution then the Americans represent in evolution what the adult represents in development, and the Germans and Filipinos are less mature stages. The Filipinos represent what I have called Hypo-phylo-morphs, the Germans Meso-phylo-morphs (?) and the Americans Hyper-phylo-morphs. In each group may be found adult individuals with varying degrees of development in head and face form, and these I would classify as Hypo-onto-morph, Meso-onto-morph, and Hyper-onto-morph, depending upon the extent of development. Crossing of races has introduced the phylo-types into nearly all peoples, therefore the six forms may be distinguished among almost all mixed races. Among the white peoples the Hypo-types are rare, but among the Filipinos the Hyper-types are abundant. More white peoples have mixed with the Filipinos than Filipinos with the white peoples.

6. *Some ears and types of men.* (*Lantern and photos*). ROBERT BENNETT BEAN, The Tulane University of Louisiana, New Orleans, La.  
Materials:

1325	American whites
2039	American negroes
73	American Indians
171	Alaskan Eskimos
94	Manila Filipinos
3702	Total

The present study is a continuation of those made previously on the external ear and physical form of man, and it is more detailed and specific than former studies. It corroborates them in general and in particular, and adds racial distinctions to type differences.

The most important result is the segregation of the Hyper-, Meso-, and Hypo-types from each group, both by the ear form and by other anatomical characteristics. The other result of importance is the differentiation of the races by their ear form. Incidentally skin lines

were discovered on all ears, lines that represent the folded over skin tip of the ear, the skin tip which should overlie the cartilaginous tip (Darwin's tubercle) but does not always do so.

The segregation of the types, Hyper-, Meso-, and Hypo-, is accomplished by determining for each ear whether the helix is prominent or not, whether the anthelix is depressed or not, whether the lower helix and lobule turn towards the head or away from it, and whether the tragus and antitragus are everted or depressed. After having determined to which type the ear belongs, then the cephalic index, nasal index and facial index of the individual are calculated. The results are found below.

*Type differences.* Hyper-: In this type of ear the helix is depressed toward the head, the anthelix is prominent—projects beyond the helix—the lower helix and lobule turn toward the head, and the tragus and antitragus are everted and prominent—project beyond their surroundings. The nasal index, facial index and cephalic index indicate that the type of individual associated with this type of ear has a long, narrow nose, a long, narrow face, and a long narrow head as a rule.

Hypo-: In this type of ear the helix is prominent, the anthelix depressed, the lower helix and lobule turn out from the head in the form of a shelf, and the tragus and antitragus are depressed below their surroundings. The nasal index, facial index, and cephalic index indicate that the type of individual associated with this type of ear has as a rule a short, broad nose, a short broad face, and a short broad head.

Meso-: In this type of ear the helix and anthelix are both prominent, thus forming a double roll near the dorsal margin of the ear, the lower helix and lobule turn out from the head in the form of a shelf, but not to the same extent as in the Hypo- ear, and the shelf, instead of being horizontal, has a gentle slope forward or may be precipitous, and finally the tragus and antitragus have an intermediate position, are neither prominent nor depressed. The nasal index, facial index, and cephalic index indicate that the type of individual associated with this type of ear has a nose, face, and head of intermediate form between the Hyper- and the Hypo-, although the face is larger than either of the two.

Each of the three types may be subdivided into onto and phylo forms, the phylo, the primordial form, and the onto, the derived form. The Hyper-onto-morph, the Meso-onto-morph, and very rarely the Hypo-onto-morph are European, or white, types; whereas the Hypo-phylo-morph, the Meso-phylo-morph, and rarely the Hyper-phylo-morph are types of the negroes, Indians, Eskimos, Filipinos, and other primitive peoples.

At birth the white child is a Hypo-phylo-morph, and as the child develops it passes consecutively through the stages of the Hypo-onto-morph, Meso-phylo-morph, Meso-onto-morph, Hyper-phylo-morph and Hyper-onto-morph, unless development stops at or between one or the other of the types.

There is little doubt that the Hyper-ontic-morph is the end product of a hyperactive thyroid gland, the result of rapid differentiation, with slight growth, resulting in a small, active, nervous individual. The Hypo-phylo-morph is probably the end product of great thymus activity, resulting in a more or less complete retention of the infantile condition, whereas the Meso-phylo-morph has great activity of the gonads. The other types are variants of the three mentioned, composites, mixtures, blends or mosaics.

*Race differences.* The race differences are of two kinds, measured and descriptive. Only the racial differences of the ear will be considered here.

Measured differences: These are divided into differences in the living and differences in the dead. The ears of only three groups of dead people were measured, American negroes, American whites and Filipinos. By measurements of the total ear length, total ear breadth,

TABLE 1

GROUP	EAR LENGTH		EAR INDEX	
	Male	Female	Male	Female
Dead				
American white.....	64.18		58.0	
American negro.....	58.58	58.32	64.0	60.8
Manila Filipino.....	58.80	57.43	56.8	57.5
Living				
New Orleans student.....	63.9		57.4	
American "old" white <sup>1</sup> .....	66.9	61.3	55.9	56.0
American Indian.....	72.3		52.6	
Alaskan Eskimo.....	73.8	67.1	54.4	53.0
American negro.....	60.8	58.0	60.9	60.2

<sup>1</sup> 'Old' whites are those who have been in this country for 3 generations or more.

Foetuses, new-born and young infants, male and female:

Ear index white.....	68.8
Ear index negro.....	64.5

ear base, true ear length (Schwalbe), concha length and concha breadth, it is found that the negro ear is short and broad, the white ear is long and narrow, and the Filipino ear is relatively longer and narrower than the white ear. The ear length and the index of the ear of both the living and the dead are shown in table 1.

No other measurements are given because racial differences are more pronounced in the ear length and the ear index. The Indian and Eskimo have long ears, the negro and Filipino have short ears and the ears of the white people are intermediate. This accounts in part for the fact that the ear index of the negro is high, that of the Indian and Eskimo is low, and that of the white is intermediate, but it

does not account for the low index of the Filipino. The reason for this is that ear of the Filipino is short and also narrow, it is a small ear. The negro ear is not only short, but it is also broad.

The ear increases in size with age, to 70 years or later, but the increase in length is greater than that in breadth, therefore the index decreases with age.

**Descriptive Differences:** The true negro ear is small, almost flat, close to the head, and the helix is broad as if much folded over. The upper part of the helix is almost horizontal and passes directly backward from the upper end of the ear base to join the vertical dorsal portion of the helix at a right or acute angle in a rounded point at the upper outer extremity of the ear. The superior and dorsal borders of the helix are separated by a depression above Darwin's tubercle, where the helix is thin or absent. The dorsal border passes downward and turns forward at an obtuse angle to form the inferior border of the ear which enters the cheek almost at right angles, with no lobule or a very small one which is nearly flat. The Satyr tubercle is well marked and Darwin's tubercle is small or absent. The skin lines formed by the overfolding of the helix are less distinct on the negro ear than on the white, and they usually converge on the negro ear over Darwin's tubercle. The true negro ear is not seen in great numbers among American negroes. It occurred 245 times among 1478 New Orleans negroes (16.6 per cent), men, women and children, chiefly of the laboring classes.

There is another form of ear that is found frequently among the negroes, but it is also found not rarely among other peoples, even among the whites, and I have called this the involuted ear, because it seems to represent an advanced stage in retrograde development and evolution. It has a gnarled appearance, as if the ear had been burned around the border and had contracted irregularly in healing, leaving a thick, irregular helix. This ear type was at first thought to be due to accidental causes, but the presence of the skin lines of the ear tip in regular order proved the ear to be a true type. It was found 601 times in 1478 New Orleans negroes (40.7 per cent) and 52 times among 857 New Orleans whites (6.1 per cent).

The details of the ears of the negro and white are different as follows: The negro ears are glabrous, the white ears are hirsute; the Satyr tubercle is large in the negro ear, small in the white; Darwin's tubercle is more difficult to find in the negro ear than in the white; the skin lines converge about Darwin's tubercle in the negro ear, and between Darwin's tubercle and the Satyr tubercle in the white; the helix is broad in the negro ear, narrow in the white; the anthelix is more prominent in the white ear than in the negro; the posterior auricular sulcus is deeper in the negro ear than in the white, and in the negro ear the sulcus dips into the concha, whereas in the white it turns out over the helix or lobule.

I wish to thank Dr. Hrdlička for some of the records of the American whites and negroes and for the records of the Alaskan Eskimo and American Indians.

7. *Absence of the vena cava inferior in a 12 mm. pig embryo, associated with the drainage of the portal system into the cardinal system.* ALEXANDER S. BEGG, Harvard Medical School.

In a 12 mm. pig embryo which appeared normal before being sectioned, I found very radical anomalies of the abdominal veins. The vena cava inferior, which arises through anastomosis of the right sub-cardinal vein with the sinusoids of the liver in pig embryos of about 6 mm. and which is very large in 12 mm. embryos, had failed to develop. Thus this embryo presented a young stage of the interesting and well-known anomaly of the adult, described as 'absence of the vena cava inferior.'

Moreover, the portal system did not empty into the liver by the usual large venous trunk, but only through capillary connections, very difficult to follow. On the other hand, the connection between the portal system and the cardinal system, which is ordinarily insignificant at this stage, is an important, if not the chief, outlet of the portal vein. Thus this embryo shows in an early stage the rather rare anomaly of the adult in which a large vein passes from the splenic vein to the left renal vein.

In order to show accurately these features, models have been made of the vessels in the abnormal pig and also in a normal specimen for comparison. Except for the decrease in the size of the portal vein as it enters the liver, the abnormalities are a persistence of earlier normal conditions.

8. *Notes on the endocranial casts of Okapia, Giraffa and Samotherium* DAVIDSON BLACK, Anatomical Laboratory, Western Reserve University, Medical School.

The superficial convolitional pattern of the convex surface of the cerebrum in the lower gyrencephalous mammalia, more especially in the Ungulates and Carnivores, is quite accurately reproduced by the corresponding irregularities of the internal surface of the skull. The major portion of the lateral surface of the neopallium is exposed in these forms—more especially in the Ungulata. In other words, no very extensive operculae are present to obscure the fundamental fissural pattern, which may thus be accurately studied in the endocranial cast.

One endocranial cast of an adult *Okapia* and one of an adult *Giraffa camelopardalis* were obtained in Manchester through the courtesy of Prof. G. Elliot Smith. The second specimen of an adult *Okapia*, together with that of *Samotherium*, was obtained through the courtesy of Dr. Smith Woodward from the Museum of Natural History, at South Kensington, London. I am also indebted to Prof. Arthur Keith for the opportunity of studying the giraffe brains in the collection of the Royal College of Surgeons, and to Dr. C. U. Ariens Kappers for the privilege of studying the Ungulate and other material in the collection of the Central Dutch Institute for Brain Research in Amsterdam.

These notes, together with the illustrations here shown as lantern slides, will form the basis of a more extensive paper in the near future.

*Giraffa*: The convolitional pattern in the giraffe is well known from the study of actual specimens of the brain, and may be readily outlined upon the surface of the cast. Dorsally the lateral sulci and suprasylvian arc are well marked. The coronal sulcus shows a caudal connection with the ansate sulcus, which is a common ungulate condition. This 'ansata' is in no way to be compared with the somewhat similarly placed cruciate sulcus peculiar to the carnivores. The olfactory bulbs are only seen in this view as small swellings projecting very slightly from beneath the frontal pole.

The whole course of the suprasylvian sulcus is shown in the lateral view of the cast. In addition to the post. horizontal limb common in Cervidae and other forms, a posterior descending ramus of the suprasylvian sulcus is to be noted. This sulcus is probably not homologous with the postsylvian sulcus of the carnivores. The posterior rhinal fissure is well marked and above it can be seen the bulging of the 'post. sylvian operculum,' the edges of which are indented by a series of small sulci.

The Ungulate sylvian, or pseudosylvian, fissure appears as a short ascending ramus from the diverging ectosylvian sulci. Between the summit of this pseudosylvian fossa and the suprasylvian sulcus there is seen a well marked arcuate fissure. This 'arcuate' constellation is in no way homologous with the ectosylvian group so evident in *Canis*, *Felis*, etc., but appears to be a characteristic feature of the giraffidae, and when present together with the posterior descending ramus of the suprasylvian, is a distinct diagnostic feature.

The anterior rhinal fissure, together with the orbital and paraorbital sulci present no peculiarities. There is a typical triradiate diagonal sulcus. Posteriorly, the area behind the descending ramus of the suprasylvian sulcus is marked by several irregular sulci, as is the case in the corresponding area behind the oblique sulcus in *Cervus*.

The cast is one of a typically macrosomatic mammal of the Ungulate type. The olfactory bulbs are very large and sessile and, together with the tractus olfactorius and tuberculum, are best seen in the ventral view. Here, as also in the lateral view, the enormous size of the combined ophthalmic and maxillary divisions and also the large mandibular divisions of the trigeminal nerve is at once evident, the optic nerves being small in comparison. The large size of the N. V. is directly related to a highly developed so-called 'oral sense.'

*Okapia*: This animal is a hornless member of the family Giraffidae, and was first discovered in the Belgian Congo in 1899 by Sir Harry Johnston. No material other than the skin and skeleton has as yet been available for scientific study.

The arrangement of the sulci appearing on the dorso-lateral surface of this cast gives evidence of a close relationship between the *Okapia* and the Giraffe. The 'arcuate' constellation, the descending ramus of the suprasylvian and the position occupied by the lateral group of sulci are essentially similar to those obtaining in the giraffe.

There are, however, numerous specific differences. The descending ramus of the suprasylvian fissure cuts the post. rhinal fissure in *Okapia*.

In *Cervus dama* and many other forms, an 'oblique' sulcus occupies the area in which this descending ramus is found in the Giraffidae. This oblique sulcus in Cervidae in many cases cuts the rhinal fissure, and it may be that this sulcus is the homologue of the descending ramus of the suprasylvian. Thus the cutting of the post. rhinal fissure by the latter sulcus may be a premature feature common to *Okapia* and *Cervus*, but absent in the more specialized giraffe.

The pseudosylvian fissure is very short and the relation of the anterior ectosylvian sulcus to the anterior rhinal fissure is obscure. The irregular sulci in the area behind the ram. desc. of the suprasylvian sulcus present essentially similar relations to those in the giraffe.

The relations of the coronal, ansate and suprasylvian sulci together with the diagonal are quite different to the conditions obtaining in the giraffe. The suprasylvian is joined to the corono-ansate sulci, as is the case in the Cervidae and often in the other Ungulates. The absence of this condition in the giraffe may thus be considered as another evidence of specialization in this form.

A constellation apparently representing the 'diagonal' sulcus of Elliot Smith appears continuous with the suprasylvian (on the right side in the specimen illustrated). On the left side of the same specimen the diagonal sulcus occupies its usual position in front of and below the coronal sulcus.

The olfactory bulbs are large and pedunculated, and project a considerable distance beyond the frontal pole, so that the olfactory stalks are visible in the dorsal view. The olfactory tracts and tubercule, together with the pyriform lobe, are well seen in the ventral view. The tuberculum is especially prominent and occupies a special little fossa on the skull floor. The total amount of neopallium as compared with the rhinencephalon appears less than in the case of the giraffe.

*Samotherium*: This animal was an Okapi-like form found in the upper Miocene deposits in the island of Samos. The cast shows evidence of considerable compression during fossilization, resulting in marked asymmetry. Notwithstanding this unfavorable condition, it is possible to trace the course of the principal sulci with but little difficulty.

The entolateral sulcus occupies its usual position, but the lateral sulcus takes a very unusual course and becomes joined to the coronal as well as with the suprasylvian, through the intermediation of the ansate sulcus. This junction of lateral and coronal is found nowhere else in the Ungulata except in the primitive hippopotamus. In other orders, however, as for example in the Carnivora, this junction between coronal and lateral sulci is a common feature. Elliot Smith has shown this feature to be a very primitive character and present in such Eocene Carnivores as *Stenoplesicites* and *Gynohyaenodon* as well as in ancestral Ungulates.

The continuity of the suprasylvian and coronal by way of the ansate sulcus is, as has been already noted, a common feature in Ungulates, but is practically never present in Carnivores.

The coronal sulcus is placed far forward, and is comparatively small as in Hyrax, while the ansate sulcus is well developed. In front of the coronal sulcus a triradiate diagonal fissure is evident in its usual position.

The orbital sulcus emerges from the anterior rhinal fossa and is placed far forward, as in many of the Cervidae and Suidae.

A sulcus recalling the Carnivore 'cruciatus,' but not to be homologized, appears emerging from the sagittal furrow and probably represents the upturned termination of the splenial.

The ramus descendens of the suprasylvian sulcus cuts the posterior rhinal fissure as in Okapia. The pseudosylvian sulcus on the left side is represented by a series of small vertical notches, the whole being related to a typical 'arcuate' sulcus, such as obtains in Okapia and Giraffa. The pseudosylvian sulcus on the right side more nearly approaches the condition obtaining in Giraffa.

*Summary:* From a study of the limited amount of material at my disposal it appears that of the three Artiodactyl forms of the family Giraffidae under discussion, the brain of Samotherium shows undoubtedly the most *primitive* arrangement of sulci, presenting as it does certain features common both to the Carnivora and the Ungulata. In other words this form is evidently most closely related to that hypothetical 'co-mammal' from which both the Carnivora and Ungulata were specialized.

In addition to this primitive feature, Samotherium presents certain *generalized* characters, common and peculiar to the Ungulata such for example as (a) the relation of the descending ramus of the suprasylvian sulcus to the post. rhinal fissure (provided the former sulcus be the homologue of the 'oblique' sulcus of Elliot Smith) and (b) the arrangement of the coronal, ansate, suprasylvian complex anteriorly. In these generalized characters Okapia resembles Samotherium and the two differ from Giraffa.

All these forms show a certain fundamental similarity in fissural pattern. These *specialized* characters are seen in the arrangement in the 'intrasylvian arcuate' complex which, taken in conjunction with the descending ramus of the suprasylvian sulcus is apparently peculiar to Giraffidae.

And finally, Giraffa differs from both Samotherium and Okapia in the possession of certain specialized features, such, for example, as the complete separation of the corono-ansate group from the suprasylvian sulcus.

#### 9. Explanation of variations of the renal artery. J. L. BREMER, Harvard Medical School, Boston.

Vessels mentioned in the text-books of anatomy as either anomalous roots or anomalous branches of the renal artery may be placed in three groups: (1) those to the mesonephros and its adjacent organs, ventrolateral branches of the aorta; (2) those to the intestinal tract and its derivatives, ventral branches; and (3) those to the body wall and dia-

phragm, dorso-lateral branches. Group 1 includes the spermatic and adrenal arteries, and branches of the iliacs and middle sacral; group 2, the coeliac axis and its branches to liver, pancreas, and colon, and the superior and inferior mesenteric arteries; and group 3, the lumbar arteries and the inferior phrenic. If horizontal anastomoses between members of the different groups can be found, and if in addition longitudinal or vertical anastomoses between members of the same group exist, any of the variations are explicable.

At the outset it was found that, whereas in man the renal artery is normally a branch of a mesonephric artery, in pig and sheep the new vessel is derived from body wall (or lateral body) vessels.

The origin of the renal artery in man from the iliacs or the middle sacral is due to the original pelvic position of the kidney, in the immediate vicinity of the vessels mentioned. Branches from them, similar to mesonephric arteries, may run to the kidney, and may continue in activity as the kidney migrates, instead of being lost or becoming uræteric arteries, as is more usual. Spermatic and adrenal branches of the renal artery are not uncommon, and are due to the fact that all are derivatives of the mesonephric arteries. Vertical anastomoses of mesonephric arteries are common, and since now one root, now another, is kept, such anastomoses account readily for the frequent asymmetrical origin of the renals from the aorta. Vertical anastomoses between dorso-lateral aortic branches are also common in the abdominal, as well as in the cervical, region.

Horizontal connections, rarer than the vertical, are found oftenest between the ventral and the ventro-lateral group, and account for the origin of the renal artery from the coeliac axis or the mesenteric arteries, and for the branches of the renal to liver, pancreas, and colon. A double anastomosis between one of the early ventral arteries and a mesonephric artery on each side, with the subsequent loss of the ventral artery and of any two of the three roots to such an anastomosis, would result in the origin of the renals from a common stem.

Horizontal anastomoses between vertebral, or dorsal, and lateral body arteries can hardly be considered anomalies, as in most animals the two sets of vessels soon come from a common stem, as in adult man. Horizontal anastomoses between lateral body and mesonephric arteries are very rare, but serve to explain the origin of the renal artery in man from a lumbar artery, or the presence of a phrenic branch of the renal.

The various anastomoses found may be so close to the aorta, when it is only an endothelial tube, that they become incorporated in its wall by the development of the muscular layer.

It will thus be seen that all the variations of origin or anomalous branches of the renal artery can be explained by the presence, in different embryos, of pieces of a periaortic anastomoses, joining the various aortic branches, vertically and horizontally. If these pieces were all developed in one individual, it would be capable of transferring the blood stream from a dorsal vessel to a ventral one, and vice versa. In

this connection it is interesting to note that the mesonephric arteries of adult selachians are said to spring normally from the segmental body wall vessels, and that in *Bdellostoma* the mesenteric arteries arise from the dorsal wall of the aorta.

10. *Comparative size of nucleus and cytoplasm in old and regenerating tissues.* E. L. BREZEE, Cornell University Medical School, New York City. (Introduced by C. R. Stockard.)

The species used for this experiment were *Fundulus heteroclitus*, tadpoles of *Rana sylvatica*, and two species of salamander, *Diemyctylus viridens* adults and *Amblystoma punctatum* larvae.

Old and regenerating tissue from both adult and larval animals were studied. The tails and arms were cut off about one-third or one-half way from the body. These parts after a few days had regenerated and the organisms were then preserved in Bouin's fluid, sectioned and stained with h amatein and eosin.

Sections were cut in such a plane as would pass through both old and regenerating tissue. Two sections of each specimen were studied and camera lucida drawings made of the nuclear outlines of portions of epithelium and mesenchyme from the old and new tissue of each section. The cell outlines could not be traced as they did not show distinctly enough. In the epithelium the cells were so closely packed that all the substance which was not nucleus could be considered as cytoplasm. In the mesenchyme, however, the cells were so scattered and the intercellular spaces so numerous that the amount of cytoplasm could not be determined in this way; hence no relation between the amount of nuclear material and of cytoplasm could be calculated, but merely the size of nuclei in old and new tissue compared.

The purpose of the experiment was three-fold: (a) to determine whether the nuclei of the old or of the new tissue were larger; (b) to determine in both old and new tissue whether the amounts of nuclear or of cytoplasmic material were greater; and (c) to compare the relative amounts of nuclear and cytoplasmic material in old and new tissue. In each case the areas traced from the old and new tissue of the same section were compared, and the results noted.

(a) By comparing the tracings from each portion of new tissue with the corresponding portion of old it was possible to determine without actual measurement in which the nuclei were larger. The results are shown in table 1.

In the mesenchyme there is a slight advantage in nuclear size shown in the old tissue; this is true also of the epithelium of the larvae but in the adult animals the size of the nuclei of the new epithelium is more often greater.

(b) The actual areas of nuclear and cytoplasmic material were found by the following method: a rectangular portion was outlined and its area found; the mean diameter of each nucleus within this portion was determined and its area found by use of the formula  $\pi r^2$ , letting  $\pi =$

3  $\frac{1}{2}$ . The sum of these areas gave the total area of the nuclei and by subtracting this from the area of the rectangle, the area of the cytoplasm was found. Then, using the ratio

$$\text{Area of nucleus} : \text{Area of cytoplasm} :: 1 : X$$

the number of parts of cytoplasm to one part of nuclear material was found for each specimen. In the majority of all cases the area of cytoplasm was greater than of nuclear material; i. e., in the ratios X was found to be greater than 1. Table 2 shows the number of cases which were exceptions:

TABLE 1

SPECIES	NUCLEI LARGER IN OLD TISSUE AS COMPARED WITH NEW	EQUAL	NUCLEI LARGER IN NEW TISSUE AS COMPARED WITH OLD
<i>Mesenchyme</i>			
Adult			
Fundulus heteroclitus tail.....	2 cases	4 cases	4 cases
Diemyctylus tail.....	31 cases	18 cases	15 cases
Diemyctylus arm.....	5 cases	3 cases	12 cases
Total.....	38 cases	25 cases	31 cases
Average.....	40 per cent	27 per cent	33 per cent
Larval			
Rana sylvatica tail.....	6 cases	2 cases	2 cases
Amblystoma punctatum tail....	10 cases	13 cases	7 cases
Total.....	16 cases	15 cases	9 cases
Average.....	40 per cent	37 $\frac{1}{2}$ per cent	22 $\frac{1}{2}$ per cent
Adult and larval			
Total.....	54 cases	40 cases	40 cases
Average.....	40 per cent	30 per cent	30 per cent
<i>Epithelium</i>			
Adult			
Fundulus heteroclitus tail.....	7 cases	No cases	3 cases
Diemyctylus tail.....	21 cases	12 cases	31 cases
Diemyctylus arm.....	2 cases	2 cases	16 cases
Total.....	30 cases	14 cases	50 cases
Average.....	32 per cent	15 per cent	53 per cent
Larval			
Rana sylvatica tail.....	4 cases	2 cases	4 cases
Amblystoma punctatum tail....	12 cases	9 cases	9 cases
Total.....	16 cases	11 cases	13 cases
Average.....	40 per cent	27 $\frac{1}{2}$ per cent	32 $\frac{1}{2}$ per cent
Adult and Larval			
Total.....	46 cases	25 cases	63 cases
Average.....	34 per cent	19 per cent	47 per cent

The cytoplasmic area was greater than the nuclear in 86 per cent of all cases, the percentage of exceptions being considerably less in the larval than in the adult animals.

TABLE 2

SPECIES	OLD	NEW
Adult		
Fundulus heteroclitus tail.....	No exceptions	No exceptions
Diemyctylus tail.....	12 exceptions	9 exceptions
Diemyctylus arm.....	2 exceptions	7 exceptions
Total.....	15 per cent	17 per cent
Larval		
Rana sylvatica tail.....	No exceptions	1 exception
Amblystoma punctatum tail.....	5 exceptions	2 exceptions
Total.....	12½ per cent	7½ per cent
Total number of tracings of epithelium.....268		
Total number of exceptions..... 38		

(c) In a small majority of the cases, 57½ per cent, the number of parts of cytoplasm to one part of nuclear material was found to be greater in the old tissue than in the new. Table 3 shows the summary of the ratios as found for each pair of tracings, the numbers representing X in the ratio

TABLE 3

*Summary of tables of areas of cytoplasm to areas of nuclei*

NUMBER OF SECTIONS STUDIED	AVERAGE OF PARTS OF CYTOPLASM TO 1 PART NUCLEUS	
	Old	New
Adult		
10	6.041	6.533 Fundulus heteroclitus tail
64	1.381	1.302 Diemyctylus tail
20	1.428	1.204 Diemyctylus arm
Larval		
10	2.694	2.266 Rana sylvatica tail
30	1.808	1.628 Amblystoma punctatum tail
Total Average.	2.585	2.587

Area of nucleus : Area of cytoplasm :: 1 : X.

Table 4 shows the percentage of cases for each species in which there is more cytoplasmic material in the old than in the new, as compared with the same amount of nuclear material:

TABLE 4

Adult	per cent
Fundulus heteroclitus tail.....	30
Diemyctylus tail.....	55
Diemyctylus arm.....	75
Average.....	53½

TABLE 4—Continued.

Larval	
Rana sylvatica tail.....	70
Amblystoma punctatum tail.....	57
Average.....	63½
Total Average.....	57½

*Summary:* From the total number of 536 tracings in this experiment the following conclusions may be drawn: (a) In the mesenchyme of both larval and adult animals and in the epithelium of the larval animals the nuclei are larger in the old tissue than in the new; but in the epithelium of the adult animals the nuclei are larger in the new tissue. (b) In 86 per cent of the tracings of epithelium the cytoplasmic area is greater than the nuclear; the larval tissues show fewer exceptions to this rule than the adult. (c) The amount of cytoplasmic material as compared with nuclear is found to be slightly greater in the old than in the new tissue in 57½ per cent of the cases.

In general then it would seem that in the epithelium of the larval animal the whole cell in the old tissue is larger than the cell in the regenerating tissue, the cytoplasm, however, being larger in greater proportion than the nucleus. In the epithelium of the adult animal the nucleus of the old cell is smaller than that of the cell in the regenerating tissue but the amount of cytoplasm is greater per nuclear area.

*11. An attempted analysis of growth.* MONTROSE T. BURROWS, Anatomical Laboratory, Cornell University Medical College, New York City.

The study of the growth of different tissues during their development, the study of regeneration, the study of animal behavior, as well as the study of cancer and the effect of physical and chemical conditions on growth and development has shown that the environment is very important in development. Further these studies have given evidence to show that the various forms of cell activity are dependent upon conditions in the environment aside from food and oxygen. Little is known, however, as to the nature of the changes brought about in the cells through this effect of environment nor has sufficient evidence been given to any one theory to cause its general acceptance.

Two years ago I made the observation that growth, division, migratory movements, rhythmical contractions and latency could be observed in heart muscle cells migrating from the same piece of tissue into the same media and in a more recent study I have found that each of these activities is associated with a particular environment. These environmental differences consisted not only in differences in the chemical composition of the medium brought about by the active cell metabolism but also in differences in the mechanical support given these cells, which in plasma clots was altered not only by the concentration and nature of the substances coming from the tissue fragment but also by the shape of the clot and the support given to the clot and the tissue

fragment. The facts in this last statement I have been able to show by direct experiment, as well as to show further that the conditions of the particular environment were necessary for the particular activity shown by the cell. By altering the environment of a cell showing one activity this cell showed the form and activity of one occupying this new environment.

In the paper to be presented before the society I wish not only to describe these observations in greater detail but to give evidence to show that the movement of tissue cells may be interpreted in terms of surface tension. In the same manner I have been able to find a relation between surface tension changes and growth and evidence to show that the organization peculiar to the contracting cells may be interpreted by similar changes.

*12. Observations of the lymph-flow and the associated morphological changes in the early superficial lymphatics of chick embryos.* ELEANOR

LINTON CLARK, Anatomical Laboratory of the University of Missouri.

The present investigation is concerned with a few of the physiological and morphological changes which take place in the developing lymphatic system, after its first appearance. Early superficial lymphatics were studied in living chicks and experiments performed to test the direction and character of the lymph-flow at various stages. The same embryos were then injected and the extent and character of the lymphatic system studied in cleared specimens. Thus an attempt has been made to correlate the structure and function of the early lymphatic system, and to determine, if possible, some of the factors which regulate a few of the phases of its development.

Eggs are opened in a warm chamber, left at incubator temperature, in a manner which has already been described. Under the binocular microscope the lymph circulation is tested by injecting a few India ink granules directly into a lymphatic capillary or duct. The fine glass canula is withdrawn carefully, so as to prevent leakage and the movement of the granules is observed through the binocular microscope. After testing the direction and character of the lymph-flow in various regions, the lymphatic system is injected and the embryo cleared by the Spalteholz method. Chicks of  $5\frac{1}{2}$  to 9 days were studied in this manner.

(1) In its primary condition (in chicks of approximately 5 to 6 days) the superficial lymphatic system is a rapidly growing, richly anastomosing plexus. A lymphatic plexus gradually extends posteriorly, from its venous connections in the neck, through the axillary region and down the side. At the same time another plexus is extending anteriorly from the coccygeal veins in the tail. It spreads out over the pelvis and eventually the two plexuses meet and anastomose over the hip. During this period of rapid extension there is no circulation in the superficial lymphatics. The side pressure in the veins with which the lymphatics connect, is higher than the pressure in the lymphatics and con-

sequently blood is continually forced out into the extending lymphatic plexus. The plexus covers a wide area and is irregular and indifferent in character.

(2) The next period in the developing superficial lymphatics is characterized by the beginning of lymph-flow, accompanied by the differentiation of definite ducts or channels in the irregular primary plexus. The flow starts in the side plexus and follows a definite path anteriorly, through the axillary region and the deep plexus, into the veins near the duct of Cuvier. Somewhat later the circulation over the pelvis begins. The flow in this region is instigated by the first pulsations of the lymph heart (still in the form of a plexus). In the earliest stage of circulation the granules move slowly and follow a narrow winding path. Injections show that the first channels are small and somewhat tortuous but quite distinct from the surrounding plexus. On this stage the blood is gradually washed out of the lymphatic system: first from the side region and later from the lymphatics of the pelvis.

(3) The development of the superficial lymphatics in chicks of 7 to 8 days is characterized by increased pressure in the lymphatics, stronger pulsations of the lymph heart, a more rapid lymph-flow and associated with this, the formation of new channels in the lymphatic plexus and the enlargement of those already formed. The exact position of a channel is not predetermined, since variations in the number and position of the main ducts are frequent at all stages.

(4) In chicks of 8 to 9 days, the pressure in the superficial lymphatics is very high. The great increase in the flow of lymph from the allantois and from the deep body lymphatics appears to interfere with the outlet of the fluid from the superficial lymphatics. At this stage the flow is rapid in certain portions of the superficial lymphatic system and very sluggish in others. Injections show that ducts or channels are present in the former regions and large sacs or lakes in the latter. The sacs always occur at a point where there are two conflicting pressures. Because of the looseness of the subcutaneous tissue at this stage, the lymphatic system encounters very little resistance from without and so expands in response to the increased pressure within the lymphatics. The sacs may be formed by the enlargement of two or more neighboring ducts and the breaking down of the walls between them, or by the enlargement of a single duct. At this stage the lymph heart first assumes the form of a sac. Its muscular walls offer an obstacle to its distension and hence it remains much smaller than some of the other sacs or reservoirs. Except for the development of muscles in its wall, the lymph heart does not differ in its mode of formation from other portions of the early lymphatic system.

In addition to the differences in pressure at various stages, the flow of lymph is influenced and altered by (a) the movements of the embryo, (b) the beating of the lymph heart, (c) changes in the blood circulation (d) development of valves at the entrance to the veins, (e) the formation of new lymphatic capillaries and ducts, and (f) by the shifting of the relationship of various organs.

In chicks older than 9 days the increased thickness of the skin and the development of feathers prevented further observation of the circulation of granules in the superficial lymphatics.

13. *Studies of the growth of blood vessels, by observation of living tadpoles and by experiments on chick embryos.* ELIOT R. CLARK, University of Missouri, Anatomical Department.

There are two views each claiming the support of active workers as to the mode of development of the main arteries and veins. According to one view, which has been largely developed by Hochstetter and recently championed by his pupil Elze, the main arteries and veins develop in definite predetermined places, and the growth of each represents merely the steady extension of a single vessel along its inherited path. The second view, which has been mainly developed by Thoma, and which has found support recently in the works of E. Müller, C. G. Sabin, H. Rabl, Mall and Evans, is that each artery and vein is preceded by an indifferent capillary plexus, any part of which is capable of developing into artery or vein, and that the selection of one or another capillary depends upon mechanical conditions inside and outside the capillary.

The studies here presented are in favor of the second view. The observations on which this view has rested have consisted of studies made by injection or reconstruction, of vessels in a selected region in embryos of different ages. In the present study the development was watched in its various stages in the same embryo. The region selected was the transparent fin expansion of the tail of the frog larva. Drawings were made while the tadpole was immobilized by chloretone, with the aid of an apparatus previously described. By alternating the periods of observation with periods in which the tadpole was returned to fresh water, it was possible to make many successive observations on the same animal, as it increased in size. The records made consisted not only of camera lucida drawings of the vessels, with records as to direction of flow, but also notes as to the comparative rate and amount of flow in each.

It was found that arterioles and venules develop from an indifferent capillary plexus, in which, at any stage, it is impossible to predict which capillary will be incorporated as a part of the advancing arteriole or venule. Thus a vessel which, at one stage, is the main channel between artery and vein, may in later stages either remain the same size, or may even become solid, and disappear by retraction of the endothelium. The factor which determines the selection of a capillary as part of the developing arteriole or venule is the relation in which it is placed with reference to the new capillaries which develop more peripherally. If favorably placed the flow of blood through it increases and its diameter increases, until it becomes a part of the arteriole or venule.

That the development of the main vessels is due to favoring mechanical factors, and not to heredity, is indicated also by the results of experiments on chick embryos. These consisted of the removal of the an-

terior cardinal vein of one side in chicks approximately two days old. This was accomplished by injecting into the vessel Berlin blue—which clumps on contact with the blood and sticks to the endothelium—and removing with forceps and needle the vein and surrounding tissue. The ear vesicle was removed along with the vein as the vein passes under it. In all the chicks in which the operation was successful, examined three or four days after the operation, there was found a well developed vein in the place usually occupied by the internal jugular. In one case this vein was larger than the vein on the unoperated side; in most cases it was slightly smaller, but in all cases it was well developed.

This would seem to show that there exist in the side of the neck mechanical conditions favoring the development of a large vein—since, after the normal vein had been removed, its place was taken by another, which could in no way be considered as inherited.

*14. Salient features of the medulla oblongata of Amblystoma embryos of definite physiological stages in development.* GEORGE E. COGHILL.

In the stage of development designated by me (*Jour. Comp. Neur.*, vol. 24, p. 163) as non-motile, root fibers of the trigeminal and lateral line ganglia of *Amblystoma* enter the medulla oblongata. In the early flexure stage the descending trigeminal tract extends to the auditory region and there is a perceptible ascending division of the root; while, in the coiled-reaction stage, the trigeminal tract becomes continuous with the spinal sensory tract, which is composed of fibers from the Rohon-Beard cells. Dorsally of the trigeminal tract in the auditory region of the coiled-reaction stage are recognized the auditory root bundle, the fasciculus communis (solitarius) and two lateral line root bundles, the fasciculus communis laying between the auditory and lateral line root bundles. In the early swimming stage the lateral line root bundles of the seventh and tenth nerves overlap and the fasciculus communis has almost if not quite become continuous with the visceral sensory root bundle of the ninth and tenth nerves. There are no longitudinal association bundles corresponding to tracts *a* and *b* of Herrick (*Jour. Comp. Neur.*, vol. 24, no. 4).

Very large tangential neurones are arranged along the mesial aspect of the root bundles as if functionally related to all of them in common, though smaller cells, located farther dorsad, appear to be especially related to the lateral line components. The axones of the large tangential cells pass mesially of the latero-ventral motor tract to the ventral commissure. This motor tract is the only longitudinal tract in the ventral part of the medulla oblongata until about the time swimming begins, when there appears a suggestion of a slightly more dorsal bundle, presumably the bulbo-spinal tract.

The functional significance of the sensory centers of the medulla oblongata in *Amblystoma* of this period can not be judged by the degree of development of the sensory root bundles alone, for these are developed entirely out of proportion to the corresponding peripheral nerves,

this being particularly true of the visceral sensory system. Also, experiments show that, in the earlier periods under consideration, the sensibility of the preauditory region to tactile stimulation is much lower than is that of the rostral portion of the trunk.

15. *On the development of the lymphatics in the lungs of the pig.* R. S. CUNNINGHAM, Anatomical Laboratory, Johns Hopkins University.

The lymphatics of the lungs are derived from three sources, the right and left thoracic ducts and the retroperitoneal sac.

In embryos 2.6 to 3 cm. long vessels bud off from the thoracic duct and grow across to the lateral wall of the trachea and form there a plexus that gradually extends over the ventral surface of the trachea and especially down over the bifurcation. From this plexus vessels pass into both lungs and into the pleura.

The right thoracic duct divides, in embryo 2.5 to 2.6 cm., one branch passes to the heart while the other breaks up to form a plexus on the right lateral wall of the trachea; some vessels from this plexus pass down into the hilum of the right lung and others anastomose with the plexus that extends up over the trachea from the other side. The development of the lymphatics within the lung depends upon the division of the vessels into two groups—those accompanying the veins and those accompanying the bronchi and arteries.

Each of the principal branches of the pulmonary vein is accompanied by a group of lymphatic vessels that anastomose freely with the plexus around the adjacent bronchus. These lymphatics grow more rapidly than those associated with the bronchi, and, after following the veins almost to the capillary bed, they pass to the pleura. In the early stages the terminal veins lie about midway between the adjacent bronchi and in this plane a sheet of lymphatic vessels develops from the vessels accompanying the vein and passes to the pleura, marking out the boundaries of the distribution of each bronchus. The first vessels to reach the pleura thus follow the veins, and they anastomose with the vessels that grow to the pleura from the hilum. These vessels reach the pleura when the embryo is about 3.6 cm. long. The bronchial vessels grow more slowly and at first are only to be found around the larger bronchi. As these structures multiply and the lung increases in size the lymphatics accompanying the main bronchi send vessels to the smaller ones, these vessels form a plexus around each bronchus—so that the bronchial tree is surrounded by a continuous series of branching tubes made up of lymphatic vessels. From every point of division of the bronchi lymphatics pass to join those following the veins, and those around the terminal bronchus leave it, near where it ends in the primitive atria, and join those of the veins, septa, or—more rarely—those of the pleura. Lymphatics also arise from the retroperitoneal sac and grow up posterior to the stomach and the diaphragm to enter the lower pole of the lower lobe of the lung. These vessels form a plexus on the median surface of the lower lobe and send branches both to the other surfaces of the pleura of the lower lobe and

into the lung along the veins, where plexuses develop similar to those above and soon the two groups anastomose (embryos 3.9 to 4.1 cm. long).

The further development consists in the multiplication of the plexuses on the bronchi and blood vessels, following the further development of these structures. As the lung increases in volume the larger veins become more closely approximated to the bronchi and only the terminal ones are separated from them, these lie in the periphery of the lobule. Thus the vessels around the veins and bronchi become closely associated, except those accompanying the terminal branches where the veins still lie in the connective tissue septa. These septa develop along the course marked out by the sheets of lymphatic vessels.

The common plexus surrounding the artery and bronchus becomes separated into two plexuses, incident to the increase in the size of the artery, they continue to have many anastomoses however. The vessels of the pleura mark out the early connective tissue septa, but later there develops a fine meshed plexus between these larger divisions, this plexus is not connected with the deep lymphatics. The valves begin to form in embryos about 6 cm. long and practically all point away from the pleura, so that the pleura is drained separately from the remainder of the lung.

In the adult there are lymphatic vessels accompanying the bronchi, the arteries, and the veins—these anastomose freely. There are also vessels in the connective tissue septa that drain chiefly into those around the veins and to some extent into those around the bronchi, near the point where the vein separates from the other structures to take its peripheral position in the lobule. All the deep vessels, together with most of the pleural vessels, drain into large trunks that end in the nodes at the hilum, but the lower half of the pleura of the lower lobe drains by a group of 4 to 6 vessels to the preaortic nodes that develop from the cephalic portion of the retroperitoneal sac. These vessels pass down through the ligament that connects the lower and median surface of the lower lobe with the tissue surrounding the aorta.

16. *The morphology of the mammalian seminiferous tubule.* GEORGE M. CURTIS, Anatomical Laboratory, Vanderbilt University Medical School.

The problems here considered may be divided into two phases: (1), dealing mainly with the purely morphologic aspects of the tubule and (2), considering more the relation of the process of spermatogenesis to the tubule.

1. *The seminiferous tubule:* (a) Blind ends. Since their description and delineation by J. Müller ('30), in the testis of the squirrel, the presence of blind ends in the course of the seminiferous tubules has been repeatedly affirmed and denied. In this work as thus far completed none of these structures have been disclosed. This statement is based upon the following evidence:

Adult albino mouse: Two complete tubules.

One isolated and reconstructed graphically and in wax.

One isolated and reconstructed graphically.

Adult rabbit: Six complete and one incomplete, tubules and tubule complexes.

Five isolated by teasing by Huber (Huber and Curtis '13).

One isolated and reconstructed graphically.

One incomplete complex isolated and partially reconstructed graphically and in wax.

Three-week dog: Two complete tubules.

Both isolated and reconstructed in wax.

From the above eleven tubules and the careful study of the material necessary to isolate them it is concluded that blind ends are not present in these three forms.

(b) Ampullae. These structures described and figured by Sappey ('88) have not been met with in any of the three above forms.

(c) Lobules. These are present in the albino mouse structurally as evidenced by the tubule modelled and by the tubule graphically reconstructed. However, no apparent lobulation is visible in examining the sections. In the rabbit and dog lobules are visible with the naked eye, each lobule being found to contain the coils of a portion of a single tubule or tubule complex.

(d) Branches and anastomoses. These were found to be infrequent in the mouse testis, more frequent in the dog and most frequent in the rabbit.

(e) Embryonic ends. In the mouse tubule, between the cessation of the active process of spermatogenesis and the flattened epithelium of the tubules rectus, was found a region where the tubule retained its embryonic structure, disclosing the sexual and sustentacular cells around an irregular lumen. It suggests itself that this may be a possible region of reserve to be used in growth or regeneration.

2. *The spermatogenic wave*: Especially through the work of v. Ebner it has been shown that the development of mammalian spermatozoa proceeds in a wave-like process along the course of the seminiferous tubules. v. Ebner ('88) states that in the rat these waves ascend from the rete and vary in length from 25 mm. to 38 mm. averaging 32 mm. Benda ('87) intimates their variability.

A study of these waves has been made in the two complete seminiferous tubules of the adult mouse, and in one complete and in a portion of an incomplete tubule complex in the rabbit. In determining the relations between wave and tubule it first became necessary to arbitrarily choose a series of eight successive stages of spermatogenesis. These were obtained from v. Ebner's figures and a study of the series. The above tubules were then reconstructed graphically, their loops corresponding to the numbered tubule sections in the serial drawings. By observing the stages of spermatogenesis present in each tubule section at definite intervals and applying them to their proper loop in the graphic reconstruction, the continuity of the stages was determined.

By comparing and numbering alike all the loops of the serial drawings,

graphic reconstruction and model, the relations of the waves to the model were determined and their actual lengths computed. Their succession was shown by plotting the successive stages occurring along the course of the tubules. By this method the following results have been obtained.

(a) Wave length. From a study of seven waves in the mouse this was computed as averaging 1.83 cm. From a study of one complete wave and a comparison of eight wave portions the average wave length in the rabbit is estimated 1.4 cm.

(b) Wave variability. In the mouse and rabbit the waves vary in length, direction of course, uniformity, and in that single stages may be out of order in a successive series.

(c) Wave reversibility. In both forms the waves may reverse their direction, often frequently in a single portion of a tubule or tubule complex.

(d) Wave direction. In the mouse the waves of five rete ends all descend from the rete. In the rabbit the waves vary, three waves ascending and two descending from the rete.

The above work was completed under Dr. Huber's direction at the Histological Laboratory of the University of Michigan, and I desire to express here my thanks for his courtesies and assistance.

*17. The structural relations of anterior hepatic arteries.* C. H. DANFORTH, Washington University Medical School.

In an earlier paper (*Jour. Morph.*, vol. 23, no. 3, 1912) the writer published a brief description of the anterior hepatic arteries of *Polyodon*. These vessels, which arise from the same trunk as the posterior coronary arteries, were found to be of constant occurrence and of fairly uniform distribution. They were often equal to, or even more extensive than the posterior (ordinary) hepatic arteries, with which they anastomose.

At the time the above mentioned paper was published, it was supposed that anterior hepatic arteries were peculiar to *Polyodon*. Further observations, however, have revealed them in several other forms. Their general relations, so far as gross methods reveal, seem to be essentially the same in all cases.

It is now possible to record a few recent observations on the development and finer relations of the anterior hepatic arteries of *Polyodon*. In this fish the connective tissue about the hepatic veins is unusually extensive. Associated with these veins, as well as with the branches of the portal system, there are considerable accumulations of lymphoid tissue. In general there is a branch of the artery running through each of these lymphoid aggregations. In young fish, less than 100 mm. in length, the thickened connective tissue sheath about the vein is not apparent and in such specimens anterior hepatic arteries have not been detected. At 123 mm. the connective tissue about the veins shows a slight thickening and the arteries may be traced in sections. But even at this stage there is no noticeable accumulation of lymphocytes.

In the adult the ramifications of the artery are usually found associated with the tributaries of the hepatic vein. Nevertheless, they are not confined to the connective tissue about the veins, but branches of considerable size may pass out into the liver parenchyma where they are for the most part surrounded by lymphocytes. Some of their branches may again become associated with veins.

These observations indicate that the anterior hepatic arteries are not to be regarded as of the nature of vasa vasorum in connection with the hepatic veins, but as independent vessels, probably of considerable functional importance.

18. *The so-called "endothelioid" cells.* HAL DOWNEY.

Pathologic conditions affecting primarily the hematopoietic organs are frequently characterized by the presence of large protoplasmic cells which are usually designated as "epithelioid" or "endothelioid" cells. Such cells are seen in generalized granulomata of the lymph nodes (tubercular lymph nodes, Hodgkin's disease), in Gaucher's disease, Banti's disease, in lymph nodes from typhoid fever patients, etc. Although these cells may show structural variations of considerable degree, most pathologists, especially American pathologists, do not hesitate to group them together under the heading of "endothelioid cells" or 'endothelial leucocytes.' They are given this name, because it is believed that they are derived from the endothelium which is supposed to line the lymph sinuses and cover the strands of reticulum of lymph nodes, or from the "endothelial" lining of the venous sinuses of the spleen, or from that which lines the blood and lymph vessels, primarily the latter.

Mallory calls all these cells 'endothelial leucocytes,' and he believes that they correspond to the so-called "large mononuclear leucocytes" of the circulating blood. Of the latter he says (Principles of Pathologic Histology, p. 21): "They are derived from the endothelial cells lining blood, and to a less extent lymph, vessels by proliferation and desquamation. They also multiply by mitosis after emigration from the vessels into the lesions." In this connection Mallory's idea of the structure of a lymph node is of interest. On page 616 he states: "Next to the cells of the lymphocyte series the most important cells of the lymph nodes are the endothelial cells. They line the blood vessels, the lymph sinuses and the reticulum of the parenchyma. Those lining the sinuses and the reticulum play a much more important part in pathologic conditions than those lining the blood vessels. They may increase greatly in number, desquamate from the walls of the sinuses and from the reticulum and form endothelial leucocytes. As a rule they exhibit marked phagocytic properties for other cells. The capsule and trabeculae are composed of fibroblasts, among which are occasional smooth muscle cells. Fibroblasts also form the reticulum in the lymph sinuses and in the parenchyma and strengthen the walls of the vessels."

From this we see that Mallory believes the entire reticulum of a lymph node to be covered by a distinct endothelium which is independ-

ent of the reticular cell, which he describes as fibroblasts. This endothelium not only lines the sinuses but also covers the reticular strands of the parenchyme. Mallory is quoted so extensively because, in the main, his views coincide with those of most American pathologists.

The writer became interested in this problem while working over the lymphoid tissue of a fish (*Folia Haem.*, Bd. 8). Here it was found that the blood and lymph spaces of the lymphoid tissue were not lined by a distinct endothelium, and that cells which might be mistaken for endothelial cells were merely portions of the general reticulum, in many cases with fibrils running through their protoplasm. The reticulum was partly fibrous and partly protoplasmic; where the fibers were present they were always embedded in the protoplasm of the reticular cells. The question was investigated again in connection with a problem on the origin of the lymphocytes in lymph nodes and spleen (*Arch. f. mikr. Anat.*, Bd. 80), and lately in connection with a study of the histology of the spleen and lymph nodes in Gaucher's disease.

The conclusions from this study of the reticulum and its supposed relations to endothelial cells are very different from those of such anatomists as v. Ebner and Stöhr, and the greater number of pathologists. Even with ordinary methods it is evident that the strands of the reticulum are composed of branched, anastomosing cells which are closely associated with the fibers. Nothing can be seen of a continuous epithelial covering. Associated with these strands, especially where the reticulum forms the wall of a sinus, are varying numbers of larger and more rounded protoplasmic cells whose connection with the fibers of the reticulum is not so evident with ordinary methods. Such cells, especially where they project out into the lumen of a sinus, might well be mistaken for hypertrophied endothelial cells. However, the use of any one of the numerous specific stains for reticular fibers (Krause's iodo-iodide of potassium—gold chloride method, the Maresch-Bielschowsky, or the older formula of Mallory's hematoxylin as used by Thomé) shows clearly that these cells are frequently traversed by fibers, and that even the large rounded cells resembling large mononuclear leucocytes are frequently attached to the reticulum and have fibers embedded in their peripheral portions. These latter cells show great phagocytic activity, especially for red corpuscles, and their nuclei are large and indented. If these cells were not attached we would not hesitate to pronounce them as large mononuclear leucocytes. Frequently large numbers of similar cells are seen free in the sinuses and in the meshes of the reticular network. It is no difficult matter to show that they have been derived from the reticulum. These same cells are very numerous in the lymph of the thoracic duct and in the lymph of the lymph vessels beyond the lymph nodes. It therefore seems proven that large mononuclear leucocytes, or at least cells which cannot be distinguished from them morphologically, may be derived from the reticulum of the lymph nodes; in fact it is possible to demonstrate all intermediate stages between ordinary reticular cells and these larger cells. These cells

are frequently seen to be dividing by mitosis both within the lymph nodes and within the thoracic duct. The resulting daughter cells will be smaller cells resembling lymphocytes in structure.

The specific stains for reticular fibers, especially when followed by a good counterstain, show further, that the reticular fibers of the strands within the parenchyme are embedded in the protoplasm of the cells. With these methods it is impossible to see an endothelial covering to these strands. Since there is no endothelium covering the reticular strands or lining the sinuses we are hardly justified in naming the large cells which are cut off from the reticulum 'endothelial leucocytes.' Whether such cells may also be derived from the endothelial cells lining lymph and blood vessels, as claimed by Mallory, still remains to be demonstrated. Theoretically there is nothing against such a view, since numerous investigators, including the writer, have shown that similar cells may be derived from the covering cells of the omentum and serous layers generally. However, Weidenreich and Schott believe that these covering cells are merely flattened surface fibroblasts (see also experiments of W. C. Clarke, *Anat. Rec.*, vol. 8, no. 2, p. 95). If this view is correct these covering fibroblasts would not be very different from the reticulum cells of the lymph nodes.

There is nothing new about the results obtained from this study of normal animals, since Thomé, Weidenreich and others reached the same conclusions. The pathologists Rössle and Yoshida, working with the Maresch-Bielschowsky method, also concluded that it is impossible to distinguish between endothelial cells and cells of the reticulum, and Ferguson, using the same method, found that the fibers of the reticulum are largely embedded in the protoplasm of the reticular cells. This literature, however, is almost unknown to pathologists; consequently statements like those quoted from Mallory are constantly reappearing in the pathological literature, and to some extent in the anatomical literature also (Evans, *Anat. Rec.*, vol. 8, no. 2, p. 101).

Gaucher's disease has already been mentioned as one of those diseases which are characterized by the presence of large numbers of the so-called 'endothelioid' cells in the spleen, lymph nodes, liver, and bone marrow. The writer was fortunate in obtaining some of this material from Dr. F. S. Mandlebaum (Pathologist, Mount Sinai Hospital, New York City). The fixation of the material is unusually good, and so it is ideal material for working out the origin of the 'endothelioid' cells. In this case these cells are large clear cells characterized by the presence of exceedingly fine fibrils in their cytoplasm. American pathologists have claimed that they were derived from the endothelium, especially from that lining the venous sinuses of the spleen, and the lymph sinuses of the lymph nodes. Several German pathologists have suspected that the reticulum was concerned in the formation of these cells, but none of them were able to prove this positively, as they could not find the necessary intermediate stages between reticular cells and the large cells of the disease. Mandlebaum's material.

however, shows the early stages in the disease, and it is not difficult to find all of the necessary intermediate stages between the characteristic cells of the disease and the cells of the reticulum, as I hope to be able to prove with the demonstrations. In the liver, in the walls of the vessels, etc., they seem to be derived from fibroblasts. From this material it was impossible to prove the origin of these cells from the 'endothelium' of the venous sinuses of the spleen. However, if such were the case it would in no way invalidate the above findings, as it is now generally conceded by anatomists that this 'endothelium' is merely a specially modified portion of the reticulum.

In Hodgkin's disease we again have cells which have been called 'endothelioid' cells. They are very different in character from the large cells seen in Gaucher's disease, nevertheless, their origin from the reticulum of the lymph nodes is easily demonstrated. Reticular fibers may be seen penetrating their protoplasm, and the same is true of the Gaucher cells while they are still attached to the reticulum.

These facts, and the results obtained from a study of normal lymph nodes, show that the large cells which are characteristic of many pathologic processes, and which are numerous in the sinuses of normal lymph nodes, in the lymph of the thoracic duct, etc., are in most cases not derived from endothelial cells. There is, therefore, no reason for naming them 'endothelial leucocytes' or 'endothelioid' cells. In most cases they could be designated as "reticular" cells. However, this would not do for a general term, because Dominici, Weidenreich and Downey among others have shown that large mononuclear leucocytes may also be derived from lymphocytes. The intermediate stages in this process are shown in one of the lantern slides. One of the slides will also show that the reverse may be true, i.e., that lymphocytes may be derived from large mononuclear leucocytes.

Those investigators (Goldmann, Evans and Schulemann, Aschoff, Kiyono, etc.) who have recently been engaged in the study of the results of vital staining by means of lithium carmine and the dyes belonging to the benzidine group will not agree with the view of the relationships between cells of the reticulum and large mononuclear leucocytes and lymphocytes expressed above. However, it must be remembered that they have not yet succeeded in showing that the cells which are able to store the dyes in the form of granules (a process related to phagocytosis—Evans and Schulemann) are *genetically* different from those which do not take up the dye. Their results are equally well explained if we assume that those lymphoid cells which are located in the tissues or which have recently been cut off from the reticulum are in a condition which is especially favorable for phagocytosis, a fact which was known long before the modern investigations with vital staining were begun. We know that reticular cells, while they are still attached to the reticulum, show special phagocytic activity, and that this activity may be increased after the cells have separated from the reticulum. This can easily be proven by an examination of the large reticular cells in the sinuses of any lymph node

which contains free red corpuscles. In the lymph of the thoracic duct or in the peritoneal fluid the phagocytic activity of these cells is still very pronounced, but it is greatly diminished as soon as they reach the blood stream. In the tissues the phagocytic activity is again very pronounced. The function, and to some extent the morphological appearance, of a lymphoid or 'endothelioid' cell depends, therefore, on the conditions under which it finds itself. It is not necessary to assume the existence of a special line of 'histiocytes' which differ genetically from the other lymphoid cells.

19. *On the anlage of the bulbo-urethral and major vestibular glands in the human embryo.* (Lantern). ARNOLD H. EGGERTH, Department of Anatomy, University of Michigan. (Presented by G. Carl Huber.)

For this investigation, the urogenital systems of four human embryos of critical ages from the collection of Dr. Huber were reconstructed. In each of the models, the urogenital sinus presents three pair of symmetrically placed lateral epithelial folds. The cephalic ends of the middle of these lateral folds bear short epithelial buds, the anlagen of the bulbo-urethral and major vestibular glands. The measurements given are for crown breech length. The model of a 32 mm. female embryo presents a short epithelial bud, 15  $\mu$  in length, on the left side only. The model of a male embryo of 30 mm. presents gland buds on both sides, whose respective lengths are 50  $\mu$  and 60  $\mu$ . A female embryo of 45 mm. shows gland buds having a length of 120  $\mu$  and 150  $\mu$ , and a female embryo of 60 mm. presents gland buds with terminal branching, having a length of 200  $\mu$  and 240  $\mu$ . The relative positions for the gland anlagen in both male and female embryos for the varying ages as reconstructed, is essentially the same.

20. *The cell clusters in the dorsal aorta of the pig embryo.* V E. EMMEL, Department of Anatomy, Washington University Medical School.

In the course of a study of hematogenesis in several regions of the mammalian vascular system, the following observations were made on the dorsal aorta of the pig embryo. The material studied consisted of about seventeen embryos varying from 6 to 25 mm. in length, together with several mouse and rabbit embryos. In the dorsal aorta of the 6 to 15 mm. specimens there occur rounded cell masses or clusters, the cytological characteristics of which identify the component cells as belonging to the mesamoeboids of Minot or the primitive lymphocytes of Maximow. Their constant occurrence at certain stages of development, their evident more or less firm attachment to the vascular surface, and their restriction, apparently without exception, to the ventral wall of the aorta, appear to necessitate relegating to these clusters a significance greater than that of agglutinated cell masses merely incidentally resting upon the aortic wall. The absence, in many cases, of evident endothelial continuity at the basal regions of these clusters, the transitional cytological characteristics from the

basal to the more peripheral cells, the changes in form and increase in number and size of the adjacent endothelial nuclei, together with the frequent occurrence of mitotic figures within the masses, is evidence strongly indicative of their active proliferation and origin *in situ* from the aortic endothelium. At the 15 mm. stage the clusters have become greatly reduced in number and are no longer to be observed in the 25 mm. embryo. During this 6 to 20 mm. period of development, there occurs in the ventral region of the aorta, in contrast to the dorsal region, an extensive degeneration of the medial and lateral intersegmental aortic arteries and a remarkable 'caudal wandering' of the coeliac and mesenteric arteries upon the aortic wall. The simultaneous appearance of these phenomena in the ontogeny of the embryo and the morphological interrelationships of the structures under consideration in the ventral aortic wall are of such a character as to be suggestive of some significant correlation between the formation of these clusters and the development of the permanent visceral arteries of the adult.

21. *Feeding experiments on rats.* J. F. GUDERNATSCH, Department of Anatomy, Cornell University Medical College, New York City.

Based upon the results obtained by feeding the internally secreting glands to amphibians, these experiments are being continued, this time with mammals. The glands are given to white rats in stated portions, at regular intervals. A preliminary account of some observations on the thyroid-treated animals may here be given.

Beef thyroid was fed in portions small enough to keep the animals in fairly good health; 1 gram a week was given, in some experiments 1 gram in 5 days. The application of even so small doses of thyroid sometimes produced slight symptoms of hyperthyroidism; however, the animals kept well enough to have offspring.

The following enumeration gives the records of 8 successful matings; in the first 4 cases the offspring are still living, while in the remaining 4 the young died, at stated dates.

*Case I:* ♂ *t* × ♀ *t*.<sup>1</sup> (a) While under treatment the father was bred to 3 treated ♀; no result. (b) After discontinuation of the thyroid treatment the father was bred to a non-treated ♀; 10 young were born after 26 days, all so frail that they died within a week. (c) One month after thyroid treatment of the father and immediately after thyroid treatment of the mother the two were mated; 3 young were born 54 days after the father and 29 days after the mother had received their last dose of thyroid. The young are much smaller than the normal rats of equal age.

*Case II.* ♂ *t* × ♀ *n*. After discontinuation of the thyroid treatment the father was bred to the non-treated mother; 4 young were born 30 days later; 2 died very soon, 2 undersized ones are living.

*Case III:* ♀ *t* × ♂ *n*. (a) While under treatment the mother was

<sup>1</sup> *t* = treated; *n* = normal.

bred to a treated ♂; no result. (b) Immediately after thyroid treatment the mother was bred to the non-treated father; 6 young were born 113 days later; 4 undersized ones are living. The mother required 3 months to recover from the thyroid influence.

*Case IV:* ♂ *t* × ♀ *n*. (a) While under treatment the father was bred to a treated ♀; no result. (b) The normal mother was bred to 2 normal ♂; 2 litters. (c) After discontinuation of thyroid treatment the father was bred to the normal mother; 3 young were born 117 days later; they are undersized. The father required 3 months to recover from the thyroid influence.

*Case V:* ♂ *t* × ♀ *n*. (a) While under treatment the father was bred to a non-treated ♀; no result. (b) While under treatment the father was bred to the non-treated mother; 5 young were born 24 days later; 1 died 9 days old, 4 very frail and undersized lived about 7 months. When 3 months old they weighed 57, 58, 60 and 66 grams respectively; (65 to 70 grams is the average weight of a rat about 60 days old).

*Case VI:* ♂ *t* × ♀ *t*. (a) While under treatment the father was bred to the treated mother; no result. (b) While under treatment the father was bred to a non-treated ♀; 5 young (see Case V). (c) The treated father and treated mother (under a) were again mated; thyroid treatment ceased 31 days later; 7 young were born 81 days later; very frail and undersized; lived 2 months. The parents required 2 months to recover from the thyroid influence.

*Case VII:* ♂ *t* × ♀ *n*. (a) While under treatment the father was bred to 3 treated ♀; no result. (b) After discontinuation of the thyroid treatment the father was mated to the non-treated mother. Ten young were born 26 days later; all died within 2 weeks.

*Case VIII:* ♀ *t* × ♀ *n*. (a) The non-treated father was bred to 2 non-treated ♀; 2 litters. (b) Before thyroid treatment the mother was bred to a non-treated ♂; 1 litter. (c) While under treatment the mother was bred to a treated ♂; no result. (d) After discontinuation of the thyroid treatment the mother was bred to the non-treated father; 5 young were born 151 days later; all died within 5 days. The mother required 4 months to recover from the thyroid influence.

The history of these cases shows that the feeding of thyroid to rats greatly interferes with their breeding qualities. Twenty-four matings, in which both parents were treated, resulted in failure, 2 in which the female alone had been treated and 4 in which the female alone received thyroid food, in all 30 matings. Yet out of these 14 males 7 had been tested and given offspring previously to the treatment, and out of the 16 females 9 had been tested and were found fertile.

Table 1 gives the enumeration of several matings, which will show that pregnancy did not set in until several weeks after the discontinuation of the thyroid treatment, except when the female was non-treated. The number of days is given that elapsed between the placing together of the parents and the birth of a live litter (or death of the female). The gestation period of the rat is from 21 to 24 days.

Thus under no circumstances will pregnancy set in during the thyroid treatment (cases 3 to 7, 15); after discontinuation of the thyroid treatment, the animals usually required several weeks to recover from the thyroid influence (cases 11 to 18).

TABLE 1

(1) ♀ dies after 2 days	} No pregnancy	} Thyroid given after mating
(2) ♀ dies after 2 days		
(3) ♀ dies after 7 days		
(4) ♀ dies after 19 days		
(5) ♀ dies after 21 days		
(6) ♀ dies after 21 days		
(7) ♀ dies after 38 days		
(8) ♀ normal; young born after 24 days; all die early	} No thyroid given after mating	}
(9) ♀ normal; young born after 26 days; all die within two weeks		
(10) ♀ normal; young born after 28 days; 2 die within a week		
(11) ♀ dies after 57 days; 6 fetuses		
(12) ♀ dies after 67 days; 7 fetuses	} Thyroid feeding continued for 33 days after mating.	}
(13) ♀ dies after 107 days; pregnant		
(14) ♀ dies after 112 days; pregnant		
(15) young born after 110 days, 87 days after thyroid treatment	} No thyroid given after mating	}
(16) ♂ normal; young born after 113 days		
(17) ♀ normal; young born after 117 days		
(18) ♂ normal; young born after 151 days		

Only one mating of both parents previously treated gave offspring after 29 days. However, the treatment of the father had ceased one month before the mating time.

The feeding to rats of fresh thyroid tissue shows its effect in three different ways:

1. When the dose is too large, all the well-known symptoms of hyperthyroidization become evident, viz.: emaciation, diarrhoea, muscular weakness and finally cachexia leading to death. The hair becomes yellowish, stands erect, sometimes falls out in patches, in short the entire coat looks ragged.

2. When the dose is so regulated, as to keep the animals in approximately good health—the fur will always become shabby—then the animals do not breed. *Not one mating of both parents treated, after the animals had been placed together, gave any result.* Pregnancy was always delayed, since fertilization did not occur until several weeks after the application of the thyroid had been discontinued.

3. Did pregnancy finally occur, it resulted (a) in abortus; (b) the young died soon after birth; (c) in very late pregnancies, the young show a diminished tendency to grow. Although they are not especially frail, they keep in relative size behind the young of normally fed rats.

22. *The development of reflex mechanisms in Amblystoma.* C. JUDSON

HERRICK AND GEORGE E. COGHILL.

The results of neurological studies of *Amblystoma* by the authors and others now afford a tolerably sound basis for the interpretation of some features of the mechanism of functional differentiation of the central nervous system of the individual and may also contribute something to the knowledge of the factors involved in the phylogenetic differentiation of the nervous system.

Most of the observations on the nervous system of *Amblystoma* and other urodeles from which these conclusions have been deduced are recorded in the following papers:

COGHILL, GEORGE E. 1902 The cranial nerves of *Amblystoma tigrinum*. *Jour. Comp. Neur.*, vol. 12, pp. 205-289.

1909 The reaction to tactile stimuli and the development of the swimming movement in embryos of *Diemyctylus torosus* Eschscholtz. *Jour. Comp. Neur.*, vol. 19, pp. 83-105.

1913 The primary ventral roots and somatic motor column of *Amblystoma*. *Jour. Comp. Neur.*, vol. 23, pp. 121-143.

1914 Correlated anatomical and physiological studies of the growth of the nervous system of Amphibia. I. The afferent system of the trunk of *Amblystoma*. *Jour. Comp. Neur.*, vol. 24, pp. 161-233.

HERRICK, C. JUDSON. 1914 The medulla oblongata of larval *Amblystoma*. *Jour. Comp. Neur.*, vol. 24, pp. 343-427.

*The swimming reflex.* The reflex mechanism essential to swimming in *Amblystoma* embryos of the youngest age in which this reflex is possible consists of three groups of neurones: (1) sensory peripheral neurones lying within the spinal cord (the transitory Rohon-Beard cells) which send their dendrites to the skin and myotomes, while their axones ascend in a dorso-lateral sensory tract of the cord; (2) commissural neurones which pass from the sensory cells of one side to the motor cells of the other through the ventral commissure; the decussation of these fibers occurring only in the upper spinal cord and lower medulla oblongata; (3) motor cells, which form a descending, ventro-lateral motor tract and innervate the myotomes by means of collaterals. It should be noted particularly that all responses of such embryos are 'total reactions,' and of the same sort regardless of the place and kind of excitation; that the peripheral sensory fibers are not specific with reference to exteroceptive and proprioceptive stimuli; that the sensory and motor peripheral neurones are not differentiated away from central neurones of longitudinal columns, and that the first central paths to appear are long and made up of chains of numerous relatively short neurones.

*Spinal reflexes in half-grown larvae.* The spinal ganglion cells and ventral horn cells are at this age fully matured, and crossed as well as uncrossed reflexes have become possible at all levels in the spinal cord. Both correlation neurones and ventral horn cells send dendrites into all parts of the cross section of the white substance, some even

crossing in the ventral commissure. The responses are still in large measure simple and 'total reactions,' but they are brought under the influence of a much greater variety of excitations than are those of young embryos, in consequence of the introduction of longitudinal tracts that are actuated by special sense organs, especially those of the head.

*The mammalian spinal cord.* Here the correlation neurones are organized into an elaborate system of distinct reflex circuits, and there is a corresponding specialization and refinement of motor functions.

*The medulla oblongata of larval Amblystoma.* Each physiological type of end organ has its own distinct ganglion or ganglia and nerve roots. Each root fiber from the several types of end organs, immediately upon entering the medulla, divides into ascending and descending branches which pass upward and downward for practically the entire length of the medulla oblongata. These bundles of root fibers constitute nearly all of the substantia alba of the dorsal half of the medulla, with the exception of two large, longitudinal correlation paths on either side. The ventral half of the white substance contains the motor roots and numerous long correlation tracts. In contrast with the sharp physiological differentiation of the sensory neurones of the first order, those of the second order are not functionally specific, for the dendrites of any one of them may establish synaptic relations with several or all of the peripheral sensory root bundles. The primary sensory centers, therefore, serve, not only as receptive centers, but also as correlation centers.

*The medulla oblongata of mammals.* The arrangement of the peripheral sensory neurones in the mammalian medulla oblongata is essentially the same as in the amphibian. The sensory neurones of the second order, however, are segregated into definite primary receptive centers, each related specifically to one peripheral system, and the secondary paths leading from these primary centers may be as specific functionally as are the peripheral root bundles themselves. The correlation of these elements into particular reflex systems is effected in centers farther removed from the first sensory neurone of the arc.

*Conclusion.* It is the prevailing belief that every form of central nervous system has arisen by the concentration of an original diffuse and relatively equipotential peripheral ganglionic plexus. Out of such a primordial nervous matrix there has been a progressive individuation of centers and pathways and a parallel progressive differentiation of specific reflexes away from the primitive type of 'total reaction.' The reflex mechanisms of embryonic and larval *Amblystoma* are in many respects primitive; and their forms suggest that they represent different stages in this process of individuation of specific reflexes. The 'typical' two-neurone, short circuit connection between dorsal and ventral root fibers is, therefore, not to be regarded as primitive. During such processes of individuation of parts of the nervous system its integrative action has been preserved through the development of correlation centers farther removed from the receptors and effectors. Thus arose such supra-segmental apparatuses as

the cerebellum and cerebral cortex. Finally, we would urge that the factors operating in either the ontogenetic or the phylogenetic differentiation of the functional mechanisms of the brain cannot profitably be investigated without a precise knowledge in each stage investigated of the peripheral relations of each of these functional systems and of the interrelations of the neurones involved at every step in the progress of the nervous impulse from periphery to center and back to the effector organs during the normal course of functional activity.

23. *The development of fibrous tissues in peritoneal adhesions.* ARTHUR E. HERTZLER, Kansas City, Mo.

The material used in this study was obtained by causing adhesions of intestines by suture or by irritants and by attaching foreign bodies to the mesentery or omentum.

When a suture of adjacent loops of intestine is placed, the space between the guts is filled with an amorphous exudate. In 10 to 30 minutes this exudate coagulates forming fibrinous bands which extend from one gut surface to the other. These bands stain specifically with Weigert and Mallory stains. By comparing series it can be noted that the bands first lose the specificity for Weigert while retaining it for Mallory. This occurs in 24 to 48 hours. In 4 days they no longer accept the Mallory stain for fibrin but do accept the Mallory fibril stain. Bands may be seen which stain in part red and in part blue with the Mallory stain. With picro-fuchsin the same transition attains.

When a disc of foreign material is sewed to the mesentery it is covered at once with an exudate. This coagulates over the entire surface and its conversion into fibrous tissue takes place simultaneously over the entire disc and does not proceed from the edges toward the center.

These fibrin bands may form in the exudate before the advent of cellular elements and the transition above noted may take place without the advent of cells.

Any process which prevents the exudate from coagulating into fibrin prevents wound healing. This is true irrespective of the means employed. Infections produce this effect permanently and peptonization of the animal prevents it temporarily.

If wound healing has been delayed by any means which prevents the formation of fibrin in the primary exudate then healing must await the advent of new exudate. This takes place only when cells find their way into the unfriendly exudate. The formation of fibrous tissue then takes place according to the methods described in the literature. The healing of wounds as described in the literature is in fact healing by second intention.

24. *On the development of the digitiform gland in Squalus acanthias.*

E. R. HOSKINS, Institute of Anatomy, University of Minnesota.

The digitiform gland in *Squalus* is evidenced first by a slight thickening of the entoderm of the dorso-lateral border of the gut just pos-

terior to the spiral valve. This may be seen in embryos 15 mm. in length, especially in those sectioned longitudinally. The thickening soon pushes laterally to form a hollow bud which turns and grows anteriorly along the gut. The form of the curved portion at the point of emergence from the gut always persists so that in older stages and in the adult this portion which becomes the duct of the gland enters both the intestine and the digitiform gland anteriorly.

In the stage of 28 mm. it may be seen that from the main part of the gland small buds resembling the original form of the gland grow laterally on all sides. These buds become tubules extending laterally and slightly posteriorly. They in turn give rise to secondary tubules which in time form irregular groups opening into the primary tubules. This condition is to be found throughout development, the gland becoming a compound tubular structure, the secondary tubules arising from the primary, close to the main lumen of the gland.

As the gland develops, it carries the mesentery of the intestine with it and is thus supported from the dorsal wall of the body cavity.

The entoderm of the digitiform gland is composed at first of four layers of low columnar or cuboidal cells with elongated nuclei, being similar to the entoderm of the gut from which it develops. As the gland increases in length, the epithelium is gradually reduced to one layer in thickness. Its primary and secondary tubules both arise as structures of an epithelium of one layer of cells. At the points of greatest growth, namely, at the distal ends of the tubules the nuclei are wider and shorter than along the main lumen, often being spherical.

The epithelium lining the main or central lumen later thickens giving us a structure of two layers of columnar cells with rounded nuclei in the full-term fetus and of four layers in the adult.

25. *The development of the albino rat, from the end of the first to the tenth day after insemination.* G. CARL HUBER, Department of Anatomy, University of Michigan.

The material on which this investigation is based was collected while the writer was stationed at The Wistar Institute of Anatomy. For the trustworthiness of the records pertaining to the time of insemination of the female rats used, he is greatly indebted to Dr. J. M. Stotsenburg. The age of the stages as given in this account is reckoned from the time when copulation was first observed, thus from the time of insemination. Carnoy's fluid was used as a fixative; paraffin embedding and staining in hemalum and Congo red was the general procedure. The process of ovulation, maturation and fertilization having been carefully studied by Sobotta and Burekhard, their account carrying the development to the pronuclear stage, my own studies of the development of the albino rat begin with this stage.

The pronuclear stage extends through a relatively long period, perhaps 12 to 15 hours. All ova obtained 24 hours after insemination present the pronuclear stage, this period presenting about the middle of the pronuclear phase. Of the two pronuclei, the female

pronucleus is slightly the larger. The nuclei lie near the centre of the ovum, are distinctly membranated, and do not fuse prior to the formation of the first segmentation spindle. By the end of the first day, the fertilized ova have travelled about one-fourth the length of the oviduct, and are found lying free in its lumen. The formation of the first segmentation spindle, and the first segmentation occur during the early part of the second day after insemination. The resulting 2 cell stage extends for a period of about 24 hours, since 2 cell stages were found in material obtained 1 day, 18 hours to 2 days, 22 hours after insemination. The first two blastomeres are equivalent cells. By the end of the second day after insemination, all the fertilized ova are in the 2 cell stage, having traversed a little over one-half the length of the oviduct. One of the cells of the first two blastomeres divides before the other resulting in a three cell stage; such a stage was obtained 2 days, 19 hours, and 2 days, 22 hours after insemination. The division of the other of the first two blastomeres soon follows, so that by the end of the third day all tubes examined contained ova in the 4 cell stage, they having traversed by this time about  $\frac{1}{10}$  of the length of the oviduct.

An 8 cell stage is reached toward the end of the fourth day after insemination (3 days, 17 hours) and by the end of the fourth day, the segmenting ova, in a 12 cell to 16 cell stage, pass from the oviducts to the uterine horns.

It will be observed that beginning with the pronuclear stage, found 24 hours after insemination, there occur three successive segmentations, spaced at intervals of about 18 hours and resulting in 2, 4, and 8 cell stages during transit of the ova through the oviducts. During the fourth segmentation, the ova pass from the oviducts into the uterine horns. Weighings of the water displaced by a series of madels made of early segmentation stages indicate that during the first 4 days of the development of the albino rat there is only very slight increase of the size of the egg mass as against the unsegmented ovum with two pronuclei.

During the early hours of the fifth day after insemination all of the segmenting ova of the albino rat are to be found lying free in the lumen of the uterus, spaced as in later stages of development, the fifth series of segmentations having been completed by this time, the resulting morula mass having an ovoid form and consisting of 24 to 32 cells and measuring approximately  $80 \mu$  by  $50 \mu$ . During the middle of the fifth day after insemination, the early stages of blastodermic vesicle or blastocele formation may be found. The segmentation cavity or blastocele begins as a single, irregularly crescentic space, arising between cells, and is excentrically placed. The early stages of blastocele formation are thus observed in morula masses composed of 30 to 32 cells, these lying free in the cavity of the uterus. The enlargement of the blastocele, after its anlage, is obtained by a flattening of the border or roof cells; to a lesser extent, by an increase in the number of these cells. By the end of the fifth day after insemination,

all the ova are found in the blastoderm vesicle stage, one pole of each vesicle, designated its floor, consisting of a relatively thick mass of cells; the other pole, its roof, consisting of a single layer of flattened cells bordering and enclosing the segmentation cavity. Cell differentiation into a layer of covering cells, a layer of ectodermal and entodermal cells, such as described by Selenka, is not to be observed at this stage. During the sixth day after insemination, at which time the ova still lie free in the lumen of the uterine horn, the blastodermic vesicles increase in size, partly as a result of further flattening of the roof cells, partly as a result of rearrangement and flattening of the cells constituting the floor of the vesicle, this portion of the vesicle now consisting of about three layers of cells, the innermost layer having differentiated to form the yolk entoderm. By the end of the sixth day the blastodermic vesicle consists of a discoidal area, the germ disc, comprising about  $\frac{1}{3}$  to  $\frac{1}{6}$  of the wall of the vesicle, and consisting of two to three layers of cells, of which the inner is differentiated to form the yolk entoderm, the remainder of the vesicle wall consisting of a single layer of very much flattened cells.

During the seventh day after insemination, the blastodermic vesicles become definitely oriented in the decidual crypts, the thicker portion of the vesicle wall, its floor, being directed toward the mesometrial border. During the early hours of the seventh day, cell proliferation, cell rearrangement, and enlargement of cells takes place in the region of the germinal disc, resulting in a marked thickening of this portion of the wall of the vesicle, manifested by an outward growth, as also a growth inward into the cavity of the vesicle, initiating the phenomena known as 'inversion of the germ layers,' or 'entypy of the germ layers.' In this thickening of the germ disc, there may be recognized on the one hand the anlage of the ectoplacental cone or 'Träger,' on the other hand, in the cell mass which extends into the cavity of the blastodermic vesicle, the anlage of the egg-plug or egg-cylinder. In the anlage of the egg-cylinder there may be recognized early a circumscribed compact mass of cells, staining somewhat more deeply, which mass of cells I have designated the ectodermal node, since it is the anlage of the primary embryonic ectoderm of the future embryo. This ectodermal node, so far as it extends into the blastocoel, is covered by the single layer of yolk entoderm, or as it is now known, the visceral layer of the entoderm. The ectodermal node is readily differentiated from the cells of the ectoplacental cone, with the base of which it is in close relation.

The more complete development and differentiation of the egg-cylinder, the anlage of which was noted during the seventh day, may be observed during the eighth day after insemination. The thin walled portion of the vesicle, its roof or antimesometrial portion, enlarges, assuming a distinctly cylindrical form. The ectodermal node with covering of the layer of visceral entoderm is forced into the cavity of the vesicle, this by reason of proliferation of the cells at the base of the ectoplacental cone, this resulting in the formation of a nearly

cylindrically formed column of compactly arranged, polyhedral cells, interposed between the ectodermal node and the base of the ectoplacental cone, but merging into the latter without sharp, demarkation. To this column of cells, the name of extraembryonic ectoderm is given. The ectodermal node and the extraembryonic ectoderm together form a cylindric structure, surrounded by a single layer of visceral entoderm, which reaches from the base of the ectoplacental cone to nearly the mesometrial end of the original segmentation cavity. During the latter half of the eighth day, a small cavity appears in the ectodermal node. This is the anlage of the mesometrial portion of the proamniotic cavity. The cells bounding this cavity, derived from the cells of the ectodermal node; constitute the primary embryonic ectoderm. Soon after the anlage of the mesometrial portion of the proamniotic cavity, several discrete spaces become evident in the extraembryonic ectoderm of the egg-cylinder, constituting the anlage of the antimesometrial portion of the proamniotic cavity, these discrete spaces quickly joining to form a single space, the antimesometrial portion of the proamniotic cavity, lined by a single layer of cells of the extraembryonic ectoderm. Toward the end of the eighth day the mesometrial portion of the proamniotic cavity, arising in the ectodermal node, and the antimesometrial portion of the proamniotic cavity, arising in the extraembryonic ectoderm, fuse to form a single proamniotic cavity, the mesometrial portion of which is lined by primary embryonic ectoderm, the antimesometrial portion of which is lined by extraembryonic ectoderm, the two types of ectoderm forming a continuous layer, their line of union, however, being readily distinguishable. This hollow ectodermal cylinder, attached to the base of the ectoplacental cone and extending to the antimesometrial end of the segmentation cavity, is surrounded by a single layer of visceral entoderm in the meantime differentiated into a portion which surrounds the primary embryonic ectoderm, which consists of flattened cells and is now known as the primary embryonic entoderm, and a portion surrounding the extraembryonic portion of the egg-cylinder, consisting of tall columnar cells with vacuolated protoplasm containing hemoglobin granules, and constituting an embryotrophic layer.

This cylindrical structure presents during the early part of the ninth day after insemination no evident bilateral symmetry, so that longitudinal sections, cut in planes at right angles to each other, present identical pictures. This is also evident in cross sections of the vesicles.

During the middle and latter part of the ninth day, active cell proliferation in the embryonic ectoderm in the region of the future caudal end of the embryo, leads to a distinct thickening of the embryonic ectoderm of this region. This thickening constitutes the primitive streak region. In it, there is developed a short axial groove, the primitive groove. From the edges of this groove, cells derived from the embryonic ectoderm, wander between ectoderm and embryonic entoderm. This constitutes the anlage of the mesoderm. There is no evidence of the participation of the embryonic entoderm in the anlage of the

mesoderm. Toward the end of the ninth day, and beginning of the tenth day after insemination, as a result of proliferation of the cells of the mesodermal anlage and further outwandering of cells from the embryonic ectoderm in the region of the primitive groove, the mesoderm extends so as to form a distinct layer situated between the two primary germ layers.

26. *The development of the lymphatic drainage of the anterior limb in embryos of the cat. Lantern.* GEORGE S. HUNTINGTON, Columbia University.

Two phases of the functional adaptation of early mammalian lymphatics are considered:

1. Since Miller's discovery in 1913 (*Am. Jour. Anat.*, vol. 15, pp. 131-198) of the haemophoric function of the avian thoracic ducts in the early stages of their development, attention has been directed toward the determination of homologous conditions during lymphatic ontogeny in embryos of the other amniote classes. In the mammal (cat) the area of the jugular lymphsac and of some of its tributaries offers in the early stages conditions corresponding to those of the bird, although they are more obscure by reason of the close association with the adjacent systemic veins, a difficulty not encountered in the avian axial lymphatic line. The interpretation of mammalian lymphatic ontogeny gained from the viewpoint of the functional adaptation of early lymphatic channels serves to clarify some heretofore doubtful points in the mutual relations of developing lymphatic and venous channels in the mammalian embryo.

The vessel which offers the least complicated and clearest view of the genetic processes involved is the primitive ulnar lymphatic, draining the lateral body wall and the anterior limb bud, and accompanying the primitive ulnar vein during the period of the latter's functional activity, prior to the establishment of the definite subclavian venous line.

The first anlagen of the developing primitive ulnar lymphatic are found in embryos between 7 mm. and 8 mm. as a disconnected series of intercellular mesenchymal spaces which form dorsal to the primitive ulnar vein and to the lower cervical nerve trunks. At first these spaces are irregular and communicate with smaller intercellular clefts in the surrounding mesenchyme. Later, in embryos of 8 mm. to 8.5 mm., they become distended and in part bounded by flattened mesenchyme cells. In embryos of 8.5 mm. to 9 mm. the originally separate individual spaces have united to form a continuous channel whose cephalic extremity effects a secondary junction with the dorsal division of the jugular lymphsac. This stage is usually completed in the 9 mm. embryo in which the primitive ulnar vein is paralleled at a little distance along its dorsal or dorso-lateral aspect by the distinct channel of the primitive ulnar lymphatic. The mesenchyme surrounding this vessel exhibits at numerous points groups of developing bloodcells. In the 9.5 mm. embryo this local haemopoiesis attains its fullest develop-

ment and the red bloodcells begin to crowd the previously clear lumen of the primitive ulnar lymphatic channel. The walls of the latter appear to be formed still in part by undifferentiated mesenchymal cells, in part by flattened cells assuming distinct endothelial characters. At numerous points the lumen of the lymphatic channel is still in open communication with smaller intercellular clefts in the surrounding haemopoetic mesenchyme. Some of these enlarge to include groups of bloodcells and become added to the main lymph channel. The majority of red cells appear to gain access to the latter through these avenues. It is of course possible that some of the blood-contents of the ulnar lymphatic are due to reflux from the jugular lymphsac, but the conditions in the 9.5 mm. embryo seem to point clearly to the inclusion of the cells in situ in the manner described.

In the 10 mm. and 11 mm. embryos the primitive ulnar lymphatic appears enlarged, lined by a definite and closed endothelium and the lumen densely crowded with red blood-cells. This is the fully developed haemophoric stage of the vessel.

In embryos of 11.5 mm. evacuation of the blood contents of the primitive ulnar lymphatic into the jugular sac and through the same into the precardinal vein occurs. This process is usually completed in the 12 mm. and 12.5 mm. stages. The proximal segment of the primitive ulnar lymphatic rapidly narrows after evacuation is completed and in embryos of 13 mm. to 14 mm. the continuity of the channel becomes interrupted a short distance caudad to its point of entrance into the jugular sac. The endothelial cells lining the lumen become larger, more rounded, stain deeply and appear to revert to the indifferent mesenchymal type, obliterating finally all trace of the former channel at this point. This may occur as early as the 13 mm. stage or be deferred to the 14 mm. or even the 15 mm. stage.

2. After the interruption of the primitive ulnar lymphatic at the level stated above, the distal segment of the channel enlarges rapidly by distension with clear fluid and by the addition of numerous new intercellular spaces forming in the surrounding mesenchyme. In this way a vast lymphatic reservoir, the axillary lymphsac, is formed which for a time receives and stores the lymph drained from the limb-bud and body wall. The former path of lymphatic drainage of this area via the primitive ulnar lymphatic *dorsal* to the nerve trunks of the anterior limb into the jugular lymphsac having been interrupted, as above described, a new *ventral* lymphatic line is now established by the concurrence of numerous originally separate lymph. spaces developed along the course of the recently established subclavian vein. The resulting channel connects cephalad with the ventral process extending caudad from the subclavian approach of the jugular lymphsac over the ventral face of the jugulo-subclavian venous angle. Distally it opens into and drains the axillary sac which subsequently becomes reduced in extent and incorporated in the permanent thoraco-appendicular lymphatic system. The axillary sac thus functions as a temporary storage reservoir for the lymph pending the completion of the change from

the *dorsal* primitive ulnar to the *ventral* permanent subclavian line of lymphatic drainage of the anterior limb and lateral body wall.

The definition of stages given above is based on observations covering a large number of closely graded embryos of the cat and represent the average. Considerable individual chronological variation is encountered. The material used comprises the following series of the Columbia University Embryological Collection in transverse section:

Cat 7.0 mm.	Serial No. 105, 108, 119, 121, 135, 137, 138, 266, 281, 487, 488, 752
Cat 7.5 mm.	Serial No. 282, 284, 486, 567, 595
Cat 8.0 mm.	Serial No. 89, 102, 485, 596, 597, 703
Cat 8.5 mm.	Serial No. 285, 466, 490, 704
Cat 9.0 mm.	Serial No. 106, 136, 265, 268, 421, 458, 459, 462, 467, 468, 489, 491, 492, 589
Cat 9.5 mm.	Serial No. 132, 133, 239, 269, 273, 461, 497, 499, 501, 598, 599
Cat 10.0 mm.	Serial No. 79, 101, 111, 112, 113, 114, 140, 237, 272, 274, 474, 477, 478, 496, 498, 500, 707
Cat 10.5 mm.	Serial No. 81, 118, 120, 479, 480, 720
Cat 11.0 mm.	Serial No. 77, 98, 213, 473, 566, 718, 719
Cat 11.5 mm.	Serial No. 251, 256, 472
Cat 12.0 mm.	Serial No. 78, 97, 100, 217, 263, 471, 744
Cat 12.5 mm.	Serial No. 264, 590, 591, 592
Cat 13.0 mm.	Serial No. 92, 107, 262
Cat 13.5 mm.	Serial No. 76, 189, 223
Cat 14.0 mm.	Serial No. 122, 127, 210, 211, 212, 214, 747

The paper is illustrated by photomicrographs of the sections and Lumière lantern slides of the reconstructions.

27. *Effect of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats.* C. M. JACKSON, Institute of Anatomy, University of Minnesota, Minneapolis.

Twenty-one well-nourished adult rats were used, initial body weights varying from 182 to 367 grams. Fifteen rats were used for acute inanition, being allowed water but no food. They were killed after 6 to 12 days, the loss in body weight varying from 25 to 39 per cent (average loss, 36 per cent). Six rats were subjected to chronic inanition, being underfed so as to reduce the body weight slowly through a period of about five weeks, and were killed when the loss in body weight reached about 36 per cent. The results below stated apply in general to both acute and chronic inanition, unless otherwise specified. The published data of Donaldson, Hatai, Jackson and Lowrey are taken as normal for comparison. On account of the great variability of some organs and the relatively small number of observations, final conclusions are in some cases uncertain.

The head and fore-limbs lose relatively less than the body as a whole. Their relative (percentage) weight therefore increases. Of the systems—integument, skeleton, musculature, viscera and 'remainder'—the in-

tegument loses relatively nearly the same as the whole body, and therefore nearly maintains its original relative (percentage) weight. The same is true of the musculature, which however undergoes a somewhat greater loss in relative weight during chronic inanition. The visceral group, as a whole, undergoes little change in relative weight, decreasing slightly in acute inanition. The individual organs, however, vary greatly, as indicated below. The skeleton retains nearly its original absolute weight, and therefore increases greatly in relative weight. There is a corresponding decrease in the 'remainder,' due chiefly to loss of fat.

The individual viscera may be classified in three groups:

(1) The brain, spinal cord and eyeballs show little or no loss in absolute weight, compared with the normal at the initial body weight, hence their relative (percentage) weight has markedly increased with the diminution in body weight during inanition. The same is apparently true for the thyroid gland in acute inanition, but in chronic inanition there is apparently a loss, though relatively less than in the body as a whole. The suprarenal glands also apparently lose less in absolute weight than the body as a whole, hence their relative (percentage) weight increases.

(2) The heart, lungs, kidneys, testis, epididymis and hypophysis undergo nearly the same relative loss in weight as the body as a whole, therefore their relative (percentage) weight remains nearly the same. The thymus has already undergone age involution, and is therefore not materially affected by inanition.

(3) The liver and the alimentary canal (both empty and including contents) usually decrease in weight relatively more than the body as a whole. The spleen is exceedingly variable; in acute inanition it usually shows a marked decrease in relative weight (although averaging higher in chronic inanition).

28. *Changes in young albino rats held at constant body weight by underfeeding for various periods.* C. M. JACKSON, Institute of Anatomy, University of Minnesota, Minneapolis.

Ten litters, including 65 rats, were used. Twenty-five rats were used as controls, including 11 at 3 weeks of age, 2 at 6 weeks, 6 at 10 weeks, 3 at 32 weeks, and 3 at 35 weeks. In addition, data previously gathered by observations upon several hundred normal rats were available for comparison. Forty rats were held at constant body weight by underfeeding for various periods, 8 rats from age of 3 weeks to age of 6 weeks; 3 rats from 3 weeks to 8 weeks; 22 rats from 3 weeks to 10 weeks; 1 rat from 3 weeks to 13 weeks; 1 rat from 3 weeks to 16 weeks; 2 rats from 6 weeks to 32 weeks; and 3 rats from 10 weeks to 35 weeks. On account of the great variability of some organs, the number of observations in some cases is insufficient for final conclusions.

As to body proportions, the relative weights of the head, trunk and extremities remain practically unchanged in young albino rats held at constant body weights.

Of the systems—integument, skeleton, musculature, viscera and

'remainder'—there is but little change in the weight of the musculature, visceral group (as a whole) and 'remainder.' There is, however, usually a marked decrease in the integument, counter-balanced by a marked increase in the skeleton. Thus under these conditions the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process, during which the musculature shows the greatest increase and the skeleton lags behind relatively.

The increase in the skeleton during constant body weight appears to involve the ligaments and cartilages as well as the bony skeleton. The skeletal growth tends to proceed along the lines of normal development, as indicated by changes in water content, by formation and union of epiphyses, and by relative elongation of the tail (compared with trunk length). The teeth also continue to develop normally (eruption of third molar).

The individual viscera may be classified in three groups.

(1) There is during the maintenance of constant body weight a well-marked increase in the weights of the spinal cord and eyeballs; usually also of the tectal alimentary canal (both empty and including contents) and hypophysis.

(2) There is no marked change in the weights of the brain, heart, lungs, suprarenal glands, kidneys and epididymi. The liver is variable, with apparently a slight tendency to increase in the younger rats and to decrease in the older.

(3) There is always a marked decrease in the weight of the thymus ('hunger involution'); usually also of the spleen (earlier stages) and probably to a slight extent of the lungs and thyroid gland.

*29. Haemopoiesis in the yolk-sac of the pig embryo.* H. E. JORDAN, University of Virginia, Va.

The yolk-sac of the 10 mm. pig embryo was found to be especially favorable for a study of the early stages in blood-cell formation. It is still sufficiently young to show the earliest steps (with the exception of the initial origin of the angioblast) and sufficiently advanced to include the more important of the later stages. For these reasons it seems desirable to limit the present description to this stage of development. It need simply be added that the haemopoietic phenomena are essentially the same in the yolk-sacs of specimens ranging from 4 to 12 mm. Still younger and older specimens have not yet been examined.

This description pertains chiefly to a specimen fixed in Zenker's fluid and stained in toto with Delafield's hematoxylin and counter-stained with eosin. The specimen is exceptional only in its unusually good preservation. Mitotic figures are abundantly present in all or the tissues of the embryo and the sac; and the mitochondrial content of cells of the liver and the entoderm of the sac are clearly shown. It would seem that the specimen may therefore be confidently regarded as perfectly normal and well preserved.

The point of special importance pertains to the evidence for the origin of primitive blood cells or haemoblasts, from the mesenchyma. This

is the link in the monophyletic view of haemopoiesis as urged notably by Saxer, Bryce, Maximow and Dantschakoff concerning which there remains perhaps the greatest doubt. In the belief that the yolk-sac offered the best material for an investigation of this particular point, this study was undertaken. Both direct and indirect evidence strongly indicates that the mesenchyma of the young yolk-sac does differentiate into blood-vascular tissue, or so-called angioblast.

Regarding the origin of the initial angioblast in the yolk-sac of the pig this material yields no data. To identify angioblast with mesenchyma, at least in part, in the yolk-sac of this stage may seem to beg the entire question. Against this objection can be brought the observation that in certain portions the entire extra-entodermal layer forms a continuous tissue, or syncytium. The continuity is complete only in the location of early blood-islands; where blood vessels appear the continuity pertains to the endothelium. Moreover, there is apparently no difference, either from the standpoint of cytoplasmic or nuclear structure or form, between endothelial cells, mesenchymal cells, and the surface mesothelial cells. And in certain regions the three are seen to be continuous. The criteria which Clark (*Anat. Rec.*, vol. 8, no. 2; 1914) used with success in the chick embryo for the differentiation of endothelial from mesenchymal cells are not applicable to this material. On the other hand, the angioblast is everywhere sharply delimited from the entoderm. The morphologic evidence seems to force the conclusion that endothelium (angioblast), mesenchyma, and mesothelium are at this stage composed of the same cell, slightly modified along different lines by chiefly mechanical factors. In mesothelium and endothelium these factors include predominantly the element of pressure forcing an elongation and flattening of the cells. This accounts for the close similarity, amounting apparently to an identity, between the endothelial and the mesothelial cell.

Since it can be readily proved, as will be shown below, that haemoblasts differentiate from the endothelium of these early blood-vessels, these observations regarding the similarity and continuity of endothelium and mesenchyma constitute the indirect evidence for the mesenchymal origin of primitive blood cells. That mesothelium and endothelium, in spite of their histologic close similarity, are however functionally different at this stage must be admitted from the fact that no evidence appears of a direct origin of haemoblasts from mesothelium. But the continuity of mesothelium and mesenchyma must again be emphasized, as well as the proliferative capacity of the mesothelium. Moreover, that mesothelium may function haemopoietically is shown in Bremer's description of mesothelial ingrowths (angioblast-cords and angiocysts) into the body-stalk of a young human embryo (*Amer. Jour. Anat.*, vol. 16, no. 4, 1914).

The direct evidence for the origin of haemoblasts (primitive lymphocytes—Maximow; mesamoeboid cells—Minot) from mesenchyma appears chiefly in the presence of certain cells in the mesenchymal syncytium with the cytoplasmic and nuclear features of true intra-vascular haemo-

blasts and megaloblasts. There can be no doubt regarding their identity. What needs to be established is their true relationship to the mesenchymal cells. It is possible that they may have wandered into the mesenchyma from the blood vessels. This is Minot's suggestion regarding the 'lymphocytes' which Maximow has described as arising in the body-mesenchyme of the young rabbit embryo; and he bases his conclusion upon the observation that such cells frequently show certain nuclear and cytoplasmic features which he interprets as degenerative (Keibel and Mall's Human Embryology, p. 511, vol. 3). The cells in question in the yolk-sac of the pig show no nuclear or essential cytoplasmic differences which seem to warrant the interpretation that they are degenerating cells.

But neither these facts nor the additional one, namely, that they appear to be continuous through numerous processes with the mesenchyma, prove that they have arisen *in situ*. The processes may be of the nature of those described by Kite (Journ. Infect. Dis., vol. 15, no. 2, 1914) for polymorpho-nuclear leucocytes under certain conditions; and these pseudopodia of a possible wandering cell may have become so intimately fused with the mesenchymal syncytium as to simulate continuity. The following observation, however, seems to establish the *in situ* origin of these haemoblasts in the mesenchyma: In the case of certain undoubted haemoblasts which are still in continuity with an undoubted mesenchymal cell, a delicate chromatic thread attached to the haemoblast nucleus extends for a considerable distance through the connecting bridge of protoplasm towards the nucleus of the mesenchymal cell. In a few instances the connection was apparently complete. This chromatic bridge is always most conspicuous at its haemoblast terminal, and looks like an evagination from the haemoblast nucleus. That this chromatic bridge indicates amitotic division is doubtful, since many of the mesenchymal cells are seen in mitosis. But whatever its interpretation in terms of cell division, it would seem to show a haemoblast origin from mesenchyme. In some instances the evidence indicates that the mesenchyme becomes arranged in the form of endothelium about such forming haemoblasts. Haemoblasts are occasionally seen in process of transit between mesenchyma and blood vessel, the direction being probably either way. Certain spaces in the mesenchyme are lined by flattened endotheliod (mesothelial) cells.

It remains to describe blood cell origin and differentiation within the blood vessels. The blood vessels are at the start simply endothelial tubes of irregular caliber. The blood cells within the vessel include haemoblasts, cyrthroblasts (megaloblasts and normoblasts) and giant cells. No evidence appears of leucocytes except in so far as the smaller basophilic haemoblasts simulate lymphocytes. All of these cells are capable of intense proliferative activity.

The *haemoblast* (mesomoeboïd stage of Minot) is a relatively small spherical cell with a relatively large nucleus, and a narrow shell of slightly basophilic cytoplasm. The nucleus contains a delicate wide-meshed chromatic reticulum with generally several larger spheroidal

chromatic masses. Occasional cells answering to this description are of much greater size.

The *megaloblast* (ichthyoid stage of Minot) phase of the developing erythroblast is a relatively much larger cell. Its nucleus, however, is approximately of the same size and structure as that of the haemoblast. Its considerable cytoplasmic body reacts to the eosin stain. It thus has a bright pink color, due presumably to the presence of a small amount of haemoglobin. These cells divide extensively; they present a large series of size variations; moreover, the larger parent cells are frequently of lenticular or bluntly fusiform shape. This peculiar shape is interpreted in terms of an endothelial origin, as will be described below. Again, certain cells of this type possess a number of blunt pseudopodia. Certain cells with bilobed nuclei suggest that the nuclei of certain of these cells may also occasionally divide by amitosis.

The normoblast (saunoid stage of Minot) is also a relatively large cell, with a relatively smaller, denser, more chromatic granular nucleus. This is the most abundant type of cell. It is very uniform from the view-point both of size and structure. Very many of these cells are seen in mitosis. The cytoplasm of this cell is peculiar; apparently the technic employed extracted the haemoglobin; the cytoplasm appears clear, with a very wide-meshed delicate reticulum. A smaller number of cells are present very similar to the normoblasts, except that the nucleus is still smaller and more chromatic. This is the *erythrocyte* in early stage of metamorphosis into an *erythroplastid*. This involves the extrusion of the nucleus together with an enveloping shell of cytoplasm, as described by Emmel (Am. Jour. Anat., vol. 16, no. 2, 1914). This stage is extremely rare, but in view of Emmel's careful work can be properly interpreted.

The *giant cells* are relatively enormous cells, and very variable from the standpoint of the number, size and chromaticity of the nuclei. The larger varieties have a more deeply staining apparently cytoplasm. A graded series can be traced from the megaloblast with one nucleus, through one with two nuclei still retaining a pink-staining cytoplasm, to cells with more nuclei (or a larger, more chromatic nucleus) and a deep-brown-staining cytoplasm. This indicates the origin of the giant cells, namely, through growth (frequently accompanied by endogenous multiplication of the nucleus) of a megaloblast (or haemoblast).

From the standpoint of nuclear material giant cells have relatively little cytoplasm. From this viewpoint they represent young or 'rejuvenated' cells. There is some evidence to indicate amitotic division of the nuclei; but mitotic figures also appear, frequently with tri- and multipolar spindles. All the evidence indicates very rapid growth of these cells. They are of two types, mono- and multinucleate. As to their function the evidence seems clear in the case of the multinucleate type. The megakaryocytes most probably subsequently become multinucleate through division of the nucleus, and thus partake of the same function. About one or several of the nuclei appear clearer courts

identical in structure with that described for the normoblast. The nuclei meanwhile also assume the features of the normoblast type. Where in a binucleate cell one of the nuclei and its surrounding cytoplasm is thus modified, while the remainder of the cell retains the features of the megaloblast, the appearance might be interpreted in terms of the ingestion of a normoblast by a megaloblast. But where both nuclei of the same cell, as is frequently the case, and their adjacent cytoplasmic areas are similarly differentiated, the interpretation is inapplicable. The polykaryocytes of the yolk-sac undoubtedly have at least as a partial functional rôle that of the formation of normoblasts as here described.

In the human yolk-sac Spee (*Anat. Anz.*, Bd. 14, 1896) described the giant cells (to which he also attributed a haemogenic function) as arising from the entodermal cells. This view is untenable for the yolk-sac of the pig. Giant cells and the entodermal cells lining the sac have no features in common except their general staining reaction. The cytoplasm of the entodermal cells contains distinct basal filaments (mitochondria). Such are absent in the giant cells. Also, the entodermal cells contain a single relatively small nucleus, with coarse chromatic net, and usually two large spherical chromatic nucleoli. Moreover, there is never an intimate spatial relationship between entodermal and giant cells, and nothing appears in the nature of transition stages.

In passing may be noted the very close similarity between the entodermal cells of the yolk-sac and those of the liver. Graf v. Spee ('96) and also Paladino ('03) have attributed to the entoderm of the yolk-sac an hepatic function on the basis of a general morphologic similarity. This similarity in the pig pertains even to the finer cytoplasmic structure and content, namely apparently identical mitochondrial threads. It should be noted that no other tissues in this specimen showed distinctly any mitochondrial elements. This statement is not meant to imply that they were not present—there is sufficient recorded evidence to prove that they are—but only that this technic did not preserve them, while it reveals very beautifully the special threads in the entodermal cells of the sac and the liver cells.

Similar deep-staining filament were described by myself (*Anat. Anz.*, Bd. 31, 00, 11, 1907, and Bd. 39, 00, 1, 1910) in the yolk-sac of a 9.2 mm. human embryo as 'mucinous masses,' and subsequently by Branca (*Ann. de Gynec. et d'Obst.*, tome 2, 1908) in yolk-sac of about the same age as 'functional protoplasm' (ergastoplasm). They are very similar to the ergastoplasmic filaments of certain secretory cells as, for example, those of the pancreas, where they have been described as segmenting distally into "secretory granules." No such segmentation is discernible in these cells from the yolk-sac of the pig. In the human yolk-sac the entodermal cell contained a generous irregular granular content but the granules showed no direct relationship to the filaments, and they were tentatively interpreted as débris incidental to degenerative changes. Mislawsky (*Arch. Micr. Anat.*, Bd. 81, 00, 4, 1913) has recently shown by aid of delicate differential staining methods that the

basal filaments of the pancreas cell are in reality condriocents, and have nothing directly to do with formation of secretion granules. These filaments of the yolk endoderm and hepatic cells are more probably of the nature of mitochondria and give evidence of the metabolic virility of these cells.

It is of importance also to note that all the types of blood cells described for the yolk-sac are present also in the liver, only the normoblasts are relatively much more abundant. Elsewhere in the embryo (including especially the heart) normoblasts and later erythrocytes are exclusively to be seen.

Finally, as to the rôle of the endothelium of the yolk-sac vessels in haemopoiesis: the matter may be summed up by simply stating that endothelial cells differentiate into haemoblasts, both intravascularly and to a slight extent also extravascularly. This involves a rounding up of the cytoplasm about an endothelial cell nucleus and a subsequent abstraction of the forming cell from the wall of the vessel. Such process is probably commonly preceded by proliferation of the endothelial cell involved. Mitosis of endothelial cells are fairly abundant. Occasional endothelial cells are binucleated. The haemoblast need not necessarily at once separate from the endothelial wall. It may retain its connection through the succeeding stages of development including the fully differentiated megaloblast; and there is some evidence to show that even a giant cell may thus remain connected. The evidence for this, not however quite complete in the case of the giant cell, consists in a graded series of developmental stages from the true endothelial cell to the megaloblast. The megaloblast of this series is of lenticular form, its pointed terminals passing into a thread of protoplasm continuous in both directions with the endothelial wall.

### 30. *Morphological evidences of intracellular destruction of red blood-corpuses.* PRESTON KYES, from the University of Chicago.

The intracellular destruction of red blood-corpuses by vascular endothelium although recognized as of frequent occurrence under pathological conditions, has not been established as a physiological process taking place under normal conditions.

It is the purpose of this communication to give morphological evidences that in the case of the pigeon, among other species, there is a constant normal phagocytosis of red blood-cells by specialized vascular endothelium in the liver and spleen.

Sections of suitably fixed tissue from the liver and spleen of pigeons display when subjected to Perl's test for iron, a distinctly differentiated type of cell marked by the Prussian blue tone of the cytoplasm due to a positive iron reaction. Combined with counter-stains, therefore, Perl's method affords a favorable technique for microscopic study of the cells in question, which cells will be interpreted later as phagocytic endothelial cells whose iron content is due to ingested red blood-corpuses. In detail, the histological technique which I have employed in studying the tissues of eighteen normal pigeons, is as follows:

Fix thin slices of tissue for 18 to 24 hours in Muller's fluid plus 5 per cent mercuric sublimate. Imbed in paraffin and section to 4 microns. Fix sections to slide and stain 20 to 40 minutes with acid carmine. Wash, and transfer to equal parts of a 2 per cent aqueous solution of potassium ferrocyanide and of a 2 per cent aqueous solution of hydrochloric acid. Remove after from 3 to 10 minutes, wash in distilled water and pass quickly through a 0.5 per cent aqueous erythrosin solution. Dehydrate in alcohol, clear in xylol and mount in Canada balsam.

Specimens of normal pigeon's liver and spleen prepared according to the above method, display an extensive content of cells possessing the distinct blue tone of the Prussian-blue iron reaction. These cells are distributed rather evenly throughout both organs but more numerously in the liver. Under low powers of the microscope their general morphology indicates that the iron-containing cells are of the same type in the two organs, and as will be seen later this is supported by a correspondence in their finer structure and physiology. Inasmuch as the relation of these cells to other structures is much more evident, however, in the liver, their first description is limited to that organ.

In liver specimens observed under medium magnification, the cells referred to above appear as blue patches sharply differentiated from the red-stained parenchyma. These cells are larger in their greatest dimension than the liver cells proper, vary much in size and form, and are often seen to contain two or three carmine—or eosin-stained bodies. In their distribution they display a constant relation to the venous capillaries, often appearing to occupy the lumen of these vessels. Under the higher powers of the microscope, it is seen that each cell is an integral part of the endothelial intima lining the capillaries; in other words a fixed tissue cell engaged by one of its surfaces upon the reticulum of the vessel wall and with a free surface bulging to a greater or less degree into the vessel lumen. The attached surface of the cell follows strictly the line of the vessel-wall be it straight or curved, often continuing around an angle of bifurcation. No processes are seen extending between the liver cells: in fact I have not seen evidence that these cells possess processes extending in any direction. The cells under discussion are clearly those described in the liver of mammals by v. Kupffer first as perivascular connective-tissue cells and finally as intimal cells. To these cells the terms 'Sternzellen,' 'stellate cells,' 'Kupffer cells,' have been applied in reference to the liver; but to include the same cell as also seen in the spleen and where not, I employ the term hemophage.

The nucleus displayed by the hemophage stains a deep garnet with the carmine used in the given technique and contains two or three very distinct and intensely stained nucleoli. In the hemophages which are more nearly flat, the nucleus appears as those of the typical endothelial cell, whereas in the protruding cells of greater bulk, the nucleus is more vesicular and is irregularly pyramidal in form. Two nuclei may be found within a single cell. This but rarely, however.

The most striking characteristic of the hemophage is the morphology of its cell-body. This is determined by the fact that within vasculo-

of the cytoplasm, are contained red blood-corpuses taken from the circulating blood stream. It is not meant that here and there may occasionally be found a hemophage which has taken up an erythrocyte, but rather, that the occurrence is general and that approximately one-third of the total intimal cells are active hemophages and that each hemophage displays evidence of containing, or having recently contained, one or more erythrocytes. The fact that the red blood-corpuses of birds are nucleated, have a definite ovoid outline, and are of relatively large size, allows clear observation as to their actual inclusion and ultimate intracellular fate. The cell-body of the hemophage has no fixed morphology but changes from time to time according to the phase of its phagocytic activity. Within a single field of the microscope may be seen all intermediate stages between the hemophage whose cell-body is greatly distended by an intact erythrocyte recently ingested and the hemophage which has so far completed the destruction of the erythrocyte as to again appear as a flat endothelial cell except for the presence of the traces of the end-products of the digestion. In the first instance the cell-body of the hemophage bulges markedly into the capillary lumen and its nucleus is crowded to one side. The included erythrocyte in this earliest stage appears in all ways the same as those of the blood-stream and displays the normal staining reaction; namely, by the technique given, its nucleus stains a deep red-brown, while its cytoplasm stains an even yellow bronze tone. In hemophages which represent the subsequent stages, the included erythrocytes are seen in various stages of disintegration and digestion while the cytoplasm of the including cell gives a constant iron reaction. The first marked change in the erythrocyte is hemolysis, the hemoglobin escaping into vacuoles of the cytoplasm of the phagocytic cell and leaving the nucleus-containing stroma distinctly outlined. Gradually both the stroma and nucleus lose their staining reaction, until finally the vacuole contracts about a small indistinct remnant of the nucleus which in its turn ultimately disappears.

Meanwhile the hemoglobin which has escaped into the cytoplasm of the hemophage is seen to undergo a series of changes. At first the greater part of the pigment does not give the iron reaction but retains its yellow-bronze tone with erythrosin and occupies vacuoles of various sizes. In hemophages representing a later stage, however the contents of the vacuoles also give the iron reaction and with great intensity, contrasting with the lighter blue of the surrounding cytoplasm. Such cells finally show no content of unmodified hemoglobin. With the disappearance of the native hemoglobin, therefore, there is a parallel increase in the iron-reacting pigment. In untreated specimens this pigment is golden-yellow and is presumably hemosiderin. The hemophages which represent the last stages in the phagocytosis and digestion, appear less and less bulky, with a fainter iron reaction and a less vesicular nucleus. The last observable stage is represented by a cell which contains no yellow pigment but which in all ways appears as a typical endothelial cell of the vascular intima except, however, that its cytoplasm gives a faint and diffuse iron reaction.

As stated above, examples of the stages just outlined are readily seen in a single microscopic field and the interpretation of the sequence of events which they represent leads to the conclusion that the cells of the vascular endothelium of the venous capillaries of the liver of birds in performing a normal physiological function, ingest intact red-blood corpuscles, hemolyse the same, destroy the stroma and nucleus, split the hemoglobin with a freeing of the iron, and finally return to their original form.

In the spleen, the hemophages are seen in distinctly fewer numbers than in the liver. For the most part they are confined to the pulp cords in contrast to the Malpighian follicles and have no such evident relation to a vessel-wall or lumen as in the liver. The hemophage, however, is morphologically in all of its details of the same type as that of the liver, and the phases of ingestion and digestion of erythrocytes form the same cycle giving the same iron reaction at corresponding points.

With the recognition of a constant normal phagocytosis of erythrocytes by the intimal cells of the venous capillaries of the liver and corresponding cells in the spleen, the question arises as to how far these cells differ from vascular endothelium in general; in other words, the extent of their specialization. In reference to this point, the evidence shows that the phagocytosis is normally accomplished by endothelium in certain locations only. Thus in the liver, the hemophages are confined to the intima of the venous capillaries, while the intima of the larger vessels displays no such phagocytic action.

In applying the same technique in a study of the livers of the frog (*Rana pipiens*), toad (*Bufo lentiginosus*), turtle (*Chrysemys marginata*), crocodile (*Alligator mississippiensis*), and opossum (*Didelphys virginiana*), I have found a similar cycle of intracellular blood destruction in the corresponding cells of the reptiles, amphibia and mammals.

It would appear, therefore, that the application of a trustworthy differential histological method, shows that the liver and spleen of many species, do contain specialized endothelial cells which have as a normal physiological function the destruction of red blood-corpuscles with a liberation of the contained iron.

31. *On the implantation and placentation in the Sciuroid rodents (lantern).*

THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

In 1902 and 1903 the writer published descriptions of the implantation of the ovum in *Spermophilus*. In this work attention was called to a method of implantation and a series of structural changes preceding the formation of the true placenta which were unlike those of any other previously described mammal, and at the same time were the first account of the implantation in any of the Sciuroidea. These observations were confirmed on the European *Spermophilus* by Rejsek. In 1905 Müller found similar conditions in the European red squirrel, *Sciurus*. In 1910 the writer described the early stages of *Cynomys* at the International Anatomical Congress in Brussels. Since 1902 the writer has been engaged in collecting early stages of various genera of

American Sciuroid rodents to determine if the peculiar conditions found in *Spermophilus* (or 'Citellus' as the taxonomists have since decided upon as the proper generic name) were characteristic of this large group of rodents. The collection of very early stages of wild rodents which breed for the most part but once a year, and whose genera are widely separated over the United States, is an extremely tedious and very expensive undertaking, but sufficient material has been secured to date of the following genera of the Sciuroidae to determine that the general method of implantation and placentation as previously described for *Citellus* (*Spermophilus*) hold true for the larger division. The genera studied include *Citellus*, 3 species, *Ammospermophilus*, *Tamias*, *Cynomys*, and *Sciurus*. The writer is preparing a more complete description and illustration of the early developmental conditions characteristic of these Sciuroidae than was possible before with the quite limited material. The greater variety of material now available enables one to point out the interesting slight divergences among the several genera.

32. *On the relationship of the endocardium to entoderm in Citellus.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

In studying the early development of the sciuroid rodent *Citellus* the writer noted the following described conditions which may be of interest to investigators working on that yet unsolved problem of the origin of the vascular system.

With the folding over and fusion of the entodermal walls of the foregut to form the pharynx region, there is to be noted a dorso-lateral angle on either side formed by the dorsal wall on either side of the chorda and the lateral wall of the pharynx and a somewhat less prominent ventro-lateral angle or groove, which will be designated in this paper as the cardiac sulcus.

Each cardiac sulcus is a groove or furrow in the free surface of the entoderm which follows the course of the lateral hearts. It begins in the lateral wall of the mid gut region and extends forwards to enter the closed pharynx region at the ventro-lateral angle and then continues along the ventral wall of the pharynx converging to unite with the opposite sulcus in the mid ventral line just above the point of fusion of the lateral hearts.

The entoderm in the line of the cardiac sulcus is considerably thicker than that on either side. This thickening, however, is not uniform; it is more pronounced in certain areas than others. There is thus produced a corresponding elevation or ridge of the entoderm in the direction of the lateral heart. This thickened entoderm constitutes the walls of the sulcus. The groove, while easily recognizable throughout its course, varies in its shape; in places it is narrow and deep, in other places it becomes widened out and quite shallow. In embryos of this stage of development, the fold of splanchnic mesoderm which will form the myocardium does not completely envelope the endothelial tube of the lateral heart, the interval being completed by the entoderm of the foregut. It is this portion of the entoderm that forms the

cardiac sulcus. The above described sulcus is not peculiar to *Citellus* but is figured by many investigators, as Köliker in the rabbit, Fleischman in the cat, Bonnet in the dog. It is a transitory structure but is probably to be found in all mammals at the proper stage of development. While this region has been figured, almost no reference has been made to it as far as I am at present familiar with the literature.

In a number of series of *Citellus* I have found interesting examples of an intimate relationship between the entoderm of the cardiac sulcus and the endocardium of the lateral heart as shown by the reconstructions and drawings that illustrate this paper.

In the *Citellus* embryo here modelled, the primary implantation attachment (previously described by the writer) is just separating while the trophoblastic attachment for the allantoic placenta is beginning at the mesometrial portion of the uterine cavity; the amnion is not yet quite complete; the foregut is closed, the ectoderm of the oral plate is fused with the entoderm but not broken through; the pharynx does not yet show the evagination of the pouches; the endocardium of the lateral heart is beginning to fuse at the anterior end; the two dorsal aortae are well outlined, the first aortic arch is not yet completed. In an embryo at this stage the endocardium of the lateral heart on either side, in the region between the junctions of foregut and midgut and the point of beginning union of the two lateral hearts, shows an intimate relationship to the thickened entoderm of the cardiac sulcus. The endocardial tube is free and separate from the myocardial fold of splanchnic mesoderm in the sections, the contour of the tube is either oval or pear-shaped with a portion of the endothelial wall extended out as a thin fold or strand of cells toward the sulcus. Examining the series section by section it will be seen that while in each there is the extension of the fold or strand of cells toward the sulcus, in certain sections there is a short interval and in others very close contact; in certain sections there is distinct continuity with the entoderm of the sulcus walls. This intimate relationship is lost in the region of the fusion of the lateral heart.

33. *The comparative embryology of the mammalian stomach.* FREDERIC T. LEWIS, Harvard Medical School.

The study here reported has been carried out in large part by Dr. C. H. Heuser of The Wistar Institute of Anatomy, and a preliminary report of it was presented by him at the last meeting of the Anatomists. The work has now been carried further, and is nearly finished. Four animals have been studied, the cat, rat, pig, and sheep. The simple lenticular stomach from which the very diverse adult forms proceed, has been modelled as a starting point (cat, 6.2 mm.; rat, 5.4. mm.; pig, 7.8 mm.; sheep, 7.2 mm.). Even at this stage there are some significant differences, notably in the decidedly convex lesser curvature in the sheep.

In the human stomach, the later development has been the subject of a paper already published by the writer. The effort is now made to obtain the stages of these other mammals most suitable for comparison. From a considerable number modelled, four stomachs of each species

have been chosen, ending with the rather definite stage in which the smooth epithelial lining has become corrugated.

In the cat, the simple carnivorous type of stomach is early manifest. There is a well-marked angular incisure separating the elongated cardiac portion from the tubular pars pylorica. The fundus is less prominent than in any of the other mammals chosen, including man, and the gastric canal, following the cardiac part of the lesser curvature, is relatively late in its development. As a whole the stomach is less differentiated than in any of the other forms. In the rat the fundus early becomes elongated, forming a capacious pouch with its tip hooked toward the oesophagus. The gastric canal is short, but well-defined, and ends at a distinct angular incisure. In the pig there is also a large fundus, which soon overhangs toward the right side. The gastric canal ends at an incisure which produces only a shallow indentation of the lesser curvature. Below it, as in the rat, the pars pylorica is at first capacious, apparently representing a pyloric vestibule, and then more tubular, forming the pyloric antrum. The sheep's stomach, at 10 mm., presents a slight angle indicating the lower end of the gastric canal, and a much deeper incisure below the rounded ventral swelling of the lesser curvature, which is the beginning of the future psalterium. The psalterium is an early and prominent subdivision in the sheep, but is scarcely indicated in the other forms. The fundus in the sheep develops into the large rumen. Even though it is lined with stratified epithelium in the adult, it cannot be regarded as a portion of the oesophagus, as some have taught. The reticulum represents the body of the stomach, and the abomasum is the pars pylorica, including both vestibule and antrum. In all of the forms examined the fundus, corpus and gastric canal are readily identified. The subdivisions of the pars pylorica require further study.

34. *Reversed torsion of the human heart.* FREDERIC T. LEWIS, Harvard Medical School, and Maud E. Abbott, McGill University. Presented by Dr. Lewis.

While preparing the chapter on congenital cardiac disease for Osler's "Modern medicine," Dr. Maude E. Abbott, curator of the Medical Museum in Montreal, visited the Warren Museum and examined all the abnormal hearts which it contains. Among them is the heart of a man who died of phthisis in 1838, when twenty-one years of age. Its interventricular septum is so slightly developed that it was overlooked in the contemporary account of the specimen. The most interesting feature of this heart, however, is the large ventrally placed artery which appears to be the pulmonary artery, but which in reality is the aorta. It passes from the left side of the imperfectly divided ventricle toward the right, crossing the ventral surface of the pulmonary artery. The latter leaves the ventricular cavity like an aorta. Although these vessels have been cut away quite close to the heart, their distal relations as to aortic arch, right and left pulmonary branches and ductus arteriosus were apparently normal. Moreover, the atria and atrio-ventricular valves are normally arranged. In fig. 1B this heart is shown beside a normal one (fig. 1A) for comparison.

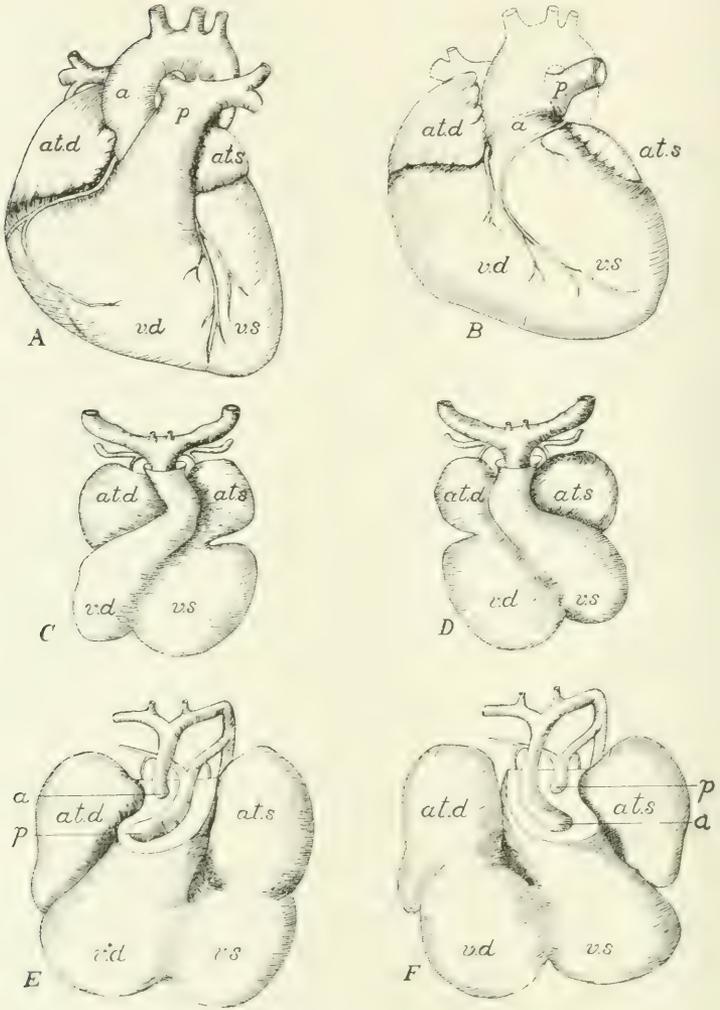


Fig. 1. *A*, ventral view of a normal adult human heart. *B*, corresponding view of an adult human heart showing reversed torsion. *C*, model of the heart of a 4.9 human embryo, which in *D* has been manipulated so as to present reversed torsion. *E*, model of the normal heart of a 10 mm. human embryo, the torsion of which has been reversed in *F*.

*a*, aorta. *at. d.*, right atrium (or auricle). *at. s.*, left atrium (or auricle). *p*, pulmonary artery. *v. d.*, right ventricle. *v. s.*, left ventricle.

Dr. Abbott brought this specimen to the writer for embryological interpretation, and he made the suggestion that in the embryo the cardiac tube had bent in the reverse direction to that which is normal, so that the aortic limb turned upward on the left side of the common ventricle instead of on the right. In order to verify this supposition, which we found had already been made by Keith, Dr. Abbott and the writer together undertook the following investigation. Normal embryonic hearts of the critical stages were selected and modelled as they occur in the embryo. Second models were then made, in which the ventricular portion of the heart was reversed, section by section. The two models of the heart of a 4.9 mm. embryo, kindly loaned to us by Dr. A. S. Begg, have been completed, and with others which are unfinished they seem to demonstrate the correctness of the interpretation. The distal part of the aortic trunk, including the roots of the pulmonary and fourth aortic arches, remains undisturbed in all its relations, and the atria and atrio-ventricular orifices are also in essentially normal position. But the reversal of the primary torsion causes the aorta to be split off from the truncus arteriosus ventral to the pulmonary artery, around the front of which it swings to the left ventricle. By manipulating the embryonic hearts in this way, the conditions in the abnormal adult specimen can be produced very satisfactorily, as shown in fig. 1 C-F.

35. *Variations in the early development of the kidney in pig embryos with special reference to the production of anomalies.* FREDERIC T. LEWIS, Harvard Medical School, and JAMES W. PAPEZ, Atlanta Medical College. Presented by Professor Papez.

In the summer of 1914, the collection of 165 series of 10 to 12 mm. pig embryos used for class instruction at the Harvard Medical School, was carefully examined to detect anomalies in the region of the developing kidneys. Although fused kidneys of the horse-shoe type are said to occur not infrequently in adult hogs, the proportion of cases is not such that a kidney of this type might be expected in the number of series examined, and none was found. However, the normal relations of the kidneys to one another varied in such a way that we may offer a new explanation of this anomaly, namely that it is due to the relation of the kidneys to the bifurcation of the aorta into the umbilical or common iliac arteries. This bifurcation forms a U-shaped crotch in which the kidneys are lodged, and from which they escape by migrating upward. The arteries, as a mechanical obstruction, tend to bring the right and left renal blastemas close together, so that fusion may readily take place. A fusion at the upper poles, making a horse-shoe kidney convex superiorly, would probably arise earlier than the fusion at the lower poles, in which case the horse-shoe would be convex inferiorly. The relations of the kidneys which seem to justify this interpretation have been demonstrated in a series of models.

The anomalies actually observed consist chiefly of diverticula of the Wolffian duct, perhaps representing abortive ureters. Eighteen of these were found, most of which are on the part of the Wolffian duct

distal to the orifice of the ureter. One is detached, forming an epithelial cyst. Two diverticula were found springing from the ureter itself. One of these which is slightly elongated, ending blindly near the renal blastema, might give rise to a divided ureter, but inasmuch as it does not enter the blastema, no renal tubules would empty into it. On the proximal side of the orifice of the ureter in the Wolffian duct, there were six diverticula, generally close to the ureter. Thus a portion of the Wolffian duct immediately below the lowest and typically rudimentary tubules of the Wolffian body is generally free from diverticula. In one case, however, an elongated diverticulum in this region extended toward the Wolffian body and ended in relation with a blastemal cyst, such as produces the glomerular end of a renal tubule. In position and structure this formation is intermediate between the Wolffian body and kidney. In the anterior end of the Wolffian body, the elongating cysts open directly into the Wolffian duct, but below, as is well shown in this instance, the Wolffian duct sends out tubules, comparable with the ureter and collecting tubules of the kidney, to join with the portion of the tubule derived from the cyst. This specimen shows also a second and much smaller outgrowth of the Wolffian duct, nearer the ureter, which brings the Wolffian body into still closer relation with the permanent kidney.

36. *Some anatomical deductions from a pathological temporo-mandibular articulation.* FREDERIC POMEROY LORD, Department of Anatomy and Histology, Dartmouth Medical School.

In a previous paper, "Observations on the temporo-mandibular articulation," certain reasons, based largely on the study of a working model of the jaw-joint, were given to prove that the mouth is opened by the combined pull of the external pterygoid muscles. It was also shown that, during opening, each condyle of the jaw moved forward nearly in a straight line, which is almost parallel to the plane of pull of the two external pterygoid muscles; and that the depth of the bony glenoid fossa is practically obliterated by the inter-articular cartilage.

Further proof of these facts has been noted in a singular skull, found in the Peabody Museum at Harvard, whose Director, Professor Putnam, kindly gave me access to its large collection of skulls.

In this specimen the left jaw-joint is virtually normal; the right has suffered from an attack of osteo-arthritis, apparently recovered from, later. During the attack the whole of the joint surfaces had been remodelled along entirely new lines, without a meniscus, and yet it gave, it would seem, equally good function with that of the other side, representing the usual condition.

The new joint shows the course taken by the advancing condyle, permanently recorded in bone, and the evidence as to the character and direction of the condylar path, thus disclosed, corroborates that previously adduced.

The joint surfaces show, also, better than those of a normal specimen, how well adapted they are to resist lateral or mesial displacement of the

condyles, in closing the mouth, either by the pull of the masseter or the internal pterygoid, and in proper proportion to the direction of their pull.

By making an artificial meniscus, exactly fitted to that especial skull, in the case of a normal specimen, the same adaptation of the joint surfaces can be demonstrated.

37. *Distribution of nervus terminalis in man (lantern).* ROLLO E. McCOTTER, Department of Anatomy, University of Michigan.

Johnston and Brookover were the first to observe the presence of the nervus terminalis in man. Apparently, the material used by them permitted only of the examination of a portion of its intracranial course. By means of gross dissections of the heads of several human fetuses the writer is able to demonstrate the intracranial course and nasal distribution of the nervus terminalis. The nerve appears on the cortex in the region of the olfactory trigone, courses as a single trunk over the medial surface of the olfactory tract and breaks up into a plexus on the medial surface of the olfactory bulb where it is associated with the vomero-nasal and olfactory nerves. From the plexus on the medial surface of the olfactory bulb the fibers of the nervus terminalis collect into several communicating filaments and course over the lateral surface of the crista galli and pass through the cribriform plate well forward. The nervus-terminalis reaches the nasal cavity as a single bundle and is distributed to the septal mucosa anterior to the path of the vomero-nasal nerves.

This article will be published, with figures, in volume 9 of the Anatomical Record.

38. *On the anatomy of the brain and ear of a fish from the coal measures of Kansas.* ROY L. MOODIE, Department of Anatomy, University of Illinois, Chicago.

The preservation of the soft parts of extinct animals has always been a matter of great interest to students of paleontology and a number of papers have appeared on this subject. There are now known from the studies of various paleontologists muscle and kidney tissues from the Devonian, alimentary canals and muscle tissues from the Carboniferous and from the succeeding formations a variety of the softer organs have been preserved in different ways. They may be mummified, carbonized or changed into mineral substances, or the form of the part may be preserved as a cast of the cavity which the organ occupied. The latter is the usual mode of formation of fossil reptilian and mammalian brains. The casts are, however, always dural casts which never repeat the exact topography of the organ, and the smaller convolutions of the brain are not represented in the average brain cast.

A study of the brain cast of a mammal would give a more accurate idea of the form of the brain than would the cast of the brain case of a reptile, since the mammalian brain more nearly fills its cavity than does the brain of a reptile, as noted by Dendy (Phil. Trans., Royal Soc.

London, Ser. B, vol. 291, pp. 227-331) for *Sphenodon*. The brain case of all recent selachians and teleosts is much larger in proportion to the size of the brain than among the reptiles, so that a cast of the cranial cavity of a fish would give no idea of the detailed anatomy of the brain. We may be sure that the organs which are described herewith are not casts but are, apparently, a transformation of brain substance, before decomposition, into some mineral, probably calcium phosphate. The walls of the brain are not shrunken but preserve a rounded contour as they probably had in life, resembling greatly, so far as details are concerned, the brain of a recently dissected and well-preserved fish. There are also preserved in their proper relations nerves and blood vessels, somewhat enlarged by the segregation of mineral matter and the subsequent formation of crystals but still preserving the normal relations. It is hard to conceive of this method of replacement of the brain by mineral matter in view of the chemical analysis of the brain which shows such a high percentage of water and soluble substances, and such a small percentage of resistant substances such as neurokeratin.

The little fossils with which we are at present concerned were collected in shales above the Kickapoo limestone in the Coal Measures near Lawrence, Kansas. The nodules containing the brains are all small, the specimens of brains themselves measuring only 15 mm. in length. The skeletal parts of the fishes have largely disappeared, so far in fact that it is not possible to determine the nature of the skull. Identification of the form being thus impossible, we are forced to use the characters of the brain to locate our form. Fortunately for this purpose Eastman has described a small brain from the Waverly of Kentucky, Iowa Geol. Surv., vol. 18, 1908, p. 267, pl. 13, very similar in many ways to the brains from Kansas. He was fortunate enough to identify the species of the fish to which the brain belonged, naming it *Rhadiniichthys deani* and placing it among the *Chondrostei* or ganoids. The fish described by Eastman was collected in the Mississippian of Kentucky, but the character of the brain is so similar to those from the Coal Measures of Kansas that we will be quite safe in locating our fish near the *Chondrostei*, to which certain characters of the brain ally it independent of any comparison.

The brain itself is very completely shown in a series of specimens and we are able to study all sides of the brain in a few cases. The spinal cord is only partly represented, if at all, by a very small portion on the edge of the nodules. The vagal lobe is single and lies far back over the region of the fourth ventricle. There are no indications of separation of the lobe into subdivisions as is so common among existing fishes. Its complete form is preserved but the one best preserved has the upper surface abraded, since it projected slightly through the surface of the nodule. The facial lobe is smaller in the fish from Kansas than it is in the Mississippian brain described by Eastman. It is separated from the vagal and cerebellar lobes by slight constrictions and from its anterior aspect there arises a tubular structure which is apparently connected with the pineal organ. This may be a vessel of some description or it

may be a fold of membrane which has been preserved. The cerebellar lobes are most unusual in being entirely lateral. If the median portion of this organ has become involuted below the surface of the huge optic lobes it is not possible to determine this. A study of the internal construction of the brain is not possible with the material at hand, if it will ever be; the formation of large crystals having obliterated all structural characters. Between the medial tips of the cerebellar lobes lies a structure which may be the pineal body. It is not present in all of the specimens, being entirely absent in one well-preserved brain. From the anterior, ventral portion of this organ runs a rounded elevation which may be either the stalk of the epiphysis or a plexiform vessel. Its morphology is uncertain. This is a very constant structure among the specimens at hand. The optic lobes are very large and indistinct, apparently, a teleostean character for the fish. They occupy one-third the full length of the brain and constitute fully one-half its bulk. The eye was large as is indicated by an impression of the orbit and the optic stalk short. The optic chiasm is evident in one specimen but the details of its nature are uncertain. The structure just anterior to the optic lobes is probably the thalamus or praethalamus. It, like the vagal lobe, has its dorsal surface somewhat abraded but other specimens show that its full form was not greatly different from that shown in the figures. The olfactory lobes are distinct and relatively large, being separated by a slight groove. The base of the olfactory tract is preserved and shows a strong olfactory development. The horizontal semicircular canal is well preserved on the right side of one specimen and on both sides of another nodule. The ampulla is large and the utriculus nearly double its size. The base of the vertical semicircular canal is preserved on the upper aspect of the utriculus. The base of the hypophysis is large and well preserved.

My thanks are due Doctor Herrick and Doctor Johnston for assistance in the determination of the characters of this little Paleozoic brain. A fuller discussion with illustrations and a review of other fossil brains will appear shortly in the *Journal of Comparative Neurology*.

39. *The growth of the vascular system as it is correlated with the development of function in the embryos of amblystoma.* JULIA S. MOORE, (introduced by GEORGE E. COGHILL).

Embryos of *Amblystoma punctatum* and *microstomum* in the physiological stages of development described by Coghill (*Jour. Comp. Neur.*, vol. 24, p. 163) as (1) non-motile, (2) early flexure, (3) coiled-reaction, (4) early swimming stage, were used in this study of the vascular system in its correlation with other organ systems and with the growth of the embryo as a physiological unit. The following correlations are made upon the basis of a close study of serial sections and of living embryos.

As in other vertebrate embryos, rhythmic contractions of the heart begin before there is any connection with the nervous system and before there is any histological evidence of the muscular nature of the myocardium. The first cardiac movements do not occur until after

the body movements, as represented by the early flexure stage, are well established. The rate of heart-beat averages in the early flexure stage 29 per minute; in the coiled-reaction stage, 49. In the early swimming stage it varied from 49 to 72. At the time that rhythmical contraction begins there is no perceptible connection between the arteries and the veins. In the coiled-reaction stage communication is established between the branchial vessels of the first gill and between the internal carotid artery and the ophthalmic vein, and a circulation of plasma may begin at this time. But corpuscles in living embryos were seen to circulate first in the late coiled-reaction or the early swimming stage, though they are found in the heart and venous system earlier in the microscopic sections. It is evident that circulation of corpuscles in the gills is started about the time that definite swimming begins, circulation in the balancer follows very soon afterwards. The evidence obtained concerning the aerating function of the corpuscles is not conclusive. From all observations thus far made it would seem that the corpuscles carry no haemoglobin until a later period than that considered in this paper.

Up to the early swimming stage no blood vessels could be seen to enter the myotomes, and no vascular connection has been made with the digestive system, which is differentiated even less than the myotomes at this time. Circulation could be seen in the vessels between the myotomes only at a later period. The mouth of the embryo does not open until some two weeks after swimming begins. At the non motile stage the cells of the ectoderm and the nervous system already show a greater degree of differentiation than those of other organ systems, as signified by the diminution of the yolk content of the cells. A similar diminution of yolk is marked in the blood corpuscle about the time that it comes into circulation. By the time that the corpuscles have used up their yolk they have approximately attained their adult size and form. The cells of the pronephros show a similar differentiation and diminution of yolk about the same time or a little earlier. The entodermal and mesodermal cells are still crowded with yolk up to a later period. A characteristic relation seems to exist in all cells between the nuclei and the yolk, which suggests a process of digestion within the cell. The relative amount of yolk thus seems to hold a definite relation to the degree of differentiation in the various parts of the embryo. The facts observed in connection with the rate of differentiation and the disappearance of yolk in the various parts of the embryo would indicate that the blood performs little or no nutritive function, as each cell of the body seems to be able to supply its own needs both for differentiation and for function, until swimming is well established.

In the early swimming stage no blood vessels are yet found in the nervous system, but the anterior cerebral vein is very closely applied to the surface of the brain, while the segmental vessels, developing later in the trunk come into close contact with the spinal cord. This close relation in development of the anterior cerebral vein with the most highly differentiated parts of the brain would indicate a correlation of the

vascular system with the early processes of function and differentiation in the nervous system. The tetanic condition of the embryo in the coiled-reaction necessitates a violent metabolism whose products must be removed. The nerve centers controlling this muscular activity must also undergo considerable metabolism. Hence it may be supposed that the early differentiation and function of these parts have stimulated the development of the vascular system, and that the vascular system has an excretory function in relation to these parts. The presence of the sacculated outgrowth of the dorsal aorta at the level of the pronephros, the ciliated nephrostome, the opening of the pronephric duct into the cloaca, and the close relation of the posterior cardinal vein to the pronephros indicate that the vascular system in conjunction with the pronephros is functional as an excretory system in the coiled-reaction stage. The communication of afferent and efferent vessels in the gills, permitting a circulation of plasma, makes possible the excretion of carbon dioxide through the blood in the coiled-reaction stage, while a distinctive aerating function can appear only later with the development of haemoglobin.

40. *A preliminary note on the septum secundum in the pig.* C. V. MORRILL, Department of Anatomy, University and Bellevue Hospital, Medical College.

In the course of a paper devoted chiefly to the development of the Purkinje fibers of the heart, Retzer (some results of recent investigations on the mammalian heart; *Anat. Rec.*, vol. 2, no. 4, 1908) briefly discusses the formation of the atrial septum in the pig. He states that the accounts of His and Born though accepted by most embryologists are incorrect on this point. In the pig Retzer considers that septum II in Born's sense does not exist and that this supposed septum is merely a fold in the atrial wall produced by the growth of the auricles around the conus arteriosus as a fixed point; and further that it never attains sufficient size to justify its being called a septum.

Since, as Retzer says, Born's account has been followed to a large extent by other writers as evidenced by the descriptions of Hochstetter in Hertwig's *Handbuch* and Tandler in Keibel and Mall's *Manual*, it seemed worth while to re-examine the development of the atrial septum in the light of Retzer's criticism.

The study of this point is based on serial sections of pig embryos of 6.8, 7.9, 8.5, 12.3, 15.2 and 21.0 mm. total length. Of these, the heart regions of the 7.9 and 15.2 mm. embryos have been reconstructed in wax and that of the 21.0 mm. is in process of reconstruction.

In the 6.8 mm. stage, the earliest examined, septum I forms an incomplete interatrial curtain. Both ostia are present. The caudal (inferior) border of septum I meets and fuses with the corresponding wall of the atria. At the ventral end of the line of fusion, a slight thickening projects into the right atrium and with this the caudal extremity of the left sinus valve blends. In the 7.9 mm. embryo, septum I has fused with the endocardial cushions for the most part,

but a narrow slit, the remains of ostium I, still connects the two atrial chambers. Ostium II has enlarged and its borders are fimbriated. The thickening in the caudal wall of the right atrium close to the ventral extremity of septum I, which was noticed in the earlier embryo, has developed into a distinct spur which extends cephalad (upward) a short distance in the ventral border of septum I, just dorsal to the still-persisting ostium I. This, I believe, represents the earliest appearance of the septum II of Born (he did not describe the earlier stages). With it, the caudal end of the left sinus valve blends. The corresponding end of the right sinus valve is lost in the caudal wall of the right atrium, close to this point. In the 12.3 mm. embryo, the spur has thickened and extends further cephalad. In the 15.2 mm. stage, it has lengthened out into a definite ridge extending from its place of origin in the caudal wall of the right atrium, first cephalad, then dorsally, arching over ostium II to reach the dorsal atrial wall, where it fades out. Its caudal end is thick and up-standing; its cephalic end narrow, pointed and not sharply marked off from the atrial wall. The caudal ends of both sinus valves are now fused with it. In this stage ostium I has entirely closed and ostium II considerably enlarged dorso-ventrally, so that the free border of septum I bordering the latter opening, now faces almost entirely cephalad (upward). In the 21.0 mm. stage, the oldest examined, septum II has become thicker and more sharply defined near its caudal extremity. Its narrow, pointed end extends cephalad and dorsally, bordering ostium II, then caudally for some distance along the dorsal wall of the right atrium in the region of the spatium intersepto-valvulare and close to the left sinus valve.

It does not seem probable that this very definite thickening is merely a fold in the atrial wall produced by the growth of the auricles around the conus, as Retzer claims. It is true that in the middle of its course it does conform to the curve of the conus, but at its caudal extremity where it is thickest and at its pointed extremity which lies in the dorsal atrial wall, it is entirely unrelated to that structure. Thyng (the anatomy of a 17.8 mm. human embryo; *Am. Jour. Anat.*, vol. 17, no. 1, 1914), has recently recorded the presence of a 'ridge or tubercle' in the caudal part of the right atrium which he considers to be the caudal end of the future septum secundum in the human heart.

A more detailed account of this structure and nearly related parts will be given in a subsequent paper.

41. *Studies on the syrinx of Gallus domesticus.* J. A. MYERS, Institute of Anatomy, University of Minnesota, Minneapolis.

The results of this work may be summarized as follows:

*Structure.* (1) The syrinx of the domestic chicken belongs to the tracheo-bronchialis type, and is quite simple when compared with the voice organ of song birds. (2) No intrinsic muscles are present in the syrinx of *Gallus domesticus*. The extrinsic paired sterno-trachealis with its caudal prolongations constitute the entire musculature of the syrinx. (3) The rigid skeleton is very highly modified. The first

four tracheal rings are imperfectly fused to form the tympanum. The four intermediate syringeal cartilages are continuous ventrally with the ventral pyramid of the pessulus, while dorsally they end unattached. The first bronchial half-rings are large and in adults are attached and fused at both ends of the pessulus. The pessulus is the largest of all skeletal parts and lies dorso-ventrally at the junction of the bronchi in a plane transverse to the long axis of the trachea. The tracheal rings, the pessulus, and the ventral ends of the first half-rings become ossified, while all other skeletal parts remain cartilaginous. (4) The external tympanic membranes appear between the fourth intermediate syringeal cartilages and the first half-rings while the internal tympanic membranes extend from the caudal borders of the pessulus to the bronchidesmus and represent merely a modified part of the medial bronchial walls. (5) The syrinx is lined with stratified ciliated columnar epithelium containing numerous simple alveolar glands. Upon approaching the tympanic membranes this columnar epithelium is transformed into a stratified squamous epithelium which becomes a single layer of flattened cells over the membranes proper. (6) The tympanum is attached to the remainder of the syrinx only by elastic membranes.

*Development.* (1) The first indication of the respiratory system was observed in a 68 hour embryo in which the laryngo-tracheal groove and the bronchi were represented. At first the trachea is much shorter than the bronchi, but with the development of the neck, it becomes, after the 140 hour stage, relatively much longer than the bronchi. The walls of the trachea and the bronchi are at first composed only of epithelium which contains two or three rows of nuclei. (2) The mesenchymal condensation common to the entire epithelial tube first becomes markedly prominent at the tracheal bifurcation in an embryo of 152 hours. (3) The anlagen of the first bronchial half-rings appear in a 176 hour embryo, those of the fourth intermediate syringeal cartilages appear 12 hours later. The anlagen of the third intermediate syringeal cartilages and the anlage of the pessulus are present at 200 hours. (4) Distinct cartilage cells were first observed in the first bronchial half-rings. (5) The first four tracheal rings have not united to form the tympanum at hatching, nor have the other skeletal elements taken the shape of those found in the adult. No bone is present at the time of hatching. (6) Ciliated cells are present in stages beyond 248 hours but were not observed in the region of the future tympanic membranes. (7) Mucous cells were first observed in 332 hour embryos and only in later stages were they found arranged in the form of simple alveolar glands. (8) Muscular tissue is differentiated in the 176 hour stage. Muscle fibers showing faint cross striations appear at 296 hours. At 452 hours the muscles are well developed. (9) At the time of hatching the tympanic membranes are thick. They are covered, however, as in the adult, with a single layer of epithelial cells.

*Function.* (1) That the syrinx is the true voice organ of the chicken is evident from the following deductions: First, structurally it is the only part of the respiratory tract capable of producing sound; Second,

when the trachea is divided and the cephalic portion tightly tied off, the chicken is still able to crow; Third, after division of the trachea, voice can be reproduced artificially by forcing air into the air sacs. (2) The upper larynx serves only to modulate the voice. (3) The sterno-tracheal muscles by their contraction shorten the trachea and modify pitch. They also draw the tympanum cephalad, thus indirectly varying the tenseness of the tympanic membranes. (4) The air sacs are necessary in voice production, for voice could not be reproduced artificially after puncturing the cervical sacs.

42. *On the presence of elastic ligaments in the middle ear region of birds.*

A. G. POHLMAN, St. Louis University.

Recent work by otologists, notably Kreidl and Mangold, has demonstrated that our knowledge of the anatomy and physiology of the middle ear region is imperfect. The Tensor tympani and Stapedius muscles are regarded as synergists rather than opponents and even the facial nerve innervation to the Stapedius is denied on an experimental basis. The problem of what these two muscles really work against is of interest not only to the physiologists but to the anatomists as well and that they may have some function relative to respiratory and atmospheric changes in the air content of the middle ear is not to be denied. The bird because of its single columella and single Tensor tympani presents a condition where the physiology may be more easily determined but Denker's work on the parrot does not consider the details of the middle ear and Breuer's article, while it takes into account the functions of the single muscle, quite avoids all mention of the ligamentous apparatus. The most recent work on ligaments is that by Smith who describes the position of Platner's ligament and the two accessory drum ligaments in the chicken quite accurately.

It was assumed that the Tensor tympani in birds must pull against elastic ligaments, and the following points were developed: (1) That the attachment of the stapedial plate to the oval window and the membrane of the Fenestra cochleae were elastic in nature. (2) That Platner's ligament, placed in direct opposition to the pull of the Tensor tympani, is also elastic and draws the columella forward to its position of rest when the muscle relaxes. (3) That the drum attachment itself is rich in elastic fibers. (4) That in some birds elastic fibers and even ligaments reach from the extra-columella over the drum into the Eustachian tube. The conditions in the mammal remain to be investigated.

43. *A genetic interpretation of the stapes, based on a study of avian embryos in which the development of the cartilaginous otic capsules has been experimentally inhibited.* FRANKLIN P. REAGAN, Department of Comparative Anatomy, Princeton University. (Introduced by C. F. W. McClure.)

The chondro-crania of all vertebrate embryos possess one essential ground plan. Around the anterior end of the notochord, is formed the parachordal cartilage, anterior to this the trabeculae. Following the

formation (invagination) of the epithelia of the three bilaterally symmetrical sense organs, the latter become more or less surrounded by prechondral cytotblastemae, later by cartilages, that surrounding the otocyst being the anlage of the otic capsule or cartilaginous labyrinth.

It occurred to the writer that this cartilage of the otic capsule evidently arises in response to the presence of the auditory epithelium, and that if this be true, an excision of the epithelium at an early stage would inhibit the stimulus to the development of an otic capsule, and that further, if this also be true, it would be possible to test to what extent the stapes can develop in the absence of a cartilaginous otic capsule, or in the absence of the stimulus which produces the latter.

Fortunately it was found that the removal of the otocyst from one side of chick embryos from five to sixty hours incubation resulted in the complete inhibition of the development of the cartilaginous otic capsules, as revealed by a study of operated embryos which were allowed to incubate from six to fifteen days.

On the operated side, the staff-like portion of the stapes resembles in shape, size and position the same portion on the uninjured side, seemingly complete except lacking the flange-like ring of cartilage which completes the stapedial plate.

It seems that the central portion of the stapedial plate is actually formed by cartilage of the hyoid arch while its periphery arises as an independent chondrification in the fenestra ovalis, distinct from the otic capsule but incapable of developing under conditions in which the latter fails to form. Both the otic capsule and the periphery of stapedial plate appear to have as their exciting stimulus the auditory epithelium.

I have reason to believe that my evidence is not merely of a negative sort, resulting from injury to the mesenchymatous anlage of the otic capsule.

44. *On the origin of the duct of Cuvier and the cardinal veins.* FLORENCE R. SABIN, Anatomical Laboratory, Johns Hopkins University.

Certain injections of young embryonic pigs brought out clearly the fact that the posterior and mesial or sub-cardinal veins arise as longitudinal anastomoses of direct lateral branches of the aorta. These lateral branches are distinct from the dorsal segmental branches which pass directly to the spinal cord. The lateral branches on the other hand alternate with the nephritic tubules and are two or three to a segment according to the number of tubules. The mesial cardinal vein in the pig forms at the same time as the posterior cardinal, but it is the posterior cardinal vein which connects with the duct of Cuvier. The specimens of injected pigs will be demonstrated at this meeting.

These studies in the pig led to taking up the subject of the origin of the cardinal veins in the chick since the material is so much easier controlled. In the chick of 36 hours incubation I injected a little India ink into the marginal vein and watched it circulate through the embryo. All of the ink simply passed through the heart and the double aorta back into the capillaries of the membranes so that there was no capil-

lary circulation within the embryo. In chicks a little older, however, from 38 to 42 hours of incubation I saw a little of the ink pass through a tiny branch from the lateral surface of the aorta opposite the venous end of the heart around to the vitelline veins on either side. As soon as this connection between the aorta and the venous end of the heart is established the embryo itself as distinct from the membranes may be said to have a circulation. This tiny vessel which is the first part of the duct of Cuvier to develop grows from the aorta just cephalic to the first cervical myotome. Subsequently direct branches from the aorta opposite about three segments grow around to the vitelline veins and these primitive branches at once show anastomoses.

At the same time that this venous return for the blood of the embryo is formed, sprouts grow to the brain from the arch of the aorta. In the early chick the arch of the aorta is just at the root of the optic vesicle and it is at this point that the brain first receives capillaries. At first these capillaries have no circulation since they have no connection with the venous end of the heart. Gradually the capillaries to the brain spread out around the base of the optic cup and over the mid-brain. From this plexus a single vessel grows caudalward along the side of the medulla ventral to the otic vesicle and just at the caudal end of the medulla turns sharply ventralward almost at a right angle and joins the cephalic part of the duct of Cuvier. I have one specimen in which the branch of the duct of Cuvier connects both with the aorta and with this vessel from the brain. Soon, however, the direct connection between the aorta and the heart is broken and the primitive head vein is established. Thus the composite nature of the head vein, suggested by Salzer in 1895 and more fully described by Grosser in 1907, is explained. The part of the head vein which lies close to the neural tube arises from the arch of the aorta and is a part of the vascular system of the central nervous system; the caudal part of the head vein arises directly from the aorta just cephalic to the first cervical myotome, it lies in the lateral groove and is analogous to the posterior cardinal vein. The part of the head vein cephalic to the first myotome may well be called the *vena capitis* as was done by Grosser while the caudal part which becomes the internal jugular vein is a true anterior cardinal vein in the sense of being analogous to the posterior cardinal vein. This caudal part of the vein is much the shorter portion of the head vein in the early chick. It should be noted that it is the entire head vein which is usually termed the anterior cardinal vein. In this use of the name it must be emphasized that the head vein has a double origin.

The posterior cardinal vein in the chick is likewise formed from direct branches of the aorta which grow lateralward to the Wolffian body and there form a longitudinal vein. In contrast to the pig these branches are for the most part segmental arteries that is they arise between the myotomes. A few lateral branches opposite the myotomes are involved in the formation of the posterior cardinal veins in the chick but they are always less numerous than the segmental vessels. The position of origin of the segmental vessels along the aortic wall varies

in the different segments and seems to follow a line corresponding to the lateral surface of the spinal cord, but the vessels grow directly lateralward to the groove just ventral to the myotomes. Moreover, when the nephritic tubule is present the longitudinal vein lies closer to it than to the myotome. The difference in the development of the posterior cardinal vein in the pig and in the chick seems to correspond to a relative difference in the time of development of the nephritic tubules.

Thus to sum up the origin of the venous system, the first vein of the embryo is the duct of Cuvier which is a direct connection between the aorta and the vitelline veins. The cardinal system in general arises as a longitudinal system of veins from direct branches of the aorta. The cardinal system proper extends throughout the zone of the myotomes and lies in the Wolffian groove ventral to the myotomes. In the chick the direct connection between the aorta and the heart occupies the zone of the first three or four myotomes. Eventually the plexus of vessels representing the duct of Cuvier in the chick covers the zone of the first seven myotomes. In the pig the posterior cardinal vein develops from lateral branches at the same time as the nephritic tubules and these lateral branches alternate with the nephritic tubules. In the chick the posterior cardinal vein develops more rapidly than the tubules and comes in part from lateral branches of the aorta which are intersegmental but mainly from dorsal segmental branches which however do not grow first to the spinal cord but rather directly lateralward to the Wolffian groove where they anastomose to make a longitudinal vein.

45. *The technique of Weber's method of reconstruction.* RICHARD E. SCAMMON, Institute of Anatomy, University of Minnesota. (Lantern slides.)

This method as used by its author was applied mainly to surfaces which were almost flat. Its extension to the study of rounded surfaces requires that a method of curvature elimination be adopted. In dealing with the surfaces of cylinders or cones this is easily done by correcting for vertical curvature alone, but where segments of spherical surfaces are involved corrections for horizontal curvature are also necessary. Vertical and horizontal corrections can be made so that the finished plat will give an approximately true representation of both the area and shape of a given outline upon a curved surface.

The method of making a finished reconstruction from the reconstruction plat has been considerably simplified by the introduction of color by means of 'Herring' papers and by building up the final reconstruction in strata.

The entire process involves the following steps: (a) Correcting section drawings for vertical curvature; (b) Scaling drawings for thickness; (c) Laying out reconstruction plat with corrections for vertical and horizontal curvature; (d) Plotting gauge lines; (e) Building up the finished reconstruction from the plat.

46. *Nasolacrimal duct diverticula and their genetic significance (a preliminary note)*. J. PARSONS SCHAEFFER, The Daniel Baugh Institute of Anatomy and Biology of the Jefferson Medical College.

It is generally believed that the wall of the nasolacrimal duct are regular and that the lumen of the duct represents a more or less uniform cylinder. Indeed, this is what one gathers from many text-books and from the average gross dissection of the channel. The common practice of passing the lacrimal probe would also lead one to think that the nasolacrimal duct has even or regular walls.

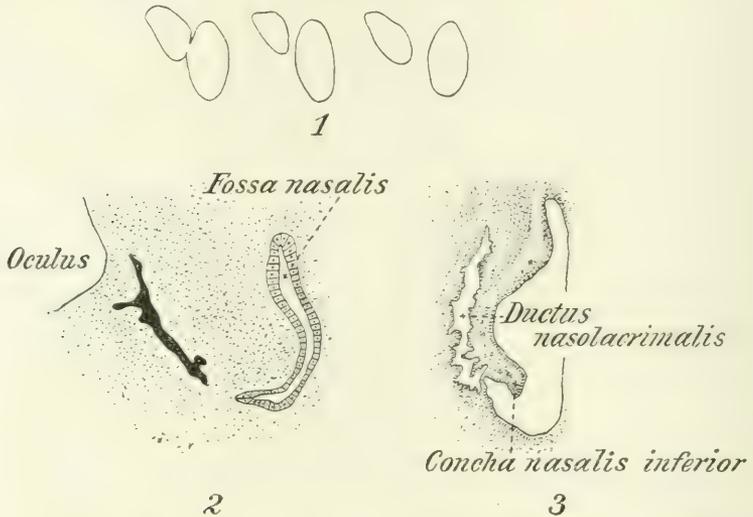


Fig. 1 Outlines of the lumen of the ductus nasolacrimalis at various levels. What appears to be two ductus nasolacrimales lying side by side at one level turns out to be the main duct and a diverticulum from it. From an adult.

Fig. 2 Frontal section through the nasal fossa of a forty-day human embryo. The anlage of the nasolacrimal passages is indicated in solid black. Note its complete isolation from its former surface connection. Note a few lateral buds from the mother cord of cells, presumably the proton of diverticula. At the ocular end of the cord the lacrimal ducts are beginning to sprout.

Fig. 3 Showing the irregular canalization of the ductus nasolacrimalis, from a term child.

Admittedly, such a condition does obtain in a certain percentage of cases and represents one of the anatomic types of the nasolacrimal duct (fig. 4). On the other hand, recent investigations by the writer indicate that many nasolacrimal ducts present lumina of very irregular contour, some even more or less tortuous in course. The irregularity and complexity of the lumen of the nasolacrimal duct is at times carried to a marked degree (fig. 4).

Minor irregularities are at times due to mere folds in the mucous membrane. In many instances, they are of little moment, again, they may form definite bridges along the walls of the duct.

In some instances two parts of the nasolacrimal duct are not in exact line and the connection between the parts a somewhat deviating cross-channel. Finally, many nasolacrimal ducts present very irregular lumina due to diverticula.

These diverticula vary from those of an insignificant size to those with relatively large dimensions. The diverticula are obviously direct extensions of the walls of the duct proper. They are lined with a mucosa

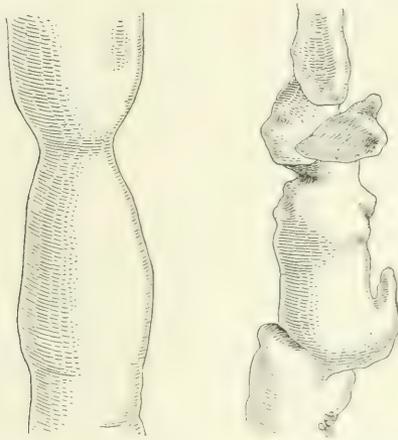


Fig. 4 Showing outline of reconstructions of lumina of mid-portion of two adult nasolacrimal ducts, one is very regular, the other every irregular and with diverticula.

similar to that lining the main duct, and at the ostia of the diverticula the mucosae of the main duct and diverticula are directly continuous, both grossly and histologically.

In studying cross-sections of the nasolacrimal duct, one is at times puzzled to explain what are apparently two ducts lying side by side. However, by following the sections serially one finds that the one cavity sooner or later communicates with the other, i. e., one turns out to be the nasolacrimal duct proper and the other merely a diverticulum from it (fig. 1).

These diverticula must be very important clinically and they need further study. Owing to the irregularity of the lumen in many instances of the nasolacrimal duct, it is obvious that false passageways are repeatedly made by operators when they pass the lacrimal probe.

Genetically, nasolacrimal duct diverticula are doubtless the result of irregular canalization in fetal life of the solid cord of epithelial cells from

which the several nasolacrimal channels develop. One must, therefore, return to the embryologic stage of the nasolacrimal duct for a proper genetic interpretation of the nasolacrimal duct diverticula.

After the strand of thickened epithelium (the anlage of the nasolacrimal passages) along the floor of the now rudimentary naso-optic groove becomes entirely isolated from its surface connection it becomes entirely surrounded by mesenchymal tissue and is for a time without a lumen (fig. 2).

This epithelial cord becomes canalized in a very irregular manner. In this canalization, one has direct evidence of the earliest stages of nasolacrimal duct diverticula. Small lateral pouchings from the main channel, due to a re-arrangement of epithelial cells, are early in evidence. *Para passu* with the growth of the main duct, the diverticula increase in size. At times one finds direct side branches from the mother cord of epithelial cells and some of them doubtless represent the proton of diverticula (fig. 2).

It must, therefore, be concluded from the evidence at hand at present that the diverticula from the ductus nasolacrimalis are of congenital origin and are not acquired in later life.

47. *On the gross morphology, topographical relations, and innervation of the human parotid gland.* S. S. SCHOCHET, Department of Anatomy, Tulane University. (Introduced by Irving Hardesty.)

The parotid gland hardened *in situ* presents an irregular three-sided inverted pyramid with base uppermost and apex below. The posterior surface of the gland presents a marked concavity with three depressions: an external, middle, and an internal concavity. In addition there are in the inferior portion of the mesial surface three grooves caused by the styloid process, the diaphragm muscle, and the sterno-cleido-mastoid respectively.

The external carotid artery was not found buried in the gland substance in any of the six specimens so far examined, and the temporo-mandibular vein lies in a more external and deeper plane. These relations differ from the descriptions given in all the standard text-books. In the illustrations of Testut these vessels are represented as buried in the gland substance.

A small oval or fusiform thickening of irregular outline of a yellowish white color has been found embedded in the auriculo-temporal nerve. Sections of this show it to contain numerous ganglion cells. Because of the position and the branches of connection of this body, it is here named the "parotid ganglion" (ganglionum parotidis). It measures from 1 to 1.5 cm. in length and from .25 to 0.5 cm. in thickness. It is supported by considerable fibrous connective tissue. It is located a short distance from a point where the two roots of the auriculo-temporal nerve fuse after encircling the middle meningeal artery, and in close relation with the temporo-mandibular articulation, the internal axillary and temporal branches of the external carotid artery, thus lying in the plane of the posterior mesial surface of the parotid gland. It should be num-

bered among the sympathetic ganglia of the head, and included, with the ciliary, sphenopalatine, otic, and sub-maxillary ganglia, as a ganglion of the cephalic sympathetic plexus.

The branches of distribution of this ganglion are mainly through the parotid branches of the auriculo-temporal nerve. This ganglion is assumed to serve as a common point of termination of visceral efferent axones from the glosso-palatine nerve (*nervus intermedius*) which axones reach it by way of the small superficial petrosal nerve, and pass uninterrupted, through the otic ganglion to terminate about its cells; these cells in turn send their axones into the substance of the parotid gland. It is also possible that some of the visceral efferent axones of the glosso-palatine are incorporated in that part of the facial nerve which passes to the parotid ganglion by the two communicating branches to the auriculo-temporal nerve.

Serial stained sections of the entire human parotid gland are examined to determine the presence of other sympathetic ganglion cells in its capsule and gland substance.

48. *Comparative study of certain cranial sutures in the Primates.* R. W. SHUFELDT, Washington, D. C.

Early in February, 1914, Doctor Ales Hrdlicka, in charge of the Department of Physical Anthropology of the United States National Museum, invited my attention to certain variations and peculiarities to be found in the sutures on the lateral aspect of the skull in man, and suggested that I should examine into the matter, with the view of publishing a report upon my subsequent studies of the subject. Doctor Hrdlicka very kindly gave me every facility to examine, compare, and photograph the enormous collection of human skulls of which he has charge at the Museum, or such of them as I intended to employ in my investigations.

Hardly had I gotten into these researches when I came to the conclusion that my work would be a far more useful contribution to anthropology were I to include in it a similar series of comparisons made with the skulls of various species and genera of apes, monkeys, marmosets, and their allies. To this end I applied to Mr. Gerrit S. Miller, Jr., Curator of the Division of Mammals of the United States National Museum, who kindly permitted me to examine the superb collection of the skulls of these animals under his charge at the Museum, and to make such photographs as I required for illustrations. In this matter I was very materially assisted by Mr. Ned Hollister, Mr. Miller's aid at the Museum. To all three of these gentlemen I am under great obligations and I have pleasure in thanking them for their assistance in my work, without which it would have been entirely impossible for me to have taken it up in any satisfactory manner whatever. Data from one or two human skulls in my own collection are included in this work, as well as those from skulls of certain monkeys and tamarins, presented me by Mr. Edward S. Schmid, of Washington, D. C.

The locations of the sutures in the human skulls have been known for a great length of time, as have also the bones between which, in any particular case, they may occur. These sutures have, further, all received names which have been bestowed upon them by the older anatomists, and the majority of these names are still employed in our present-day works on human anatomy.

In examining large series of skulls, it will be found that some of their sutures vary but little, as for example, most of those found between the bones of the face; while on the other hand, a very considerable amount of variation is to be observed in some of the cranial sutures, and especially those at the lateral aspect of the cranium. These sutures are caused to vary in accordance with the mode of articulation of certain of the cranial bones, which later, in their turn, present various differences in their articulations that are responsible for those sutural variations. Again, as is well known, certain sutures present variations which are due to the presence of certain supernumerary or epactal ossifications which are intercalated between the cranial bones in the lines of their sutures, examples of which are the Wormian segments and the epipterics—the former usually occurring in the lambdoid suture connecting the parietal and occipital, as well as in the sutures between the parietals and other bones. On the other hand, the adventitious epipterics occur in the spaces of the lateral fontanelles, and are subject to marked variations with respect to number, size and position.

It was these epipterics and the sutures among the bones at the lateral aspects of the cranium, as they are found to vary in the various races of man and in the different families, genera and species of the *Quadrumania*, that I gave my especial attention, and to which my contribution to the subject is devoted, of which this paper is a brief abstract.

In the course of my work I compared several thousand skulls of men, women, and children of all ages, except the very young. The vast majority of these were of prehistoric Peruvians collected by Dr. Hrdlicka, in addition to which there were a sufficient number from other races of the world to satisfy me that I had obtained in my researches all the known sutural variations worthy of record in the aforesaid region of the cranium, and that variations presented on the part of the epipterics were practically endless in nearly every respect. I made a large number of sketches to show this, the majority of which will appear in my paper when it is published. I also made thirty-six photographs of the lateral aspects of skulls of men, women and children, and of various species of the *Quadrumania*. These, for the most part, are more than half natural size, and show most interesting variations of the sutures at the two sides of the cranium in the *Primates*, and, taken in connection with my other data, probably present the most extensive study of these sutures and epipterics up to the present time.

Thousands of figures of photographs of the lateral aspect of the human skull, of both sexes and all ages, have been published, and thousands of descriptions of the sutures in that region of the cranium have ap-

peared. There have also been an enormous number of similar illustrations and descriptions given in works upon comparative anatomy for the *Quadrumania*. In the vast majority of these figures and descriptions the fact is pointed out that the normal articulatory arrangement of the frontal, parietal, temporal (squamous portion) and sphenoid (greater wing) is, apart from the facial articulations, that both the frontal and parietal bones, separated as they are by the coronal suture, articulate in nearly equal proportions with the superior margin or border of the greater wing of the sphenoid, where we find the spheno-parietal and spheno-frontal sutures; that the squamo-parietal suture is the bounding line between the temporal and parietal, and, lastly, that the squamo-sphenoidal suture occurs between the sphenoid and squamous portion of the temporal. These are the only sutures to be considered here and indicate the remaining articulations.

These articulations may or may not be the same on the two sides of the same skull—indeed, they may exhibit very considerable variation. Moreover, they are to be found in the skulls of all Primates, irrespective of various forms of disease, as hydrocephalus, or in distorted skulls, whether the distortion be congenital or induced.

Craniologists long ago bestowed names on some of the principal points of meeting of certain of these sutures, or where they cross prominent ridges, as the stephanion, where the coronal suture crosses the temporal bridge, and pterion, the point where the temporal, frontal, parietal and greater wing of the sphenoid either are in contact or approach each other.

As already pointed out, the squamous portion of the temporal fails to meet the frontal through the intervention of the spheno-parietal articulation. This spheno-parietal suture varies greatly in length, and when it is reduced to zero, all four of the above-named bones are in contact. This may occur upon both sides of the cranium or only upon one. It may be designated by the word 'contact,' and it occurs only in a certain percentage of skulls, whether they be normal, abnormal, or pathological. Contact of these four bones also occurs in the crania of the Primates below man; this is shown in one or more of my photographs, and I shall probably meet with others, later on, when this paper is entirely completed.

So far as my studies carry me at present I find, and especially among the higher simians, that among the *Quadrumania* the sutures, at the lateral aspects of the cranium, have all the variations as they occur in man. The occurrence of epipterics of any size in the skulls of the *Quadrumania*, however, are rare; indeed, up to the present time, I have failed to meet with any such bones in them beyond those of very minute size.

As to the percentage of the occurrence of contacts in the crania of various races of men, I can at this writing only say that it is very small; and how this percentage compares with a similar percentage, taken from the crania of any genus or family of the *Quadrumania*, I am not, at this time, prepared to say. For the human species, I have prepared

a great quantity of data on this subject, which can not be well presented in a brief abstract, such as is here submitted.

In man, the true epactal epipteries vary greatly with respect to number, form, size, and position. With respect to their relation to the pterion, these epipteries, on one or both sides, may be anterior or posterior, or they may be, as I found them to be in the case of an adult male Cinco Cerros Indian (Peru), anterior, posterior, superior, and middle of the left side, while they were multiple also on the right side, but some of the pieces were here lost. An epipteric may be co-extensive with the pterion, in which case it is total. Sometimes the two sides of cranium agree in these respects—that is, there may be an anterior or a posterior epipteric on both aspects of the skull, while they *never* exactly agree in the matters of form and size. Some epipteries are nearly round, some are triangular, others squarish, while still others are very elongate and narrow. They do not occur any more frequently in the skulls of adolescents than they do in adults, and they are very frequently absent entirely in the former.

As to why these adventitious ossifications occur in one skull and not in another, I have, up to the present time, been unable to discover. They occur with equal frequency in small, contracted skulls, as in unusually large crania, or in the skulls of hydrocephalics, where their presence would seem to be more in demand to insure ossific filling in of the lateral fontanelles.

It is still more puzzling to find a reason for from one to four epipteries occurring on one side and none on the opposite side of any particular cranium in man. We see a similar difficulty for solution to account for no Wormian bones in the lambdoid suture of one human skull, and upwards of an hundred in another, with no apparent reason for their being there.

When published, my paper will take up all these questions more fully, illustrating my researches by various sketches presenting unusual conditions as to the epipteries and the sutures at the lateral aspect of the skull in the Primate.

49. *An experimental study of the origin of blood and vascular endothelium in the Teleost embryo.* CHARLES R. STOCKARD, Cornell University Medical College, New York City.

The study of the origin and development of the blood and vascular endothelium has proven a difficult problem, mainly on account of the fact that the circulating fluids of the embryo usually begin to flow before the cellular elements of the blood are completely formed. These early developing cells are thus quickly washed from their places of origin and diffusely scattered throughout the embryonic tissues. All types of corpuscles are therefore found in intimate association, whether their origins may have been from a common center or from distinctly separate sources. The study of no other tissue presents this obstacle. It has thus seemed highly advantageous to obtain material in which the circulation of the body fluids might be prevented without seriously altering the normal processes of development.

Several years ago I observed that the eggs of the fish, *Fundulus*, when treated with weak alcohol, chloroform, ether and other solutions developed embryos in which the blood failed to circulate although the heart pulsated in a feeble manner. During the last three years a systematic study of the origin and development of the blood and vessels in embryos with a heart beat but without a circulation has been conducted. This investigation of the experimental material has at all times been controlled by a study of the blood in the normal embryos.

*Such material permits the analysis of the following propositions: Do blood corpuscles and vascular endothelium have a common origin from definite anlagen, or does the one arise from a localized anlage and the other from widely distributed sources? Does vascular endothelium ever give rise to any type of blood corpuscles? Do all types of blood corpuscles arise from a common anlage? Do certain organs such as the liver have a true hematopoietic function or simply serve as a seat for the multiplication of blood cells derived from other sources? What rôle does circulation and function play in the normal development and history of blood corpuscles? Finally, the specific question, is the bony fish an exception to the rule that all eggs with meroblastic cleavage develop blood islands in the yolk-sac? All recent workers claim that there are no blood islands in the Teleost yolk-sac.*

In many instances the embryo develops in a fashion closely approximating the normal when the heart beat is fairly strong. The blood fails to circulate, however, on account of the fact that the heart is either blind at one or both ends or fails to connect with the veins. In a normal embryo the plasma begins to flow from the vessels of the embryo out into the sinusoids of the yolk-sac, and there establishes a complex vitelline circulation. In individuals in which there is no circulation the plasma accumulates in the pericardial sinus and also in Kupffer's vesicle at the posterior end of the body.

The distended pericardial vesicle forces the head end away from the yolk sphere and thus stretches the heart out into a long straight thread-like structure. These hearts present a great variety of forms depending upon the extent of the pressure in the pericardium. When studied in sections such hearts are in some cases actually solid strings of cells, an inner endothelial string surrounded by a single layer of myocardial cells. In other cases they are slender endothelial lined tubes, while in still others the endothelial cavity is distended and filled with plasma although both ends are closed so that in life the plasma is churned up and down by the pulsation of the heart yet is prevented from escaping or flowing out through the aorta.

The dorsal aortae are in certain specimens almost impossible to identify with high power since their lumina are obliterated—while in other specimens the aortae are well formed endothelial tubes. Yet invariably the aortae never contain any trace of blood corpuscles. The yolk vessels and cardinal veins of such embryos are also distinctly lined with vascular endothelium. It must be concluded from abun-

dant observations that the endothelial vessel linings are present and may arise in all parts of the embryo and do not arise from a local anlage situated in some limited part of the embryo or yolk-sac.

The blood of the Teleost has been found to arise from the so-called 'intermediate cell mass'—Swaen and Brachet, Ziegler, Sobotta and others. These authors differ, however, as to the origin of the cells of the 'intermediate cell mass,' claiming them to be separated from the myotomes, schlerotomes or to be an accumulation of mesenchymal cells. This cell mass in the material here studied is always found to be distinctly connected with and derived from the mesenchyme. Early workers claimed that the blood of the Teleost arose in blood islands on the yolk-sac—but more recent investigators have held that the Teleost forms the marked exception to the rule that in all meroblastic types of eggs the blood arises in islands on the yolk-sac. In the Teleost they hold that the entire blood anlage is within the 'intermediate cell mass.' In *Fundulus*, however, it is found that blood islands do exist in the yolk-sac and continue their development in this position to give rise to well differentiated masses of erythrocytes when there is no circulation. Normal embryos show these islands distinctly in life and when the circulation is established the corpuscles are swept away in the same fashion as those arising from the intermediate cell mass. The erythrocytes in *Fundulus* embryos have, therefore, two distinct and limited places of origin, first, in the stem vein or conjoined cardinal veins, and, second, from the blood islands of the yolk-sac.

The stem vein may be single or double, separate cardinals. The blood forming portion is posterior, behind the anterior portion of the kidney and extending into the tail. The yolk islands are always on the posterior and ventral yolk surface and do not extend over the anterior surface.

It is clearly shown by these embryos that the vascular endothelium is of almost universal distribution arising from the mesenchyme, while the blood corpuscles arise from a limited area. The vascular endothelium never gives rise to blood cells. So that the heart, aorta and vessels of the anterior end of the body, although invariably lined with endothelium do not contain a single corpuscle in embryos of any age. Embryos have been studied up to 20 days old; (the normal embryo hatches and is free swimming after the 12th day).

The erythroblasts developing from the 'intermediate cell mass' and from the splanchnic layer of mesenchyme in the yolk-sac are not at first surrounded by endothelium. As development proceeds the cells surrounding the mass which are of the ordinary embryonic mesenchymal type differentiate or flatten out to form an endothelial layer surrounding the blood cell mass.

All of the corpuscles arising in the stem vein and yolk-sac develop into erythrocytes. Numerous cells closely resembling poly-morpho-nuclear and polynuclear leucocytes are found to arise chiefly in the head region and later such cells are found throughout the body. These cells are often very degenerate in appearance and it cannot be definitely

stated what their nature actually is. They occur in the tissue and not within the vessels and are abundant in normal embryos as well as those without a circulation.

The further development of the erythroblasts is significant in embryos without a circulation. These cells reproduce rapidly by mitosis and finally give rise to well formed erythrocytes, the cytoplasm of which contains a normal amount of haemoglobin. The haemoglobin forms in the cells within the stem vein and in the blood islands and attains a bright red color in life. As the embryo grows in size the erythrocytes within the stem vein are further removed from the surface supply of oxygen and after about the eighth day of development they begin to degenerate, and in embryos of sixteen days only a few cells of the erythrocyte type are present in the stem vein along with numerous more or less degenerate mesenchymal cells. The blood islands are better supplied with oxygen and the erythrocytes persist and present a bright red color. When compared with the erythrocytes of a normal embryo those of the blood islands in an old noncirculating specimen show an interesting condition, instead of the healthy, slightly granular nucleus of the fish corpuscle the nucleus is more compact and darkly stained resembling in a striking way the reptilian type of corpuscle or 'Sauroid type' of Minot's classification.

Circulation and normal function seem therefore necessary in order to maintain the typical appearance and structure of the red blood cells in these fish, although such cells originally attain a perfectly typical structure without having circulated.

Finally, these embryos in which the blood cells arise in a normal manner, yet are never permitted to circulate, furnish material for answering in a conclusive way the long contested question—whether the so-called hematopoietic organs such as the liver do actually contain cells which give rise to blood cells, or serve merely to harbor multiplying blood cells in their sinusoids. An examination of the liver of a normal *Fundulus* embryo of seven or eight days shows it to be very vascular and numerous erythroblasts in mitotic division are often seen. The organ presents the usual hematopoietic appearance.

A similar examination of the liver at any stage of development of an embryo in which the blood has not circulated shows a marked contrast to the normal. The organ is perfectly compact scarcely a vessel is to be found with the highest power on thin sections. *Such a liver does not contain a single erythroblast or erythrocyte in any condition.* The liver of the bony fish has no true hematopoietic function.

50. *Experiments on the amphibian ear vesicle.* G. L. STREETER, Carnegie Institution, Johns Hopkins Medical School, Baltimore, Md.

At the last meeting of the Anatomists the results of a series of experiments were shown in which the ear vesicle in amphibian larvae was transplanted in other specimens and intentionally placed in an abnormal posture. It was shown that there is a subsequent spontaneous

correction of the posture and that the resulting labyrinth has normal relations to its environment.

Since then the attempt has been made to determine the time at which this spontaneous rotation of the ear vesicle occurs and lantern slides will be shown representing this period and showing the histological conditions under which the rotation occurs.

51. *Comparative studies of the neck muscles of vertebrates.* R. M. STRONG, Department of Anatomy, The University of Mississippi.

During the past five years, I have been engaged in comparative anatomy studies which have concerned birds especially, but have included *Necturus* and the alligator also. Publications are in process of preparation for all of these animals, and a work on the anatomy of the Tubinares (albatrosses, petrels, etc.) is approaching completion.

For this occasion, I have selected one of the sections I found less satisfactorily treated in the literature. I have been especially impressed with the need of work on the neck muscles, and I have been unable to find satisfactory illustrations or descriptions of some of these structures. Even the publications of Owen, Gadow, Fürbringer, and Shufeldt have omitted much, and Fürbringer has considered the neck muscles only incidentally. The present confusion in terminology has impressed on me more than ever before the great need of action by a commission to extend the B N A to comparative anatomy.

As the structures described in this paper can not be satisfactorily described without illustrations, no account of them appears in this abstract. Lantern slides and demonstration.

52. *Further observations of the origin of melanin pigments.* R. M. STRONG, Department of Anatomy, The University of Mississippi.

In two previous publications the writer has presented evidence supporting the position maintained by a number of writers that epidermal pigments are produced *in situ*, i.e., are not of dermal origin. A little over two years ago, I assigned to Miss Katherine Knowlton, a graduate student at The University of Chicago, some work on the pigments of feather germs from Plymouth Rock and Brown Leghorn fowls.

In the course of the studies, some interesting evidence concerning the origin of melanin pigments was obtained. Chromatophores were found in the dermal pulp near its proximal end as well as in their usual location in the epidermal cylinder of the feather germ. We found no evidence, however, that these dermal chromatophores wander into the epidermis. They differ in form and size from the epidermal chromatophores. They were also never seen in positions that would indicate migration into the epidermis, although a few were found crowded against the basement membrane which was not penetrated at any point. These dermal chromatophores occur mostly at a level lower, i.e., earlier in the development of the feather elements, than the chromatophores which supply the melanin pigment located in the feather elements.

It seems probable that these chromatophores are comparable to those of the skin dermis. They were found in a more or less continuous series leading to the inferior umbilicus, and it is probable that this continuity would have been complete with the chromatophores of the skin if the preparations had included the tissue which surrounds the feather follicle. A more complete account of this work will be published later. Demonstration of microscope slides with sections of feather germs which show dermal pigment.

53. *The date and clinical significance of fusion of the costal element with the transverse process in the seventh cervical vertebra.* T. WINGATE TODD, from the Anatomical Department, Western Reserve University, Cleveland, Ohio.

In a paper presented at a meeting of the Anthropological Society of Paris (Bull. et Mém. de la Soc. Anthropol. de Paris, 1914) on the variations of the transverse process of the seventh cervical vertebra in Homo, I discussed at length the evidence afforded by the examination of some three hundred vertebral columns regarding the precise disposition of the costal element and its relation to the foramen transversarium (B N A). While many accounts mention the appearance of an ossific centre in the costal element of the transverse process of this vertebra, they do not agree as to the precise date of its appearance, from which we may reasonably gather that the date varies greatly from individual to individual. Concerning the date of fusion of the costal element with the true transverse process, information seems to be even more scanty. That the matter has some importance, however, is apparent when one considers the confusion existing in the minds of many clinicians and anatomists regarding the mutual relations of the transverse process of the seventh vertebra and its cervical rib or costal element.

So much more frequently are cervical ribs observed in children than in adults that Dr. Gilbert Scott has been led to state his belief, for which he says he has some evidence and is collecting more, that the osseous tissue of the cervical rib is absorbed as the child grows (Brit. Med. Journ., 1912, vol. 2, pp. 483-84). This extraordinary statement led me to investigate carefully the date of fusion of the costal element with the transverse process in the belief that such fusion is the explanation of the so-called absorption. Skeletons of suitable age are, however, not readily obtained, but that fusion may be delayed until comparatively late in adolescence is shown by the presence of an unfused costal element, which by no stretch of the imagination could be called a cervical rib, in an Austrian male of 18 years. Fusion is not always delayed so late as this, for in a negro male 17 years of age I found the fusion already complete. In neither of these skeletons had the epiphysis of the spinous processes yet fused with the main part of the bone. The striking difference between the two skeletons is that while the seventh vertebra in the negro approximates the sixth in type, that of the Austrian more nearly resembles the first thoracic. It may be that such a difference in type is closely allied with the date of fusion.

It is plain, however, that fusion may be delayed until the approach of maturity.

Extending my observations to the Primates, I found further evidence in favor of the foregoing conclusions. In a female gorilla which I estimate to be 14 years old, since the third molars are just being cut, the costal element of the seventh vertebra had fused with the transverse process on the left side, but was still incompletely fused on the right. In a specimen of *Ateles belzebuth* which I believe to be about five years old, as the permanent dentition is almost completed (i.e., the third molars are emerging from the alveolar process and the canines are not quite fully erupted) while the epiphyses of the limbs are still ununited with the shafts of the bones, the fully ossified costal element of the seventh vertebra is distinct and separate from the true transverse process. Both of these animals were on the verge of maturity.

The examination with the Röntgen rays of fresh skeletons of children, which exhibited distinct costal elements unfused with the transverse process in the seventh vertebra, clearly showed that the skiagram is not to be trusted on the point. Its evidence may be equivocal and too indefinite to decide whether fusion has occurred or not.

Hence one may feel justified in stating that so-called absorption of infantile cervical ribs as the child grows is probably explained by the fusion of the fully ossified costal element with the transverse process, an occurrence which may be delayed till after puberty, and the date of which cannot be indicated with certainty by the aid of the Röntgen rays.

*54. Is function and functional stimulus a factor in producing and preserving morphological structures?* EDUARD UHLENHUTH.

I propose to show you some preparations of a series of experiments, which I have made with the transplanted eyes of *Salamander maculosa*. These experiments are designed to show whether or not organs of a typical functional structure, after transplantation to an abnormal site, require function or functional stimulus, in order to be preserved at this new site. In short, I wished to investigate whether function and functional stimulus is a factor in preserving functional structures in transplanted organs.

After cutting through the optic nerve the eyes of amphibians undergo considerable degeneration of the retina. Later, however, the optic fibres regenerate and the trunks of the optic nerve unite. At the same time the retina which had undergone disintegration becomes restored to a normal condition.

By transplanting the eye to a place far removed from the normal position such a reunion of the optic nerve with the central nervous system is inhibited and the eye is permanently prevented from functioning. Partly, as might be supposed, the functional stimulus, namely light, which influences the retina even after transplantation, could bring about this result. I therefore used two series of larvae with transplanted eyes, both consisting of more than 100 specimens. One of the

series was kept in ordinary daylight and the other series in a dark room, and in the latter case, no light, not even red light, was admitted while the animals were being cared for, by which means the retina could neither functionate nor be affected by functional stimulus. The two series were then compared.

I will show you preparations of these two series. Those of the daylight series, as you will see, clearly show a regular increase in degeneration during the time immediately following upon transplantation, succeeded by a second period of a gradual increase in regeneration. To each preparation of the light series belongs one preparation of a transplanted eye of the same age but kept in darkness, and this is, as you will find, always much more normal than that of the light series. If function or functional stimulus were to favor regeneration the relation would be the reverse.

But I might have selected for each light eye a less normal dark eye of the same age, instead of a more normal one. As in both series the same factor, but in a contrary sense, has equally influenced every transplanted eye, these variations of the eyes of the same age and series cannot be explained as an effect of this factor, but must be the result of another factor which is variable and not controlled and which is perhaps produced by slight variations of the position of the eyes in the new place. Anyway, by having a large number of experiments, one is able to take the average of every age of both series, by means of which I obtained curves of the rapidity of regeneration in every series. Compared with the other they do not show any differences which can be ascribed to the influence or absence of light.

If the function or functional stimulus does not influence the rapidity of regeneration it might be impossible to keep the transplanted eyes permanently in a normal condition without these factors; but you will find fairly old transplanted eyes, if you will look at the preparations. Some of them were made after the eye had been left for fifteen months in its new position. You will see that even those old eyes were perfectly normal.

The conclusions, therefore, which we must raise from these facts is the following:

The regeneration of the structures of vision following destruction caused by the transplantation of the eyes can by no means be considered functional regeneration, and even the rapidity of this regeneration is not influenced by functional stimulus. The permanent preservation of the retina, when deprived of all function or functional stimulus and kept in perfectly abnormal conditions, is not a process which is governed by functional adaptation.

*55. On the early development of the inguinal region in Mammalia.*

JOHN WARREN, Harvard Medical School, Boston.

The early development of the gubernaculum and processus vaginalis was studied in the human embryos and in the embryos of the cat, sheep, rabbit, and rat in the Harvard Embryological Collection. As far as

possible different stages in each form were described so as to show the more important changes in the early development of this region.

In human embryos the first sign of a connection between the Wolffian body and the abdominal wall can be seen in an embryo of 13.6 mm., transverse series no. 859. From the cells in the wall of the Wolffian duct a fairly distinct mass of cells may be traced dorso-laterally immediately beneath the primitive peritoneum covering the Wolffian body to join the abdominal wall at a slight elevation, the inguinal crest. This cell mass invades later the abdominal wall, and is the anlage of the gubernaculum. A fossa is formed in the peritoneal cavity dorsal to this primitive gubernaculum. In an embryo of 16.6 mm., transverse series no. 1707, the cells in the gubernaculum have become much concentrated, and have begun to invade the ventro-lateral abdominal wall, where as yet there is no differentiation of the abdominal musculature. The gubernaculum steadily increases in density and length, and in an embryo of 19. mm., transverse series 819, it extends well through the abdominal wall, where its peripheral end expands and blends with the cellular constituents of the wall. At this stage the musculature ventrally forms a general cell mass, but more dorsally has differentiated into its three layers. In embryos of 22.8 mm., frontal series no. 757, and 24 mm., transverse series no. 38, the musculature is completely formed and grows around the peripheral part of the gubernaculum. An indistinct prolongation of the latter can be traced through the external abdominal ring into the subcutaneous tissue of the abdominal wall, forming a primitive ligamentum scroti. The processus vaginalis appears soon after this stage in embryos of 37 mm., transverse series no. 820, and 42 mm., transverse series no. 838, and develops downward and inward chiefly on the mesial side of the gubernaculum.

In the Carnivora, the primary point of contact between the Wolffian body and the abdominal wall can be distinguished in a cat embryo of 12 mm., transverse series no. 399. Here the anlage of the gubernaculum resembles the earliest form in human embryos, and a shallow fossa is formed also on its dorsal side. In an embryo of 15 mm., transverse series no. 436, the abdominal portion of the gubernaculum has increased in length, while the inguinal or muscular part has already passed through the abdominal musculature into the subcutaneous tissue over the external ring. The musculature is fairly well differentiated, and the external ring can be clearly seen with the peripheral end of the gubernaculum projecting through it. An embryo of 31 mm., transverse series no. 500, gives an excellent view of the whole length of the gubernaculum. The abdominal portion is now very long and slender, and the scrotal part appears as a large oval mass projecting through the external ring. The processus vaginalis has just begun to develop, but is growing downward on the lateral side of the gubernaculum. In an embryo of 39 mm., the processus vaginalis surrounds the gubernaculum except on its dorsal side, and can be traced well into the large expanded scrotal portion beyond the external ring. This represents the most advanced stage of any of the embryos studied.

The sheep embryos offered the best view of the development of the region in ungulates. The earliest appearance of the gubernaculum was seen in an embryo of 18 mm., transverse series no. 1238. It forms a thick connecting bar of tissue, similar to the corresponding stage in the cat and in man. Its extension through the abdominal wall, the development of the abdominal musculature about it, and the earliest trace of the processus vaginalis follow closely the course already described in the cat. The processus vaginalis develops first on the lateral aspect of the gubernaculum and then seems to grow downward chiefly in its substance. This is rather strikingly shown in the oldest embryo, transverse series no. 1696, 48.4 mm., where the lower end of the processus vaginalis is completely embedded in the large expanded distal end of the gubernaculum, into and about which the cremaster fibers can be seen growing. The relation between the processus and the gubernaculum is different at these stages from any of the other forms.

In rodents, a rabbit embryo of 11 mm., transverse series no. 1327, shows a primary point of contact between the abdominal wall and the Wolffian duct exactly similar to the cat embryo of 12 mm. A rat embryo, transverse series no. 1823, 9.3 mm., shows the early gubernaculum at a stage a little further advanced and comparable to that of the human embryo of 16.6 mm., and to the sheep of 18 mm. In all cases the presence of the retro-gubernacular fossa in the peritoneal cavity is striking. The development of the gubernaculum and processus vaginalis is very precocious in rat embryos and seems to advance more rapidly in the earlier stages than in those of the other mammalian embryos studied. A rabbit embryo of 21 mm., transverse series no. 738, gives an excellent view of the whole extent of the gubernaculi of both sides and the three parts—abdominal, inguinal or muscular, and scrotal—are clearly differentiated. There is no sign yet of the processus vaginalis which can be seen however in a rat embryo of 15.2 mm., transverse series no. 1801, appearing on the lateral aspect of the gubernaculum. The processus vaginalis begins to develop in rabbit embryos of between 21 and 29 mm., and in the latter arises from the large funnel-shaped fossa dorsal to the gubernaculum and extends downward on the dorso-lateral side of the gubernaculum. This ends in a dense but narrow ligamentum scroti that fuses in the subcutaneous tissue with the one of the opposite side. The cremaster fibers are well marked and arch over and blend with the gubernaculum as in the older sheep embryos.

56. *Is defective and monstrous development due to parental metabolic toxæmia?* E. I. WERBER, Department of Biology, Princeton University.

The problem of the causes underlying defective development has recently received masterly treatment by F. P. Mall. In his extensive study based on 163 pathological ova he supports the conclusion arrived at by the experimental embryologists, namely, that the human monsters are—with the exception of the hereditary 'merosomatous' terata—due to injurious influences of atypical environmental factors. He

makes the specific suggestion—which seems justified in the light of evidence brought forth by him as well as by clinical data—that the monstrous development of some ova may be due to their inadequate nutrition owing to the imperfect implantation in a diseased uterus. It would seem that this hypothesis will hold good at least for many pathologic embryos aborted during the first two months of pregnancy. At any rate, the principle advocated by the hypothesis, viz., the influence of unusual environmental factors, seems to be correct beyond any doubt.

Mall's interpretation could not, however, be applied to monstrous fetusses of the later months of pregnancy or to monsters after full-term births. Some environmental factors must be looked for other than faulty implantation of the ovum, to account for the occurrence of such cases. The results of investigations in experimental embryology and teratology by Dareste, Roux, Hertwig, Féré, Morgan, Tur, Stockard, and others, who obtained monstrous development of ova which had been subjected to the action of physical and chemical modifications of the environment, suggested to me that the human as well as other mammalian monsters may be due to the physical or chemical action of some substances in the blood of one of the parents on either one of the germ cells or on the fertilized ovum respectively. The toxic substances found in the blood of individuals afflicted with some diseases of metabolism, I think, might be the ones which could be made responsible for the origin of monstrous development.

To test this hypothesis it would be necessary to breed mammals in which certain diseases of metabolism had been produced experimentally, for the spontaneous occurrence of these disturbances in animals is too rare to permit of conclusive breeding experiments. On the other hand, to produce these diseases experimentally, at least as far as this can be done by the present rather inadequate methods of experimental pathology of metabolism, requires some facilities which, so far, are beyond my reach. I have thus had to confine myself to a preliminary step in the investigation.

This consisted in subjecting the eggs of an oviparous vertebrate to the action of solutions of substances found in the circulation of man under certain pathological conditions of metabolism. The eggs of a *Fundulus heteroclitus* were chosen as the object of experimentation and they were subjected in early (1 to 2 or 2 to 4 cells) cleavage stages to the action of sea-water solutions of various strengths of urea, butyric acid, lactic acid, acetone, sodium glycocholate and ammonium hydroxide, respectively. Positive results permitting of definite conclusions were so far obtained only with butyric acid and acetone.

For butyric acid 10 cc. of a  $\frac{1}{2}$  to  $\frac{1}{4}$  gram molecular solution, added to 50 cc. of sea water was found to give the best results, that is, the greatest number of monsters, while very much stronger solutions of acetone, namely, about 35 to 40 cc. in 50 cc. of sea-water were found to be necessary to obtain like numbers of monsters. In the case of butyric acid a long exposure was found to kill most eggs and it was necessary to limit it to 20 hours, while in the case of acetone the eggs could be kept

in the solution up to 48 hours, exposures longer than this increasing their mortality. It was also found that the eggs were more susceptible to the influence of these chemical modifications of the environment during the first and second than during the third and fourth cleavages.

The results obtained with butyric acid and acetone solutions are very much alike. Great numbers of cyclopean monsters were found in both these series of experiments. I have recorded in my observations the occurrence of transition from two normal eyes in the typical position in the head all the way down through the more or less closely approximated eyes or eyes of a double composition and through true cyclopia to complete anophthalmia as described by Stockard in his experiments with magnesium chloride and alcohol. Stockard has also described a peculiar change in the form and position of the mouth in the cyclopean embryo. The mouth in such embryos has the appearance of a snout, a proboscis-like structure and is pushed down below the cyclopean eye. This displacement into the ventro-lateral position Stockard attributes to the circumstance that the cyclopean eye, being frontally located, has caused the mouth to move downward. I have observed this occurrence in most of the cyclopean monsters found in my experiments as well as in many cases of dorsal microphthalmia or even in some cases of asymmetric monophthalmia.

Besides the cyclopean, asymmetrically monophthalmic, microphthalmic and anophthalmic embryos there was found a very great variety of monstrosities in which practically the entire bodies of the embryo were more or less involved in the malformations. Thus curiously misshapen dwarfs with vestigial eyes or blind or very elongate, greatly malformed embryos, often with many waistlike constrictions were of not infrequent occurrence. Eggs in which only an anterior part of the embryo ('meroplastic embryos'—Roux), as if the rest of the body had been mechanically removed, were found in great numbers. Of these the ones in which an approximately anterior one-half of the body was present would correspond to the 'hemibryones anteriores,' which Roux obtained experimentally in the frog by injuring one of the blastomeres after the first cleavage. These hemibryos found in my own experiments have defective or sometimes very rudimentary eyes, they often exhibit evidences of oedema and are as a rule extremely misshapen. Eggs were also recorded in considerable numbers in which only a malformed head or small anterior part of it could be observed. These 'meroplasts' are so deformed that only by the presence of rudimentary eyes is it possible to determine that they are heads.

But the most curious and most significant of all meroplasts recorded were eggs in which all that could be observed on the yolk-sac was a very small fragment of brain tissue with a *solitary eye*, which was sometimes somewhat defective—of the "coloboma" type. The fragment of brain tissue is usually smaller than the eye it has given rise to. Since the eggs were fixed by Child's sublimate-acetic method and preserved in formaline, the embryos are white while the yolk-sacs are transparent; yet nothing at all can be seen in the transparent yolk-sac to indicate that the embryo had sunken into it leaving one of its

eyes on the surface where it might have been constricted off. Moreover, in order to establish the fact of the occurrence of the solitary eye beyond any possibility of skeptical criticism, I have sectioned one of these eggs on which besides the solitary eye several very small fragments of tissue could be observed in the living as well, as in the fixed specimen in various places, distant from each other, on the yolk-sac. The interpretation proved to be perfectly correct. *For*, besides the referred to few, small amorphous fragments of tissue scattered over the yolk-sac *there can be seen only an eye typical in all its structures*, while nothing can be found, to indicate the presence of an embryo. *This is, as far as I am aware, the first case on record of the independent development ('self-differentiation'—Roux) of the eye.*

Another instance of apparently independent development of the eye in these experiments has occurred in some cases where an eye appeared at a considerable distance from a monophthalmic embryo.

The ear vesicles are often involved in the malformations of embryos. This can be readily seen by their sometimes enormous size. Some asymmetrically monophthalmic embryos after hatching could not swim directly forward, dropping to the bottom of the dish in which they were kept, if forced to do so. They could only move in circular or spiral lines which would indicate some injury to the semi-circular canals.

There is a wide range of variation in the deformities of the blood vascular system. The heart is almost perfect in some embryos in which cyclopia is the only superficially noticeable defect. In cases of more extreme malformation the heart may be only a very delicate, straight tube in some embryos while it may be absent altogether in others. In this connection it may be of interest to note that I have found some eggs in which all that was present of the embryo was a functioning heart and some rudimentary blood vessels. The degree of malformation of the blood vessels is subject to a great deal of variation. There may be merely blood-islands scattered on the yolk-sac, rudimentary, imperfectly connected, or in some instances more or less normal vessels.

The tendency of butyric acid and acetone solutions to produce twins seems to be only slight for I have observed only a few of such cases. I have only one case comparable to the "Siamese twins" type of the human. In this egg two deformed embryos with a common heart had developed on opposite sides of the egg. Other cases of twin formation found in these experiments belong to the type known as 'duplicitas anterior,' which was produced experimentally by mechanical means by Speemann.

Not infrequent is the occurrence of amorphous embryos or small amorphous fragments of tissue on the yolk-sac as the only evidence of development.

The mechanism involved in the action of butyric acid and acetone in bringing about these effects will have to be taken up as one of the further steps of this investigation. At the present time I can only state that there seems to occur in the eggs when subjected to the action of butyric acid and acetone solutions an elimination of substance of the blastomeres or possibly of the germ-disc. This elimination may be brought about either by precipitation or by the solvent effect respectively of the chemicals used in the experiments. Whatever parts of the

blastoderm survive that process of destructive elimination, may go on developing to form an isolated organ or a part of the body or a complete embryo with defects in some organs.

57. *The ileo-jejunal artery.* C. R. BARDEEN, University of Wisconsin.

From the superior mesenteric artery opposite the origin of the ileo-coecal artery there arises a branch which passes to the free small intestine near the junction of the upper with the middle third of the gut as measured from the duodenum to the caecum. This branch may be called the ileo-jejunal artery. In a previous article (C. R. Bardeen, "The critical period in the development of the intestines," *American Journal of Anatomy*, vol. 16, p. 427) I have shown the probability that the region supplied by this artery represents the junction between that portion of the small intestines the primitive coils of which develop within the umbilical cord, the ileum, and that portion the primitive coils of which develop within the abdominal cavity, the jejunum. A study of the blood supply of the intestines in a number of fetuses and adults has shown that the portion of the intestines proximal to the center of the region supplied by the ileo-jejunal artery, the 'jejunum,' varies from 26 to 44.1 per cent of the total length of the small intestines as measured on the side opposite the mesentery and from 27.4 to 41.2 per cent measured on the mesenteric border.

In 10 fetuses measuring from 40 to 280 mm. in length (vertex breach) the average proportional length for the jejunum opposite the mesenteric border was 37.1 per cent with extremes of 32.2 and 44.1 per cent. In only two cases was it 40 per cent or over.

In 18 adults varying in age from 14 to 74 years the average proportional length of the jejunum was 33.5 per cent with extremes of from 26 to 40.3 per cent. In five cases it was under 30.7 per cent and in two cases 40 per cent or over. In eleven instances the jejunum as measured in the mesenteric border averaged 33.3 per cent with extremes of from 27.4 per cent to 41.2 per cent. In four instances it was less than 30 per cent; in two instances 40 per cent or more.

In the adult specimens examined the length of the intestines varied from 138 to 294.5 inches but I have been able to trace no correlation between the length of the free intestines and the proportion between the proximal and distant portions. Aside from the average greater proportional length of the jejunum in the fetuses examined as compared with the adult I have found no correlation between age and the proportional length of the jejunum and with a greater number of specimens examined this difference might disappear. In 8 women the average length of the gut was shorter than that in 10 men, although I have found no certain correlation between length of gut and length of body. In the 8 women the average length of the jejunum was 31.96 per cent (extremes 26, 40 per cent) and in the 10 men, 35.31 per cent (extremes 27.3, 40.3 per cent). This may indicate a tendency on the part of women to have a longer ileum than men have and may possibly be related to the greater tendency in women to constipation.

## THE FOLLOWING DEMONSTRATIONS WERE SHOWN:

1. *Certain aspects of hematogenesis in the pig embryo.* V. E. EMMEL, Department of Anatomy, Washington University Medical School.  
The microscopical demonstrations and drawings are to illustrate data relating: (a) to the cytological structure and morphological relations of the cell clusters in the dorsal aorta (cf. also abstract of paper on the same subject) and the macrophags and mesamoeboids in the liver sinusoids and coelomic cavities, with reference to the problem of the relation of endothelial tissue to hematogenesis in these regions, and (b) to certain cytological characteristics of erythrocytes during their cytomorphosis.
2. *Sections, models and drawings showing the development of the chondrocranium in Felis domestica.* R. J. TERRY, Washington University Medical School.  
The following points of interest have been selected for demonstration:
  - (1) Basal plate: the position of the notochord, flexures of the basal plate.
  - (2) Occipital region: basal and lateral moieties of occipital condyle, hypoglossal canal, dorsal root and ganglion of the hypoglossal nerve, the occipital hypochordal arch, neural arches of the atlas and atlantal foramen.
  - (3) Otic region: evidence of independence of chondrification of otic capsule, relations of suprafacial commissure, independent chondrification of the parietal plate, development of the tegmen tympani, course of the facial nerve, formation of the internal acoustic meatus, foramen cochleae and aquaductus cochleae, cavum supracochleare, supraganglionic cartilage, distribution of the acoustic nerve.
  - (4) Orbito-temporal region; hypophyseal cartilage, development of the dorsum sellae, foramen hypophyseos, early relations of the ala orbitalis, primary elements of the ala temporalis, origin of the foramen lacerum, epipteric cave, relations of ocular muscles to chondrocranium.
3. *Microscope slides showing feather germs with dermal pigment.* R. M. STRONG, The University of Mississippi, Oxford.
4. *Photographs of plates illustrating the anatomy of the albatross (Diomedea).* R. M. STRONG, The University of Mississippi, Oxford.  
The original drawings were made by Mr. Kenji Toda, artist for the Department of Zoology at the University of Chicago. About one-third of the plates are represented in the exhibit.
5. *Photographs, drawings and charts illustrating (A), the morphology of the mammalian seminiferous tubule and (B), the relation of the stages of spermatogenesis to the tubule.* GEORGE M. CURTIS, Vanderbilt University Medical School, Nashville.

6. *Demonstrations of 'endothelioid' cells.* HAL DOWNEY, University of Minnesota, Minneapolis.

1. Normal lymph node of guinea-pig; Helly, methyl green and pyronin. The sinuses contain many of the so-called 'endothelioid' cells, both attached and free.

2. "Endothelioid" cells in lymph node from normal cat; Helly, May-Giemsa. They are large protoplasmic cells which form a part of the reticulum. The wall of the small lymph sinus is in part composed of processes from these cells. One of the cells has almost completely separated from the reticulum. Its nucleus is indented and it has phagocytosed a red corpuscle. This method does not bring out the reticular fibers. The reticulum is not covered by an endothelium; if it were it should be possible to see the nuclei of its cells. The large cells will separate from the rest of the reticulum and form the 'endothelioid' cells of pathologists.

3. Lymph node from normal cat, stained by one of the methods for reticular fibers. The 'endothelioid' cells contain fibers in their peripheral portion.

4. Spleen from Mandelbaum's case of Gaucher's disease; Orth's fluid, iron-hematoxylin, fuchsin S, orange G, toluidin blue. 'Endothelioid' cells very numerous in the pulp and venous sinuses.

5. Lymph node from Mandelbaum's case of Gaucher's disease; alcohol fixation, stain as for (4) above. This field shows that the large, characteristic 'endothelioid' cells are derived from the reticulum and not from endothelium. The long strand in the center of the field is a part of the modified reticulum, and the long strand of reticulum approaching the center from the right gradually assumes the character of the protoplasm of the Gaucher cells.

6. Lymph node from a case of Hodgkin's disease; Helly, Weigert's iron-hematoxylin, fuchsin S, orange G, toluidin blue. In many cases the fibers of the reticulum can be seen to penetrate the 'endothelioid' cells.

7. *A differential counterstain for vertebrate embryos.* W. A. WILLARD, University of Nebraska, College of Medicine.

Pig embryos designed chiefly for class study of organogenesis are first deeply stained *in toto* with borax carmine then cut into serial sections 20 micra or more in thickness and counterstained on the slide with a dilute solution of Lyons blue in absolute alcohol which has been rendered a blue-green color by the addition of a few picric acid crystals. The strength of the solution and the time required to stain is best determined by experiment with any particular lot of material. The result of the counterstain is to add brilliancy and transparency to the whole section slightly decolorizing the carmine and giving a selective stain of light green to the developing nerves and certain portions of the central nervous system. Blood cells and to a certain degree epithelial structures are differentiated. By this method short series are made available with a minimum amount of handling, recommending it as a practical laboratory method.

8. *A double embryo of the spiny dogfish (Squalus acanthias)*. W. A. WILLARD, University of Nebraska, College of Medicine.  
This is an example of two normally developed embryos attached by separate yolk stalks to a common yolk-sac. The embryos are in the second year of their intra-uterine development, in what is known as the 'pup' stage, one measuring 16 cm., the other 14.5 cm. in length. The larger of the two is supplied from a larger yolk-sac area as indicated by the vascularization by the vitelline vessels. An exposure of the viscera does not disclose any modification of the normal arrangement of organs, such as transposition or reversal of the normal symmetry. As the embryos had slipped from the cloaca of the female before they were noticed no data were obtainable as to position in the uterus or as to other embryos of the same brood.
9. *Slides of yolk-sac of 10 mm. pig embryo*. H. E. JORDAN, University of Virginia, University.  
Technic (1): Zenker fixation, hematoxylin-eosin stain; slides of yolk-sac of 4 mm. pig embryo. Technic (2): Helly fixation, Giemsa stain.
10. *Injections of the lymphatics of the lung*. ROBERT S. CUNNINGHAM, Johns Hopkins Medical School, Baltimore.
11. *Injections of the vascular system in early pig and chick embryos*. FLORENCE R. SABIN, Johns Hopkins Medical School, Baltimore.
12. *Cell groups of the hypothalamus in man*. EDWARD F. MALONE, University of Cincinnati, Cincinnati.  
(1) Nuclei tuberis laterales; (2) Ganglion opticum basale; (3) Nucleus paraventricularis hypothalami; (4) The three cell groups of the corpus mammillare.
13. *Microscopic preparations showing the reactions of transplanted eyes in Amphibia*. EDWARD UHLENHUTH, Rockefeller Institute, New York City.
14. *A human embryo of 22 somites (models and figures)*. FRANKLIN P. JOHNSON, University of Missouri, Columbia, Mo.
15. *Models of the liver veins of pig embryos*. FRANKLIN P. JOHNSON, and T. F. WHEELDON, University of Missouri, Columbia, Mo.
16. *Models of the heart of a 20 mm. pig*. T. F. WHEELDON, University of Missouri, Columbia, Mo.
17. *Dissections showing origin, course and distribution of nervus terminalis in the human fetus*. ROLLO E. MCCOTTER, University of Michigan, Ann Arbor, Mich.

18. *Models of the early development of the inguinal region and of the pelvic outlet in human embryos.* JOHN WARREN, Harvard Medical School, Boston.
19. *Demonstration of reconstructions of lateral hearts and foregut in Citellus to show connection of endocardium to entoderm.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

20. *Models showing the development of the hypophysis in Squalus acanthias.* E. A. BAUMGARTNER, Washington University Medical School, St. Louis.

The study of the hypophysis, begun at the University of Minnesota and completed at Washington University Medical School, is represented in part by a series of ten models.

A model of a 19 mm. embryo shows Rathke's pouch extending obliquely forward and dorsalward from the mouth. In a 21 mm. embryo a part of the wall of the oral cavity ventral to Rathke's pouch has begun to evaginate to form the anterior end of the hypophysis. From these two out-pouchings are formed the hypophysis of the adult. The upper lateral portions of Rathke's pouch are somewhat dilated. In a model of the hypophysis of a 22 mm. embryo, the anterior end is distinctly evaginated; while in a model of the hypophysis from a 28 mm. embryo this part is constricted off from the mouth except for a small stalk connected to its caudal side. Most of the first out-pouching, or Rathke's pouch, will form the caudal end of the anterior lobe. The lateral dilated portions are separated from the median part by two furrows which have appeared on the anterior side of the upper part of the hypophysis. The extreme tip of Rathke's pouch is somewhat enlarged, the anlage of the superior lobe of the hypophysis. A model of the hypophysis of a 33 mm. embryo shows a short anterior end connected by a narrow mid-part to the wider caudal end. A slight ridge, superior to the hypophyseal stalk, connects the lateral portions which, in the adult, form the inferior lobes. In a 48 mm. embryo the model shows that the hypophyseal stalk has disappeared. The superior lobe has two lateral wings extending forward and slightly dorsally. In a model of the hypophysis of a 95 mm. embryo the anterior lobe is very long and the wider anterior and caudal extremities are marked. The inferior lobes project laterally and from their median connection a slender duct connects them to the caudal extremity of the anterior lobe. In the pup stage ridges indicate the beginning glandular structure. These are present on the ventral wall of the anterior extremity of the anterior lobe and on the roof of the inferior lobe. A model of some of the glands of the anterior lobe shows them connected to the ventral wall. They are short-branched tubular outgrowths showing anastomoses. The lumina may not be continuous throughout the anastomosed tubules.

21. *Wax models in verification of the nucleus-plasma relation of nerve cells.* DAVID H. DOLLEY, University of Missouri (introduced by E. R. CLARK).

As a result of the averages of measurements, for the most part on the crayfish and the dog, the nucleus-plasma norm of functionally resting nerve cells of the same type has been found to be represented by a close numerical constant in all individuals within any particular species.

For final verification, calculations were made for individual Purkinje cells of several dogs from serial sections at 2 and 1 $\mu$ . From these serials wax models were reconstructed and the data were afforded for the mathematical application of the prismoid formulas. In the case of the wax models, the proportion by weight of wax nucleus to wax plasma is identical within very narrow limits, whatever the size or shape of the cell or whatever the size of the animal or its age between the full development of the relation and senescence. The uniformity of the results after all three methods, with the support of certain collateral evidence, has led to the induction of a law of species identity of the nucleus-plasma norm for corresponding nerve cell bodies (*Jour. Comp. Neur.*, vol. 24, October, 1914).

The shifts in absolute and relative size in nucleus and plasma which result from function, as determined by average measurements, are also confirmed by the application of the wax reconstruction and the prismoid formulas to the individual cell.

22. *On the use of orcein as a bulk stain for elastic fibers.* A. G. POHLMAN, University of St. Louis.

Blocks of tissue are run through to 95 per cent alcohol and placed in Unna's orcein solution for 2 to 12 hours according to size. Remove to absolute alcohol slightly acidulated with HCl for 2 to 3 hours and then into an excess of absolute alcohol for 6 to 12 hours. The tissue may now be handled in the usual way for paraffin and celloidin technique. If tissues are not sufficiently differentiated after sectioning use 5 per cent oxalic acid. The stain is very resistant and will not be affected by ordinary laboratory methods.

Demonstration I. (1) Plain bulk orcein; (2) Paracarmine followed by orcein; (3) Orcein followed by hematoxylin-eosin; (4) Orcein followed by hematoxylin-eosin and orange G.; (5) Orcein followed by hematoxylin and picrofuchsin; (6) Orcein followed by picrofuchsin; (7) Differentiation in cleared bulk specimen; (8) Differentiation shown in uncleared bulk specimen.

Demonstration II. (1) Length section of Platner's ligament; (2) Length section through drum ligament in chick; (3) Section through attachment of Stapedial plate and membrane of the Fenestra cochleae; (4) Dissection of the gross relations of the columella in chicken, duck, goose and turkey. Platner's ligament shows as a delicate fiber running forward from columella to quadrate bone.

23. *Plaster casts of the sphenoid, maxillary and frontal sinuses; the cubical capacity and superficial area of these sinuses.* HANAU W. LOEB, St. Louis University.

The casts are made by joining together plaster moulds of those portions of the sinuses lying adjacent to one another in serial sections of the head. The casts are then boiled in paraffin and the cubical capacity determined by ascertaining the amount of water displaced by them. To determine the superficial area, the casts are covered with strips from a known amount of adhesive plaster. The difference between the known amount and that remaining gives the superficial, subject to whatever error results from lack of complete approximation of the strips. This is exceedingly small indeed.

24. *A method for handling paraffin sections.* IRVING HARDESTY, Tulane University.

A method for staining and issuing paraffin sections for mounting by the students in large classes in histology. Thin sheets of a modified form of celluloid may be obtained under the commercial name, "la cellophane." These sheets are quite thin, perfectly transparent and resist the action of water, alcohol of all strengths, ether, chloroform, and all the oils commonly used in clearing and differentiating, including creosote and oil of cloves. La cellophane of thickness No. 253 may be obtained in sheets  $17\frac{1}{2}$  by 25 inches. These may be cut into sheets of desired size. The paraffin sections of a specimen, in sufficient number to supply the class may be placed upon the sheets, straightened out and fixed by the usual albumen-water method. After drying, the entire sheet is treated for staining, clearing and mounting, just as is a slide with a single section. After clearing, the sheet is clipped into small pieces each bearing a section and these pieces issued to the class. La cellophane No. 253 is sufficiently thin for the purpose; No. 252 however, is, said to be of thinner weight. For work with oil immersion objectives under cover—glasses of ordinary thickness, the student should be advised to mount the pieces with the sections uppermost. The sheets retain practically none or very little of the stain after hematoxylin and anilin dyes commonly employed.



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A COMPARATIVE STUDY OF THE SHOULDER  
REGION OF THE NORMAL AND OF  
A WINGLESS FOWL

W. B. KIRKHAM AND H. W. HAGGARD

*From the Osborn Zoölogical Laboratory, Yale University*

ELEVEN FIGURES (THREE PLATES)

INTRODUCTION

Among a lot of Rhode Island Red chicks hatched in an incubator by Dr. Fred Sumner Smith, of Chester, Connecticut, appeared one male individual entirely destitute of wings. This was reared to maturity, and lived for nearly two years, forming the subject of an extensive breeding experiment by Prof. Wesley R. Coe. (The results of this experiment will be the subject of a separate paper.) After the death of this bird his body was preserved in alcohol, and later handed over to the writers for a study of the modifications of the muscular and skeletal structures which might be correlated with the congenital absence of wings.

The writers knew of no connected, detailed account, in the literature, of the anatomy of *Gallus domesticus*, so, with the aid of the brief references available, a study of the normal musculature and skeleton has been carried out on normal fowls of the breed of the wingless rooster. This study has been limited to the shoulder and thoracic regions, as examination of the wingless specimen revealed no abnormalities elsewhere.

No special study of the nerves and blood vessels of the shoulder region was made, but as on the wingless rooster a number of large nerves and blood vessels were found coming from the body cavity and breaking up into small branches to enter the thick

fascia and skin which covered the area where the wings would normally have been, it may be safely assumed that these were the blood vessels and nerves normally destined to go to the wings, in the absence of which they terminated in the skin and underlying fascia. The normal innervation of the shoulder region of the hen has been described and the nerves figured by Fürbringer ('88). The muscles have been described in the present paper in an order based on their nerve supply, a scheme used by Gadow ('91).

All the variations appearing on the wingless rooster and described below were symmetrical on the two sides of the body unless otherwise stated.

#### MUSCULATURE

##### *Rhomboideus superficialis* (figures 6 and 7; 10 and 11)

*Normal.* This muscle takes its origin from the rib-bearing cervical vertebrae and from the crests of all of the fused thoracic vertebrae except the most posterior one. It is a very broad muscle, but made up of short fibers, its insertion being along the dorso-lateral surface of the scapula, from the posterior extremity to a point almost directly above the scapular tubercle of the coracoid, and extending ventrally to the dorsal margin of the origin of the scapuli-humeralis posterior.

*Wingless.* The superficial rhomboid muscles of the wingless rooster were much thinner than the normal, and showed decided variations as to origin and insertion on both sides. On the right side this muscle had its origin limited to the median third of the fused thoracic vertebrae, and its insertion on the dorsal border of the scapula was limited in a similar way. The left rhomboideus superficialis had an origin extending from the crest of the most posterior cervical vertebra to the posterior extremity of the crest of the fused thoracic vertebrae, while its insertion extended from the anterior extremity of the scapula to within a quarter of an inch of the posterior extremity of that bone.

*Rhomboideus profundus* (figures 7 to 11)

*Normal.* The deep rhomboid muscle arises immediately beneath the line of origin of the superficial one, but the origin of the deeper muscle extends one vertebra further, both anteriorly and posteriorly. Its insertion is on the medial surface of the scapula from the posterior extremity of that bone to a point about opposite the anterior limit of the superficial rhomboid, and from the dorsal margin ventrally for about the same distance as the superficial muscle.

*Wingless.* On the abnormal bird the chief variations in the rhomboideus profundus were the extension of its origin posteriorly to the anterior margin of the ilium, and a restriction of its insertion on the scapula to an anterior limit opposite the space between the first and second cervical ribs. These variations were bilateral.

*Serratus profundus* (figures 7 to 11)

*Normal.* The serratus profundus is a very narrow, thin muscle whose fibers arise from the tip of the first cervical rib, and thence pass in an antero-dorsal direction to insert on the medio-ventral border of the scapula. The posterior margin of this muscle is closely applied to the anterior margin of the serratus superficialis anterior.

*Wingless.* This muscle on the wingless rooster was normal as to position and relations, but was at least twice as broad and thick as on the normal bird.

*Serratus superficialis anterior* (figures 6 to 11)

*Normal.* The anterior superficial serratus muscle is a broad, thin band, arising from the lateral side of the ventral extremity of the second cervical rib, and inserting just posterior to the serratus profundus on the medio-ventral border of the scapula. The fibers run in an antero-dorsal direction.

*Wingless.* On the wingless rooster the origin of this muscle was more extensive than usual, and, on account of the second cervical rib of this bird having retained its embryonic sternal

segment, the origin was along the antero-lateral border of the vertebral segment of this rib. The upper limit of origin was opposite the base of the uncinatè process. The muscle itself possessed more than the usual number of fibers, rendering it much thicker than the normal, but without increasing its breadth. The insertion on the scapula was normal.

*Serratus superficialis posterior (figures 8, 10 and 11)*

*Normal.* The posterior superficial serratus muscle consists of two slips, one arising from the dorsal third of the postero-lateral border of the second cervical rib, the other arising in approximately the same place on the first thoracic rib. Both slips are broad and thin. The fibers run in a postero-dorsal direction, those of the two slips becoming indistinguishable where they lie side by side. The insertion of this muscle is along the median ventral border of the scapula from a point opposite the anterior margin of the second thoracic rib posteriorly to a point opposite the anterior margin of the third thoracic rib.

*Wingless.* The origin, insertion, and topographical relations of this muscle were normal, but the muscle fibers were so few in number that it was almost reduced to a fascia.

*Sterno-coracoideus (figures 10 and 11)*

*Normal.* This is a very short, broad muscle taking its origin from almost the entire lateral surface of the anterior lateral process of the sternum and inserting on the adjoining margin of the coracoid. It lies mediad of the membrane stretched between the coracoid and the anterior lateral process of the sternum, and some of its fibers inosculate with this.

*Wingless.* Same as normal.

*Latissimus dorsi (figures 2, 6, 7, 9 to 11)*

*Normal.* The latissimus dorsi arises (1) from the mid dorsal line, starting with the neural crest of the most posterior cervical vertebra and extending posteriorly over the crest of the first thoracic vertebra; (2) this muscle also has a line of origin from the anterior margin of the ilium. The fibers from the iliac origin

run almost straight anteriorly over the surface of the superficial rhomboid muscle until they inosculate with those coming from the vertebral origin; the combined fibers then run in an antero-ventral direction to their insertion on the ventral surface of the humerus, somewhat posterior to the pneumatic foramen and alongside of one origin of the triceps.

*Wingless.* The wingless rooster showed the two separate points of origin of the latissimus dorsi, but the fibers from the two places never united, both sets running in a general ventral direction to inosculate separately with the pectoralis muscles. Furthermore, both points of origin were unusually extensive.

*Deltoid major (figures 2, 6 and 10)*

*Normal.* The deltoid major takes its origin from the highest point of the humeral tubercle of the coracoid, and extends a very short distance, as a thick, round bundle of fibers to its tendinous insertion on the superior tubercle of the humerus.

*Wingless.* The wingless specimen showed no trace of this muscle unless a fascial mass covering the point of union of the three members of the shoulder girdle should be considered as the rudiments of this and the following muscle.

*Deltoid minor (figures 2, 6 and 10)*

*Normal.* The deltoid minor arises from the posterior border of the furcular tubercle of the coracoid, and like the deltoid major it is a very short muscle, having its insertion on the lateral surface of the humerus under the superior cristas and embraced by the insertion of the pectoralis major.

*Wingless.* As mentioned above, in connection with the deltoid major, a thick fascial mass of doubtful significance may represent on this bird both the deltoid muscles.

*Scapuli-humeralis anterior (figures 2, 6, 8 and 10)*

*Normal.* This is a small, round muscle arising from the ventro-lateral border of the scapula just anterior to the origins of the subscapularis externus and the scapuli-humeralis posterior. The fibers run almost at right angles to the surface of the scapula to

insert on the ventral surface of the humerus just distal to the pneumatic foramen.

*Wingless.* On the wingless rooster the scapuli-humeralis anterior was entirely absent on both sides.

*Scapuli-humeralis posterior (figures 2, 6, 7, 9 to 11)*

*Normal.* The scapuli-humeralis posterior is a broad sheet of muscle arising from the ventral three-fourths of the lateral surface of the scapula, from the posterior extremity of that bone to a point opposite the anterior margin of the most posterior cervical vertebra without a rib. Its fibers run in an antero-ventral direction, and when about in line with the anterior limit of origin they abruptly join a tendon which inserts on the lateral border of the pneumatic foramen between two origins of the triceps. The anterior border of this muscle is overlaid by the latissimus dorsi.

*Wingless.* On the abnormal rooster the origin of the scapuli-humeralis posterior extended much nearer the dorsal margin of the scapula than on normal specimens. The fibers ran in the usual direction, but ended by fusing with those of the coraco-brachiales and subcoraco-scapulares.

*Subcoraco-scapulares (figures 2, 6 to 10)*

*Normal.* The subcoraco-scapulares are three muscles, external and internal subscapular and the subcoracoid, arising separately, but grouped together because all three possess a common tendon of insertion. The subscapularis externus arises from the ventro-lateral margin of the scapula, ventral and slightly posterior to the anterior limit of origin of the scapuli-humeralis anterior. The subscapularis internus arises (1) from the medial surface of the scapula from a posterior limit corresponding to the origin of the externus on the lateral surface, anteriorly to the coracoid; (2) from the medial border of the coracoid for a distance of three-quarters of an inch from the ventral margin of the scapula; (3) a few fibers arise from the membrane separating this muscle from the coraco-brachialis anterior. The fibers of the subscapularis externus run ventrally, those of the subscapularis

internus converge to join the tendon common to the three subcoraco-scapular muscles.

The subcoracoideus arises (1) from the ventral third of the medial border of the coracoid; (2) from the medial surface of the furculo-coracoid membrane, which separates it from the coraco-brachialis anterior; and (3) from the posterior surface of the anterior median process of the sternum together with the margins of the body of that bone.

The common tendon of these three muscles passes upward across the lateral surface of the scapula to insert on the humerus proximal to the pneumatic foramen, and between the humeral origin of the biceps and the insertion of the coraco-brachialis posterior.

*Wingless.* The subcoraco-scapulares were represented on the wingless rooster by the subcoracoid and the internal subscapular, the external subscapular being absent on both sides. The origins of the two muscles present differed from the normal in being unusually extensive, the subcoracoideus arising from the whole posterior border of the coracoid, and the origin of the subscapularis internus extending along the medio-ventral margin of the scapula from the anterior extremity of that bone to the normal posterior limit of its origin. Fibers from both these muscles inosculated with fibers of the coraco-brachiales, and there was also a common tendon, not shown on our diagrams, which inserted on the humeral tubercle of the coracoid.

*Pectoralis major (figures 2, 6, 7, 10, 11)*

*Normal.* This muscle takes its origin (1) from the ventral quarter of the keel of the sternum throughout its entire length; (2) from the ventral part of the lateral surface of the membrane connecting the furcula and the sternum; (3) from the entire lateral surface of the furcula with the exceptions of a very short distance at its dorsal extremity and of the postero-dorsal angle of its enlarged ventral extremity; (4) from that part of the furculo-coracoid membrane adjoining its origin on the furcula; (5) from the external and internal posterior lateral processes of the sternum and from the membrane joining them to each other,

to the keel, and to the body of that bone; (6) some fibers of the pectoralis major arise from the fascia covering the dorsal border of the pectoralis minor.

The insertion of the pectoralis major is on the upper end of the humerus, under the superior crista.

*Wingless.* On the wingless rooster the origin of the pectoralis major was considerably reduced, the reduction being in different regions on the two sides of the body. On the right side the origin of this muscle failed to reach the posterior tip of the keel of the sternum while anteriorly it crossed the membrane from the sternum to the furcula, ran up the latter bone for only a short distance beyond its enlarged ventral extremity, and that, except for the membrane stretched between the posterior lateral processes of the sternum is the whole extent of its origin on that side. The left side of the wingless bird showed a normal origin from the keel of the sternum, and from the membrane between that bone and the furcula; on the furcula the origin extended dorsally further than usual but was everywhere limited to the anterior margin of the bone. The only other point of origin of the pectoralis major on the left side was from the dorsal angle of the posterior lateral process of the sternum.

The origins of the pectoralis major muscles of the wingless bird were, however, nowhere near as unusual as were the insertions. On the right side the muscle was saddle-shaped, its fibers inosculating with both the posterior and the anterior portions of the latissimus dorsi. The left side showed a more complex anomaly, the slip of the pectoralis major arising from the external posterior lateral process of the sternum inosculating with the posterior portion of the latissimus dorsi, while the main part of the pectoralis major inosculated with a slip from the anterior portion of the latissimus dorsi, the two parts of pectoralis major appearing to be entirely separate from each other.

On both sides of the body the fibers of the pectoralis major and of the pectoralis minor inosculated anteriorly, and sent dorsally a common tendon which bifurcated just before it inserted on the dorso-lateral border of the scapula, anterior to the origin of the scapuli-humeralis posterior.

*Pectoralis minor* (figures 2, 6 to 11)

*Normal.* The pectoralis minor arises (1) from the postero-dorsal angle of the ventral extremity of the furcula, and from the lateral surface of the membrane stretched between that angle, the ventral end of the coracoid, the body and keel of the sternum; (2) from the ventral border of the body and the dorsal three-quarters of the keel of the sternum; (3) from the ventral margin of the membrane between the keel and the internal posterior lateral process of the sternum. The fibers of this muscle converge toward its midline, and its tendon of insertion passes with that of the coraco-brachialis anterior through the foramen triosseum—between the anterior extremities of the furcula, coracoid, and scapula—to attach to the upper end of the humerus at the base of the dorsal surface of the superior crista.

*Wingless.* On the wingless rooster the pectoralis minor, like the major, showed an origin over a smaller area than the normal, the reduction being more marked on the right side, where no fibers arose from the sternum, the origin being limited to the ventral extremity of the furcula and the membrane stretched between that bone and the sternum. On the left side the origin of the pectoralis minor was practically confined to the ventral half of its normal limits, with the further abnormality of a dorsal extension along the posterior border of the furcula to the extremity of that bone. Inosculation with the pectoralis major were present on both sides, and on both sides of the body there was a small tendinous insertion on the scapula near the scapular tubercle of the coracoid. The posterior portion of the left pectoralis minor inosculated with a slip from the latissimus dorsi.

*Coraco-brachialis anterior* (figures 2, 6, 8 to 11)

*Normal.* This muscle arises (1) from the lateral surface of the anterior median process of the sternum, (2) from the ventral half of the medial border of the coracoid, (3) from the lateral surface of the furculo-coracoid membrane bordering the coracoid. The muscle narrows at the dorsal end and its tendon accompanies that of the pectoralis minor through the foramen trios-

seum; it inserts on the anterior dorsal margin of the superior crista of the humerus.

*Wingless.* On the wingless rooster the usual place of origin of the coraco-brachialis anterior was covered with a heavy fascia, possibly representing this muscle, which ran posteriorly to inosculate with the coraco-brachialis posterior.

*Coraco-brachialis posterior (figures 2, 6, 8 to 11)*

*Normal.* The coraco-brachialis posterior arises (1) from the fascia of the pectoralis minor at a point on the dorsal border of that muscle near the sterno-coracoid articulation; (2) from the lateral surface of the coracoid with a ventral limit at the line of articulation of that bone with the sternum and a dorsal limit just ventral to the capsule of the humeral joint; (3) by a narrow slip from the body of the sternum starting anteriorly from the line of articulation of the coracoid and terminating posteriorly at the base of the lateral processes of the sternum.

This muscle runs abruptly into a tendon in the axillary space, and thereafter it joins by slips with the tendon of the subcoraco-scapulares, the two tendons finally separating and that of the coraco-brachialis posterior passing posterior to that of the subcoraco-scapulares and inserting on the anterior end of the humerus proximal to the pneumatic foramen, and embraced by the tendon of insertion of the subcoraco-scapulares.

*Wingless.* The coraco-brachialis posterior, and possibly some of the anterior as well, was represented on the wingless rooster by a large mass of muscle fibers which arose over almost the entire lateral surface of the coracoid, and passed in a posterodorsal direction to inosculate with fibers of the scapuli-humeralis posterior.

*Biceps brachii (figures 2, 6 and 10)*

*Normal.* This muscle arises by two heads, one a long tendon from the fureular tuberosity of the coracoid, the other a short tendon from the proximal end of the humerus along a lateral line starting distal to the caput, a little ventral to the dorsal margin of the bone, and extending around to the ventral surface,

anterior to the pneumatic foramen, to where the tendon of the subcoraco-scapulares inserts. The head of the biceps brachii which has its origin on the coracoid, passes first over the deltoid minor, then over the base of the broad ligament uniting the coracoid and humerus, to finally join the head arising from the humerus. The insertion of this muscle is on the radius and ulna.

*Wingless.* Absent.

*Triceps cubiti s. anconeus (figures 2 and 10)*

*Normal.* The triceps muscle arises by three heads of which the two tendinous ones, attached to the scapula along its ventro-lateral border just caudad of the capsule of the humeral joint, are sometimes (Beddard '98) described as a separate muscle, the anconeus longus. These two tendons, which are oval in section, unite at once, but do not join the third head until near their common insertion on the olecranon process of the ulna and on the capsule of the neighboring joint. The third head of the triceps arises fleshily from a limited area on the humerus just proximal to the pneumatic foramen, and from the greater part of the ventral surface of that bone, exclusive of the articular portions.

*Wingless.* This muscle was absent from the wingless rooster, but attached to both scapulas in the position of its posterior tendinous origin from that bone were tendons connected with the pectoralis muscles.

SKELETON

The skeleton of the wingless rooster (figs. 4 and 5) differs in its entirety from any of our normal specimens (fig. 3) in being more heavily built; the individual bones are broader and thicker than usual. One notices also that the sternum is more nearly parallel to the vertebral column than in the normal bird of this species, a condition apparently correlated with the more perpendicular position of the coracoid. Aside from these peculiarities the abnormalities of the wingless skeleton are limited to the thoracic region and will be taken up individually.

The vertebrae present no abnormalities.

The ribs show slightly different anomalies on the two sides of the body, the cervicals being the ones affected. On the left side the first cervical rib is unusual in possessing an uncinata process, while the second cervical rib on this side has an incomplete sternal segment. The first cervical rib on the right side likewise has an uncinata process, and the second shows a condition further removed from the normal than its mate on the left side, possessing a complete segment uniting it to the anterior lateral process of the sternum.

The most posterior thoracic rib on the left side appears at some time to have had its vertebral and sternal segments separated so that they slid past each other, and their ends formed a new ligamentous connection between the ventro-median extremity of the vertebral segment and the dorso-lateral extremity of the sternal segment.

The sternum of the abnormal bird is quite aberrant in form, showing a very gradual, instead of an abrupt, transition from the vertical to the horizontal plane at the dorsal margin of the keel, being unusually broad just anterior to its caudad extremity, and having a thick and very short anterior median process. The anterior lateral processes of this sternum are also unusual in their perpendicularity. The dextral curvature of the anterior end of the keel is probably due to many falls the rooster had while this bone was still somewhat cartilaginous.

The shoulder girdles of the wingless bird are both normal to the extent of each possessing the usual three members, in their normal positions. The abnormalities are in the proportions of the separate bones, and in their articular relations or absence of them. The two sides require separate attention. On the left side the scapula is essentially normal except for a much reduced humeral process, the coracoid is shorter than usual and has its ventral extremity faced more laterally than the normal, while the furcular tubercle of the coracoid is absolutely lacking and the humeral tubercle is rudimentary. The left half of the furcula is normal except for the absence of an articular enlargement at its dorsal extremity. A movable joint exists on the

left side between the scapula and the coracoid. The right shoulder girdle is decidedly more aberrant than the left, the scapula fails to narrow at its anterior extremity, faces more laterally than usual, bears no trace of a humeral process, and is completely fused with the coracoid along the entire dorsal margin of the latter bone. The right coracoid resembles the left in general form and the absence of a furcular tubercle, but differs from its mate in having no humeral tubercle and only a very rudimentary scapular tubercle. The right half of the furcula is like the left except for a decided median convexity, perhaps, like the asymmetry of the sternum, due to falls in early life.

The foramen triosseum, bounded by the antero-dorsal extremities of the scapula, coracoid, and furcula, is absent on the right side and decidedly reduced in caliber on the left side of the wingless skeleton. On both sides of the wingless bird the angle between the scapula and the coracoid is almost twice the normal, while the angle between the coracoid and the furcula has been correspondingly reduced, carrying the ventral extremity of the furcula almost against the anterior margin of the sternum.

Absolutely no trace of wing bones, nor indication of a humeral joint capsule, is present on either side of the skeleton of the wingless rooster.

#### CONCLUSIONS

It now remains to consider the cause and the significance of the already described abnormalities of this wingless rooster.

Two unpublished instances of wingless hens are known to us. Concerning one of these wingless hens we possess no details; the other was an incubator-hatched bird which was killed when only a few weeks old and lost to science.

It is well known to those who use incubators extensively that whenever, through some error of the regulating mechanism, the temperature rises a few degrees above the optimum, abnormalities result which in the majority of cases prevent the embryos from developing to the hatching stage. In view of this, and also of the evidence set forth below, we believe our wingless

rooster to have been produced by unusual temperature conditions in his environment, probably at the end of the first week of incubation.

The muscular anomalies fall into two groups: (1) Muscles which were present on the wingless bird but which differ from the normal in origin, insertion, or number of fibers; (2) muscles which were absent. A complete list of the muscles having unusual origins would include almost all of those present on this bird, but without more extensive knowledge of the normal limits of variation it is impossible to tell just how far abnormal these origins are, and furthermore the causes of such abnormalities must in most instances be far too complex for us to analyze them. The pectoralis muscles, however, are so evidently outside the normal limits of variation that attention should be called to them, and the partial explanation may in this case be hazarded that the reductions in origins are due to disuse, there being no wings present for them to move. The same is true of size of muscles, as of origins.

The abnormal insertions of muscles of the wingless rooster are of great interest and importance, and they can be collectively described as an inosculation between the fibers of the muscles arising along the dorsal side of the body, with normal insertions on the humerus, and the fibers of muscles, likewise with normal insertions on the humerus, but with origins on the ventral side of the body, the specific unions being between muscles of the same plane. This condition exactly coincides with what might be expected to occur in case the wings failed to develop while the rest of the body grew normally, for in the embryo the wing buds starting to grow out from the body wall carry with them the tissue destined to form their musculature.

The absent muscles would normally all have had their origins in the vicinity of the antero-dorsal angle of the shoulder girdle, where very evidently the focus of disturbance was located, and their insertions on the humerus, which is absent. What the immediate cause was of their failure to develop or of their subsequent atrophy, there is no evidence to show, but in the case of the deltoids and the biceps their place of origin is absent,

while the triceps origin may be represented by an anomalous tendon from the pectoralis minor.

The entire skeleton of the wingless rooster is heavier than any of our normal specimens of the same breed. Miss Lindsay ('85) states that in the chick, early in the sixth day of incubation, both cervical ribs are attached to the sternum at their ventral extremities while the scapula and coracoid are fused into a continuous cartilaginous plate. She further states that on the seventh day of incubation the cervical ribs have lost their connection with the sternum. The wingless rooster of the present paper has therefore on his right side the persistent embryonic condition of the sixth day of incubation as regards the second cervical rib and the relation between the coracoid and scapula. On his left side the sternal segment of the second cervical rib started normally to atrophy but never completed the process, while the coraco-scapular plate developed a normal joint. The fact of these abnormalities being normal for the six day embryo is our reason for believing that the disturbance which produced these abnormalities must have occurred at about that time. Why no traces of any wing bones are present it is impossible to say, but the complete absence of any capsule for a humeral joint goes to prove that they never started to develop.

In a word, all the evidence derived from a study of this wingless rooster, including the failure of extensive breeding experiments to produce any wingless offspring, indicates that his abnormalities were all instances of arrested development.

No extensive bibliography of the literature on avian anatomy accompanies this paper as such lists can be found in the works of either Gadow ('91) or Furbringer ('88).

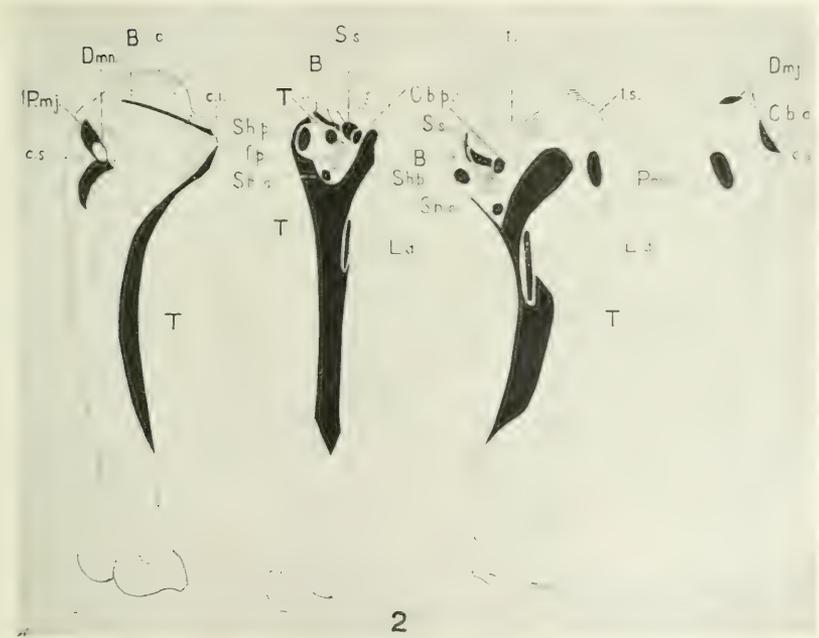
The drawings used to illustrate this paper were, with the exception of those of the humerus, built up on photographs of skeletons; the parts desired being outlined in ink on the photographs, the latter then bleached with hypo and red prussiate of potash, and after they were dry the details put on in ink.

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Fig. 1 Photograph of the wingless rooster when about one year old.

Fig. 2 Normal left humerus showing topography of the bone and points of origin and insertion of muscles belonging to the shoulder region. Four views, from left to right, lateral, ventral, medial, and dorsal. *B.*, biceps brachii; *c.*, caput; *Cb.a.*, coraco-brachialis anterior; *Cb.p.*, coraco-brachialis posterior; *c.i.*, inferior crista; *c.s.*, superior crista; *D.mj.*, deltoid major; *D.mn.*, deltoid minor; *f.p.*, pneumatic foramen; *L.d.*, latissimus dorsi; *P.mj.*, pectoralis major; *P.mn.*, pectoralis minor; *Sh.a.*, scapuli-humeralis anterior; *Sh.p.*, scapuli-humeralis posterior; *S.s.*, subcoraco-scapulares; *T.*, triceps cubiti; *t.i.*, inferior tuberele; *t.s.*, superior tuberele.



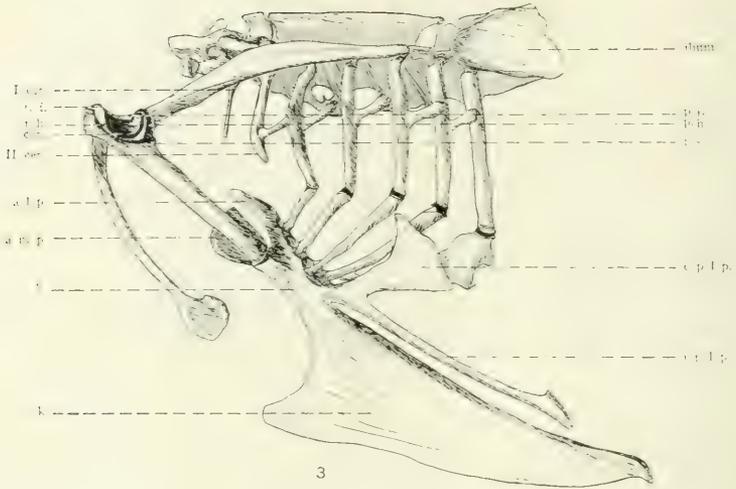
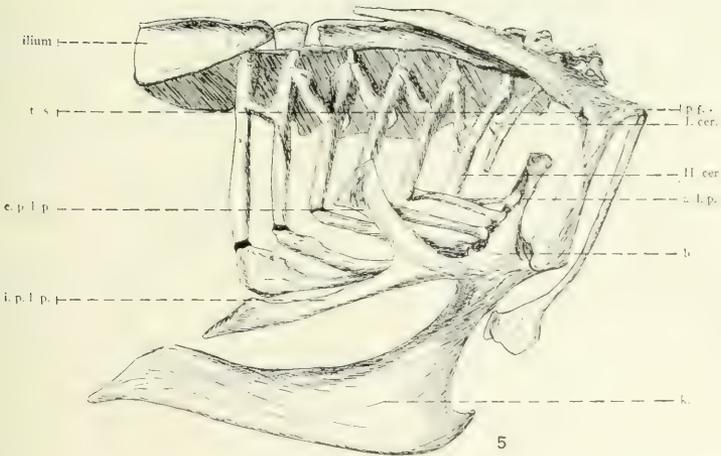
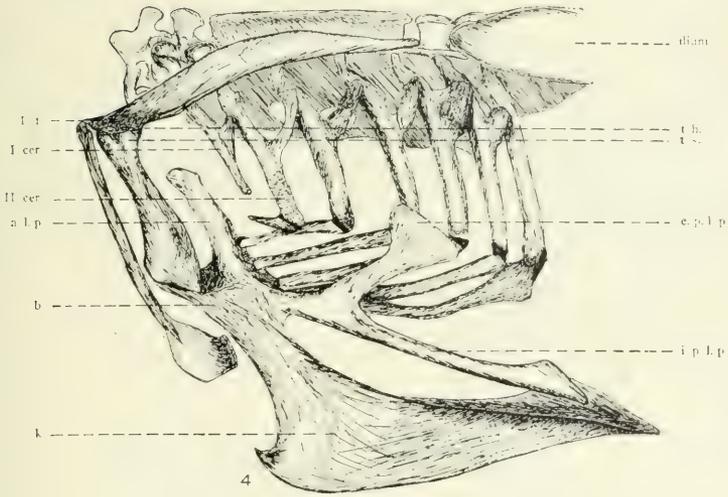
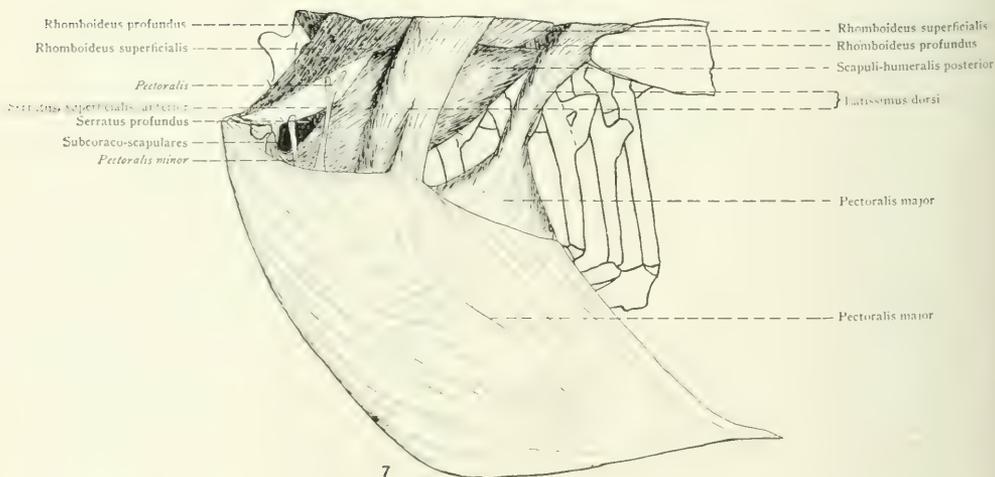
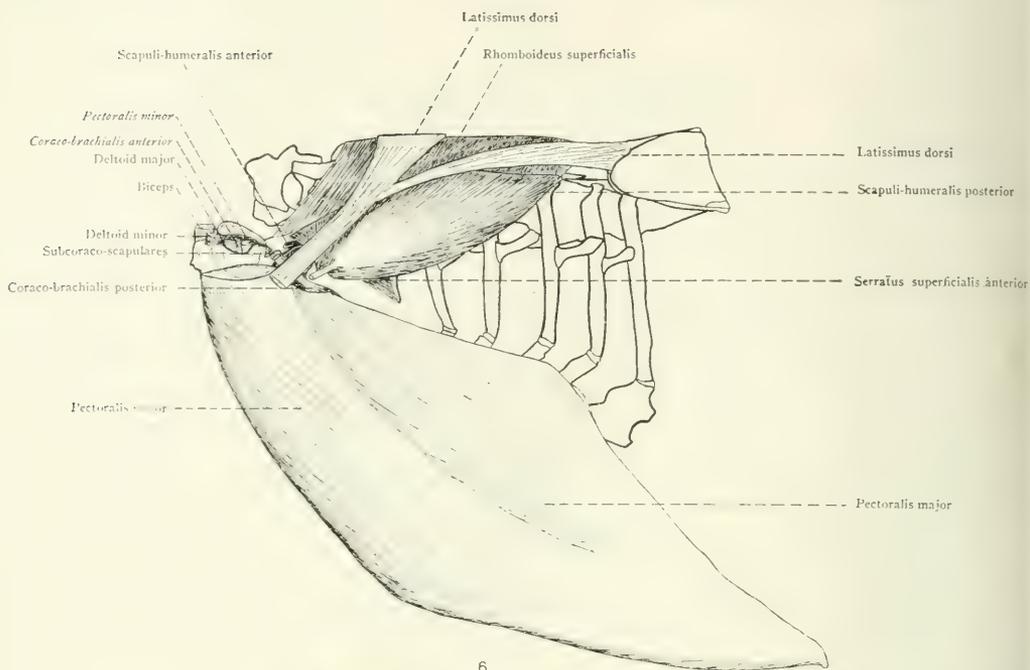


Fig. 3 Thoracic region of normal skeleton viewed from left side. *a.l.p.*, *a.m.p.*, *b.*, anterior lateral and median processes, and body of sternum; *cap.*, capsule of humeral joint; *e.p.l.p.*, *i.p.l.p.*, *k.*, external and internal posterior lateral processes, and keel of sternum; *p.f.*, *p.h.*, furcular and humeral processes of scapula; *t.f.*, *t.h.*, *t.s.*, furcular, humeral, and scapular tubercles of coracoid, *I. cer.*, *II. cer.*, first and second cervical ribs.

Fig. 4 Thoracic region of wingless skeleton, viewed from left side. *a.l.p.*, *b.*, *e.p.l.p.*, *i.p.l.p.*, *k.*, anterior lateral process, body, external posterior lateral process, internal posterior lateral process, and keel of sternum; *p.f.*, furcular process of scapula; *t.h.*, *t.s.*, humeral and scapular tubercles of coracoid; *I. cer.*, *II. cer.*, first and second cervical ribs.

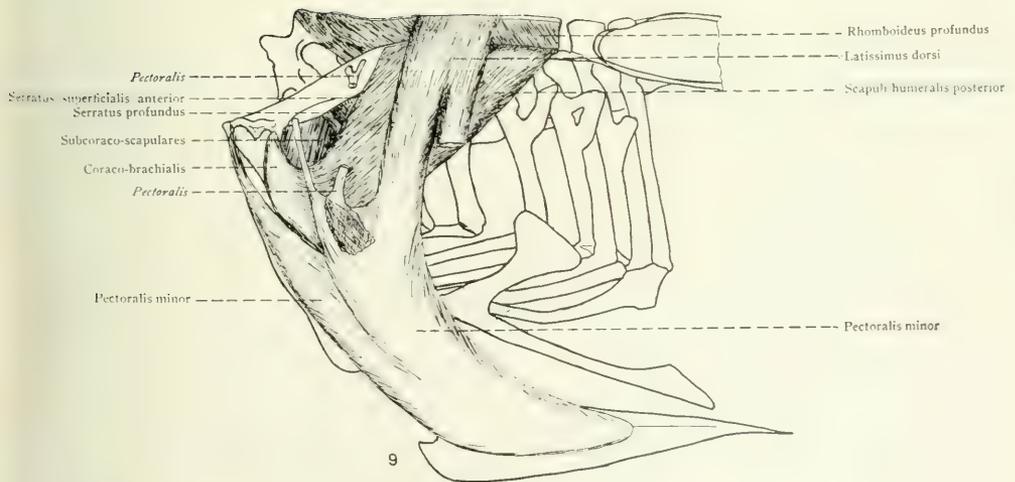
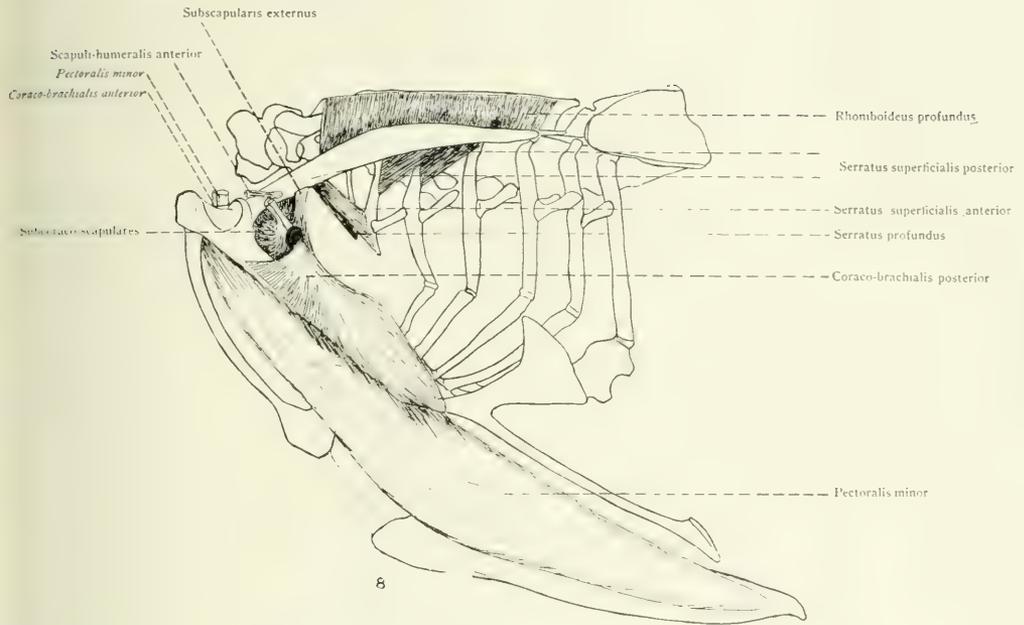
Fig. 5 Thoracic region of wingless skeleton, viewed from right side. *a.l.p.*, *b.*, *e.p.l.p.*, *i.p.l.p.*, *k.*, anterior lateral process, body, external posterior lateral process, internal posterior lateral process, and keel of sternum; *p.f.*, furcular process of scapula; *t.s.*, scapular tubercle of coracoid; *I. cer.*, *II. cer.*, first and second cervical ribs.





EXPLANATION OF FIGURES

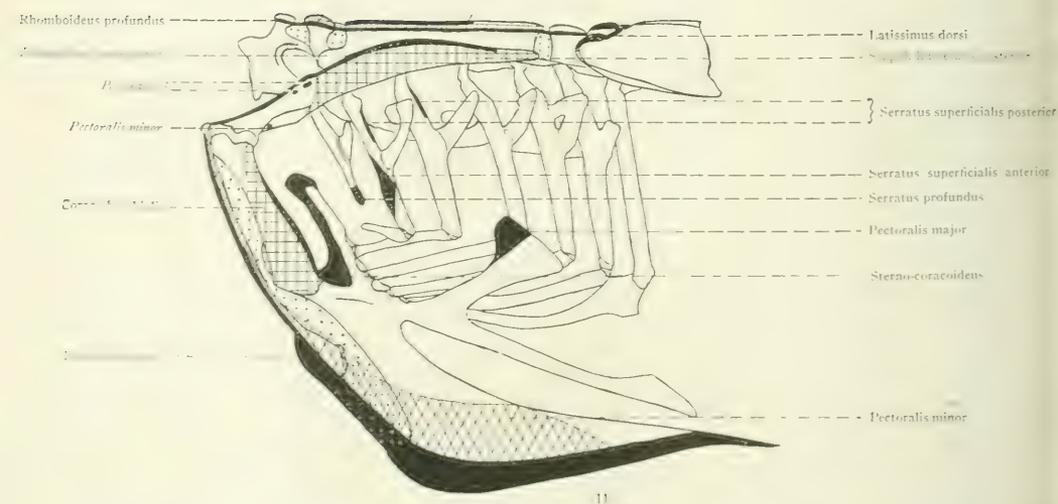
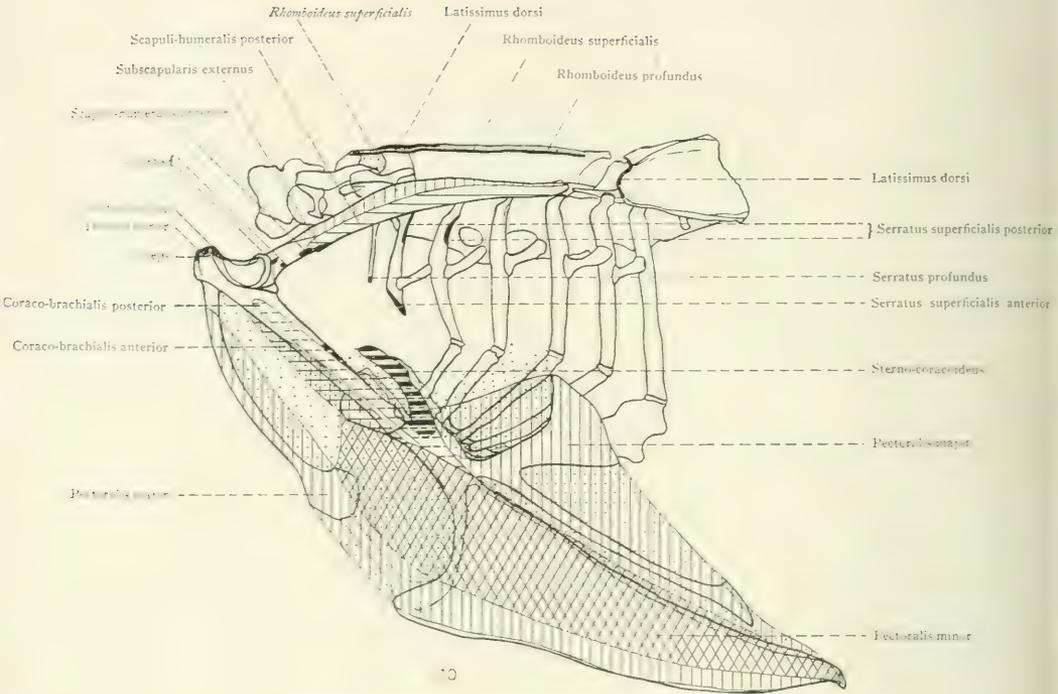
- 6 Diagram of superficial muscles of shoulder region; normal rooster. Insertions in italics.  
7 Same as above; wingless rooster.



EXPLANATION OF FIGURES

8 Diagram of deep muscles of shoulder region; normal rooster. Insertions in italics.

9 Same as above; wingless rooster.



EXPLANATION OF FIGURES

- 10 Diagram of origins and insertions of superficial and deep muscles of shoulder region; normal rooster. Insertions in italics.
- 11 Same as above; wingless rooster.

## REPORT OF THE ANOMALIES IN A SUBJECT WITH A SUPERNUMERARY LUMBAR VERTEBRA

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### SIX FIGURES

The specimen here reported was obtained in the dissecting room of the School of Medicine, University of Pittsburgh. The body was that of an adult white male.

In the vertebral column there are thirty-three vertebrae arranged as follows: seven cervical, twelve thoracic, six lumbar, four sacral and four coccygeal. In the cervical region there are no anomalies. In the thoracic region there is a right scoliosis with lordotic tendency, corresponding to the bodies of the fourth to seventh vertebrae and the spines of the fourth to sixth vertebrae. The ribs on the right side are so bent as to narrow and deepen the costo-vertebral groove. The twelve ribs are in normal position but the lower ribs, especially the twelfth pair, are longer than usual, measuring 18 cm. and reaching bilaterally to within 4 cm. of the iliac crest.

There are six lumbar vertebrae. The first vertebra carries a lumbar rib bilaterally. This is short, 2.5 cm. long by 1.2 cm. wide, and articulates by a facet on the upper half of the lateral aspect of the body of the vertebra, as well as by a facet on the transverse process. It resembles in every way except for its articulations the transverse process or costal element of the other lumbar vertebrae. The vertebra itself resembles morphologically a lumbar rather than a thoracic vertebra. It has the quadrate horizontal spine, the larger body, the well-defined mammillary process. Of the other lumbar vertebrae, the sixth only is anomalous. It presents a spine rather narrow and clubbed at the end, which is deflected 0.7 cm. to the left of the median line.

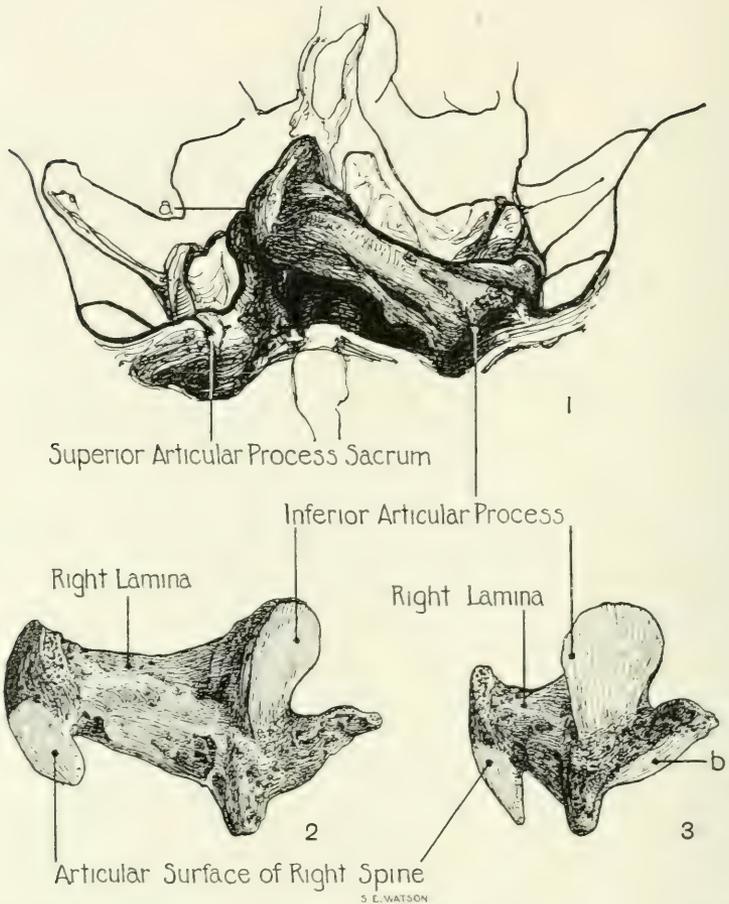


Fig. 1 Diagram of VIth lumbar vertebra from behind, showing cleft in spine at *a*.

Fig. 2 Drawing of detachable portion of neural arch of VIth lumbar vertebra from in front.

Fig. 3 Drawing of detachable portion of neural arch of the VIth lumbar vertebra, lateral aspect. Articular surface in pedicle shown at *b*.

The spine itself is split into two unequal portions with an articular surface between. The left portion is shorter, 1.2 cm. long, more or less pointed, and is attached to a lamina 1.5 cm. broad, and with the lamina is directed backward and outward so as to be slightly concave laterally. The right portion embraces most

of the spine, is club-shaped, and is attached to a lamina 1.6 cm. broad, which is directed almost horizontally outward (fig. 1). Another defect in the neural arch occurs in the right pedicle just behind and below the superior articular process, where there is a narrow irregular articulation, 2.3 cm. by 0.6 cm. long, looking upward and slightly forward. The separation of the pedicle is not complete, however, for below this joint, between it and the inferior articular process there is definite bony continuity. In other respects the vertebra is normal (figs. 2-4).

The sacrum presents only four vertebrae, and the coccyx four. The first coccygeal vertebra is ankylosed to the last sacral. The anomalies of this vertebral column then, summarized, are the presence of an extra lumbar vertebra, the absence of one sacral segment, the addition of a lumbar rib, a thoracic scoliosis, and an ununited neural arch in the sixth vertebra.

The intervertebral foramina correspond to the number of segments in each region. Thus there are six in the lumbar region and four in the sacral region. Through the foramen between the sacrum and the sixth lumbar vertebra, the spinal branch of the iliolumbar artery passes in, as it does normally, between the fifth vertebra and sacrum, while an extra nerve which may be designated as the sixth lumbar nerve, passes out to join the fifth lumbar and a communication from the fourth lumbar and first sacral to form the sacral plexus (figs. 5-6). Through the foramen between the fifth and sixth vertebra passes a spinal branch of the fifth lumbar artery, which in turn is of large size and given off in normal fashion from the middle sacral artery. Through the first four foramina lumbar branches of the abdominal aorta enter (fig. 5). There are no muscular or ligamentous anomalies.

Special mention is made of the arterial and nervous arrangement because in the usual description of anomalous vertebral columns found in the literature no record is made of the disposition of the soft parts.

From this description it will be noted that the specimen presents both numerical and morphological variations. Numerical variations in the experience of all investigators are most frequent

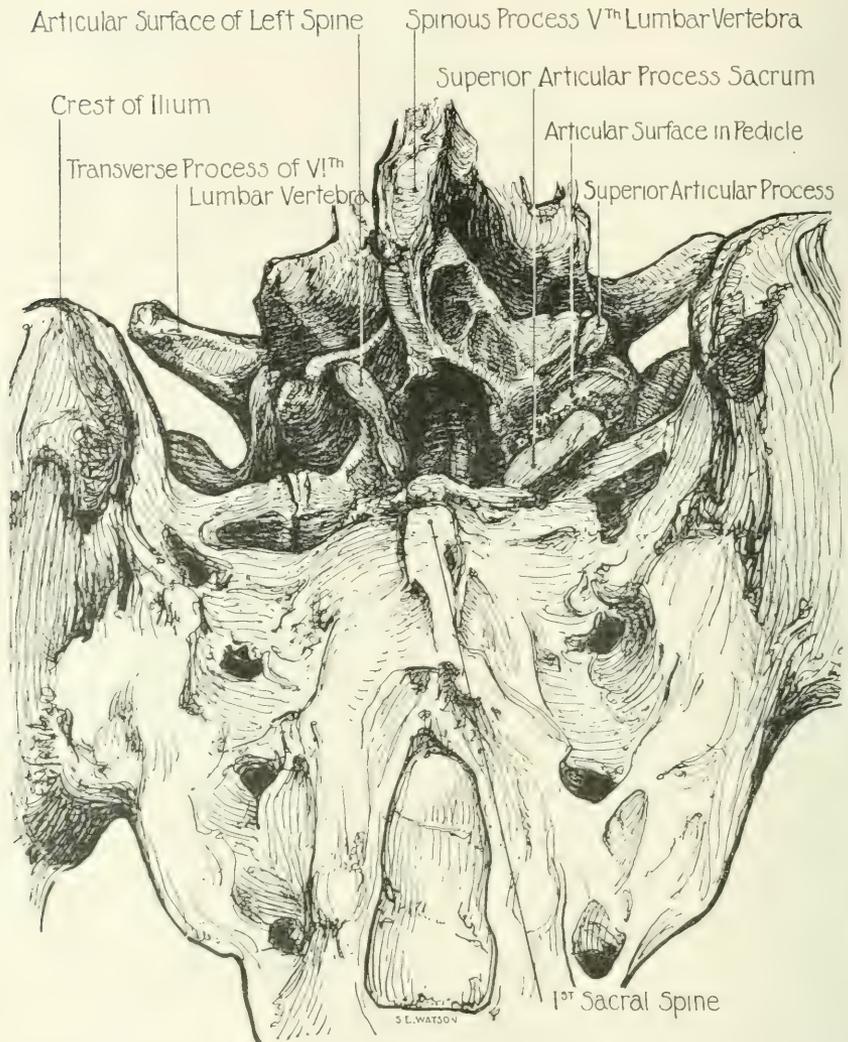


Fig. 4 Drawing of VIth lumbar vertebra with detachable portion of neural arch removed.

in the coccygeal region, since here a certain number of original embryonal segments fail to persist. In the adult there may be as few as three, the others having been lost or fused. Steinbach

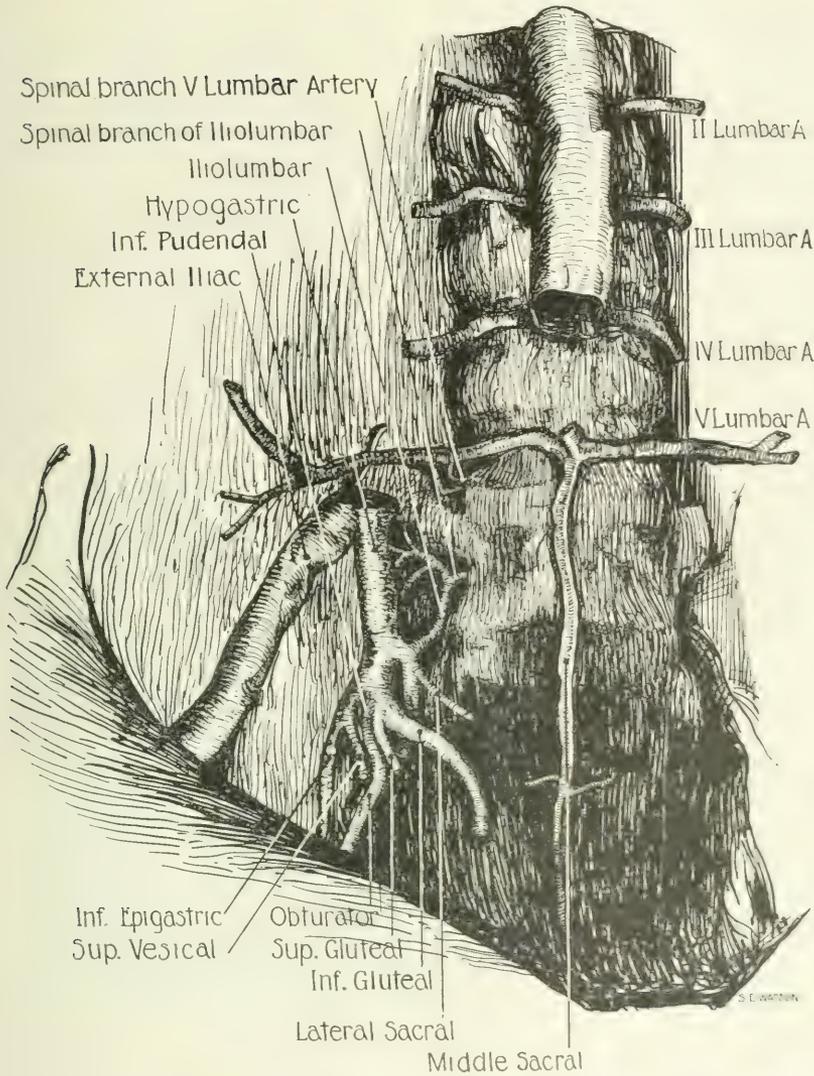


Fig. 5 Diagram of arterial distribution in region of VIth lumbar vertebra.

('89) believes that five are normal for the male and four for the female, while Bardeen ('04) thinks that normally not more than four sacral vertebrae persist. Variations are less frequent in the

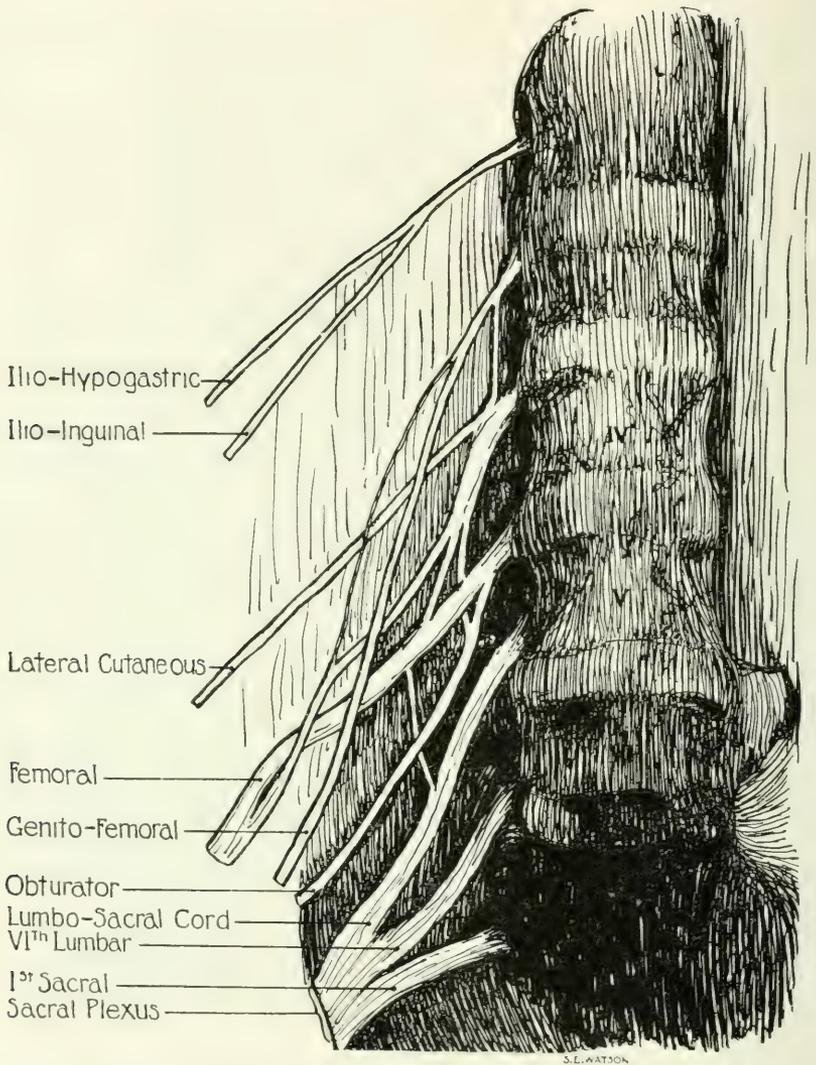


Fig. 6 Diagram of lumbosacral plexus in region of VIth lumbar vertebra.

sacral region, and the frequency diminishes as we ascend the vertebral column, so that in the cervical region numerical variation is rare.

In the presacral region there are normally twenty-four vertebrae, numerical variations here arranging themselves into two groups; first, where the total number of segments is either increased or diminished without compensation from another region; secondly, where the total number of presacral vertebrae is twenty-four but increase or diminution in one region occurs at the expense of an adjacent region, as in the specimen here described, where a sacral deficiency is compensated for in the lumbar region. Variation of this type seems to be concomitant with the development and attachment of the costal element. Thus in the lumbosacral region the last lumbar vertebra may be sacralized by fusion of its costal element in the lateral mass, or the first sacral vertebra may be set free and lumbalized because of lack of fusion of its costal element.

Numerical variations have been studied by a number of investigators, notably Bianchi, Steinbach, Paterson, Ancel and Sencert, Topinard, Dwight, Rosenberg and Bardeen. Bardeen ('04) after a study of reports of 1059 specimens including 75 of his own, found that numerical variations occur in about 16 per cent of vertebral columns of which 7.3 per cent have compensated variations and 8.7 per cent uncompensated, equally divided between an increase and decrease of segments.

Morphological variations are comparatively infrequent, although there are no actual figures. Anomalies of form may exist in any part of a vertebra and may be unilateral or bilateral. The common types are defects in the neural arch, defects in the body, deviation in the normal alignment of parts, and synostoses. Variations in the size of spinous processes, laminae, articular processes, bodies, from hypertrophy to dwarfing are not uncommon. When morphological defects occur they are usually in the lumbar region.

Defects in the neural arch may occur at any place, but commonly are seen in the pedicle between the superior and inferior articular processes, so that a segment consisting of spinous process and laminae may be lifted away when the soft parts are divided. They may be, as in the specimen here described, asym-

metrical and incomplete. Complete absence of part of the neural arch as occurs in spina bifida is not uncommon. Anomalies of this type are easily explained by the failure of centers of ossification to develop fully and unite.

When one comes to account for numerical variations, explanations are not so easy to give, leading to the development of a number of theories which are well discussed by Bardeen ('04) and by Testut ('11). It is not within the scope of this report to discuss these further than to state that numerical variation can be reasonably explained on the simple basis of errors in segmentation.

From a clinical standpoint it would seem that numerical variation is not an important factor. There is no ground for believing that one vertebra more or less regionally or otherwise is of disadvantage to an individual. Morphological anomalies, on the other hand, may easily be the cause of or be associated with definite disturbance in body function. Defects in the neural arch or defects in normal proportions of a vertebra may tend to render that vertebra more unstable, its articulations more insecure, and to permit abnormal motion between the bodies or adjacent articular processes. When such a vertebra is subjected to acute or to long-continued force, muscular strains, joint sprains, spinal curvature, and even dislocation may happen. Much backache may be due to vertebral defect. If the last lumbar vertebra be the one affected, instability may be given to the lumbo-sacral articulation, and a true projection forward of the promontory of the sacrum may occur to such an extent as to interfere with normal parturition. Lack of fusion in the neural arches in spina bifida is of course a distinct clinical entity, as is often the presence of a cervical rib. Just what was the exact clinical aspect of the case here reported, is not on record.

I desire to acknowledge many suggestions received from Professor Sheldon in connection with the preparation of this article.

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## INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with Vol. 18, price per volume, \$7.50; foreign, \$8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price per volume, \$5.00; foreign, \$5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, beginning with Vol. 25, price per volume, \$7.50; foreign, \$8.00.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

36TH STREET AND WOODLAND AVENUE

PHILADELPHIA, PA.

ON THE ANLAGE OF THE BULBO-URETHRAL (COW-  
PER'S) AND MAJOR VESTIBULAR (BARTHOLIN'S)  
GLANDS IN THE HUMAN EMBRYO

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FOUR FIGURES

The first observations on the development of Bartholin's glands were published in 1840 by Tiedemann, who saw them in embryos of five, six and seven months. Huguier found the glands in an embryo of four and a half months. Hoffman determined that the anlage of Cowper's glands first appeared in the tenth to eleventh week, on both sides of the urogenital opening, near the anlage of the penis. Toldt recorded that both Cowper's and Bartholin's glands originated as outpouchings of the urogenital sinus. Debierre saw Cowper's glands in a seven-months' fetus, and declared its anlage to be an outpocketing of the epithelium of the urethra. Beigel observed Bartholin's glands in a six-months' fetus, and Swiecicki in one of 99 mm. Van Aekeren states that he found Bartholin's glands in an embryo at the end of the fourth month, and records that he observed the gland duct as entering the lowest portion of the urogenital sinus. He further states that the blind end of each duct bore five epithelial twigs, separated from each other by connective tissue. Cadiat indicated the anlage of Cowper's glands in his figure of a 3.5 cm. embryo; the validity of his interpretation, however, is questioned by v. Müller. Tourneaux found in a 4.4 cm. embryo the anlagen of Bartholin's glands in the form of epithelial buds having a length of 120  $\mu$ .

The observations of Vitalis Müller deserve fuller consideration. An embryo of 27 mm. length did not show any trace of Bartholin's glands. The next embryo in his series was one of 6.5 cm. length, though one of 8 cm. length showed an earlier stage of the gland anlage. In the latter embryo, the two epithelial buds were respectively 133  $\mu$  and 166  $\mu$  in length, and without lumen. A male embryo of 6.75 cm. showed buds of 300  $\mu$  and 100  $\mu$ , with a lumen in the longer bud. In a female embryo of 6.5 cm. length, he observed the left bud as having a length of 400  $\mu$  and presenting two end branches, and the right bud as having a length of 750  $\mu$  with three end branches, both buds showing lumina. From these and other embryos, Müller concluded that the first appearance of these glands is irregular as to time, and may take place in embryos of from 4 to 8 cm. in length. This author states, in substance, that the

anlagen of Cowper's and Bartholin's glands arise as solid buds from thickenings of the epithelium of the urogenital sinus. The solid anlagen later acquire a lumen, the ends extending as solid sprouts, the beginnings of division. Between the distal buds there is found a cellular mesenchyme. He further notes that in cross-sections of the urogenital sinus, this has the form of a five-rayed star, the gland anlagen always arising from the two lower or ventral rays; in younger embryos growing laterally, in embryos of 10 to 11 cm., latero-dorsally. Müller also studied ox embryos of 6 cm. length. In these he observed that the urogenital canal presents in transverse section the form of a cross with side arms running to a point. On the crest of these side arms, as seen in cross-section, he found the anlagen of Cowper's glands as two solid buds of 100  $\mu$  to 150  $\mu$  in length.

Nagel observed the anlagen of Cowper's glands in a 4 cm. embryo, these appearing as solid tube-like epithelial buds on the sides of the urogenital sinus, somewhat above its external opening. The epithelium of the gland buds he found to be of a high cubic variety. He found end branches in the gland anlagen of embryos of 5 to 6 cm., rump length. In older embryos, the hollowed-out ducts of the gland anlagen were lined by a low cubic epithelium, as in the *canalis urogenitalis*; while the branches had a high, almost cylindrical epithelium, as in the early stages of the anlagen.

Robert Mayer, in his account of the development of the glands of the vagina and the vulva, gives consideration to the Bartholin's glands as observed in embryos of five months and older. He notes that in the vestibule of the female embryo, longitudinal folds are formed at an early stage; in embryos up to about five months old, these folds appear, in cross-sections of the vestibule, arranged in the form of a star having five principal rays on each side. As described by him, the first pair of rays runs in front from the urethral opening parallel to the clitoris. The second pair of rays passes on both sides of the papilla urethralis, passing diagonally backward from what has been termed the '*suleus paraurethralis*.' This pair of folds, which early bears relatively large glandlike recesses, remains backward in growth, and is poorly developed in the newborn, though still recognizable. The third pair of folds or rays—which as seen in cross-section of the urogenital sinus, forms the middle of the star—is characterized by the ducts of Bartholin's glands. The fourth pair, situated just behind the third pair and running parallel to the ducts of Bartholin's glands, have a direction which is obliquely backward, and support, in the newborn, glandular anlagen which are similar to those of Bartholin's glands, except that shorter tubules are found. The fifth pair of folds is situated in the *fossa navicularis*, beside the midline, having a direction which is upward rather than backward. Mayer points out that it is along these five rays or folds that the glands of the vulva first develop, and it is here that they form in the greatest numbers.

Keibel's observations on *Echidna* embryos have influenced the more recent investigators who have considered the anlage of Cowper's gland

in Homo. Keibel found in his echidna embryo 45 a, length 7.7 mm. a thickening of the ectodermal epithelium to the right and left of the midline which, to use his own words, "may perhaps be ascribed to the anlage of Cowper's gland." This region is stated to be at the cranial end of the just forming ectodermal cloaca. In his Echidna embryo 46, the Cowper's gland anlage is present as an elongated solid bud arising from the ectoderm at the base of the penis; that is, from the cranio-lateral wall of the ectodermal cloaca (see Keibel's text figures 53 a and 53 b, and plate figures 17, 19 and 20). The upper end of the gland anlage is invested by a muscle complex that is continuous with the muscle of the skin.

Van der Broek established a similar origin for Cowper's glands in the embryos of Marsupia. In a Halmaturus embryo of 17.5 mm., he observed what he took to be the anlage of Cowper's glands, arising from the ectoderm of the ectodäum (Keibel's ectodermal cloaca) on both sides of the urethral plate (Phallusleiste). In later stages of the embryos of Halmaturus and other Marsupia, he found Cowper's glands as solid epithelial buds, arising from the urogenital sinus at the boundary between the ectoderm and the entoderm.

Lichtenburg, in a comprehensive investigation on the development and structure of the urogenital canal in man, in the course of which he made free use of reconstruction methods, accepts without verification the account given by Keibel and Van der Broek of the ectodermal origin of Cowper's glands as observed in the Monotremata and Marsupia, and makes their findings applicable to man. In a 48 mm. human embryo, the youngest stage described by him, Lichtenburg finds the anlagen of Cowper's glands "at the typical place on the dorsal wall of the urogenital sinus." It appeared to him, to quote further, "as if the tubules were not naked, but that a kind of compressed embryonic connective tissue formed a capsule around them." In a 65 mm. embryo, the gland buds were found to be unbranched, though both buds possessed a lumen; small side buds indicated future branching. Lichtenburg's figure (p. 143) shows the gland buds lying side-by-side near the mid-line and dorsal to the urogenital sinus. In an embryo of 68 mm. length, terminal branching of the gland buds was evident, while one of 70 mm. length showed an accessory Cowper's gland.

Felix, in his account of the anlage and development of the urogenital organs as given in Keibel and Mall's "Human Embryology," makes the following observations concerning Cowper's glands: "They arise as paired solid epithelial buds from the pars pelvina of the urogenital sinus" . . . "and are therefore of entodermal origin. The solid epithelial buds grow upward almost parallel to the urogenital sinus, and lie from the beginning in the compact mesenchyme which is the anlage of the corpus cavernosum urethrae. The glands grow through this mantle, and only when they have reached the looser mesenchyme between the rectum and the sinus are they able to enlarge." Felix observed the anlagen of Bartholin's glands in an embryo of 36 mm.;

the first evidence of branching in the gland-buds of Bartholin's glands were observed in an embryo of 80 mm. length.

Broman states that Bartholin's glands are first evident in female embryos of the third month, 4 to 8 cm. long, as paired outgrowths from the epithelium of the urogenital sinus, basing his statement on the observations of Vitalis Müller. Cowper's glands arise as buds from the entodermal urogenital sinus epithelium, in 4 to 5 cm. embryos. His figure 397 reproduces a model of the urogenital sinus and Cowper's glands of a male embryo of 6 cm. head-breech length.

This brief review of the literature may serve to show that while the embryology of Cowper's and Bartholin's glands has received consideration by numerous investigators, there is as yet no unanimity as to the period when their anlagen in the human embryo may first be recognized, nor has the region of their anlagen been definitely determined, and, with the exception of the figure of Broman, there exist no comprehensive figures showing their relation to the urogenital sinus, mesonephric ducts, and Müllerian ducts. At the suggestion of Dr. Huber, models of the epithelial portions of the genital tubercle, urogenital sinus to base of bladder, ureters, mesonephric and Müllerian ducts, and rectum of human embryos, both male and female, at the critical period of their development, were undertaken. The models thus obtained, while in part duplicating certain of the Keibel models, seem worthy of reproduction, in that the anlagen of Cowper's and Bartholin's glands are portrayed in their relation to other structures. The question of the ectodermal or entodermal origin of these glands is not considered, as a solution of this question is not to be obtained from embryos of the age of those used in this investigation. For this, much younger stages showing early cloacal development would be necessary. The problem confines itself to a study of the anlage of Cowper's and Bartholin's glands in their relation to the urogenital sinus and associated structures, as shown in a series of reconstructions of critical stages.

The human embryos from Dr. Huber's collection (table 1) were placed at my disposal, the measurements referring to crown-breech length, as obtained in fixed material (formalin fixation). With the exception of Embryo 48, in which there is evidence of

maceration, the embryos are all well preserved. The series are double stained in hematoxylin and congo red. Reconstructions were made from the first four of the embryos listed; the other three were studied without being modelled. The drawings for the reconstructions were made with the aid of an Edinger projection apparatus, at a magnification of 100 diameters. In all cases, the epithelium alone was reconstructed. These models, which form the basis of the study, are figured as seen from the left side. The figures were made by photographing the models and using the negative for lantern slide projection, accurate outlines being thus obtained. By placing the drawing paper at the right distance from the lantern, it was possible to give a definite magnification to the figures. My own account consists mainly of a description of the models made.

TABLE 1

No. 47	32 mm.	female	sagittal	15 $\mu$ sections
No. 15	30 mm.	male	sagittal	10 $\mu$ sections
No. 18	45 mm.	female	sagittal	10 $\mu$ sections
No. 23	60 mm.	female	sagittal	10 $\mu$ sections
No. 39	39 mm.	male	sagittal	10 $\mu$ sections
No. 49	47 mm.	female	sagittal	15 $\mu$ sections
No. 48	48 mm.	female	sagittal	20 $\mu$ sections

The model made from Embryo 47 is shown in figure 1. This embryo was secured from the service of Professor Peterson, from a case of hysterectomy for large fibroma; it was fixed while still warm, and is in an excellent state of preservation. In the model are reproduced the lower part of the much distended bladder, the mesonephric and Müllerian ducts and ureters. These structures present the typical, normal arrangement, with as yet no degeneration of the mesonephric ducts. Externally, the phallus shows, between its distal and middle thirds, a shallow coronal groove, the sulcus coronarius glandis. A small depression marks the anal pit, and a similar one the ostium urogenitalis, a shallow groove connecting the two depressions. The anal membrane is still unbroken.

Each side of the wall of the urogenital sinus presents three epithelial ridges or folds. These may be designated as the upper,

## ABBREVIATIONS

*bl.*, bladder  
*b.ur.gl.*, bulbo-urethral glands (Cowper's glands)  
*l.f.ur.si.*, lateral folds of the urogenital sinus  
*m.vs.gl.*, major vestibular glands (Bartholin's glands)  
*m.n.d.*, mesonephric duct

*M.d.*, Müllerian ducts  
 utero-vaginal canal  
*pa.u.gl.*, paraurethral glands (Skene)  
*rect.*, rectum  
*s.cor.*, sulcus coronarius  
*s.ny.l.*, sulcus nympho-labialis  
*u.*, ureter  
*ur.pl.*, urethral plate

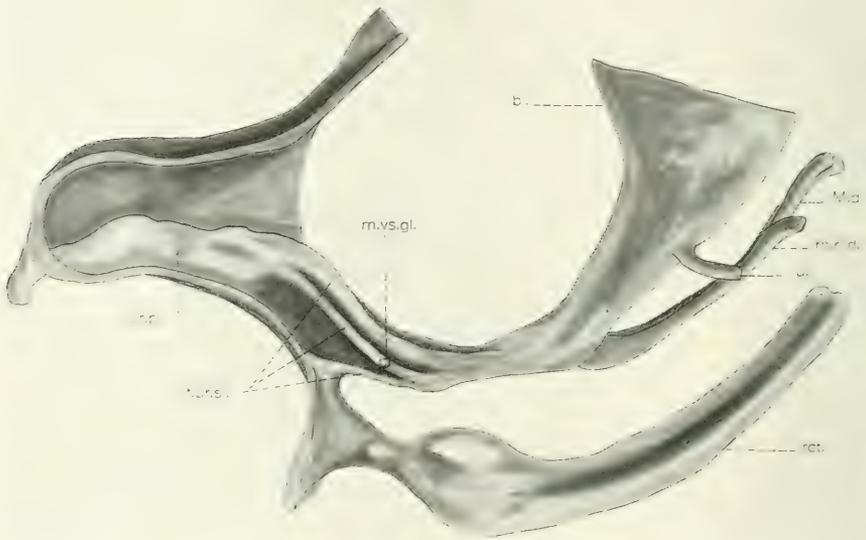


Fig. 1 Model of epithelial portion of urogenital system of human Embryo 47 (Huber collection); female, 32 mm. crown-breech length.  $\times 20$ .

middle, and lower lateral folds of the urogenital sinus. These folds begin in a region which is 0.7 mm. distal or caudal to the entrance of the mesonephric ducts into the urogenital sinus, and spread out radially. Of these folds, the upper lateral folds are the most prominent. When the model is viewed from above, it may be seen that they enclose a relatively deep fossa before blending in the sagittal plane to form the uppermost portion of the urethral plate. The middle lateral folds, shorter than the

upper ones, run nearly parallel to them, and are separated from the upper folds by relatively deep, narrow grooves. In their proximal portion, each middle fold presents an elevation of about  $75 \mu$ ; distally or caudally, each middle fold slopes downward, becoming broader and lower, and is ultimately lost in the urethral plate. The lower lateral folds taken together form a thickened ridge along the bottom of the urogenital sinus, blending distally with the surface epithelium. They are the smallest of the three folds, both in length and elevation. That portion of the urethral plate found in the angle between the middle and the lower folds is relatively thin. Of the three sets of lateral folds observed in this embryo, only in the middle ones can a lumen or extension of the cavity of the urogenital sinus be at all clearly traced, and this as a narrow slit having a depth of about 20 to  $30 \mu$ .

On the left side of the model, as shown in figure 1, the middle lateral fold presents, near its cephalic end, a small projecting bud of epithelium, which passed through only one of the sections, having a thickness of  $15 \mu$ . The relative position of this epithelial bud is shown in this figure at *m.vs.gl.* Looking at the model from below, it may be observed that about 0.3 mm. from the cephalic end of the middle lateral fold, there is evident an abrupt rise or shoulder, and that the epithelial bud above referred to, caps this shoulder. From the older stages modelled, it is evident that this epithelial bud may be regarded as the anlage of Bartholin's gland. A similar bud is as yet lacking on the other, the right side of the urogenital sinus, though three lateral folds, similar in extent and arrangement to those figured for the left side may be seen in the reconstruction. The cells of the Bartholin's gland anlage of the left side, like those of the lateral folds and the wall of the urogenital sinus, may be characterized as of the cuboidal variety and stratified. The short epithelial bud is surrounded by a membrana propria, the surrounding mesenchymal cells having in the immediate vicinity a concentric arrangement.

The model made from Embryo 15 is shown from the left side in figure 2. This is a male embryo having a crown-breech length of 30 mm., and shows a slightly older stage of development than the female embryo, the model of which is shown in figure 1.

The anal membrane is perforated, and the anal pit is a distinct fossa. The perineum has a length of only 0.15 mm. The cephalic ends of the Müllerian ducts present evidence of beginning degeneration.

The three lateral folds of the urogenital sinus present essentially the same relative positions as those described in connection with the model shown in figure 1. By reason of the thickening

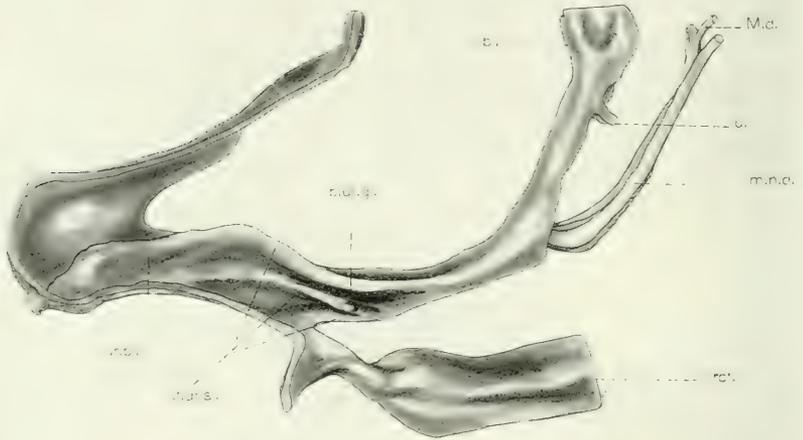


Fig. 2 Model of epithelial portion of urogenital system of human Embryo 15 (Huber collection); male, 30 mm. crown-breech length.  $\times 20$ .

of the epithelial plate in the region between the middle and lower lateral folds, the lower folds are less conspicuous than in the preceding model. The middle fold is also somewhat shorter than in Embryo 47 (fig. 1). The anlage of Cowper's gland, present on both sides of the urogenital sinus, is observed in essentially the same relative position as given for the anlage of Bartholin's glands, namely, in the middle fold, capping an abrupt shoulder seen on this fold near its cephalic end, about 1 mm. below the

entrance of the mesonephric ducts into the urogenital sinus. The bud of the left side is shown in figure 2 at *b.ur.gl.* The gland anlagen are in the form of solid epithelial buds, composed of cells of a cuboidal form; the left bud having a length of 50  $\mu$ , the right bud a length of 60  $\mu$ . Both buds project laterally into a dense mesenchyme with a direction which is perpendicular to the long axis of the middle lateral folds. The gland anlagen are surrounded by a membrana propria, the surrounding mesenchymal cells having a concentric arrangement.

The shape of the sinus lumen for the region of the lateral folds differs from that described for Embryo 47. In Embryo 15 (fig. 2) the pars phallica sinus urogenitalis (Felix) has been extended by central desquamation of the cells of the urethral plate, the sinus lumen having invaded the upper lateral folds and that portion of the urethral plate found between the middle and the lower folds.

Embryo 39, male, crown-breech length 39 mm., was studied with reference to the urogenital sinus region, but not modelled. The lateral folds of the urogenital sinus were determined, the lower lateral folds being, however, quite inconspicuous. The anlagen of Cowper's glands were found near the cephalic ends of the middle lateral folds, as solid epithelial buds with as yet no peripheral branching. The bud on the right side passed through five 10  $\mu$  sections; that on the left, through nine 10  $\mu$  sections.

Embryo 18, from which the model shown in figure 3 was made, is a female embryo having a crown-breech length of 45 mm. In this embryo the mesonephric ducts present marked evidences of degeneration. When compared with Embryo 47, the general advance in the development of the urogenital structures may be noted. The phallus (clitoris) and the perineum are relatively shorter. A well marked sulcus coronarius glandis is present; a shallow groove, the urethral groove, extends to it from the ostium urogenitalis. Laterad, the sulcus nympho-labialis separates the phallus from the genital swelling. The ostium urogenitalis has increased in size, both in length and in width. The pars phallica sinus urogenitalis has developed to such an extent that it now

forms the widest part of the urogenital sinus caudal to the junction of the Müllerian duct with the urogenital sinus.

The model made from this embryo is shown from the left side in figure 3. The three lateral folds of the urogenital sinus are evident, and present some points of difference when compared with the three lateral folds as modelled from male Embryo 15.

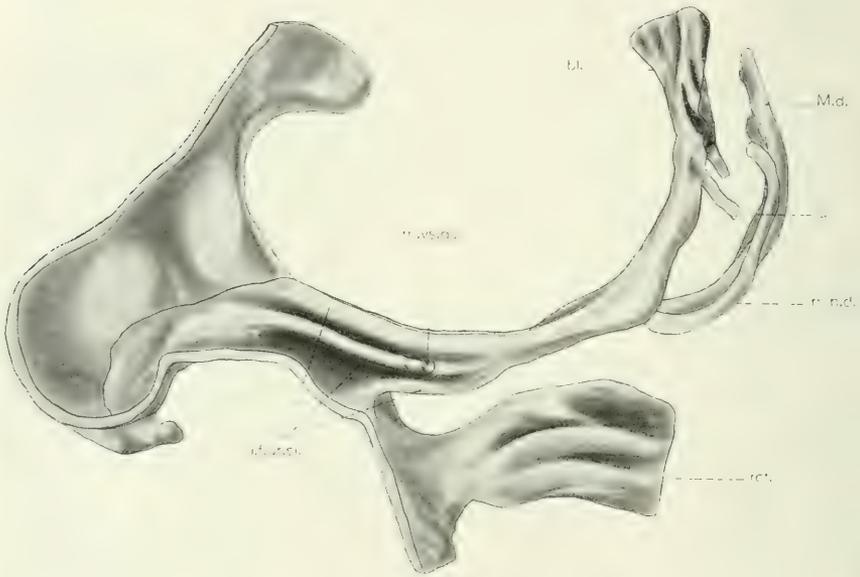


Fig. 3 Model of epithelial portion of urogenital system of human Embryo 18 (Huber collection); female, 45 mm. crown-breech length.  $\times 15$ .

Only the upper lateral folds have not materially altered their form and relations. As previously stated, when viewed from above, these enclose a deep fossa at their cephalic end, blending caudally in the sagittal plane to form the ridge-like crest for the urethral plate. The middle lateral folds have developed so as to be relatively long, being now continuous caudally with the lateral expansion of the sinus wall in the region of the ostium uro-

genitalis. The most distinctive change, however, is noticed in the lower lateral folds. These, though still the least conspicuous of the three sets of folds, have increased in length and elevation. The sinus lumen of this region presents a distinct difference from that found in Embryo 15. The lumen has invaded the upper and middle lateral folds, but the plate between the middle and lower folds is still solid, while in the region of the lower folds, there is a narrow lumen, blind at its cephalic end, but opening independently into the urogenital sinus.

The epithelial buds which form the anlagen of Bartholin's glands have the same relative positions as in younger stages (*m.vs.gl.*). Measured on the model, these buds are situated 1.2 mm. caudal to the point of junction of the Müllerian duct with the urogenital sinus, and spring from the cephalic portion of the middle lateral fold. The bud on the left side has a length of 120  $\mu$ , and presents a short, solid side bud at its distal end. In the middle third of its length, a loosening of the central cells suggests the beginning of a lumen. The bud on the right side has a length of 150  $\mu$ , presenting an expanded knob-like end, probably the anlage of side branches. A narrow lumen is present, just proximal to this expanded end; this lumen is surrounded by a layer of quite regular cells of a compressed cuboidal shape. In this embryo, both gland buds extend obliquely laterad and dorsally in the general direction of the middle lateral fold.

Embryo 48 is a female embryo of 48 mm. crown-breech length, and was not modelled. It presents the same general arrangement of lateral folds as described for Embryo 18. The lower lateral folds are distinct, and possess a narrow lumen which no longer ends blindly at its cephalic end, but here communicates with the lumen of the urogenital sinus. On the right side, the gland anlage passes through seven sections of 20  $\mu$  thickness; on the left side, through three such sections. However, the real difference in length of the two gland anlagen is not so great as this would seem to indicate, as the sections are not cut parallel to the mid-plane, and the left gland anlage is consequently cut very obliquely. The gland buds are still solid, with no evidence of branching.

Embryo 49 is a female embryo of 47 mm., crown-breech length, and was not modelled. The lateral urogenital sinus folds, as also the gland anlagen for Bartholin's glands, present the same general relations as in Embryos 48 and 18. The gland bud on the right side passes through eight sections having a thickness of  $15 \mu$ . The bud on the left side was very obliquely cut and loosened from the surrounding mesenchyme, so that its length was not determined.

Embryo 23, a female embryo having a crown-breech length of 60 mm., represents the oldest stage modelled. This model, as seen from the left side, is shown in figure 4. The model shows the downward (caudal) projection of the clitoris anlage, characteristic of this stage. When compared with the three preceding embryos, it is seen that the clitoris has become relatively, though not absolutely, smaller. A well-defined sulcus coronarius glandis is present, as also a deep sulcus nympho-labialis, bounding the base of the clitoris laterad. The urogenital sinus opens externally by two ostia; the larger one near the sulcus coronarius, the smaller one at the base of the clitoris. Externally, the two ostia are united by a deep groove. A continuation of this groove leads toward the anus, disappearing at about the middle of the perineum. The mesonephric ducts have degenerated to such an extent that they are incomplete on both sides.

The utero-vaginal canal (Müllerian tubes) is well developed, though its lumen does not appear to have joined that of the urogenital sinus. At the place of its fusion with the urogenital sinus, there may be observed, in the model, projections of the sinus epithelium, regarded as the anlagen of the paraurethral glands of Skene; three are to be observed on the right side, two on the left.

On each side, all of the three lateral folds have extended cephalad so as to reach the region of the utero-vaginal canal. In this embryo, the folds are complicated by the appearance of secondary folds. The upper lateral folds present each a secondary fold extending caudad to meet the middle lateral fold. The middle folds also present secondary folds, beginning just below

the anlagen of Bartholin's glands, and extending caudad. It seems possible to relate this model with the account given by R. Mayer, quoted in preceding pages of this communication. This observer has described five lateral folds or rays. Of the three



Fig. 4 Model of epithelial portion of urogenital system of human Embryo 23 (Huber collection); female, 60 mm. crown-breech length.  $\times 15$ .

primary lateral folds here described, the upper, with a secondary fold imperfectly developed, seems to correspond with Mayer's first and second rays; the middle lateral fold as here designated, with its secondary fold, forms the third and fourth rays of Mayer's account; the lower fold corresponds with his fifth ray.

In Embryo 23, the gland buds forming the anlage of Bartholin's glands are shown in figure 4 at *m.vs.gl.* They arise from the middle lateral fold, 1.2 mm. from the caudal end of the utero-vaginal canal, extending from this fold obliquely laterad and dorsad. The gland bud on the left side has a length of 200  $\mu$ ; on the right side, of 240  $\mu$ . Both gland buds show a knob-like end, with a slight constriction proximal to the terminal enlargement. The end of the left gland bud shows partial division into four branches, so that the cross section of this portion resembles a shamrock leaf; there is as yet, no mesenchyme separating the anlagen of the branches. The neck or stalk of this gland bud presents an interrupted lumen. Each of the branch anlagen shows a compact arrangement of the cells at the periphery, with loosely arranged central cells, precluding a lumen. The gland bud on the right side is similar to that on the left, except that only three branches are indicated, and one of these is completely surrounded by mesenchyme. Both gland anlagen are surrounded by dense mesenchyme, the beginning of a capsule. In the region of the gland anlagen, the sinus lumen has invaded the secondary fold found between the middle and lower lateral folds. The independent lumen of the lower fold was not to be observed in this embryo.

The figures of the models seem to portray the relations and extent of the lateral folds of the urogenital sinus so clearly, as also the anlagen of Bartholin's and Cowper's glands, that extended description was deemed unnecessary. The following summary and conclusions seem warranted.

#### SUMMARY AND CONCLUSIONS

1. Human embryos, both male and female, of the ages studied in this investigation, 3 to 6 cm. crown-breech length, present three pairs of lateral folds on the wall of the urogenital sinus. In the younger stages, these folds extend from the ostium urogenitalis to a point about halfway to the place of entrance of the mesonephric ducts into the urogenital sinus. In the older stages, they extend more cephalad; in the 6 cm. female embryo reconstructed, to the caudal end of the utero-vaginal canal.

2. These three folds first appear as solid epithelial ridges, symmetrically arranged on the two sides of the urethral plate. Keibel's models would indicate that they do not appear until the embryo has reached a length of more than 28 mm.

3. The upper lateral folds are at first the more prominent; later the middle lateral folds are relatively larger. The lower lateral folds remain inconspicuous.

4. A male embryo of 5 to 6 cm. length was not available, but the figures of Lichtenburg, Van der Broek, and other investigators would indicate that in the male, these folds become obliterated. The model figured by Broman (fig. 397) of a 6 cm. male embryo, does not show clearly whether lateral folds are present or not.

5. The anlagen of Bartholin's and Cowper's glands may be first recognized in embryos having a crown-breech length of 3 cm., as solid epithelial buds arising from the middle lateral fold near its cephalic end; in the younger stages extending laterad, in slightly older stages extending obliquely laterad and dorsad.

6. When the embryo has reached a crown-breech length of about 4.5 cm., the distal end of the gland bud presents a knob-like end with a narrower proximal portion in which a lumen is precluded. After attaining a crown-breech length of 5 to 6 cm., evidence of distal branching of the gland anlage may be observed.

7. The development of the glands on the two sides is not symmetrical, neither as to time of anlage, nor as to extent of development. This table 2 may serve to show.

TABLE 2

CATALOGUE NO.	CROWN-BREECH LENGTH IN MM.	LENGTH OF GLAND AND ANLAGEN, LEFT SIDE,	LENGTH OF GLAND AND ANLAGEN, RIGHT SIDE,
		IN $\mu$	IN $\mu$
47	32		15
15	30	60	50
17	39	50	90
18	45	150	120
48	48	140	60
49	47	120	?
23	60	240	200

In conclusion, I desire to express my sincere thanks and appreciation to Professor Huber, who, in addition to suggesting the problem, has given me material aid at every step in the making of the reconstructions and in their interpretation.

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VOLUMETRIC DETERMINATIONS OF THE PARTS OF  
THE BRAIN IN A HUMAN FETUS 156 MM.  
LONG (CROWN-RUMP)

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In the present communication there is reported a study of the volume of the main divisions of the brain as they are found in a fetus about four months old. The work was undertaken as a step in the history of the growth of the individual parts of the brain under the premise that a knowledge of their volume priorly would indicate in a general way the functional priority of these parts. By means of the wax-plate reconstruction method it is possible to make an accurate enlarged model of the brain that can be separated into its chief component parts. Since such a model is made of wax of a uniform composition the relation by volume and by weight of the different parts can be determined both as to each other and as to the brain as a whole. This same method was used in determining the volume of the different parts of the opossum brain, by Professor Streeter and by Mr. H. A. Tash, who reported their results at the meeting of the American Association of Anatomists at Ithaca.<sup>1</sup>

The brain measured was taken from a male fetus measuring 156 mm. crown-rump, and 201 mm. total, length. The head measurements were: Bitemporal, 48 mm.; occipito-frontal, 58 mm. These measurements were made on the fresh specimen. Its weight was 296 grams. The specimen was preserved in 10 per cent formalin, the skull having been opened to facilitate the penetration of the fixative. Subsequently the brain was removed, embedded in celloidin and prepared in serial sections 50  $\mu$  thick.

<sup>1</sup> Streeter, G. L., 1911, Volumetric analysis of the brain of the opossum. Proc. Amer. Assoc. Anat.; Anat. Rec., vol. 5, p. 91.

every other section saved and stained with alum-cochineal. From this series a model was made enlarged five diameters after the well known Born method. Serial drawings were made with a projection apparatus on papers which were then incorporated in wax plates of such a thickness that the enlargement in all planes was the same ( $\times 5$ ). The drawings were then cut out from the plates and filed. This gave a model of the whole brain with the ventricles removed. The plates were then gone through a second time and the various parts cut away from each other so that their individual weights and volumes could be separately determined. It was found that this could be done with considerable accuracy, and having the stained sections as a guide, it would have been possible to have carried the subdivisions further. But, having in mind both younger and older stages, it was decided that the adopted subdivision would prove most practical in the end. The results are given in table 1. In the first column of the table is given the weight in grams of the whole model and of its parts. In the second column is given the percentage of the total weight formed by each part, which would hold true for

TABLE 1

PARTS	MODEL WGT. IN GMS.	PER CENT OF TOTAL WGT.	MODEL VOL. IN CC.	ACTUAL VOLUME
Rhombencephalon.....	72.135	4.973	80.565	0.644
Medulla and pons.....	32.325	2.228	36.500	0.292
Cerebellum.....	39.810	2.744	44.065	0.352
Mesencephalon.....	20.960	1.441	23.659	0.189
Diencephalon (inc. epiphysis)	69.761	4.809	78.970	0.631
Telencephalon.....	1287.673	88.776	1457.658	11.661
Basal ganglia.....	110.610	7.625	125.210	1.001
Caudate nucleus (inc. parolf. body and amygdaloid nu- cleus).....	73.150	5.043	82.805	0.662
Putamen.....	33.161	2.286	37.538	0.300
Globus pallidus.....	4.299	0.296	4.867	0.039
Archipallium.....	33.865	2.334	38.341	0.306
Fornix and hippocampus.....	26.075	1.797	29.522	0.236
Paraterminal body.....	5.815	0.401	6.583	0.052
Olfactory bulbs.....	1.975	0.136	2.236	0.017
Neopallium.....	1143.198	78.815	1294.100	10.345
Total brain.....	1450.468	100.000	1641.929	13.135

the actual brain just as for the model. In the third column is given the volume in cubic centimeters of the whole model and of its different parts. Instead of determining the volume of each part separately it was found more practical to determine the specific gravity of the wax plates and then calculate the volumes from the weights given in the first column. In the last column is given the volume of the brain itself and of its parts. This was obtained by dividing the volume of the model by the amount of the enlargement, i.e., the cube of five diameters. It is to be remembered that this is the volume of the brain after it has been embedded and prepared in serial sections. The volume of the fresh brain could be obtained only by calculating the amount of shrinkage the specimen experienced in this process.

The subdivisions that were used follow as far as possible the embryological subdivisions adopted by His. Their boundaries could in most cases be determined by the cell structure of the sections. In some cases it was necessary to depend on the surface configuration of the model. The landmarks utilized in carrying out this subdivision are herewith detailed:

*Rhombencephalon.* This was separated from the spinal cord as nearly as possible at a point post cephalic to the first cervical nerve. The cephalic boundary was determined by a plane just skirting the inferior colliculus and passing out ventrally just in front of the pons. Laterally this plane passes just in front of the brachium connecting the cerebellum and pons.

*Cerebellum.* This is plainly demarcated by its surface outline, while the pons is determined more by its internal structure, the main characteristic being the densely massed nuclei. The cerebellum at this time consists of a well fissured vermis and the two lateral lobes which are fissured dorsally but are still smooth ventrally. In removing it the floccular margin was included and also the brachium pontis on each side to the point at which it meets the pons. The removal of the cerebellum leaves the medulla and pons, whose weight and volume are given together.

*Mesencephalon.* The caudal limit of the mesencephalon is the same as the plane marking the cephalic border of the rhombencephalon, which has already been given. Its cephalic limit is a

wedge-shaped plane that projects in between the masses of the diencephalon. At the median line its boundary is marked dorsally by the posterior commissure and ventrally by a point post caudal to the mammillary bodies. From this median line the plane of division on each side extends backward so as to include the red nucleus with the midbrain and comes to the surface at a groove marking the antero-lateral margin of the superior colliculus. Owing to the advanced development of the colliculi and the retarded development of the peduncular portion, the mesencephalon is V-shaped as regards its ventral aspect, as well as its cephalic boundary.

*Diencephalon.* Its separation from the mesencephalon we have already indicated. From the telencephalon it is separated bilaterally by the internal capsule, and a sharp line of demarcation on the surface is afforded by the stria terminalis. Ventrally where this is not present the line of division is continued along the anterior margin of the optic tract. By this manner of subdivision there is comprised in this portion the optic tract and thalamus including the habenular nuclei and epiphysis and also the whole hypothalamus with the exception of the hypophysis, which had been removed.

*Telencephalon.* This includes all the remainder of the brain. It was subdivided into three main divisions as follows:

*Basal ganglia.* At the end of the fourth month these structures are clearly defined and bear a relation that closely approximates the adult. The putamen and globus pallidus are easily recognized in transverse sections. As for the lamina of capsule fibers that surround them, the incisions were made half-way, so that part of the fibers would go with the globus pallidus and part with the caudate nucleus. The caudate nucleus throughout its greater extent is likewise clearly defined. At its head and tail ends, however, it is complicated by fusing with the parolfactory body and amygdaloid nucleus respectively. On this account these latter were included with it.

*Archipallium.* This includes, in the first place, the olfactory bulbs, which were removed at a transverse line at the point where

they become free from the brain wall. This corresponds to both the bulb and stalk of the adult. The paraterminal body includes the gray substance where the olfactory bulb is attached and the region of the future septum pallucidum and the pillar of the fornix, which could not be easily separated from it. The remainder of the archipallium is made up of the body of the fornix and its fimbriated extension into the hippocampus. The hippocampus is easily recognized by its histological structure and by the way it bulges into the lateral ventricle. With it was included the dentate fascia and the uncinata body. The corpus callosum was included with the neopallium.

*Neopallium.* This includes the remainder of the telencephalon and represents what we know in the adult as the convoluted cortex, together with the subjacent white matter and includes the corpus callosum, as we have just pointed out.

In conclusion I wish to acknowledge the courtesy of Professor Streeter, who kindly put the resources of the Anatomical Laboratory of the University of Michigan at my disposal for the purpose of this investigation, and gave me many helpful suggestions as the work progressed.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

A LABORATORY MANUAL AND TEXT-BOOK OF EMBRYOLOGY. By Charles W. Prentiss, A.M., Ph.D., Professor of Microscopic Anatomy in the Northwestern University Medical School, Chicago. Octavo, 400 pages, 368 illustrations, many of them in colors. Philadelphia and London: W. B. Saunders Company, 1915.

Preface. This book represents an attempt to combine brief descriptions of the vertebrate embryos which are studied in the laboratory with an account of human embryology adapted especially to the medical student. Prof. Charles Sedgwick Minot, in his laboratory textbook of embryology, has called attention to the value of dissections in studying mammalian embryos and asserts that "dissection should be more extensively practised than is at present usual in embryological work. . . ." The writer has for several years experimented with methods of dissecting pig embryos, and his results form a part of this book. The value of pig embryos for laboratory study was first emphasized by Professor Minot, and the development of my dissecting methods was made possible through the reconstructions of his former students, Dr. F. T. Lewis and Dr. F. W. Thyng.

The chapters on human organogenesis were partly based on Keibel and Mall's Human Embryology. We wish to acknowledge the courtesy of the publishers of Kollmann's Handatlas, Marshall's Embryology, Lewis-Stöhr's Histology and McMurrieh's Development of the Human Body, by whom permission was granted us to use cuts and figures from these texts. We are also indebted to Prof. J. C. Heisler for permission to use cuts from his Embryology, and to Dr. J. B. De Lee for several figures taken from his Principles and Practice of Obstetrics. The original figures of chick, pig and human embryos are from preparations in the collection of the anatomical laboratory of the Northwestern University Medical School. My thanks are due to Dr. H. C. Tracy for the loan of valuable human material, and also to Mr. K. L. Vehe for several reconstructions and drawings.

C. W. PRENTISS.

Northwestern University Medical School,  
Chicago, Ill., January, 1915.

## ON THE WEIGHT OF THE ALBINO RAT AT BIRTH AND THE FACTORS THAT INFLUENCE IT

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In the course of an extensive series of breeding experiments with the albino rat a large amount of data has been collected regarding the body weight of these animals at different stages of their growth. The records dealing with the weight at birth are given in the present paper: those of postnatal growth will be published later.

Two sets of observations on the body weight of very young albino rats have already been recorded. In a paper published by Donaldson in 1906 the average weight of 40 young male albino rats is given as 5.4 grams, and that of 17 females is stated to be 5.2 grams. The more extensive records of Jackson ('13) give the average weight of 107 young male albino rats as 5.1 grams, and that of 109 females as 4.8 grams.

The body weights, as given above, are those of animals that were 'newborn' when weighed. The term 'newborn,' as Jackson states, covers the period in the life of the animal from the time of birth up to one day. As a rule, young rats begin suckling very soon after their birth, and not infrequently part of a litter will have suckled before the rest have been born. The weight of 'newborn' animals, therefore, is probably not the same as the birth weight in many cases. To obtain the birth weight it is necessary that the animals be weighed before they have suckled, since the amount of food consumed during the first few hours of postnatal life very appreciably increases the body weight. One can tell very easily whether or not the young rats have suckled, as the skin of the young animals is quite

transparent and if milk is present in the stomach or in the intestines it can be seen very clearly through the body wall.

In the course of this investigation 113 litters of rats were obtained at or soon after birth and weighed before any of the individuals had taken food. The same course of procedure was followed in making the records for each of the litters. The young rats were first separated according to sex by the method devised by Jackson ('12). Animals of the same sex were then weighed together to a tenth of a gram and the average weight for each individual computed; if, however, there was a very marked difference in the size of the individuals of the same sex the rats were weighed separately and the weight of each recorded. In addition to recording the number of young, the sex distribution and average body weight of the members of each litter, the exact age of the mother at the time the litter was born was noted, also her body weight after the birth of the litter and her general physical condition.

The complete series of records comprise data from five different strains of rats that are being bred in The Wistar Institute animal colony at the present time:

1. Stock albinos: Members of the general colony that supposedly represent the normal albino type as it exists at the present time.
2. Inbred albino rats: Animals, originally taken from the general stock colony, that have been closely inbred for many generations.
3. Extracted albinos: A strain of rats descended from albinos cast by  $F_1$  hybrids of the albino and the wild Norway rat (*Mus norvegicus*).
4. Piebald rats: A strain derived from  $F_1$  hybrids of the albino and Norway rat.
5. Extracted grays: A strain also derived from the  $F_1$  hybrids of the albino and the Norway rat.

Table 1 gives a general summary of the birth records arranged according to the strain of rats from which the litters were obtained. The data in this table show that, regardless of strain, the

weight of the albino rat at birth is considerably less than that of newborn animals as given by Donaldson and by Jackson. As a rule the male rat at birth is somewhat heavier than the female, as is the case in many other mammals including man.

TABLE 1

*Showing the birth weight data for various strains of rats*

STRAIN OF RATS	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
Stock albinos	12	95	47	48	215.6	216.1	4.59	4.50
Inbred albinos	73	644	311	333	1409.5	1410.8	4.53	4.23
Extracted albinos	8	43	21	22	88.2	88.2	4.20	4.00
Piebalds	19	147	80	67	386.1	324.3	4.82	4.84
Extracted grays	1	9	4	5	22.9	27.7	5.72	5.54
Total	113	938	463	475				

On comparing the data for the various strains of rats it is found that stock albinos, both males and females, weigh slightly more at birth than do the inbred rats. The differences between them are not sufficiently great to have much significance, especially as the number of stock litters that was weighed was relatively small. Extracted albinos weigh considerably less at birth than either the stock or inbred rats. This fact is not surprising considering that these animals grow much less rapidly than stock or inbred albinos and that many of them, particularly the females, fail to attain the average adult size of stock animals. Piebald rats, both males and females, have a birth weight that is greater than that of any of the albino strains.

The average weight of the piebald females, as given in table 1, is slightly greater than that of the males. This is probably a chance variation, since in 12 of the 19 litters that were weighed the average weight of the males exceeded that of the females. From the single litter of extracted gray rats weighed at birth

one can obtain but little idea of the birth weight for the strain, yet the records are significant in that they show an average weight for both sexes that is close to the mean between the birth weight of the wild Norway rat, which is about 6.4 grams for both sexes according to Miller ('11) and that of the albino rat.

In any litter of rats, as a rule, individuals of the same sex are practically of the same size and body weight at birth. Occasionally, however, very marked exceptions to this rule are found. In one of the litters of inbred rats the difference in the weights of the various individuals was so unusually great as to call for more than a passing notice.

The litter in question contained eleven individuals, four males and seven females. One of the males, which was the largest rat yet obtained at birth, weighed 7.5 grams; the other three males were nearly uniform in size, weighing 4.9 grams, 5.0 grams and 5.1 grams, respectively. The females in this litter also showed considerable variation in body weight. The largest of the seven females weighed 4.6 grams, the smallest weighed 2.9 grams. The latter is not the smallest birth weight for the rat that has been obtained, however, as in one case in which the mother of the litter was in the last stages of pneumonia when the litter was born, two of the three males in the litter weighed 2.6 grams each; the third male weighed 2.9 grams which was also the weight of the one female in the litter. Female rats do not seem to show as great a variation in body weight at birth as do the males. The largest female yet obtained weighed 5.9 grams at birth; the smallest weighed 2.7 grams.

It seems most probable that such marked variations in the size of the different members of a litter at birth as are shown above must be due to a difference in the age of the embryos at the time that parturition occurs, not to causes acting late in gestation. Evidence already presented (King '13) indicates that, under certain conditions, ovulation in the rat may extend over three or four days, possibly longer. Ova liberated at various intervals for several days would probably all be fertilized, as the period of heat in the rat exists for about one week (Miller '11). The body growth of the embryos is very rapid during the latter part

of gestation, and embryos that developed from the ova first set free might be expected to have a greater body weight than the embryos that developed from ova liberated late in the period of ovulation, since they would have a longer time in which to grow. Rats born at one period of parturition, however, must be very nearly the same age, as the smaller individuals show no evidence of immaturity other than in their smaller size. If there is considerable difference in the age of the embryos developing simultaneously in the uterus the more mature ones are born first, and the remaining ones are born from one to several days later when they have reached the proper stage of development (King '13).

The variations in body weight found among rats at birth are seemingly greater than those in 'newborn' animals. The largest male in Donaldson's series weighed 6.5 grams, the smallest weighed 4.3 grams: corresponding figures for the females give 6.2 grams as the heaviest weight and 4.2 grams as the lightest weight. In Jackson's series the weights of the males range from 3.4 grams to 6.6 grams and those of the females from 3.5 grams to 6.3 grams.

There is a possible explanation for the narrow range of variation in the weights of 'newborn' rats besides the obvious one that the series of animals weighed was too small to contain the extreme variates in body weight. Individuals that are very small at birth may increase in body weight and in size more rapidly during the first few hours of postnatal life than the members of the litter that have a heavier birth weight. This would tend to equalize the size of the individuals and so give all of them approximately the same chances of obtaining food. The early growth changes in the rat have not been studied sufficiently as yet to give evidence on this point.

In analyzing the data collected in connection with the birth weights with a view of ascertaining, if possible, some of the factors that help to determine the weight of the rat at birth, it has been considered advisable to make use only of the records for the 85 litters of stock and of inbred albino rats. The average weight of the young in the piebald and in the extracted gray litters is so much greater than that of the albinos that the effects

of these records on the general averages, when taken in small groups, would be altogether disproportional to the number of individuals involved. The birth weights for the extracted albinos, on the other hand, fall so far below those for the other albinos that they seem properly to belong in a class by themselves. It does not seem worth while to analyze the data for these strains otherwise than in the manner shown in table 1. The records are as complete as for the stock and inbred albinos, however, and are filed at The Wistar Institute.

#### THE EFFECTS OF THE AGE OF THE MOTHER ON THE WEIGHT OF HER YOUNG AT BIRTH

Slonaker ('12) states that the age of the mother affects not only the number of young rats in a litter but also their weight at birth, young mothers being less prolific than older ones. He makes no mention, however, of the extent of the data on which this conclusion is based.

Under the conditions existing in The Wistar Institute animal colony the female albino rat usually has her first litter when she is about three months old, and she is capable of bearing young until she is about fifteen months old. In order to study the effects of the age of the mother on the weight of her young at birth the reproductive period in the life of the albino female has been arbitrarily divided into the four following periods:

1. From 90 to 120 days: This is the age when young females are growing very rapidly and the time when the great majority of them cast their first litters.

2. From 120 to 180 days: During this time the female reaches the end of the rapidly growing period and becomes fully mature.

3. From 180 to 300 days: The female is at the height of her reproductive powers during this period and has attained full growth.

4. From 300 to 450 days: In this period there is a dying out of the reproductive power and little, if any, growth.

Table 2 shows the data for the 85 litters of stock and of inbred albino rats arranged in four groups according to the age of the

mother at the time that the litter was born. The data, as arranged in this table, do not show the gradual increase in the body weights of the young from the first to the fourth group that one would expect to find if the age of the mother is the dominant factor in determining the birth weight of her young. If, however, we compare the average birth weight of the rats in litters cast by females during the first reproductive period with that of the individuals belonging to litters born when the reproductive power of the mother is waning, it is found that the average body weights in the first group are considerably less than those in the last group. The difference between them, amounting to 0.3

TABLE 2

*Showing the birth weight data for 85 litters of stock and inbred albino rats arranged according to the age of the mothers at the time that litters were cast*

AGE OF MOTHER	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
(1) From 90 to 120 days	27	232	112	120	494.9	494.6	4.41	4.12
(2) From 120 to 180 days	36	326	155	171	712.1	736.9	4.59	4.30
(3) From 180 to 300 days	17	143	70	73	318.5	321.5	4.55	4.40
(4) From 300 to 450 days	5	38	21	17	99.2	73.3	4.71	4.31

gram in the case of the males and 0.2 gram in the case of the females is sufficiently great, I think, to warrant the conclusion that the weight of a litter at birth depends, to a certain extent, on the age of the mother. During the first reproductive period young females are growing very rapidly both in body size and in body length and presumably, therefore, the growth processes consume a considerable part of all available energy. Litters cast by females at this time contain, as a rule, few individuals and these are of relatively small size. In older females growth has practically stopped and more energy can be used for the production of larger litters containing individuals that have a heavier weight at birth.

THE INFLUENCE OF THE BODY WEIGHT OF THE MOTHER ON THE  
BIRTH WEIGHT OF HER YOUNG

Under normal conditions body weight and age are closely correlated in the rat, as Donaldson's investigations have shown. Body weight, however, being easily affected by changes in environment or in nutrition is, to a certain extent, independent of the age factor and indicates very clearly the physical condition of an animal. Rats that are heavy for their length and age are usually in excellent health; those that are light in weight are generally ill, as certain diseases—for instance, 'pneumonia'—may be shown by a rapid drop in the weight of the animal before any other symptoms of illness are manifested. The body weight of a female, as indicating her general physical condition irrespective of age, may possibly, therefore, be a factor that would tend to influence the birth weight of her young.

As age is the factor that so largely determines body weight in the rat, it has seemed advisable to group the records for the body weights of the females according to age. To do this it is necessary to know the body weights that are normal for various ages. Table 3, compiled from an extensive series of unpublished data collected in the course of my breeding experiments, gives the normal weight of stock and of inbred albino females that correspond with the age groups used as the basis of analysis in the previous section. Inbred albino females are slightly heavier

TABLE 3

*Showing the body weight of stock and of inbred albino rats normal for different age groups*

AGE OF FEMALES IN DAYS	NORMAL WEIGHT OF STOCK ALBINO FEMALES	NORMAL WEIGHT OF INBRED ALBINO FEMALES
90 to 120 days	148 to 173 grams	156 to 175 grams
120 to 180 days	173 to 195 grams	175 to 199 grams
180 to 300 days	195 to 219 grams	199 to 221 grams
300 to 450 days	219 + grams	221 + grams

for a given age than are stock albino females, as is shown in table 3. Since the great majority of the birth weights recorded are of litters belonging to the inbred strain, the body weights

of the inbred females have been used as the basis for the grouping of the data in the present instance.

Table 4 gives the various birth weight records arranged according to the body weight of the mothers at the time that parturition occurred. If the body weight of all the females had been normal for the age at which their litters were cast, table 4 would be practically a duplicate of table 2, where the data are arranged according to the age of the mothers. A comparison of the two tables shows, however, a very different distribution

TABLE 4

*Showing the birth weight data for stock and inbred albino rats arranged according to the body weight of the mothers at the time that the litters were cast*

BODY WEIGHT OF FEMALE	NUMBER OF LIT- TERS	NUMBER OF INDI- VIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
To 175 grams .....	27	209	98	111	423.8	440.7	4.46	3.86
175 to 200 grams .....	23	222	108	114	491.3	506.8	4.55	4.47
200 to 220 grams .....	25	233	111	122	508.7	524.7	4.58	4.30
220 + grams .....	10	75	41	34	201.7	158.0	4.91	4.64

of litters in the corresponding groups. The first group in each table happens to contain the same number of litters, but in table 4 this group comprises 9 litters from females that were over 120 days of age when parturition occurred. These females had fallen below the normal weight for their age and were, presumably, not in especially good physical condition. The second group in table 4 contains 6 litters from females younger than 120 days, and 4 litters from females that had passed 180 days of age when the litters were cast. In this group, therefore, 10 of the 23 litters belonged to females that did not have a normal body weight. Eighteen of the 25 litters belonging to the third group of table 4 were cast by females younger than 180 days of age and two of them by females older than 300 days. In the last group only three of the 10 litters came from females that had a body weight normal for the age of which the litters were cast.

According to the records as given in table 4, the average weight of young rats at birth increases directly as the body weights of the mothers increase. When the body weights of the females are below 175 grams the average weight of the young males in the litters is 4.46 grams. This average rises to 4.91 grams for the males in the litters cast by females weighing 220 grams or more. Records for the female young do not show quite such uniformity as in the case of the males, since the weights of the individuals belonging to the third group are less than those of the individuals in the second group. The weight of the females in the fourth group, however, is greater by 0.78 grams than that of the females belonging to the first group. This difference is considerably greater than that shown by the males in the two groups.

That these results depend to a considerable extent on the age of the mothers of the litters there can be little doubt, since body weight is closely associated with age and in the normal animals the range of variation is not very great.

If, however, we disregard the age factor and take the body weights of the mothers as indicative of the physical fitness of the animals, it is evident that the heavier females, being in excellent condition, tend to produce young that are larger at birth than the young cast by females that are relatively light in weight and probably, therefore, not in very good condition.

From this analysis of the data it follows that it is not the body weight of the female in itself, but the factors on which the weight largely depend, i.e., age and physical condition, that have a pronounced influence on the birth weight of the young.

#### THE INFLUENCE OF THE LITTER SIZE ON THE WEIGHT OF THE YOUNG AT BIRTH

The size of a litter of albino rats depends, seemingly, on a number of different factors, one of which is undoubtedly the age of the mother. Very young females and those that have passed their prime have smaller litters, as a rule, than females at the height of their reproductive powers. The physical condition of the mother is also a factor that apparently affects the litter size,

as females in poor condition rarely have a large litter, and if the number of young exceeds the average for the species several of them are usually stillborn. Litter size therefore is another factor so inseparably linked with the age and physical condition of the mother that its influence on the birth weight of the young must be considered in connection with the other factors involved.

Unpublished data for over 1000 litters of stock albino rats show that the average litter contains seven young. The size of the litter varies greatly in different cases, the range being from one to thirteen.

TABLE 5

*Showing the birth weight data, for stock and inbred albino rats, arranged according to the size of the litter. G, litters cast by females in good physical condition; P, litters cast by females in poor condition*

SIZE OF LITTER	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS	
5 or less	4(G)	18	9	9	45.1	39.6	5.01	4.33	4.40
	5(P)	24	10	14	37.3	50.8	3.73		
6 to 8	30	217	111	106	512.6	464.7	4.61	4.38	
9 or more	46	480	228	252	1030.0	1075.7	4.51	4.26	

In order to analyze the birth records on the basis of litter size, the litters have been arbitrarily divided into three groups: small litters, containing five or less young; medium sized litters, containing six to eight individuals; large litters with nine or more young.

The arrangement of the data according to the above classification is shown in table 5. The data, as shown in this table, seem directly opposed to the generally accepted view that animals belonging to small litters weigh more at birth than those belonging to large litters, since the average weight of both males and females is considerably less for the small litters than for the very large ones. The record cards show, however, that five of the nine small litters were cast by females that were in poor physical condition. In these five litters the average weight

of the males was 3.73 grams and that of the females was 3.62 grams; in the four litters from females apparently in good condition the average weight of the males was 5.01 grams and that of the females was 4.40 grams. Only seven of the 85 litters weighed were cast by females in poor condition, and five of them, as shown above, belong in the small litter group. The other two were litters of medium size, and if their records were omitted from table 5 the average birth weight of both males and females in this group would be raised slightly.

It seems justifiable, therefore, to disregard the data for the five small litters cast by females in ill health and to take the records for the four litters cast by females in good condition as representing the birth weights for the small litter group. If this be done, the data in table 5 show that the average weight of the young in small litters greatly exceeds that for the individuals in large litters: individuals in medium sized litters weigh close to the mean between the weights of the animals in the small and in the large litters. This rule holds true for animals of both sexes, and seems to indicate that the birth weight of young rats depends to a considerable extent on the size of the litter, irrespective of the other factors that may be involved.

Additional evidence that the size of the litter influences the birth weight of the young is furnished by the records for the largest litter of albino rats as yet obtained. This litter, which belonged to the inbred strain, contained 17 individuals, 10 males and 7 females. The young rats in this litter were several hours old when first examined and they had all suckled, so that it was not possible to obtain their birth weights. The average weight of the 17 individuals at the time they were found was 2.7 grams. At birth, therefore, these rats probably weighed not more than 2.5 grams each. The smallest birth weight for the rat that has as yet been taken is 2.6 grams. In this litter, therefore, the very small size of the individuals was undoubtedly due to the exceptional size of the litter, as the mother of the litter was large for her age and seemingly in the best of condition when the litter was born.

In a study of the weight of guinea-pig Minot ('91) found that the size of the pigs at birth depends to a considerable degree on the number of young in a litter; the larger the litter the smaller the pigs at birth. According to Minot, the litter size influences the birth weight by changing the length of the gestation period.

When the litter is large the gestation period is shortened and therefore the young pigs do not have as long a time in which to grow as is the case when the litter is small and consequently the gestation period longer. The birth weight of guinea-pigs, therefore, does not depend, according to Minot, on "the ratio of food supply and demand" but on the length of gestation.

In the rat, as in the guinea-pig, the length of the gestation period varies considerably in different cases. Normally it is from 21 to 23 days, but in lactating females it may be extended to 34 days (King '13). As far as I am aware, no attempt has been made as yet to ascertain the relation between the number of young in a litter and the duration of gestation in the rat. When a lactating female becomes pregnant the number of young in the second litter is certainly a factor of considerable importance in determining the length of the gestation period, for gestation is prolonged from one to thirteen days if the number of young carried is very large. How the birth weight of the young is affected by this prolongation of the gestation period remains to be determined. Growth is so rapid during the latter part of gestation that the extension of the period for even one day might be expected to materially increase the size of the embryos unless other factors tend to check growth at a certain stage of development.

#### THE RELATION OF THE LITTER SERIES TO THE WEIGHT OF THE YOUNG AT BIRTH

To arrange the records for the birth weights according to the position of the litters in the litter series would seem to be merely another way of testing the effect of the age of the mother on the weight of her young at birth, since it is impossible to eliminate the factor of age in carrying out such a plan. Female rats show very great individual differences, however, in regard to the

time when their litters are produced. Some rats begin breeding before they are three months old; others do not have their first litter until they are four or six months old. Certain females will cast a litter every month for several succeeding months; others never have a litter oftener than every two months and not infrequently three or four months will intervene between litters even when the females are apparently in excellent condition.

The plan of the breeding experiments at present under way requires that every breeding female shall have four litters. Some females have the required number of litters by the time they are six months old; others not until they are a year or more old. While, therefore, the range of variation in the age of the mothers is comparatively slight for the first and for the second pregnancies, it may be extended over several months before the third and the fourth litters are produced.

The litter series must always be, in a sense, an age series, yet the individual variations in the frequency of the litter production are sufficiently great, I think, to make it worth while to study the birth weight records from the point of view of the number of the pregnancy.

Table 6 shows the records for birth weights arranged according to the litter series. An analysis of the litters from several hundred females has shown that the first of a rat's four litters

TABLE 6

*Showing the birth weight data for stock and inbred albino rats arranged according to the position of the litters in the litter series*

LITTER SERIES	NUMBER OF LITTERS	AVERAGE NUMBER OF YOUNG IN LITTER	NUMBER OF INDIVIDUALS	MALES	FEMALES	TOTAL WEIGHT OF MALES IN GRAMS	TOTAL WEIGHT OF FEMALES IN GRAMS	AVERAGE WEIGHT OF MALES IN GRAMS	AVERAGE WEIGHT OF FEMALES IN GRAMS
1.....	22	8.5	187	91	96	400.0	390.2	4.39	4.06
2.....	21	8.9	188	82	106	370.8	452.7	4.52	4.27
3.....	25	9.4	236	125	111	576.6	492.5	4.61	4.43
4.....	17	7.5	128	60	68	278.6	296.5	4.64	4.36

is usually the smallest, the second and the third the largest, and the fourth a little larger than the first. This rule, however, does not happen to apply in the case of the litter series shown in table 6. Not only is the average number of young in the first group considerably above that which is normal for the species, but it greatly exceeds that for the fourth group; the second and third groups are fairly normal in size. Litter size is, in all probability, a factor that in the present instance does not materially affect the birth weights of the young rats, since the average size of the litters varies but little for the four groups.

Table 6 shows that the birth weights of the males increase with the ascending scale of the litter series; for the females there is a slight irregularity in the figures for the third and fourth groups, but the average weight of the young females in the fourth group is 0.3 grams greater than that in the first group. A comparison of these birth weights with those given in table 2, where the age of the mother formed the basis of the classification of the data, shows that the figures for corresponding groups are much the same, the deviation in no case being greater than 0.07 grams. No other tables given show such close agreement, and, therefore, it appears that the position of a litter in the litter series influences the birth weight of the young chiefly because it so closely involves the factor of age.

#### THE EFFECTS OF THE PHYSICAL CONDITION OF THE MOTHER ON THE WEIGHT OF HER YOUNG AT BIRTH

One accustomed to the care and handling of rats can quickly tell from the general appearance and weight of an animal whether or not it is in good physical condition. A hunched back, labored breathing, dark red eyes and a relatively light weight indicate internal disorders from which the rat rarely, if ever, recovers. On the other hand, a heavy weight for body size and pronounced vigor in action shows that the animal is in excellent health.

Seven of the 85 females whose litters were weighed were noted as in exceptionally good condition when their litters were born, and seven others showed unmistakable signs of ill health. Records for the weights of the litters from these 14 females are given in

table 7. In the litters from females that were in excellent condition when their litters were born the average birth weight of the young for both sexes is much greater than the normal, and it exceeds the average weights of the young in the litters cast by females that were in ill health by over 1.1 grams, as is shown in table 7. This result indicates that the physical condition of the mother, irrespective of her age, is a very important factor in determining the weight of her young at birth, probably through its action on the nutritive conditions to which the embryos are subjected.

TABLE 7

*Showing the birth weight data for 14 litters of stock and of inbred albino rats arranged according to the physical conditions of the mothers at the time that the litters were cast.*

CONDITION OF MOTHER	NUMBER OF LIT-	NUMBER OF INDI-	MALES	FEMALES	TOTAL WEIGHT OF	TOTAL WEIGHT OF	AVERAGE WEIGHT	AVERAGE WEIGHT
	TERS				VIDUALS	MALES IN GRAMS	FEMALES IN GRAMS	OF MALES IN GRAMS
Excellent .....	7	66	37	29	183.2	136.6	4.95	4.71
Poor .....	7	39	14	25	53.4	89.5	3.81	3.58

Another way of studying the same problem is to compare the physical condition of the mothers of the litters in which the individuals were unusually heavy at birth with the condition of the females that cast litters having a very light weight. Data for such a comparison are given in table 8, where the body weights of the mothers of the litters are taken as indicative of the physical condition of the animals. There was a total of 13 litters in which the average weight of the young of both sexes was 5 grams or over at birth. The records show that in ten cases the mothers of the litters weigh considerably more than the amount normal for their age; each of the remaining females had a body weight that corresponded exactly with her age. All 13 litters, therefore, were cast by females that were in very good physical condition as far as one could judge from the general appearance and weight of the animals.

TABLE 8

*Showing the body weights of the mothers of those litters in which the birth weights of the young were above or below the normal weight*

AVERAGE WEIGHT OF YOUNG IN GRAMS	NUMBER OF LITTERS	BODY WEIGHT OF MOTHERS
5 or more.....	{ 10	above normal
	{ 3	normal
4 or less.....	{ 9	below normal
	{ 9	normal

No definite conclusions can be drawn from the records of the 18 litters in which the young had a birth weight of 4 grams or less since the results are, in many cases, complicated by the factor of litter size. In 9 cases the body weight of the mother of the litter was below the weight normal for the age indicated; in the remaining cases the weights of the females were normal for their age, but all of the litters were very large (containing from 9 to 14 young) so litter size was doubtless a factor that had lowered the weight of the young to a certain extent. As far as the evidence from this set of records goes it seems to indicate that, in some cases at least, the small size of rats at birth is due to the poor physical condition of the mother which prevents the proper nourishment of the young and so inhibits their growth.

## SUMMARY

1. Data for 113 litters show that the body weight of the young at birth differs considerably in various strains of rats. Stock and inbred albino rats weigh about the same at birth; the average weight of the males being 4.54 grams and that of the females 4.27 grams in the 85 litters that were weighed. Extracted albinos have a very low birth weight; while piebalds and extracted grays weigh much more at birth than do any of the albino strains (table 1).

2. The male rat at birth usually weighs about 0.2 grams more than does the female.

3. There is a wide range of variation in the birth weights of albino rats. The weights for the males range from 2.6 grams

to 7.5 grams: those for the females vary between 2.7 grams and 5.9 grams.

4. In any litter, as a rule, individuals of the same sex are practically of the same size and body weight. Marked exceptions to this rule are probably due to a slight difference in the age of the embryos at the time that parturition occurs.

5. The body weight of rats at birth depends upon a number of different factors that are more or less closely related:

(a) The age of the mother. Individuals in litters cast by older females weigh more at birth, as a rule, than do the individuals in litters belonging to very young females (table 2).

(b) The physical condition of the mother. Rats in good physical condition bear young with a birth weight considerably above that of the young cast by females in poor condition (tables 7, 8).

(c) The body weight of the mother. The body weight of a female influences the birth weight of her young chiefly because it depends on the two more important factors of age and physical condition. Rats that have a very heavy body weight are older and in better physical condition than rats with a light body weight, and their litters comprise individuals with a corresponding greater weight at birth (table 4).

(d) The size of the litter. Individuals in small litters weigh more at birth than do individuals in large litters (table 5).

(e) The position of the litter in the litter series. The birth weight of young rats increases directly with the ascending scale of the litter series. But, as the litter series is always an age series, it is probable that the number of the pregnancy affects the birth weight only because it involves the factor of age (table 6).

(f) The length of the gestation period. The evidence regarding the influence of this factor is slight as yet. It is very probable that the prolongation of the gestation period for even one day materially increases the weight of the young at birth.

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# OBSERVATIONS ON THE ORIGIN OF THE MAST LEUCOCYTES OF THE ADULT RABBIT

## PRELIMINARY NOTE

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The investigations of Maximow have shown that in mammals the connective tissue mast cells are very different from the mast cells of the blood. Maximow and Weidenreich believe that the only feature which the two types of cells have in common is the presence of basophilic granules in the cytoplasm, which stain metachromatically with basic aniline dyes. The two types of cells, however, represent independent lines of leucocyte differentiation and development, with their own peculiar nuclei and granules.

Maximow ('06) found histogenous mast cells in all the mammals he investigated, even in the rabbit where most investigators have failed. He calls attention to the fact, that where there are relatively few histogenous mast cells, the deficiency is made up by increased numbers of haematogenous mast cells, and vice versa. That such a close compensatory relationship exists between the two types of cells is shown very well in the adult rabbit, there being comparatively few histogenous mast cells, but numerous mast leucocytes.

Within the past few years the origin of the haematogenous mast leucocyte has been the subject of considerable haematological investigation. The earlier investigators, including Ehrlich, assumed that mast leucocytes were represented in the bone-marrow by certain characteristic myelocytes and evolved like the other granular cells. Weidenreich, however, has recently shown that this is not the case with the human mast leucocyte. He believes that human mast leucocytes are formed from de-

generating lymphocytes within the circulation. He derives the mast granules from the fragmenting nucleus and not from the protoplasm of the degenerating cell. Various other investigators in working on the mast leucocytes of the rabbit have come to similar conclusions with reference to the origin of these cells within the blood stream.

In 1909 Pröscher concluded from his observations that the mast leucocytes of the blood of the rabbit are merely lymphoid cells of various types whose 'spongioplasm' has undergone a special form of mucoid degeneration, which results in the formation of granules which are closely related to mucin. Mast leucocytes of the rabbit are, therefore, not true granulocytes and are not derived from myelocytes of the bone-marrow.<sup>1</sup>

Pappenheim's students Benacchio ('11), Kardos ('11), and St. Szécsi ('12) came to similar conclusions with reference to the mast leucocytes of the guinea-pig and the rabbit. They could find no mast myelocytes in the marrow of either of these animals; they therefore concluded that the mast leucocytes are not true granulocytes. They believe that the mast leucocytes of the guinea-pig are merely eosinophil leucocytes whose granules have remained in an unripe basophilic condition. They claim that they can find all the intermediate stages between these so-called mast leucocytes and the ripe eosinophil leucocytes whose granules have an acid staining reaction. Benacchio concluded that all of the myelocytes with basophilic granules in the marrow of the guinea-pig and rabbit were either unripe eosinophiles or special cells. In other words, he believed that all of the granulocytes with basophilic granules were destined to differentiate either into eosinophiles or into special cells, and that mast cells are not present in the marrow of these animals.<sup>2</sup>

<sup>1</sup> Pappenheim came to similar conclusions. His views, however, are based largely upon the work of Pröscher.

<sup>2</sup> Pappenheim and St. Szécsi also believe that mast leucocytes are not represented in the marrow of the rabbit. "Die sog. Blutmastleukozyten stammen natürlich aus dem Knochenmark, aber z. T. sind sie keine eigentlichen Mastzellen, sondern nur unreifkörnige sonstige Granulocyten, deren Granula andere chromophile Reaktion hat, z. T., soweit sie eigentlichen Blutmastzellen sind, bilden sie sich aus Lymphoid-zellen wohl erst im Blut selbst oder unter pathologischer Einwirkung (Myelose)."

In a recent paper Maximow ('13) has shown that mast myelocytes are present in the bone-marrow of man, and that they are actually seen undergoing mitosis. Maximow, therefore, believes that the granules of haematogenous mast cells cannot be products of the degenerating nucleus or spongioplasm. He could never find any evidence for the degenerative processes described by Weidenreich and Pappenheim. Maximow was able to trace the differentiation of the granules and the evolution of the cells from the typical myelocytes and believes, therefore, that the haematogenous mast cells are true granular leucocytes which are equivalent to the other types of granulocytes of the blood and marrow. Maximow is also the chief exponent of the theory that the mast leucocyte of the rabbit is a true granular cell, which is in all respects equivalent to the human mast cell. He found nothing that would lead him to conclude with Benacchio, Kardos, and others that the mast leucocyte is not differentiated in the bone-marrow.

Maximow's observations on the origin of the mast leucocytes are of the greatest importance, but his observations should be confirmed by further studies, since he maintains that the mast leucocytes do not arise in the circulating blood from altered lymphocytes, but are differentiated in the marrow from certain specific, characteristic, basophilic granulocytes. Downey's<sup>3</sup> recent studies ('13) on the mast leucocytes of the guinea-pig have resulted in the complete corroboration of Maximow's findings. He finds that the granules of mast leucocytes can always be distinguished from those of eosinophil and special myelocytes, even though they are subject to slight changes in size and shape. My observations on the mast leucocytes of the rabbit, which were carried on under the direction of Professor Downey, and to whom I wish to extend my most sincere thanks, are also a further confirmation of Maximow's results.

It is a well known fact that the early myelocyte stages of eosinophiles and special cells have a primitive or 'prodrumale' granulation which is decidedly basophilic when first differentiated.

<sup>3</sup> A preliminary report was published in the Proceedings of the American Association of Anatomists, The Anatomical Record, 1914, vol. 8, no. 2.

According to Pappenheim ('12) this primitive granulation is supposed to have nothing to do with the final eosinophilic or special granulation which is developed later. Pappenheim believes that this primitive granulation is basophilic, but that it disappears when the specific granulation develops later. The latter is also basophilic when it first forms. According to Maximow ('13) the primitive granulation is azurophilic. Downey has shown that in the guinea-pig histogenous mast cells are derived from a type of cell similar to the clasmatocyte with a primitive granulation. Whether the primitive granulation disappears or becomes the final mast cell granulation is not known.

Maximow and Pappenheim have called attention to the very decided basophilic quota of young eosinophil and special granules in the eosinophiles and special cells of the rabbit. Bone-marrow of the rabbit, prepared according to Pappenheim's<sup>4</sup> method, show the preponderance of basophilic granules in eosinophil and special myelocytes very well. The granules are seen to vary in size, but are generally rounded or slightly irregular and show no definite arrangement within the cell body. All of the granules when first formed have a strong affinity for basic aniline dyes, in which respect they resemble the basophilic granules of mast cells. Other cells, however, whose general character is similar to these contain a few granules which are intermediate in staining reaction, having an affinity for both the acid and basic component of the staining mixture which gives these granules a mixed tone. Cells can also be found in which the number of basophilic granules is greatly reduced with a corresponding increase in the number of the intermediate granules. This change of staining reaction in the basophilic granule suggests that the early myelocyte with basophilic granules is being differentiated into a cell in which the granules are acidophilic, and shows that granules of this type are not true mast granules.

Benacchio has made similar observations; however, he goes further and concludes that the myelocytes with basophilic granules, similar to those described above, are the only type of

<sup>4</sup> *Folia Haem, Archiv., Bd. 13.*

basophilic myelocyte present in the marrow of the rabbit; in other words, that all of the myelocytes with basophilic granules are destined to differentiate into eosinophiles and special cells.

Kardos, in working with sections of bone-marrow fixed in 100 per cent alcohol and Helly's mixture, found neither mast cells, nor cells of any kind which contained basophilic granules. Paraffin sections and smears were studied in the present investigation, but with decidedly different results from those obtained by Kardos. In sections (material fixed in 100 per cent alcohol and stained in alcoholic thionin) basophilic myelocytes are just as numerous as they are in the bone-marrow smears prepared according to Pappenheim's method. The alcoholic material shows practically the same conditions as are seen in the bone-marrow smears. Sections stained in May-Giemsa show many cells which contain basophilic granules only, while others contain both basophilic and eosinophilic granules, and in still other cells all the granules are decidedly acidophilic. Furthermore, and in direct opposition to the findings of Benacchio and Kardos, it is possible to demonstrate in these same preparations and in smears also, a second type of basophilic myelocyte in the marrow of the adult rabbit. This is the mast myelocyte or the precursor of the mast leucocyte. Scattered throughout the section one sees numerous cells which contain a variable number of granules; the granules have a remarkable avidity for basic aniline dyes. These granules are metachromatic as well as basophilic, in fact, the metachromasia of the granules is so pronounced that they can neither be over-looked nor interpreted as the ordinary basophilic granule of the eosinophil and special myelocyte. The size and shape of the metachromatic granule, and the configuration of the nucleus are very suggestive of the mast leucocyte of the blood. On closer investigation and observation their identity is at once apparent.

In the marrow of the adult rabbit, in addition to the fully differentiated mast leucocytes with a more or less polymorphous nucleus, all intermediate stages between them and their myelocytes can be followed out. In the early myelocyte stages the nucleus is round, but later it becomes polymorphous. A dis-

tinctive feature of the mast myelocyte, as pointed out by Maximow, is its very thick nuclear membrane. The writer can also add in further support of Maximow's statement, that eosinophil and special myelocytes usually occur in groups, while mast leucocytes and mast myelocytes appear more or less scattered throughout the section.

Mast myelocytes are well preserved in bone-marrow smears fixed in lucidol-acetone and stained in either alcoholic thionin or May-Giemsa. For fixation the solution devised by St. Szécsi<sup>2</sup> is used. Smears of fresh bone-marrow were made by rolling a small piece of marrow over a chemically clean cover-slip. Without allowing the smears to dry in the least they were immediately placed into a covered dish containing the lucidol-acetone fixative. At the expiration of fifteen minutes the smears were removed from the lucidol-acetone mixture, transferred without drying to another covered dish containing a mixture of acetone and xylol, three parts of the former to two parts of the latter. St. Szécsi states that the object of using this mixture is to dissolve the lucidol crystals, and clear the preparations, ten minutes are sufficient to complete the process. Finally the smears are placed in methyl alcohol, from one-half to one minute. Bone-marrow smears, provided that the smear is not too thick, are well fixed after being subjected to the action of the lucidol-acetone.

In view of the fact that several modern haematologists have denied the presence of mast leucocytes in the bone-marrow of the rabbit, the lucidol-acetone preparations are of particular value and interest. After seeing a single preparation there can be no doubt as to the presence of mast myelocytes and mast leucocytes in the marrow of this animal. A single preparation usually shows great numbers of mast cells. In a single field I have often counted as many as six fully differentiated mast leucocytes.

The mast myelocyte is such a distinctive type of cell that it is easily distinguished from eosinophil and special myelocytes. In lucidol-acetone preparations stained with May-Giemsa the granules of mast leucocytes stain an intense bluish black, while the

<sup>2</sup> His method of procedure appeared in the *Deutschen Medizinischen Wochenschrift*, no. 33, 1913.

basophilic granules of eosinophiles and special cells are of a reddish black tinge. The sharp contrast in the staining reactions of the mast myelocyte as compared with the eosinophil and special myelocyte is so pronounced and so characteristic that every mast cell is easily separated from the eosinophil or special myelocyte.

Of the various methods tried none gave sharper pictures for the demonstration of mast cells than did the lucidol-acetone fixation. The basophilic granules of the mast leucocyte are very well preserved. In some instances the cell body is so filled with the basophilic, metachromatic granules that the outline of the nucleus is extremely difficult to follow.<sup>6</sup> In cases, however, where the exact outline can be seen, I find that the nucleus is typically polymorphous and shows no similarity to a lymphocyte nucleus, neither does it possess lymphocyte characters nor show signs of degeneration. My preparations showed nothing to support Pröscher's theory that the mast leucocyte is derived from a lymphocyte and that the nucleus remains practically identical with the lymphocyte nucleus. In all probability Pröscher based his theory on the early, basophilic, mononuclear myelocytes of eosinophiles and special cells. At any rate, the technique which he used was such that the granules of mast leucocytes would not be preserved.

The lucidol-acetone preparations show further that the basophilic granules of mast leucocytes vary in form, size, and in number as previously stated. In the rabbit the granules are fine, usually rounded or slightly irregular. As far as the mast leucocyte of the rabbit is concerned there is no evidence to show that the nucleus is concerned in the elaboration of the granules, as is claimed by Weidenreich for the mast leucocyte of man. Pröscher also claims that the nucleus takes no active part in the elaboration of granules.

Maximow's method of fixing bone-marrow in 100 per cent alcohol followed by staining in alcoholic thionin or May-Grünwald was also tried. These preparations also show that the

<sup>6</sup> A more detailed description of these cells, with figures, will appear in the final publication (*Folia Haematologica*).

mast leucocytes are present in the marrow and that the staining reactions are very characteristic. It is not the object of the writer to re-describe Maximow's results with this method.

In previous work on the mast leucocytes of the rabbit, Maximow has called particular attention to the fact that the granules of these cells are extremely soluble in water, and has cautioned against using watery fixatives and watery stains. The writer found that after fixation in Helly's mixture no mast granules could be detected with any of the various stains used. This would indicate that the mast granules are soluble in water. However, after alcohol and lucidol-acetone fixation, the granules are able to resist the short exposure to water to which they are subjected while being stained in the Giemsa solution. In the material fixed in Helly's mixture, however, the granules are exposed to the action of water for a long period of time which is sufficient to dissolve them. It is obvious that only those methods of technique which preserve the granules will be of real value in determining the origin of mast leucocytes, since the cells are difficult to recognize after their granules have been dissolved out. Little heed has been given to Maximow's repeated warning as to the solubility of the granules in water, and in all probability this accounts for the fact that Benacchio and others have failed to find mast leucocytes in the marrow of the rabbit.

#### SUMMARY

The bone-marrow of the rabbit contains true mast myelocytes with basophilic granules in addition to the myelocytes of eosinophiles and special leucocytes whose granules are also basophilic. With ordinary methods all of the myelocytes with basophilic granules seem to belong to the latter two types of leucocytes, but after fixation in alcohol, or better in lucidol-acetone, the granules of the true mast leucocytes are also preserved. Their distinctive characters are such that they can always be distinguished from the basophilic granules of the eosinophil and special myelocytes.

The general life history of the mast leucocyte runs parallel to that of the other granular leucocytes of the bone-marrow. Their granules are differentiated gradually out of the basophilic cytoplasm of mononuclear cells. The granules are strongly basophilic from the moment of their first appearance and remain so throughout the life-history of the cell. As the number of granules increase the nucleus gradually changes shape, becoming distinctly polymorphous in the fully differentiated cell.

Fully differentiated mononuclear mast leucocytes are never found in the blood or marrow of the adult rabbit. These cells, therefore, do not show the relationships to lymphocytes of the circulation described by Pröscher and others, and they are never differentiated from the lymphocytes of the circulating blood in the normal animal.

When the proper methods of fixation have been used the mast leucocytes of the rabbit show no evidence whatever of degenerative changes. Their granules are, therefore, not products of a mucoid degeneration of the spongioplasm of lymphocytes (Pröscher, Pappenheim and others), but are formed by the progressive differentiation of the cytoplasm of mononuclear cells of the bone-marrow.

The haematogenous mast cells of the rabbit form a distinct and independent line of granulocytes which is in no way related to the eosinophil or special leucocytes excepting through the non-granular parent-cell of the bone-marrow.

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# A NOTE ON THE COURSE AND DISTRIBUTION OF THE NERVUS TERMINALIS IN MAN

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TWO FIGURES

Johnston ('13) was the first observer to determine the presence of the nervus terminalis in man. He first reported its occurrence in human embryos and later ('14) described the nerve for the adult. Brookover ('14), working independently, also observed the presence of this nerve in adult man. Apparently the material used by these authors permitted only of the examination of a portion of the intracranial course of this nerve. It is the purpose of the present paper to report observations on the intracranial course and nasal distribution of the nervus terminalis in man.

The observations about to be reported are based on gross dissections of prepared specimens of the heads of several human fetuses varying in age from ten weeks to the newborn. Two adult heads were examined. The nervus terminalis was identified in all the specimens. Drawings were made from the two most favorable dissections. Figures 1 and 2 represent such drawings. The former represents the medial sagittal dissection of the head of a six-months human fetus, the latter a similar dissection of a ten-weeks human fetus. For purposes of dissection the specimens were prepared as described by the writer ('12) in a previous communication.

The intracranial portion of the nervus terminalis, as shown in figure 1, appears on the surface of the brain in the region of the olfactory trigone and courses anteriorly over the medial surface of the olfactory tract and bulb and on to the lateral surface of the crista galli, to pass through foramina in the cribri-

form plate well forward. In its course over the medial surface of the olfactory tract it will be seen that the nerve forms a compact bundle of nerve fibers. On the medial surface of the olfactory bulb, however, it breaks up into a close plexus of fibers intimately associated with the fila olfactoria. It forms

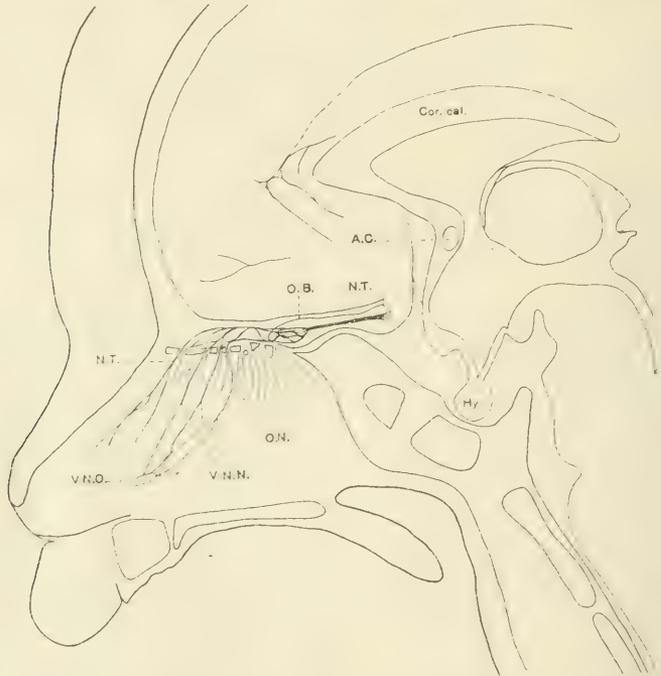


Fig. 1 Medial section of the head of a six-months human fetus with the nasal septum removed, showing the origin, course and distribution of the nervus terminalis. *Cor. Cal.*, corpus callosum; *A.C.*, anterior commissure; *N.T.*, nervus terminalis; *O.B.*, olfactory bulb; *O.N.*, olfactory nerves; *V.N.N.*, vomero-nasal nerves; *V.N.O.*, vomero-nasal organ; *Hy.*, hypophysis.

a loose plexus on the lateral surface of the crista galli imbedded in the layers of the dura mater. In this position the separated filaments of the nervus terminalis lie some distance dorsal to the cribriform plate of the ethmoid bone instead of lying directly on its upper surface as do the fila olfactoria. In the specimens examined the height to which the nerve attains on the lateral surface of the crista galli or the amount of arching upward of

the filaments of the nervus terminalis in this region, depends apparently upon the degree of development of the crista galli.

The distribution of the nervus terminalis to the nasal septal mucosa is similar to that described by Huber and Guild ('13) for the rabbit. Within the cranium filaments of the nervus terminalis join the olfactory and the vomero-nasal nerves and

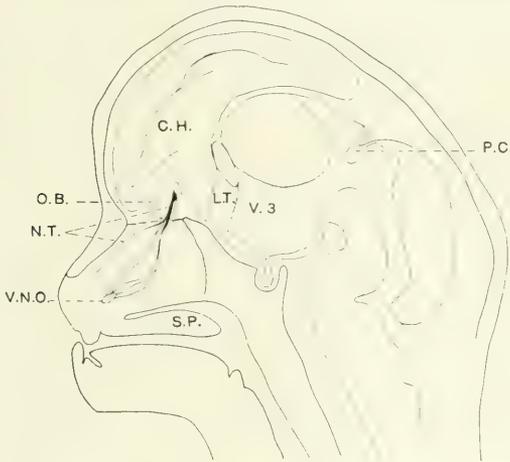


Fig. 2 Medial section of the head of a 4.5 cm. human embryo, with the nasal septum removed to show the origin, course and distribution of the nervus terminalis. *C.H.*, cerebral hemisphere; *L.T.*, lamina terminalis; *N.T.*, nervus terminalis; *P.C.*, posterior commissure; *O.B.*, olfactory bulb; *S.P.*, soft palate; *V.3*, third ventricle; *V.N.O.*, vomero-nasal organ.

apparently pass to the septal mucosa with them. The majority of the fibers, however, form a single strand and pass through the cribriform plate anterior to the exit of the vomero-nasal nerves. Upon reaching the nasal cavity the nervus terminalis takes a path anterior to that of the vomero-nasal nerves, lying just posterior to the antero-superior border of the nasal septum. In figure 1 it is represented as breaking up into three main filaments which can be traced downward nearly to the level of the vomero-nasal organ. In the first part of its nasal course it is joined by a small filament from the medial nasal branch of the anterior ethmoid nerve.

In figure 2 the long axis of the olfactory tract and bulb occupies a plane approaching the perpendicular instead of the horizontal, as is shown in figure 1. The nervus terminalis appears on the surface of the brain in relatively the same position as in figure 1 and passes directly downward to the cribriform area where it lies in close proximity to the vomero-nasal nerves. After sending a few strands to accompany the vomero-nasal nerves the larger portion of the nervus terminalis passes through the cribriform area and is distributed to the septal mucosa anterior to the path of the vomero-nasal nerves.

In conclusion it may be stated that on account of the relation of the nervus terminalis to the crista galli, where the latter is sufficiently developed to cause a stretching out, as it were, of the overlying dura mater with its contained nerve, the continuity is here usually lost in gross dissections and the fibers associated with the vomero-nasal and olfactory nerves alone remain to determine its distribution to the septal mucosa.

The distribution of the nervus terminalis in man as in the rabbit is mainly to the mucosa of the nasal septum anterior to the path of the vomero-nasal nerves. Their ultimate terminations could not be determined.

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## ON WEBER'S METHOD OF RECONSTRUCTION AND ITS APPLICATION TO CURVED SURFACES

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FIVE FIGURES

In embryological work it is sometimes a matter of importance to determine with accuracy areas of epithelial thickening which are not demonstrated satisfactorily by the ordinary methods of graphic or plastic reconstruction. Placodal thickenings of the skin ectoderm, thickened zones in the neural tube, and the thickened areas found in the early archenteron are examples of structures which are not well demonstrated by these customary methods. A method of reconstruction which brings out graphically the extent and comparative thickness of such areas was devised some years ago by A. Weber,<sup>2</sup> and was used by him with much success in the study of the very early development of the great glands of the digestive tract. Weber first published an account of his method in 1902,<sup>1</sup> and again a shorter summary in connection with the final report on his work in the following year.<sup>2</sup> Apparently the method has not been employed elsewhere. I have found it of such interest in the study of the earlier stages of the pancreas and liver that it has seemed desirable to present it here with some modifications which may be found useful, particularly in its application to curved surfaces.

Weber's method is based upon that of graphic reconstruction from transverse sections. An outline (from either lateral or dorsal view, as desired) of the organ to be reconstructed is plotted

<sup>1</sup> Une méthode de reconstruction graphique d'épaisseurs et quelques-unes de ses applications à l'embryologie. *Bibliog. Anat.*, T. 11.

<sup>2</sup> L'origine des glandes annexes de l'intestine moyen chez les vertébrés. *Arch. d'Anat. Micr.*, T. 10.

out on transverse section lines in the customary manner. Instead, however, of completing the reconstruction by indicating the *contour* of the surface thus outlined (as is commonly done), the *thickness* of the wall which forms that surface is measured in the transverse sections, and the variations in this thickness are plotted out upon the reconstruction. The reconstruction will then bear a number of lines which mark the boundaries between areas of epithelium of different thicknesses. To finish the reconstruction, the areas thus outlined are filled in with different shades of a single color, the darker shades being used for the thicker areas of the epithelial wall. The finished reconstruction then will exhibit the outline of the structure and the variation in the thickness of the part of its wall which is shown in this particular view. It will give no conception of the surface modeling of this wall aside from what can be determined from the outline alone.

The picture obtained is much the same as would be secured were it possible to remove the wall of the structure, render it translucent and, magnifying it greatly, hold it before a bright light. The thicker portions of the wall would then appear to the observer as darker areas, as they do in the reconstruction.

Figure 1 is a graphic reconstruction of the left side of the archenteron of an embryo of *Torpedo ocellata*, 4.0 mm. in length (No. 765 of the Harvard Embryological Collection). The portion of the gut lying on either side of the anterior intestinal portal and included between the lines *A* and *B* has been reconstructed by Weber's method and is shown in figure 4. The latter figure shows by means of its coloring a broad band of thickened epithelium extending dorso-ventrally across the gut at the level of the anterior intestinal portal. The lower and thickest part of this band represents the anlage of the gall bladder and liver, while the upper part includes the future pancreatic region. A thickened spur extends backward from this zone, and marks off the line along which the intestine will eventually separate from the yolk-stalk ventral to it.

The method of preparation of such reconstructions can best be explained in detail by following an example of the process.

For this purpose I will use the reconstruction shown in figure 4 which has just been described.

In preparing this reconstruction, drawings were made of each section of the portion of the gut involved, although fairly satisfactory results can be secured with drawings of every other section. These drawings should be made at a high magnification, preferably over 300 diameters. As the sections are drawn, they show of course the curvatures of the contour of the archenteron wall. It is desirable to eliminate these curvatures in the reconstruction and to present the gut wall as an approxi-

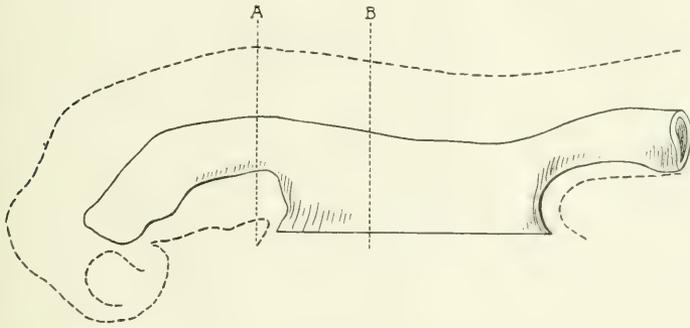


Fig. 1 A graphic reconstruction (lateral view) of a portion of the archenteron of an embryo of *Torpedo ocellata* 4.0 mm. long (H.E.C. 765).  $\times 30$ . The outline of the embryo is represented in broken line. The archenteron is drawn in solid line. The portion of the archenteron lying between the dotted vertical lines A and B is shown in a Weber's reconstruction, at higher magnification, in figure 4.

mately flat plane. If this is not done, areas which project sharply from the general plane of the archenteron will be represented in the reconstruction as much narrower than they actually are in the specimen or, if small, may be lost entirely. To eliminate these curvatures, it is necessary to divide the outer margin of each section into a number of short cords or segments, each of which will be comparatively straight, and to lay off segments of an equal length on the transverse section lines of the reconstruction. This can be done with a pair of small screw compasses. For work at a magnification of 300 diameters and over,

segments of 1 cm. are small enough to eliminate most of the error from curvature. Figure 2 is of a section (No. 104) of the reconstruction under discussion. The short lines on the inner side of its margin represent the boundaries of these centimeter segments.

The thickness of the epithelium forming the gut wall is now to be measured in each drawing. For this purpose a unit of measurement must first be determined. Working with drawings made at a magnification of 300, it has been found that the 1.5 mm. is the smallest unit practicable for such a scale. By using this unit one is able to measure variations of less than 5 micra in the thickness of the gut wall, and errors of projection and drawing would probably render more refined measurements of little value. A scale of 1.5 mm. units is laid out upon a stiff card, or better, a piece of transparent celluloid. This scale or gauge is then passed over each section, care being taken to keep its graduated margin at right angles to the *axis* of each segment of the drawing and its zero point at the inner margin of the epithelium. This process is begun at the dorsal median line on each section and is carried laterally or ventrally from that point. At the first place where the thickness of the epithelium is found to correspond to a graduation point on the scale, a fine line is drawn out to the side of the section and the thickness noted at its end. The scale is carried along the section until a point is reached where the thickness of the epithelium corresponds with the next graduation (either above or below the former one) on the scale. Again a line is drawn out from the section at this point and the thickness noted. This process is continued until the entire side of the section has been gauged and marked off into segments in which the variation in thickness is not over 1.5 mm. on the drawing or 5 micra in the corresponding section. An example of a section thus gauged is shown in figure 2. As shown by the gauge lines, the epithelium immediately below the dorsal median line is between 30 and 25 micra (6.5 mm. at  $\times 300$ ) in thickness. The thickness falls to 25 micra at the point indicated. This is followed by a broad zone which is less than 25, but over 20 micra thick. Ventral to this zone, the epithelium increases to

a thickness of between 35 and 40 micra, and then again decreases to less than 10 micra as it approaches the blastoderm. The arrows seen in the figure point towards the thinner edge of the segment and are used to avoid confusion in mapping out the gauge lines on the reconstruction at a later time. It is impor-

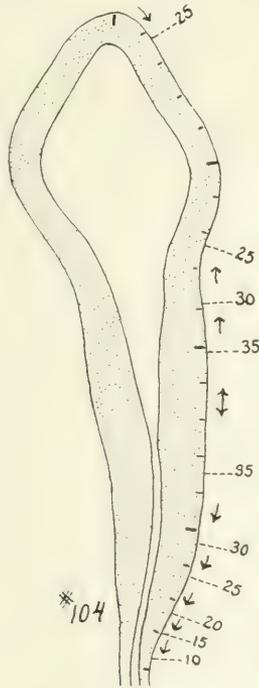


Fig. 2 Drawing of a transverse section of the archenteron of a *Torpedo* embryo 4.0 mm. long (H.E.C. 765) showing the method of measuring sections for reconstruction by Weber's method. The short lines extending into the section mark the centimeter segments used in eliminating lateral curvature from the section. The longer lines extending out to the right from the section are the gauge lines. The figures at the end of the gauge lines indicate in micra the thickness of the epithelium at the points touched by them.

tant that in gauging the section drawing the scale be held at right angles to the long axis of that particular part of the wall which is being measured rather than at a similar angle to either the inner or outer surface of the epithelial strip. This holds particularly when gauging the thickness of an epithelial band which

is rapidly changing in caliber, or when gauging a portion of a band which forms a sharp curve. Failure to observe this precaution causes noticeable error both in the gauge readings and in the position of the gauge points on the drawing. There will often be encountered considerable portions of the wall, the thickness of which corresponds exactly to the gauge unit or a multiple of it. My rule, made arbitrarily, has been to mark as the gauge point the first (i.e., most dorsal) level at which such a zone is encountered.

A reconstruction outline is now made on section-lined paper in the usual manner, except that only the dorsal margin of the gut is mapped out. Using this margin line as a base, the transverse section lines are divided into centimeter segments corresponding to those made on the margins of the drawings of the sections. In practice it is convenient to mark the point separating each block of 5 cm. segments in a different color to aid in plotting. The ventral margin of the gut and the gauge points, which have been determined on the cross section drawings, are now plotted on the transverse lines of the reconstruction in the usual manner, except that instead of measuring the distance of each point from the dorsal margin of the section and transferring this measurement to the corresponding section line as is customary, one measures the distance of the point from the nearest centimeter mark in each case. The ventral outline of the reconstruction and the gauge lines indicating the thickness of the epithelium are established by connecting all the points of the same order as is done in an ordinary graphic reconstruction. The reconstruction will now have the form seen in figure 3. There the base and transverse reconstruction lines are lightly drawn and the centimeter segment points are indicated as small dots on the latter. The outline of the reconstruction is indicated in heavy line. The ventral margin posterior to the anterior intestinal portal has been cut away arbitrarily in a straight line. The gauge lines indicating the thickness of the epithelial wall of the organ are represented in heavy broken line. The vertical figures placed at the termination of the gauge lines indicate their values in micra.

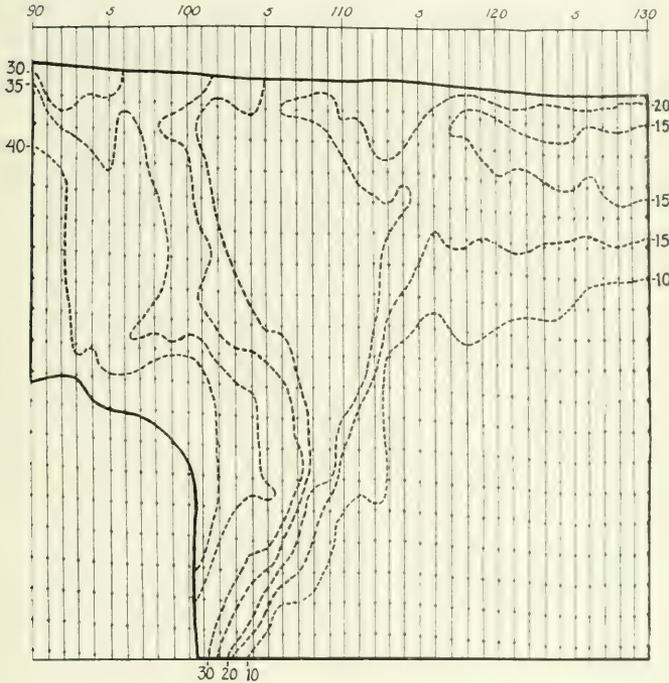


Fig. 3 Reconstruction plot of a Weber's reconstruction (lateral view) of the portion of the archenteron bounded by the lines *A* and *B* in figure 1. The light vertical lines represent the transverse sections. The outline of the reconstruction is represented in heavy solid lines. The gauge lines bounding areas of epithelium of different thicknesses are represented in heavy broken lines. The dots on the transverse lines represent the boundaries of the centimeter segments described in the text and shown in figure 2. The vertical figures at the margins of the reconstruction give the value of the gauge lines in micra. The figure is reduced to one-half the size of the original reconstruction, which was made at a magnification of 350.

There remains but to color in the areas which are marked off by the gauge lines. Weber did this with water-color and secured excellent results, as his figures show, but a much simpler and quite as satisfactory a method is to use papers of different shades of gray for the different areas. Such papers should be as near 'pure' mixtures of black and white as it is possible to secure. The only kind which I have found satisfactory is the 'Herring' series which has been prepared for color work in psychological

laboratories. The shades numbered 3; 4, 7, 10, 16, 20, 30, 42 and 46 make a satisfactory series of marked but fairly equal gradations.

To build up the final colored reconstruction gray papers are selected, equal in number to the areas mapped off on the reconstruction by the gauge lines. The entire outline of the reconstruction is now traced upon the lightest colored paper. This area is cut out and pasted firmly upon a piece of compo or plaster board. Upon the paper of the next darker shade there is traced an outline similar to the preceding except that the space representing the thinnest area is cut away. This second sheet is pasted upon the first one so that the similar angles and sides correspond. In this way the area of thinnest epithelium is represented by the lighter colored paper and all thicker areas by the darker one. This process is continued, all thinner areas being cut away from each new outline until the darkest and final shade of paper will have the shape and will represent the area of the thickest epithelium only. This method of building up the papers of different colors in strata will be found much easier than to cut out each separately and then attempt to fit them together in a mosaic. Figure 4 is a half-tone made directly from such a colored reconstruction and based upon the plotting illustrated in figure 3.

The example just described is of a lateral view reconstruction. Reconstructions may be made by Weber's method to show dorsal or ventral views of epithelial structures as well. For this purpose the transverse sections of the structure are drawn and measured in the same manner as that described. A reconstruction plat is then laid out as follows. A vertical line is drawn in the middle of the sheet to represent the dorsal median line of the structure and transverse section lines are drawn on either side at the proper distance apart and at right angles to the median vertical one. The centimeter points, which on lateral view reconstructions must be measured off on each transverse section line, can be located on the plat in this case by simply ruling vertical lines parallel to and at centimeter intervals from the median one. The gauge points and lines marking the boundaries



Fig. 4 A finished Weber's reconstruction (lateral view) made from the plat represented in figure 3 and including that part of the archenteron lying between the lines *A* and *B* in figure 1. The thicknesses of the epithelium in several parts of the reconstruction are represented by the various shades of gray. Starting with the lightest, these several shades represent the following epithelial thicknesses; (1) below  $10\mu$ ; (2) 10 to  $15\mu$ ; (3) 15 to  $20\mu$ ; (4) 20 to  $25\mu$ ; (5) 25 to  $30\mu$ ; (6) 30 to  $35\mu$ ; (7) 35 to  $40\mu$ ; (8) above  $40\mu$ . This figure is reduced to three-fifths the size of the original reconstruction.

between areas of different epithelial thickness are mapped out as in the lateral-view reconstruction. Should the reconstruction be of a tubular structure, the ventral median line must be determined in each cross section drawing. In reconstructing, the tube is then represented as split along its ventral median line and flattened out laterally; i.e., the lateral margins of the reconstruction represent in fact the ventral median line of the original tube. Figure 5 is an example of such a reconstruction plat made from the same object employed for the lateral view reconstruction already described. The method of representation and lettering are the same as used for figure 3.

Several of the possible uses and advantages of this method of reconstruction have been mentioned. It remains to speak of a few disadvantages and sources of error. In the first place the outlines of the reconstructions, aside from the one used as a base line, are not strictly such as would be secured by the ordinary graphic or plastic methods. In eliminating the lateral curvatures from the transverse sections, the dimensions of the figure are increased dorso-ventrally or laterally, as the case may be, without a similar increase antero-posteriorly. Weber made no attempt to eliminate the curvature seen in cross sections, regarding it in fact as of some value in indicating contour. I have already pointed out the disadvantage in reconstructing without making this correction, which I think should be done even at the expense of some accuracy of outline, which can easily be determined by the other reconstruction methods.

While the error introduced by this method is small when dealing with structures having surfaces approximating those of cones or cylinders, it is considerable when applied to spherical surfaces. Spherical surfaces do not admit of being spread out into planes as do those of cones and cylinders. Cartographers, who have much this same problem in representing large areas of the globe, have developed a number of methods of projection to meet, in part, this difficulty. These are, however, too complex for our work and are all based upon representations of the perfect sphere. For the purpose at hand surfaces which approximate spherical ones can best be treated by first compensating for the vertical curvature by the method of segmenting the outline of the section already described; and, second, by allowing for longitudinal or horizontal curvature by increasing the distance separating the cross section lines on the reconstruction plat. The latter can be done by determining the actual length of the outline of the structure to be reconstructed and dividing this distance by the number of sections which the structure contains. Multiplying the figure thus secured by the magnification at which the reconstruction outlines are drawn gives one the distance which should separate the section lines on the reconstruction plat. This practice differs from that of ordinary reconstruction in

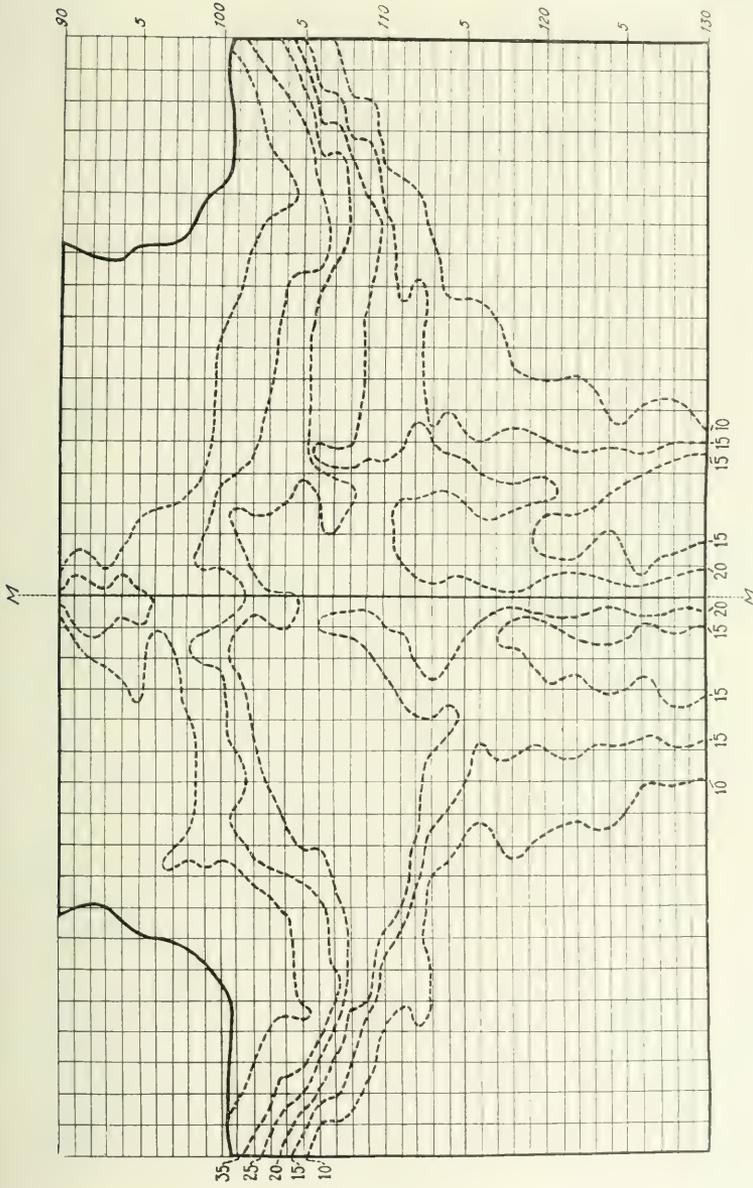


Fig. 5 Reconstruction plot of a dorsal view reconstruction of the same object as shown in figures 1, 3 and 4. The line *M-M* represents the dorsal median line of the archerfish. The lighter lines parallel to it are the boundaries of the centimeter spaces. Scheme of drawing and lettering corresponds to that of figure 3. The scale of magnification is the same as for figure 3.

that the longitudinal axis of the structure is used in the customary method instead of the actual length of the outline as in this case. Reconstructions made with these corrections will show approximately the area and shape of any given outline upon a curved surface, although neither will be strictly accurate. As a rule such correction will be unnecessary unless one is dealing with surfaces which curve very abruptly. Measurements of the thickness of epithelial plates which curve longitudinally or horizontally will always be a little exaggerated because such plates are cut somewhat obliquely by transverse sections. There seems to be no practical way of eliminating this error.

Finally, the reconstruction will represent the variations in thickness of the epithelium as occurring in definite steps and not as gradual transitions as in nature. Most of this artificial distinction can be eliminated by using the smallest unit of measurement practicable and thus increasing the number of shades present in the reconstruction while decreasing the degree of their difference. Great reduction of figures in their reproduction also aids in securing the effect of gradual transition.

# ON THE STRUCTURE OF THE ERYTHROCYTE

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FOUR FIGURES

This paper was undertaken with the hope of contributing something as to the structure of the so-called stroma of erythrocytes and as to the presence and character of a capsule or cell-membrane belonging to them.

The fact that the mammalian red blood corpuscle upon losing its nucleus becomes biconcave, its peripheral ring remaining thicker than its central region from which the nucleus has been lost, has always suggested the existence of a supporting framework. Were the corpuscle merely a hemoglobin-carrying sac borne in the blood stream, the natural tendency would be for it to assume a spherical form, the pressure on all sides being equal. The mammalian erythrocyte after being carried through capillaries smaller than its diameter, and after being drawn around angular curves of capillaries, which may stretch and modify its form considerably, always resumes its original form when returned to larger capillaries. This not only suggests a supporting frame-work for its content but also that this framework is plastic or even possesses certain elasticity.

The question of a membrane about the corpuscle is most disputed in the discussions. The most frequently advanced theory against the existence of such is that the corpuscle possesses no membrane at all but is merely covered by a film of lecithin or other lipid substance acquired from its environment, and Norris (*Physiology and pathology of blood '82*) even suggests evidence that fluid droplets enclosed by lipoids (myelin) tend to assume flattened shapes, while when enclosed by films of ordinary fats they are invariably spherical. The very probable presence of a film of some lipid or fat surrounding the entire corpuscle is

seldom disputed and is accepted here. This is indicated by and explains the phenomena of forming into rouleaux and is supported by other arguments as well. But the presence of such a film need not disprove the existence of a membrane or capsule as a structure belonging to the corpuscle itself and which may be covered without by a film of lipid substance. Fluid droplets enclosed in lipid or fat films when shaken will become divided into smaller droplets and droplets of greatly varying size, whereas the erythrocytes of normal blood are strikingly uniform in size and while shaking them may break up or burst many of them, fragments resulting do not assume the form of the original corpuscle. Further, it seems very improbable that a film of lipoids alone would result in the folded and crumpled appearances shown by the so-called 'crenated corpuscles' resulting from extraction of a portion of the content. On the contrary, it would seem that such a film, still in the blood plasma, would merely thicken instead of becoming folded. Also, crenated corpuscles have lost the biconcave form, do not have the flattened shape suggested by Norris for fluid droplets surrounded by a film of lecithin. Crenation is suggested here as indicating the presence of a membrane intrinsic to the corpuscle which has become folded due to partial extraction of the original content by exosmosis, the crenated corpuscle becoming approximately spherical, due to a disturbance or destruction of its original internal structure by the process. The chemical compound, hemoglobin, so far as is known, has no anatomical structure. It is a complex organic compound in solution capable of various degrees of dilution. The loss of the biconcave form in crenation and the assumption of the spherical form in swelling from the action of distilled water, for example, suggest the destruction of a framework in which the hemoglobin was supported and by which the biconcave form of the nonnucleated corpuscle was maintained.

The original cell, the typical cell, possesses a framework of spongioplasmic filaments, a cytoplasmic and karyoplasmic reticulum, in the meshes of which are the more fluid portions (hyaloplasm) and the various forms of granules comprising the structure and content of the cell. In the functional differentiation

of certain cells a marked increase in the evidence of a cytoplasmic reticulum is well known. The neuro-fibrillae of the nerve cell, for example, consist of anastomosing filaments more abundant and more evident than in the germinal or neuroblast stage of this element. Certain functioning gland cells show a reticulum in their cytoplasm. Hardesty ('05) and Nemiloff ('10) have shown that the emulsion comprising the medullary sheath of the nerve fiber has its component globules suspended and supported by a delicate reticulum, Hardesty showing this reticulum to be continuous into the membrane or neurilemma without and into a thinner bounding membrane (axolemma) about the axone of the nerve fiber, and that this framework and these two membranes, and not the fat of the sheath, maintain the shape of the sheath. He considered the membranes of the sheath as condensations of the internal framework. It is very probable that the corpuscle of adipose tissue, the fat cell, possesses an internal framework continuous with its capsule, both maintaining its general shape. Schäfer (vol. 2, part 1, Quain's Anatomy, 11th edition, '12) cites the fact that an erythrocyte, that of the newt for example, may be cut into two without resulting exudation of its content. Like the medullary sheath, the muscle fiber, the nerve cell or the fat corpuscle, the erythrocyte is an example of an especially differentiated arrangement for the performance of a special function.

The findings in the literature dealing with the structure of the red blood corpuscles seem to vary according to the methods of preparation used by the various authors and according to the different interpretations of the results obtained by them. Of the very voluminous literature, many of the papers consulted indicate a lack of familiarity with histology in general and an incompleteness of investigation to an extent that they seem of no value here. However, some authors give definite statements as to what they deem the structure of the erythrocyte and a number have considered the subject thoroughly.

Renant in his Histology ('89-'93) says red blood corpuscles possess no true cell-membrane, but merely a peripheral condensation of the cytoplasm such as that formed about a cell when

exposed to air. Von Ebner (in Kölliker's Handbook, '02) states that a membrane must be assumed on the surface of the erythrocyte, a membrane insoluble in water and which allows osmosis, but which cannot be called a membrane in the ordinary sense and may be similar to the exoplasm of other cells. Löhner ('07) believes that a true histological membrane, similar to a cell membrane, is improbable. His method of preparation of the corpuscles for study consisted in crushing mammalian corpuscles on the slide. He also tried drying them in indifferent substances. He describes them as jelly-like and elastic in character with a thinner, more compact peripheral layer and a broader less compact inner region, similar to the exoplasm and endoplasm of Protozoa. If the outer compact layer is referred to as exoplasm, he thinks the superficial part of this may be termed a physical membrane or plasma-film. Dehler ('95) studied the red corpuscles of the chick, fixing them in sublimate solution and staining with iron-hematoxylin. He described the convex cells as possessing a sharp border or rind of .25 to .5  $\mu$  in thickness. Heidenhain ('96), working with the red blood corpuscles of *Proteus*, using the same method as Dehler, found the same appearances. Nicholas ('96), likewise using the same procedure, found the sharp border manifest on the erythrocytes of the chick, salamander, triton and viper.

Meves ('04 and '06) studied amphibian red blood corpuscles (frog and salamander). After condemning the technique used by Weidenreich and showing the results obtained by the latter were artifacts, he describes a very complicated procedure used by himself. In general this consisted of dried smears on the slide subjected to warmth for 30 minutes, then subjected to Flemming's fluid (weak formula) plus 1 per cent sodium chloride, washed in running water, stained with safranin, hematoxylin and safranin and Gentian orange, decolorized with neutral alcohol, and cleared and differentiated in clove oil. He describes the corpuscles as showing circumferential fibrils, which were disposed either in an arrangement parallel to the surface or in the form of a continuous skein, and radial fibrils running, some from the periphery toward the center of the corpuscle and some crossing each other, form-

ing a net. In some cases the net-work arose only in part from the peripheral arrangement. He refers to the circumferential fibrils as a membrane which is continuous with the fibrils of the network within, and he interprets the fibrils as resulting from linear arrangements of mitochondria, granules having fused to form them. He thinks the arrangement of the fibrils is similar to that described by Heidenhain for Krause's membrane in muscle fibers, the fibrils continuing into the membrane (sarcolemma) in a regular system. Schäfer ('05) suggests that Meves' circumferential or peripheral fibrils merely represent a part of the reticulum of the corpuscle.

Růžička ('03 and '06) worked with frog, guinea-pig and human blood. He stained with a dilute solution of methylen blue both without and after the action of 1 per cent pyrogallie acid, which latter he thought dissolved hemoglobin. The drop of blood was mounted in normal salt solution and the methylen blue solution (0.5 gram to 1000 cc. of water) added at the edge of the cover-glass. When used, the pyrogallie acid was applied followed by the methylen blue solution. Blood from the three sources gave the same results. All showed a fine meshed reticulum with occasional knobs at the junction of its filaments. He did not think an actual membrane is present but that the corpuscle is bounded by the reticulum. The knobs at the junction of the fibrils of the reticulum being found smaller and fewer after the action of pyrogallie acid, he assumed them to represent hemoglobin. He denied the possibility that the reticulum represented a coagulation product, thinking the meshes too fine and uniform and not arranged as coagulum filaments.

In the mammalian erythrocytes, Růžička also observed the previously described large granules dispersed in the center of the corpuscle, the so-called "nucleus of the mammalian corpuscle." Quoting Löwit ('87) as claiming a character for these granules in the rabbit similar to nuclear chromatin, he claims they are only present in case of incompletely dissolved hemoglobin and are thus analagous to the larger of the knobs described at the junctions of the filaments of the reticulum and therefore are not of chromatin nature.

Bryce ('04) studied the erythrocytes of the larvae of *Lepidosiren paradoxa*. He fixed the tissues with the blood vessels containing the erythrocytes *in situ* and used sections of  $10\ \mu$ . He used sublimate-acetic as a fixing fluid. This was tried in this laboratory but was found unsuited for the study of adult erythrocytes in that it precipitates hemoglobin. Bryce's illustrations show that at least in the erythrocytes of these larvae, not having acquired sufficient hemoglobin to color them, there is a reticular structure in the cytoplasm connecting with a membrane at the periphery, and he describes a meshwork or reticulum in them which was radially arranged from the nucleus to the periphery, the meshes of which in section were 3 to  $4\ \mu$  in size. At the nodal points or junctions of the filaments forming the meshes he found "strongly refractile granules of considerable size" and he states that in some of the corpuscles the filaments near the nucleus appear as arranged in parallel threads, extending from the nucleus a short distance toward the periphery. He does not pass judgment on the nature of the membrane observed, but states that he was dealing with very young corpuscles.

#### MATERIAL AND METHODS

As is known, the Amphiumae carry the largest red blood corpuscles of any vertebrate as yet examined. One of the species of this animal, the *Amphiuma* means (the 'blind eel') being easily obtainable in the ditches of New Orleans, a study of the structure of its corpuscle was suggested by Professor Hardesty. Measurements of its corpuscles in the fresh gave, measured on the flat, an average of  $72.9\ \mu$  in length by  $44.5\ \mu$  in width. With the same technique as finally employed for those of the *Amphiuma*, erythrocytes from the alligator, frog, snake, guinea-pig and human were also prepared and studied in comparison.

Obviously, a study of the internal structure of the erythrocytes of *Amphiuma* and those of other animals, or the study of membranes probably existing, could not be accomplished in any detail except with very thin stained sections. If a structural framework existed and if a membrane possessed visible structure, to observe such, it was equally obvious that the hemoglobin car-

ried must be wholly, or at least partially removed from the specimens. Therefore any accomplishment of the purpose in mind depended largely upon the technique employed.

The greatest difficulty was encountered in finding a fixing fluid suitable for the purpose. A fluid was desired in which hemoglobin is dissolved rather than precipitated. Hemoglobin not removed or precipitated within the corpuscle of course obscures whatever other cytoplasmic structure it may possess. Further, a fixing fluid was necessary whose osmotic action results in neither appreciable shrinkage nor swelling of the corpuscles, and one the diffusion currents resulting from whose action does not break up the structural content.

Osmic acid, bichloride of mercury, chromic acid and its potassium salts precipitate hemoglobin, and fluids in which any of these act of themselves were found impossible for the results desired. It is doubtful whether, according to Růžička ('03), pyrogallie acid is a solvent of hemoglobin. It was deemed necessary here to fix, embed and section the corpuscles, which he did not do, and no suitable fixing fluid containing pyrogallie acid has been devised. Hemoglobin is dissolved in alcohol, distilled water, acetic acid and formic acid, and formalin does not precipitate it. The action of alcohol alone distorts the corpuscle and produces shrinkage and rupture, and acetic acid, formic acid and formalin not only produce swelling but in themselves are very poor fixing agents for the structure of cells. Van Gehuchten's (Carnoy's) fluid, containing absolute alcohol, acetic acid and chloroform, was tried and found to rend the fresh corpuscles into small fragments.

Bryce obtained his results after the action of sublimate-acetic, but he was dealing with corpuscles of larvae which probably contained considerably less hemoglobin than the corpuscles of the adults whose study was here desired. After trying a number of fluids containing corrosive sublimate and acetic acid, it was decided that the action of the sublimate in all precipitated the hemoglobin. Suggestive but incomplete results were obtained with corpuscles fixed in a mixture containing 5 cc. saturated aqueous solution of bichloride of mercury, 2 cc. glacial acetic acid, 5 cc. 40 per cent formaldehyde, and 88 cc. 95 per cent alco-

hol. Preparations of nucleated corpuscles after this fluid showed a cytoplasm more or less transparent in places and a distinct membrane bounding the periphery. In the clearer places in the cytoplasm a fairly well marked reticulum could be discerned. In preparations of mammalian (non-nucleated) corpuscles such were much less indicated.

Of all the fixing fluids tried, the most satisfactory results were obtained after using a well ripened mixture containing the following parts:

Aqueous 3 per cent potassium bichromate.....	100 cc.
Commercial (40 per cent) formaldehyde.....	4 cc.
Glacial acetic acid.....	5 cc.

When first made, this fluid has the color of the bichromate solution, but if allowed to stand or if warmed it becomes a dark greenish brown, due chiefly to the oxidation of the bichromate in the resultant reactions. Our best results were, or maybe happened to be, obtained with a mixture which had been standing several weeks. The formation of formic acid is one of the results of the ripening process.

In using this fluid (and all those tried) a fairly large shell-vial was filled about half full of the fluid and, gently shaking it, the blood was dripped, drop by drop, directly from the animal into the fluid. Even by agitating the mixture while adding the blood, some coagulated clumps cannot be avoided. The corpuscles in these clumps were found not so good for study as those floating free in the fixing fluid. All finally settle upon the bottom of the vial and the fluid may be removed with a pipette or decanted. The fluid was allowed to act for twelve hours. Changing it once or twice was thought to result in better extraction of the hemoglobin.

This fluid does not require a preliminary washing of the material in water. Small paper boxes were made from ordinary thin letter paper, labels written on the sides in pencil, and the accumulated corpuscles transferred to the boxes by pipette. The boxes nearly full of fixing fluid and corpuscles were then placed in a stender dish containing 30 per cent alcohol to a depth of about

half the depth of the boxes. In 5 to 10 minutes the corpuscles settle to the bottom of the box and some of the fixing fluid may be pipetted away. Gradual dehydration is accomplished by the transfusion of the alcohol through the paper walls of the boxes. If several boxes are carried in a small stender dish, the 30 per cent alcohol should be changed during the hour. Then the 30 per cent alcohol in the stender dish was replaced with 40 per cent alcohol and so on with grades of alcohol progressively increasing by 10 per cent in strength up to 90 per cent, which was replaced with 95 per cent alcohol and this in turn by absolute. To insure complete transfusion of the different grades and the action of each upon the corpuscles, the paper boxes should remain in each alcohol at least one hour with the stender dish covered.

From the absolute alcohol, the boxes were transferred to equal parts absolute alcohol and xylol for about 30 minutes and then placed in pure xylol to complete clearing. The boxes were next placed in a dish of melted paraffin in the thermostat for two hours. Owing to the xylol present, it was found necessary to change the paraffin or transfer the boxes to another dish of paraffin during the first hour. When first in melted paraffin, the corpuscles float about and show a tendency to adhere to the sides of the box, and to avoid much of this in the final the boxes should be gently shaken a few times during the first half-hour.

The specimens were embedded, paper box and all. The corpuscles were settled in a layer at the bottom of the box, so, when the paper was pulled off, a paraffin block was obtained with them accumulated in one side.

For the sections, the ordinary Minot rotary microtome was used, set at  $1\ \mu$ . The large corpuscles especially were found to have settled for the most part on the flat, or with their widths parallel to the bottom of the boxes. To obtain sections cut in this plane, the paraffin block had to be arranged with its bottom surface parallel with the edge of the knife. The paraffin ribbon was of course much packed and crumpled and, of course, few if any of the sections could have been of  $1\ \mu$  in thickness, but setting the microtome at  $1\ \mu$  was thought to give thinnest sections

possible. The sections were straightened and fixed on the slide by the usual water-method, without using albumen fixative. After drying, the paraffin was removed with xylol, the slides transferred to absolute alcohol and then passed through the gradually descending grades of alcohol, 3 to 10 minutes in each, down to water.

Of the staining methods tried, including gentian violet with safranin, the best results were given by alizarin and toluidin blue. To distilled water was added enough of a saturated solution of the sulphalizarinate of soda in 70 per cent alcohol to make the water a straw yellow, and in this the sections were immersed for about 12 hours. The sections were then rinsed in distilled water and some slides were placed in a 0.5 per cent aqueous solution of toluidin blue to stain nuclear structures. Other slides, in order to study the region occupied by the nucleus in greater detail, were not stained with the toluidin blue at all. The stained sections were then rinsed with distilled water and dehydrated by passing through the gradually increasing strengths of alcohol, 3 to 5 minutes in each, up to absolute, cleared with xylol and mounted.

The action of the fixing fluid and the technique of embedding, etc., when carefully applied, seemed to have produced little change in the shape and size of the corpuscles of the *Amphiuma*, frog, alligator and snake. Measurements of the sections of those of the *Amphiuma*, judged truly sagittal by the shape and the position and size of the nuclei, gave an average of 69  $\mu$  long by 38.6  $\mu$  wide as compared with the average 72.9  $\mu$  long and 44.5  $\mu$  wide given by the fresh corpuscles. For the blood of all four of the animals mentioned, sections showing the evenly oval contours characteristic of the fresh corpuscles were frequent on the slides and no disturbances of interior arrangement seemed evident in these. Fragments of corpuscles and fragments of sections of them were abundant, but most all such, from their form, were evidently produced by crushing and breaking by the knife in cutting and by the crumpling of the paraffin ribbon. Fragments of sections were often more favorable for the study desired than sections of whole corpuscles. In the sections containing the

mammalian corpuscles (guinea-pig and human), there showed much more evidence of distortion both as to contour and internal arrangement.

#### OBSERVATIONS

The nuclei of the nucleated corpuscles could be best observed as to their position, size, form, and arrangement of chromatin in corpuscles stained whole after fixation, without embedding. The nuclei in the corpuscles of the *Amphiuma* and alligator especially appear to consist of a tangled coil of one or more coarse rods of chromatin supported in non-chromatin substances. The coiled and tangled chromatin rods in *Amphiuma* are very much larger than those in the alligator and, in *Amphiuma* especially, the peripheral loops of the rods produce a scalloped and lobulated contour of the nuclei quite evident in whole specimens. In the thin sections of stained nuclei, the chromatin rod or rods appear cut into short segments, as shown in figures 1 and 3. A delicate membrane about the nucleus was evident in all the nucleated corpuscles examined, but could be seen only in the thin sections and best in those in which the nucleus was not stained (figs. 2 and 3, B).

A membrane about the entire red corpuscle, after the technique here employed, was distinctly present in the blood of all the animals used. In proportion to the size of the corpuscle, it appeared relatively thicker and more condensed as possessed by the mammalian corpuscles (fig. 4, human). In the thin sections of corpuscles of *Amphiuma*, this membrane, actually thicker than that of smaller corpuscles, could be resolved under oil immersion objective into an apparently parallel arrangement of very delicate threads. With the fragments of corpuscles, broken and torn by the knife in sectioning and frequently found in the preparations, the nature of the membrane could be better observed than with intact sections. Torn and broken membranes often appeared slightly frayed in the tearing and close study led to the conviction that in *Amphiuma*, at least, the membrane consists not of a condensation of concentrically arranged parallel threads, and certainly not of concentric lamellae, but instead, of a very deli-

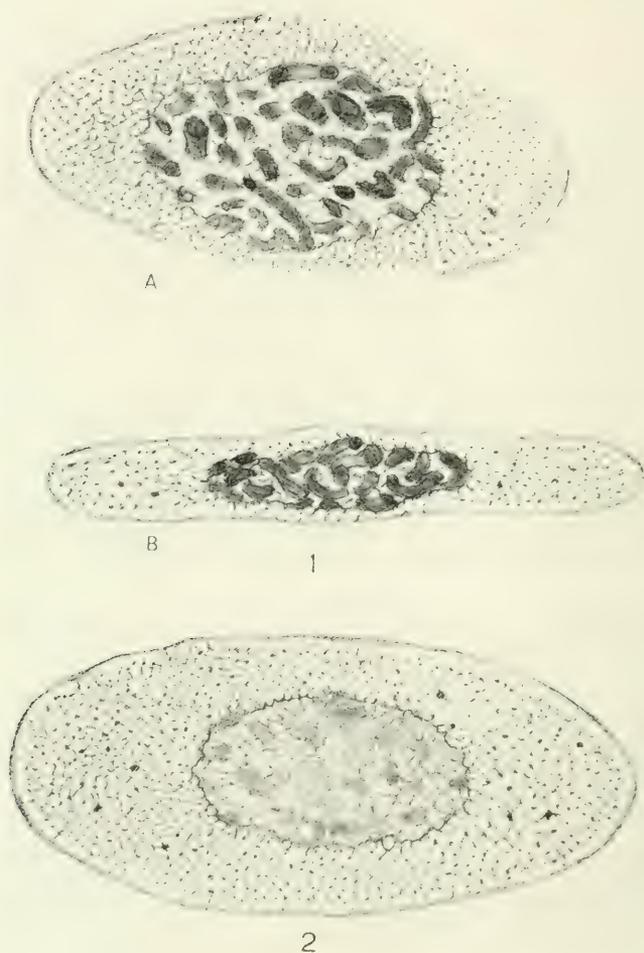


Fig. 1 Drawings from very thin paraffin sections of erythrocytes of *Amphiuma means*, fixed in the bichromate-formalin-acetic acid mixture and stained with sodium sulphalizarinate and toluidin blue, showing the capsule, reticulum, perinuclear membrane and the coiled rods of nuclear chromatin in stained section. *A*, corpuscle sectioned on the flat; *B*, corpuscle sectioned in profile.

Fig. 2 Erythrocyte of *Amphiuma* sectioned on the flat and slightly tangentially. Same technique as in figure 1 except that the toluidin blue was omitted. Given to show the full shape of the fixed corpuscle and, especially, the perinuclear membrane and reticulum within the nucleus.

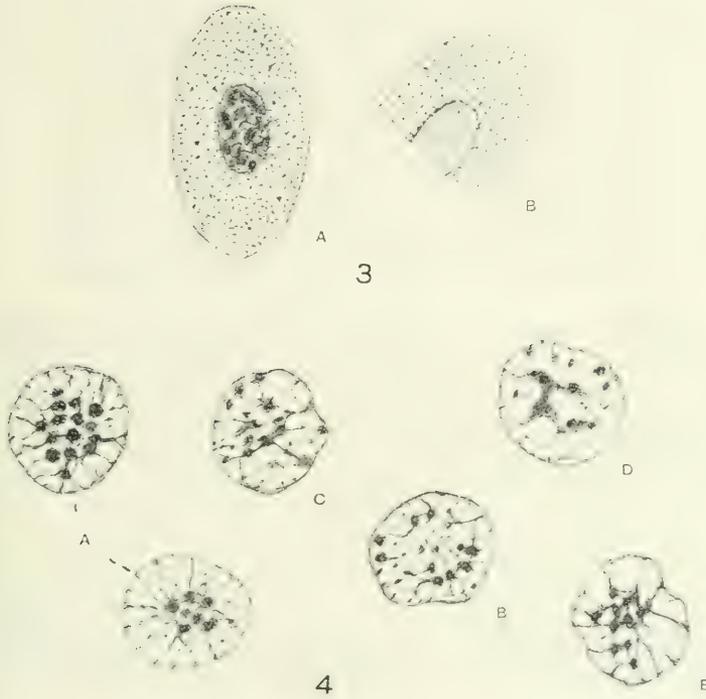


Fig. 3 From sections of erythrocytes of the alligator (*Alligator mississippiensis*). Prepared with same technique as figure 1 and drawn in same scale as figures 1 and 2 and to represent the same structures. *A*, section with nucleus stained by toluidin blue; *B*, piece of section from preparation in which toluidin blue was omitted.

Fig. 4 From sections of human erythrocytes prepared with same technique as figure 1, except that toluidin blue was not applied to the sections used for the drawing. Drawn in somewhat larger scale than figures 1 to 3. *A*, two sections of erythrocytes considered as representing more nearly the normal condition of the reticulum, capsule and central knots, with hemoglobin for the most part removed. *B*, erythrocytes with knots more distributed. *C*, *D* and *E*, varying degrees of rupture of the reticulum, presumably produced by diffusion currents set up by the reagents.

cate reticulum so condensed or compressed that its meshes are much elongated and thus produce the impression of a parallel arrangement (fig. 1, A). Meves, describing red blood corpuscles of frog and salamander as showing circumferential fibrils arranged either parallel to the surface or in a continuous skein, must have obtained the same appearances.

A very distinct reticulum is evident in the cytoplasmic areas of the sections and the threads of its meshes are continuous into the membrane. The meshes of this internal (cytoplasmic) reticulum, or stroma of the corpuscle, appear larger in the corpuscles of *Amphiuma* than in those of the alligator (fig. 3), frog and snake. Corpuscles of the frog and snake, not figured here, gave appearances practically identical with those given by the alligator. In the more transparent sections, those supposedly more free from hemoglobin, the fibrils of the reticulum, though very delicate and varying somewhat in size, appeared distinctly thread-like, and the meshes made by them were angular in form. That the threads of the peripheral meshes grade directly into the peripheral membrane, which itself appears as a condensed reticulum, supports the conclusion that the membrane is nothing more than a peripheral condensation of the internal reticulum and that the membrane could better be called a 'capsule' of the corpuscle. In one of his papers, Meves referred to it as a 'feltwork membrane.' This, and likewise the statement of Růžička that the corpuscle possesses no actual membrane but is bounded by the limits of a fine-meshed reticulum, seem warranted.

In preparations of corpuscles, of *Amphiuma* especially, in which the hemoglobin was obviously not so completely removed, the component threads of the internal reticulum (stroma) appeared coarser and gave the impression of being rod-like in form, and the meshes of the reticulum appeared oval instead of angular. The oval form of the mesh was due in part to larger knobs or accumulations of substance at the points of junction ('nodal points') of the threads in forming the meshes. Růžička described these knobs in his preparations of frog and mammalian blood as round in form and concluded they represented undissolved hemoglobin. Their larger size in certain preparations here and the

apparently larger size of the threads of the reticulum accompanying them is interpreted as due to unremoved hemoglobin adhering to or precipitated upon the reticulum throughout, for such preparations were always less transparent and coarser in appearance and the membrane or capsule of the corpuscle appeared dark and homogeneous as compared with those from which the hemoglobin was considered more completely removed.

All the figures here given are attempts to represent corpuscles considered as having been rendered most free from hemoglobin. The knobs, or junctions of the filaments making the reticulum, appear quite small in these, usually about as large as would be possible were two or more plastic filaments to cross each other in contact and fuse giving an increased amount of substance at their junctions, a knob, or knot of the mesh of the net. In the most clear of the preparations, occasional larger knobs occurred at the junctions of the threads. Several such are shown in figure 2. These larger knobs were usually angular or stellate with their points extending upon the threads joining in them and are interpreted as representing small masses of unremoved hemoglobin adhering upon junction points of a greater than usual number of threads.

In the corpuscles of the *Amphiuma*, alligator and frog, the threads which join or grade into the capsule at the periphery of the reticulum appear for the most part to join with the capsule at right angles and thus present here a somewhat radial arrangement. The meshes formed by these threads in joining the capsule average somewhat larger in size than elsewhere in the cytoplasm of the nucleated corpuscles. The greater amount of light admitted by these larger meshes compared with the greater density of the capsule gave the impression of a narrow, clear zone about the corpuscle just under the capsule. This was especially true with the corpuscles of *Amphiuma* (figs. 1 and 2) in which the meshes are larger throughout than in the other nucleated corpuscles examined. Meves observed this radial arrangement of the peripheral filaments of the reticulum in the corpuscles of the frog and salamander, and thought them in regular system similar to the relation of Krause's membranes in the muscle fiber.

Bryce likewise observed it in the corpuscles of lepidosiren larvae, but he considered it a part of a general radial or parallel arrangement of the filaments extending throughout from the nucleus to the periphery of the corpuscle. Our preparations do not show these peripheral filaments to join the capsule in a definitely regular system but at various angles and to form meshes of varying size and shape: nor do the threads of the reticulum extend from the nucleus to the capsule in definitely radial, and certainly not in parallel, arrangements. Only in the thinnest region of the cytoplasm, in the middle of the corpuscle on the flat where the nucleus is nearest the capsule, can threads be traced directly from the nucleus to the capsule (fig. 1, B, section in profile). Here the peripheral zone comprises practically the entire cytoplasm. As Schäfer (1905) suggests, these peripheral threads are only a part of the general reticulum of the corpuscle. Continuous into and stretched from the capsule or membrane, they happen to appear more sparse and regular than the threads of the remainder of the reticulum.

For the larva of *Lepidosiren paradoxa*, Bryce described in each end of profile views of the corpuscles a small area free of reticulum but occupied by a number of fine dots, and he interpreted these dots as transverse sections of circumferential filaments running in the plane of the flat dimension. Our preparations showed no differences in this respect between sections cut on the flat and profile sections (fig. 1).

As noted above, the chromatin in the nuclei of the corpuscles of the *Amphiuma* and alligator appears collected into definite coiled rods instead of being scattered in granules of varying size throughout the nucleus. Preparations in which the nuclei are stained differentially do not show a sharp, dark-staining membrane bounding the confines of the nucleus as is found in certain other tissue cells where such is probably due to chromatin material being involved in or adhering upon the nuclear membrane. On the contrary, the nuclear membrane appears here to be another but thinner condensation of the reticulum or general framework, being comparable in origin and structure with the capsule, or membrane, about the corpuscle. The threads of the cyto-

plasmic reticulum grade directly into it and it stains in the same way as the threads of the reticulum. It could best be studied in preparations to which a nuclear stain had not been applied (fig. 2 and fig. 3, B). Figure 2 represents a slightly tangential section of a corpuscle of *Amphiuma* on the flat. In such corpuscles, with nuclei not differentially stained, it could be noted that the threads of the reticulum grade directly into the nuclear membrane, that the meshes become suddenly smaller or more dense to produce it and that the membrane carries the same stain-reaction as the reticulum. In other words, it is here suggested that the nuclear membrane is but a peri-nuclear condensation of the general framework of the corpuscle. Sections of corpuscles in profile (fig. 1, B) show the threads to serve as continuations between the capsule and the nuclear membrane.

Furthermore, our preparations suggest that the general reticulum, or framework, is continuous into and throughout the nucleus, contributing to the support of its structures. Figures 1, A, and 2 and 3, B, are attempts to show this suggestion. In such corpuscles, the very thin sections showed the delicate filaments extending from the nuclear membrane and forming a reticulum throughout the nucleus. The meshes of this intranuclear framework seemed somewhat larger than the average of those in the cytoplasm, and especially large when containing a segment of the coiled chromatin rod. The threads stained just as those of the cytoplasm and the membranes. Knobs at the junction points of the threads could not be observed as so definite nor so large as in the cytoplasm, probably due to some extent to the necessarily more obscured nuclear area. The suggestion that the cytoplasmic reticulum is continuous and identical with the nuclear reticulum is somewhat supported by the frequently presented view that the spongioplasmic reticulum of the general cell structure is identical to (stains the same) and is continuous with the karyoplasmic reticulum, or nuclear linin.

The chemical composition of either or both of the reticula, including the membranes, may be that of a nucleo-proteid or lecithin or cholestrin, but in our preparations it occurs in the form of a network in whose meshes is supported the remaining cell load,

including the hemoglobin and the nuclear structures. Schäfer states that substances dissolving lecithin or cholestrin will produce an increase in the permeability of the membrane. Bryce thinks that the peripheral capsule ("peripheral ring or band," he calls it) is due to a condensation or massing of the meshes of the reticulum, and he suggests that the filaments of the reticulum are not necessarily fixed fibers but that they may be of colloidal nature. Taking all into consideration, he thinks the reticulum is not an artefact but an actual protoplasmic framework. Citing Bütehli's "Foam theory" of the structure of protoplasm, and noting that the meshes of the reticulum are larger than the limits given for the protoplasmic alveoli of this theory, and much larger than the meshes described for the cytoplasm of leucocytes, Bryce thinks that if the protoplasm of the erythrocyte may at one time have been alveolar in structure, the reticulum could be later derived from a vacuolated condition in which the hyaloplasm of the cell is greatly reduced and the alveolar arrangement lost by the breaking through of the walls of the alveoli. Růžička thinks the observed reticulum is not artefact but an actual structure, that its meshes and the knobs at the junctions of the threads are not coagulation products, for the meshes are too fine and uniform and the threads are not arranged as are coagulum filaments; that the hemoglobin is carried dispersed in the meshes of the net to the periphery of the corpuscle, that the net is the vegetative part of the corpuscle and the hemoglobin the functional part.

Whatever the chemical character, our preparations seem to support the conclusion that the structures observed are not artefact, that the corpuscle is pervaded by a true reticulum, a network of threads joining each other throughout and extending in all the planes of space. That the threads of the reticulum observed serve as a supporting framework of the corpuscle and possess a certain amount of elasticity is suggested by several observations on the living red blood corpuscle: (1) the mammalian corpuscle, after losing its nucleus, remains thinner in its center from which the nucleus was lost; (2) a living corpuscle may be cut in halves and neither half suffer exudation of its content; (3) corpuscles in the circulation may be elongated and their usual

shape considerably distorted in passing through the smallest capillaries but always resume their shape upon reaching the larger vessels; and (4), in this laboratory, by tapping under the microscope the cover-glass upon fresh mounts of corpuscles of the *Amphiuma*, the nuclei could be made to shift considerably from their normal position, moving back and forth with the tapping, sometimes being slightly spread by the pressure, but, the pressure removed, they would resume their normal position and size in the center of the corpuscle floating in the plasma.

With human corpuscles, the technique here employed was not altogether as successful as with the nucleated forms. The stained sections showed more distorted contours and internally ruptured corpuscles than those of *Amphiuma*, alligator and frog blood prepared in the same way. In the latter preparations, evidences of rupture produced by the reagents used were somewhat rare. Figure 4 is given to represent appearances found in the preparations of human corpuscles. Blood from the guinea-pig gave the same appearances. The two corpuscles farthest in the left (fig. 4, A) represent the form most common in the preparations and, showing less distortion in contour, were the form considered as most nearly representing the normal. The remaining four corpuscles were selected as an attempt to illustrate, progressively, appearances of injurious effects produced by the reagents.

It may be noted that the capsule, the sharp peripheral border of the corpuscle frequently mentioned in the literature, is here relatively thicker and more densely staining than that of the *Amphiuma*. This may be due in part to a less complete extraction of the hemoglobin considered evident in the interior. The threads of the reticulum, while grading into the capsule and continuous with each other throughout the corpuscle, do not present so nearly uniform size as in the other forms studied. Filaments much thicker than others appear to radiate from the central region, while attached to these are numerous smaller threads joining throughout and completing a general reticulum. At the junction of the larger filaments with the capsule there usually appears a visible knob, or hillock of attachment. One gets the impression in close study that the smaller, probably the

normal, meshes are themselves crossed by still finer threads, while a larger mesh may appear perfectly clear. Fine knobs show at the junction or nodal points of the threads, varying in size with the size of the threads.

In the corpuscles considered more nearly normal (fig. 4, A), the frequently described large knobs dispersed in the central region of the corpuscle comprised the most prominent feature in our sections. These appeared approximately spherical in shape and uniform in size and the number observed varied from 17 to 6, a variation due no doubt in part to the planes in which the corpuscles were sectioned. In sections in which the knobs were less darkly stained, one could get the impression, under highest magnification, that these knobs themselves are fibrillar, that they are knots or condensations of very fine threads. If they carry any nuclear material (they have been called the nucleus of the mammalian erythrocyte), our sections suggested it very doubtfully that any stain reaction of a chromatin character is retained in them. Aniline nuclear dyes, such as gentian violet, and even hematoxylin may be retained in them longer than in the threads of the reticulum, but the color will wash out, and the fact that it is retained in them more deeply at times is considered due (1) to their greater compactness of mass holding more of the dye than the smaller and looser structure, but indifferently nevertheless, and (2) to unremoved hemoglobin being entangled within them. They were always darker than the filaments, but this is to be expected, due to their greater density obscuring the light. Occasionally the knobs or knots appeared more scattered throughout the corpuscle and then to vary more in size, as shown in B of figure 4.

The larger filaments, those usually described and which appear more or less radially arranged, are here considered as artefact. Corpuscles manifestly ruptured internally by the action of the reagents (fig. 4, E) show large, clear, vacuole-like meshes and these are always bounded by the thick filaments. It is suggested that these large clear meshes represent areas of the reticulum ruptured by diffusion currents of the fixing agent or alcohols in preparation, and that the broken threads of these

areas have been washed together or condensed to form the thicker filaments. Often the latter give the impression that they themselves are composed of finer threads. They occur more or less radially between the centrally placed knots and the capsule probably because these knots are the firmest fixation points.

As to the knobs or knots ('nuclei') of the central region, we beg to suggest the possibility that they may represent remains of an originally existing membrane about the nucleus and of a reticulum within the nucleus of the erythroblast, or nucleated stage of the mammalian corpuscle, similar to those found in the nucleated corpuscles of the *Amphiuma* and alligator: that the knots represent the remains of these resulting from or after the disturbance of the central reticulum at the time the nucleus disintegrated or was extruded, and that their density may be added to by hemoglobin retained in them.

The membrane or capsule of the erythrocyte, derived as a peripheral condensation or massing of the reticulum, must be the most resistant part, the structure last to rupture under stress. It is permeable, as is well known, allowing a ready diffusion through it. The fact that the mammalian erythrocyte assumes a spherical form before bursting, when swollen and distended by excessive endosmosis in the action of distilled water or weak acetic acid, for example, may be explained as due to its less resistant internal framework being first torn asunder and destroyed by the diffusion currents and the stress. The erythrocyte then becomes a mere turgid sac, the capsule itself rupturing at continued pressure. Both the capsule and framework are dissolved by alkalies.

The red blood corpuscle is a tissue element extremely differentiated in structure for the performance of an extremely specialized function. In the mammal it is more differentiated than in the lower vertebrates, being one of the two bodies possessed that, containing no nucleus, can no longer be called a cell. From the above studies it is concluded (1) that it normally possesses a framework in the form of a fine threaded, somewhat elastic reticulum in the meshes of which its hemoglobin is supported so intimately that its content partakes of the physical characters of

gelatin: (2) that in the nucleated forms, this same reticulum is continuous into the nucleus, supporting its structures; (3) that it possesses a peripheral membrane or capsule into which the threads of the reticulum grade and which is derived from and consists of a peripheral condensation or massing of the reticulum; (4) that there is a similar but thinner perinuclear condensation of the reticulum bounding the confines of the nucleus and forming a nuclear membrane of the nucleated forms, and (5) that the central knots, or "nucleus of the mammalian corpusele," are masses of the material of the nuclear membrane and reticulum originally existing in the central part and result from or after the disturbance of the interior produced by the disintegration or extrusion of the nucleus.

Finally is due an expression of appreciation of the kindness of Professor Hardesty at whose suggestion and with whose guidance and collaboration this study was made.

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## ON THE PROVISIONAL ARRANGEMENT OF THE EMBRYONIC LYMPHATIC SYSTEM

AN ARRANGEMENT BY MEANS OF WHICH A CENTRIPETAL LYMPH  
FLOW TOWARD THE VENOUS CIRCULATION IS CONTROLLED  
AND REGULATED IN AN ORDERLY AND UNIFORM MANNER,  
FROM THE TIME LYMPH BEGINS TO COLLECT IN THE INTER-  
CELLULAR SPACES, UNTIL IT IS FORWARDED TO THE VENOUS  
CIRCULATION

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SIX FIGURES

One of the most interesting problems of the lymphatic system is the determination of the manner in which the continuous centripetal lymph flow is established in the embryo, in relation to the developing lymphatic vessels by which it is subsequently conveyed to the venous circulation.

It is well known that the anlagen of the lymphatic system do not normally make their appearance in the embryo until after the haemal vessels have been established. As soon, however, as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo and, as we know, is subsequently collected by a set of newly-formed vessels, the lymphatics, which convey it to the venous circulation.

Those who maintain that the lymphatics sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of closed and hollow outgrowths from the veins, the lymphatics, before it can be received into any portion of the lymphatic system. Such continuous outgrowths from the veins would necessarily take place in a centrifugal direction which is opposed to that of the centripetal flow of lymph they would receive.

It has always proved a difficult matter for some of us to reconcile the view that the direction of the growth of the vessels

in which the flow takes place should be opposed to that of the flow. One might expect a continuous centripetal lymph flow toward the venous circulation to be established in a gradual manner in the embryo, and to be regulated from the time lymph first made its appearance in the intercellular spaces, until it was continued on to the venous circulation. In fact, one might expect to find some provisional condition of the embryonic lymphatic system which should exactly accord with the maintenance and regulation of such a centripetal flow. *Such a provisional condition of the embryonic lymphatic system I believe I have been able to demonstrate in a positive manner in the living embryo of the trout.*

One of the most salient features noticeable in the development of the lymphatic system of the trout, as well as in that of mammals, is that the main lymphatic channels are formed through a gradual conerescence of independent and discontinuous anlagen or lymph vesicles. These independent lymph vesicles make their appearance in the embryo in a progressive manner along the lines subsequently followed by continuous lymphatic vessels, and, in certain districts of the mammalian embryo, they utilize the static line vacated by degenerating veins, so that certain lymphatic vessels of the body subsequently follow the course of abandoned veins. These independent lymph vesicles of the embryo first become conerescent to form continuous channels contiguous to the points at which the lymphatics establish typical communications with the veins. With these points of lymphatico-venous entry, the vesicles continue to become conerescent in a progressive manner, so that the outlying or peripheral lymph vesicles are the last to establish a communication with the veins.

The view which calls for the development of the main lymphatic channels through a confluence of independent and discontinuous anlagen was advanced by Huntington and McClure<sup>1</sup>

<sup>1</sup> Huntington and McClure. The development of the main lymph channels of the cat in their relations to the venous system. *Anat. Rec.*, vol. 1, 1907. Read before the American Association of Anatomists at the meeting held in New York in December, 1906.

in 1906. Since a recognition of this fact is a necessary corollary to the main issue involved in the present paper, we will first consider what constitute the main lymphatic channels in the embryo of the trout and show how, in their development, they follow this plan.

Figure 1 represents a ventral view of a reconstruction of the main lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo, on the twenty-second day after fertilization. This embryo was developed at a temperature of about  $10.5^{\circ}\text{C}$ . and its lymphatic system is represented, for the most part, by a continuous system of vessels which drain into the veins at typical points. The typical points of lymphatico-venous communication in the regions of the head and pharynx occur in the cardino-Cuvierian district (9); with the precardinal (jugular) vein near the caudal end of the otocyst (13); and with the precardinal, near a point where the latter leaves the cranial cavity (2). The first and last mentioned points of communication appear invariably to be retained in the adult.

The principal or main lymphatic vessels found in the regions of the head and pharynx of a twenty-two day rainbow trout embryo are as follows:

1. *The subocular lymph sacs (saccus lymphaticus subocularis, 1 in figure 1)*

The subocular lymph sacs of the trout embryo consist of two relatively huge sacs or vesicles, each of which lies ventro-medial to the eye. They are more or less triangular in form, with their apices directed forward. In the twenty-two day trout under consideration, they extend between the hyoidean artery (15) and the olfactory invagination (21), as shown in figure 2. At its postero-lateral angle, each subocular lymph sac (1) communicates directly with the lateral pharyngeal lymphatic (3 in fig. 1) and it is solely through the latter vessel that the subocular lymph sac drains into the veins.

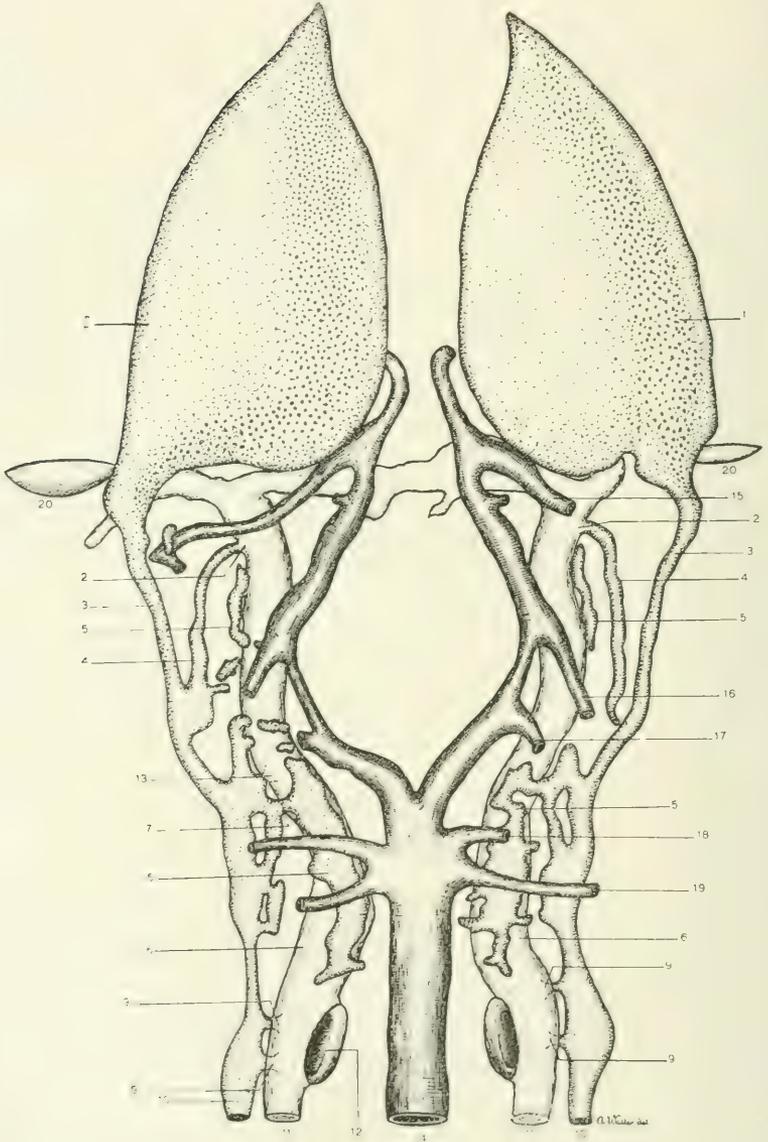


Fig. 1 Reconstruction of the main lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-two-day rainbow trout embryo; ventral view. P. E. C. series 648. Reconstructed after the method of Born at a magnification of 200 diameters.

## REFERENCE NUMBERS

- |   |                                 |
|---|---------------------------------|
| 1, Subocular lymph sac  | 11, Postcardinal vein           |
| 2, Medial pharyngeal communication  | 12, Duct of Cuvier              |
| 3, Lateral pharyngeal lymphatic   | 13, Otic communication          |
| 4, Medial pharyngeal lymphatic  | 14, Dorsal aorta                |
| 5, Precardinal (jugular) lymphatic  | 15, Hyoidean artery             |
| 6, Precardinal (jugular) vein   | 16, First efferent aortic arch  |
| 7, Communication between the pre-<br>cardinal lymphatic and the lat-<br>eral pharyngeal lymphatic | 17, Second efferent aortic arch |
| 8, Caudal end of otocyst  | 18, Third efferent aortic arch  |
| 9, Cardio-Cuvierian communication   | 19, Fourth efferent aortic arch |
| 10, Lymphatic of the lateral line of the<br>trunk   | 20, Caudal end of eye           |
|   | 21, Olfactory invagination      |
|   | 22, Carotid artery              |



Fig. 2 Sagittal section taken through the head and pharynx of a twenty-two-day rainbow trout embryo, showing the relations of the subocular lymph sac to the eye, the hyoidean artery and the olfactory invagination; 1, subocular lymph sac; 15, hyoidean artery; 21, olfactory invagination. P. E. C. series 816.

2. *The lateral pharyngeal lymphatic (truncus lymphaticus pharyngeus lateralis, 3 in figure 1)*

This vessel occupies a superficial position in the lateral wall of the pharynx and forms, on each side of the body, the direct anterior continuation of the lymphatic of the lateral line of the trunk (*truncus lymphaticus longitudinalis lateralis, 10* in fig. 1). The lymphatic of the lateral line of the trunk is completely developed in the twenty-two day rainbow trout and can be followed caudad to the region of the caudal lymph heart. The lateral pharyngeal lymphatic drains the subocular lymph sac and, at a slightly later stage of development, also the dorsal region of the head and pharynx, the operculum and the lower jaw. It communicates with the veins in the cardino-Cuvierian district (9) in common with the lymphatic of the lateral line of the trunk (10).

3. *The medial pharyngeal lymphatic (truncus lymphaticus pharyngeus medialis, 4 in figure 1)*

This vessel occupies a more central position and is more deeply situated than the lateral pharyngeal lymphatic. It follows an oblique course, in a postero-anterior direction, from about the middle of the lateral pharyngeal lymphatic with which it subsequently communicates, to open into the precardinal vein just caudal to the point where this vein emerges from the cranial cavity (2 in fig. 1).

4. *The precardinal or jugular lymphatics (truncus lymphaticus precardinalis vel jugularis, 5 in figure 1)*

These vessels develop along the line of the precardinal veins. They are not completely established on the twenty-second day in the form of continuous channels and are represented by such vessels only near the caudal end of the pharynx, where they communicate with the lateral pharyngeal lymphatic (7 in fig. 1).

All of the continuous lymphatic channels described above, as occurring on the twenty-second day in the embryo of the rain-

bow trout, can be readily injected from the veins, through any one of the typical points of communication which are established between the lymphatics and the veins. By injecting into the subocular lymph sacs it is also possible to fill the continuous lymphatic vessels with injecta, as well as the veins. At this particular stage of development, in the absence of lymphatico-venous valves, blood may flow freely into the lymphatics from the veins, at the typical points at which the lymphatics communicate with the veins. In rainbow trout embryos slightly older than twenty-two days, and in which lymphatico-venous valves have been formed, the application of chloretone apparently vitiates the normal function of these valves. Blood may then also flow freely from the veins into the lymphatics and fill up completely all of the continuous lymphatic channels, including the subocular lymph sacs. If such embryos are removed to water in which no chloretone is present, the blood will flow back from the lymphatics into the veins.

The age of the rainbow, steelhead or brook trout embryo in which a continuous system of lymphatic channels, as shown in figure 1, is met with for the first time, naturally varies with the temperature of the water in which development takes place. Much variation is also met with in the rate at which the lymphatic system of the trout develops in different embryos of the same age, as well as upon opposite sides of the same embryo. When developed at a temperature of about  $10.5^{\circ}$  C., a continuous lymphatic system, as shown in figure 1, is usually found in rainbow and steelhead trout embryos on the twenty-second day after fertilization, which is six or seven days after its earliest anlagen can first be observed in sections.

In all stages of development, prior to the establishment of such a continuous system of lymphatics as shown in figure 1, a condition is invariably met with in the embryo in which the lymphatic system is represented by a progressively appearing series of discontinuous anlagen, that present varying degrees of concrescence with one another to form continuous lymphatic vessels which communicate with the veins, only at the typical points at which the lymphatics establish communications with the

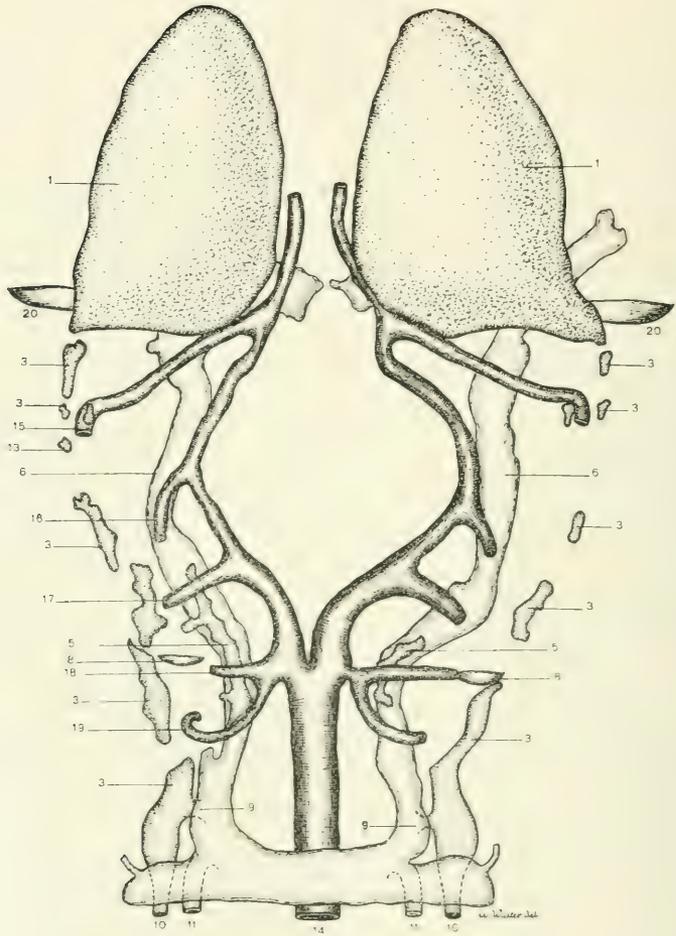


Fig. 3 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-day rainbow trout embryo; ventral view. P. E. C. series 668. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

veins. Two such stages of development are herewith presented in illustration of this fact.

Figures 3 and 4 represent, respectively, reconstructions of the lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo on the twentieth and

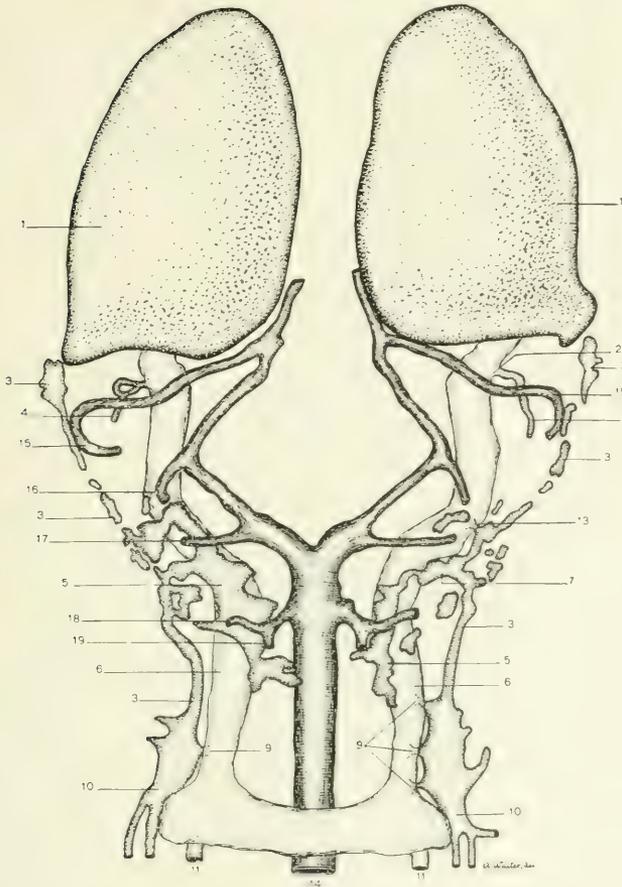


Fig. 4 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-one-day rainbow trout embryo; ventral view. P. E. C. series 646. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

twenty-first days after fertilization. These embryos were developed at a temperature of about  $10.5^{\circ}\text{C}$ . When compared with the conditions found in the embryo on the twenty-second day (fig. 1), it is seen that the subocular lymph sacs (*I* in figs. 3 and 4) are entirely independent of the veins and of other independent lymph vesicles, and that a series of discontinuous

and independent lymph vesicles lie exactly in the line subsequently followed by continuous lymphatic channels on the twenty-second day (fig. 1). It is also seen that these lymph vesicles present varying degrees of concrecence beginning at the points at which the lymphatics establish typical communications with the veins. It may also be observed that none of these independent lymph vesicles communicate with the veins except those which lie contiguous to the points at which the lymphatics establish typical communications with the veins.

For the purpose of the present paper reconstructions of the twenty and twenty-one day trout are sufficient to illustrate the general principle of development followed by the main lymphatic channels.

The main question involved in the present issue is, however, what functional significance does the presence in the embryo of such an independent and discontinuous series of lymph vesicles imply? *A study of one of these lymph vesicles, the subocular lymph sacs, as observed and experimented upon in the living trout embryo gives, I believe, a positive answer to this question.*

When rainbow and steelhead trout embryos are developed at a temperature of about 10.5°C., the development of the anlagen of the subocular lymph sacs is initiated on about the sixteenth day after fertilization. These anlagen first appear as small clear vesicles which lie in the mesenchyme in an area between the hyoidean artery and the eye and just dorsal to the maxillary ridge. These small vesicles finally become confluent to form a larger vesicle which gradually increases in size. Injection experiments upon the living trout embryo, controlled by sections, have shown that the anlagen of the subocular lymph sacs never establish a communication with the veins in the neighborhood of the sacs. They have also shown that the subocular lymph sacs cannot be injected from the veins at any stage of their development, until after the independent and discontinuous anlagen of the lateral pharyngeal have become concreseent with one another to form a continuous vessel and until a communication has been established between this vessel and the subocular

lymph sac (compare figures 3 and 4 with figure 1). In other words, at no stage of development has it been possible to inject the subocular lymph sac of the trout embryo except by way of the lateral pharyngeal lymphatic, which signifies that the subocular lymph sac of the trout embryo is entirely independent of the veins and of other lymphatics, until this communication has been made.



Fig. 5 Photograph of the ventral aspect of the head of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs. Spalteholz preparation. 1, subocular lymph sacs.

Injection experiments have also proved that the subocular lymph sacs of the trout embryo do not grow caudad and that they are invariably bounded posteriorly by the hyoidean artery: (compare 15, hyoidean artery, with 1, subocular lymph sac, in figures 2, 3 and 4). Figure 5 is a photograph of a Spalteholz preparation of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs (1 in fig. 5). The figure shows the position occupied by the subocular lymph sacs

in the living embryo and, since the sacs alone filled with the injecta, is illustrative of an experiment which proves that the subocular lymph sacs have not grown caudad, nor do they communicate with the lateral pharyngeal lymphatic, nor with the veins at the stage of development presented by this twenty-day trout.

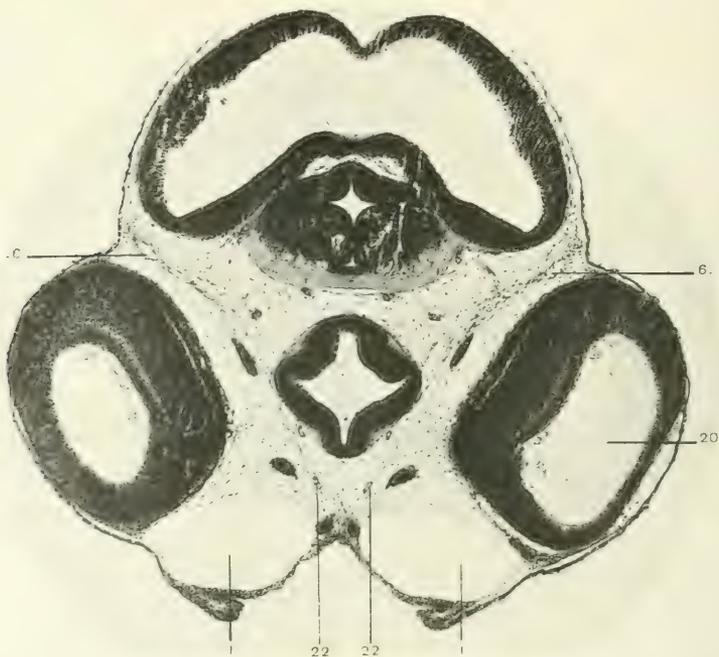


Fig. 6 Transverse section taken through the subocular lymph sacs of a twenty-one-day rainbow trout embryo in which, as independent structures, the sacs have reached the maximum stage of their development. P. E. C. series 646. Injected embryo. *1*, subocular lymph sac; *6*, precardinal (jugular) vein; *20*, eye; *22*, carotid artery.

The subocular lymph sacs of the trout embryo are non-pulsatile in character and are lined by an endothelium around which no muscular coat is formed. During the stage of their independence they become gradually distended with lymph which must necessarily enter them in a centripetal direction from the

intercellular spaces of the head. As this lymph gradually increases in amount the subocular sacs of the trout can be easily observed in the living embryo, and are especially prominent in the rainbow trout between the nineteenth and twenty-first days. By the time the subocular lymph sacs have attained their maximum size as independent structures, on the twenty-first day (figs. 4 and 6), a considerable pressure must be exerted by their lymph upon their walls, which would account for the distended appearance presented by the sacs (1) at this stage (fig. 6). As soon, however, as the subocular lymph sacs establish their communication with the lateral pharyngeal lymphatic, so that their lymph can flow continuously and centripetally to the veins, this pressure against their walls is immediately released, and the sacs then appear less prominently in the living embryo, due to a partial collapse of their walls.

*It is thus seen that, during the period of its independence, the subocular lymph sac of the trout embryo serves as a local and independent reservoir for the reception of lymph which enters it in a centripetal direction from the intercellular spaces; that it retains this lymph only temporarily, until the sac establishes a communication with the lateral pharyngeal lymphatic, through which a continuous centripetal lymph flow may then pass from the intercellular spaces to the venous circulation.*

*The subocular lymph sacs of the trout embryo therefore furnish us with a striking example of the fact that, not only do independent and discontinuous anlagen of the lymphatic system actually exist, but, that they can also be observed and be experimented upon in the living embryo.*

The functional rôle played by the subocular lymph sacs of the trout embryo, during the stage of their independence, as well as after they have established a communication with the venous circulation, undoubtedly gives us the clue to the function assumed by the independent lymph vesicles of the embryo in general. It also explains the manner in which a continuous centripetal lymph flow is established in the embryo, between the intercellular spaces and the venous circulation, in relation to the developing lymphatic vessels.

*On the basis of the functional rôle played by the subocular lymph sacs—and this can be actually demonstrated in the living trout embryo—it is highly probable that the independent lymph vesicles, of the embryo in general, also serve as local reservoirs for the temporary retention of lymph which enters them in a centripetal direction from the intercellular spaces; that these lymph vesicles become progressively conerescent with one another to form continuous channels, through which the lymph collected and temporarily retained by them is then forwarded to the venous circulation. In this manner the centripetal flow of lymph which continuously enters these independent lymph vesicles from the outlying intercellular spaces, is continued on to the venous circulation.*

It may be mentioned here, incidentally, that the functional rôle played by the subocular lymph sacs of the trout embryo during the stage of their independence, is also evidence of the fact that the lymphatics of fishes function solely in the capacity of lymphatics at the time of their inception, and that they are therefore not transformed veins.

In case the independent lymph vesicles of the embryo should fail to become conerescent with one another and with the veins, at the typical points of lymphatico-venous entry, an oedematous condition of the body would undoubtedly arise, and the ontogenetic condition, in which only independent and discontinuous anlagen are present, might then be retained in the adult. That such might actually be the case seems to be borne out by the conditions observed in an oedematous human foetus already described by Smith and Birmingham.<sup>2</sup> These investigators have described a case in which that peculiar and rare condition known as oedematous foetus was found to depend upon the complete absence of the thoracic duct, lymphatic glands and lymphatic trunks in general, and in which the lymph was stored in what they described as "greatly distended tissue spaces" which neither communicated with one another, nor with the veins.

<sup>2</sup> Smith and Birmingham. Absent thoracic duct causing oedema of a foetus. Jour. Anat. and Physiol., vol. 23.

Only two possible conclusions can be drawn regarding the significance of the complete absence of continuous lymphatic trunks and of the presence only of independent and discontinuous lymph-containing tissue spaces in this human foetus: Either there has been a complete failure on the part of the lymphatic system even to initiate its development, so that these 'tissue spaces' are in no sense related to the true lymphatic system, or, the presence of these spaces signifies a condition in which the normal development of the lymphatic system has been arrested at an early ontogenetic stage.

Huntington and the writer<sup>3</sup> have repeatedly described and figured the presence of independent lymph vesicles or lymph spaces in the mammalian embryo, and Huntington<sup>4</sup> has recently made a more extensive and detailed study of these structures, as they occur in the subclavian and primitive ulnar regions of the cat. Although one is not given the opportunity of studying these independent lymph vesicles in the living embryo of the mammal, as is the case in the trout, it would now seem quite a waste of time to parley further over the question of their presence in the mammalian embryo, or, that it is through the condescence of such independent vesicles that the main lymphatic channels of mammals are formed.

The oedematous human foetus described by Smith and Birmingham appears to present us with the most striking evidence, not only of the fact that the presence of independent lymph vesicles may actually be demonstrated, in certain circumstances, as functional structures in mammals, but, also, that the functional rôle played by them is similar to that played by the subocular lymph sacs of the trout during their independent stage. It is

<sup>3</sup> Huntington and McClure. The anatomy and development of the jugular lymph sacs in the domestic cat (*Felis domestica*). *Amer. Jour. Anat.*, vol. 10, 1910, fig. 66.

<sup>4</sup> Huntington. The development of the mammalian jugular lymph sac, of the tributary primitive ulnar lymphatic and the thoracic ducts from the viewpoint of recent investigations of lymphatic ontogeny, together with a consideration of the genetic relations of lymphatic and haemal vascular channels in the embryos of amniotes. *Amer. Jour. Anat.*, vol. 16, 1914. Also, The development of the lymphatic drainage of the anterior limb in embryos of the cat. *Proc. Amer. Ass. Anat., Anat. Rec.*, vol. 9, 1915.

highly probable, therefore, that the conditions found in this human foetus indicate that the development of the lymphatic system had been arrested at a normal ontogenetic stage; that its oedematous condition was due to the circumstance that the independent and discontinuous anlagen of the lymphatic system had failed to become conerescent with one another and with the veins, in order to establish a continuous system of channels, through which a continuous centripetal lymph flow could pass from the outlying tissue spaces to the venous circulation.

# THE PYRAMIDAL TRACT IN THE GUINEA-PIG (*CAVIA APEREA*)

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TEN FIGURES

## INTRODUCTION

The pyramidal tract (*fasciculus cortico-spinalis*) in rodents, so far as it has been examined in this order, is crossed and runs in the dorsal column of the spinal cord, but there are exceptions to this rule. In the family *Leporidae*, including the rabbits and hares, it lies in the lateral columns, and in the Canadian porcupine there is a dorsal column, a lateral column and a ventral column tract (Simpson '14). In view of the fact, therefore, that such wide variation exists between closely related species, it is desirable that as many as possible of these be examined.

In the guinea-pig, the animal with which this paper deals, Spitzka ('86), Bechterew ('90) and Wallenberg ('03) have found that the pyramidal tract decussates into the posterior column.

Ranson ('13) states that in the albino rat the tract consists of a mixture of medullated and non-medullated fibers, and by the use of the pyridine-silver method of Cajal (modified), the non-medullated fibers are stained, so that the course of the tract can be followed by this means.

According to Linowiecki ('14), who worked in Ranson's laboratory, also with the pyridine-silver method, the same obtains in the guinea-pig. In this animal the tract lies in the posterior column, but it does not form such a compact uniform area when stained by this method as is found in the rat, indicating, apparently, that the proportion of medullated to non-medullated fibers is greater in the guinea-pig.

The pyridine-silver method may be regarded as the complement of the Marchi method since the latter stains only medullated fibers in the process of degeneration.

#### PRESENT INVESTIGATION

The object of the present research was to trace the fibers of the pyramidal tract in the guinea-pig from their origin in the cerebral motor cortex to their termination in the lower levels of the brain and spinal cord. The method of secondary degeneration was employed, with Marchi staining.

Eight animals (adults) in all were used. The cerebrum was exposed on the left side, under ether anesthesia, and the motor cortex removed. At the end of periods varying from twelve to sixteen days after the operation they were killed by ether or coal gas, when the brain and spinal cord were removed and placed in 3 per cent potassium bichromate. After three weeks in this fluid, with frequent changing, the tissue was cut into slices 3 to 4 mm. thick and placed in Marchi's fluid (3 per cent potassium bichromate, 4 parts, 1 per cent osmic acid, 1 part). At the end of eighteen days the pieces were removed, washed in running tap water for twelve hours, and taken through the alcohol-xylene-paraffin series into paraffin in which they were imbedded and cut. Sections from all levels of the brain and from most of the segments of the spinal cord were mounted and examined.

#### COURSE OF PYRAMIDAL TRACT FOLLOWED BY SECONDARY DEGENERATION

The course of the pyramidal tract through the midbrain, pons and upper part of medulla oblongata is similar to that found in the higher mammals such as the cat, dog, monkey and man, and is so well known that no detailed description need be given. In the midbrain it occupies the middle three-fifths of the crusta, more or less, and is continued downwards as the pontine bundles, which unite at the lower border of the pons to form the anterior pyramid of the medulla. Above the level of

the general decussation, in the lower part of the medulla oblongata, there is no evidence of any crossing of fibers; all the degeneration appears to be confined to the side of the lesion.

Sections through the lower or closed half of the medulla oblongata, about 1 mm. below (caudal to) the calamus scriptorius, show the beginning of the pyramidal decussation. The

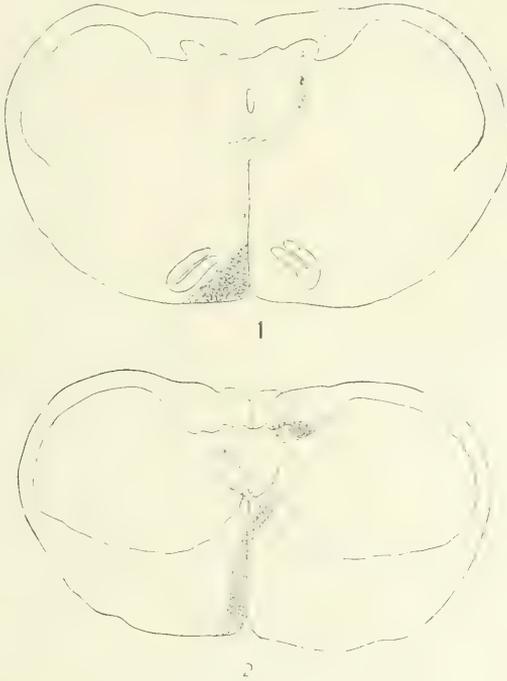


Fig. 1 Transverse section, medulla oblongata through upper extremity of pyramidal decussation.  $\times 10$ .

Fig. 2 Transverse section, medulla oblongata through middle of pyramidal decussation.  $\times 10$ .

pyramid, in transverse section, is triangular in outline at this level, and from the dorso-mesial angle a few fibers can be seen passing backwards along the median raphé. They cross the raphé close to the central gray matter and curving outwards, in front of the hypoglossal nucleus, turn backwards in the gray substance. One or two small bundles reach the posterior column but most disappear in the gray matter (fig. 1).

In sections at a lower level, about the middle of the decussation, the fibers cross in great numbers and in more or less well defined bundles which follow an undulating course, interlacing with corresponding bundles from the sound side. After crossing the raphé the fibers turn outwards and then curve backwards and inwards through the gray matter, most of them passing into the funiculus cuneatus, where, cut transversely, they form a distinct and compact tract (fig. 2). Along the dorsal margin of the gray matter a few small bundles are seen on the mesial side of the main crossed tract.

At this level a single small strand of degenerated fibers runs backwards through the gray matter on the same side, close to the central canal, and then makes a sharp bend outwards; it disappears in the gray matter before it reaches the dorsal column. It is a very small bundle, with a horizontal course, since it is present only in four consecutive sections.

At the junction of the medulla with the spinal cord (fig. 3) practically all the fibers have crossed and the tract formed lies in the funiculus cuneatus. It is more or less triangular in outline, but a few small detached bundles extend from its mesial angle along the dorsal margin of the gray matter, as described in the last section. The homolateral bundle, seen near the middle of the decussation, is absent at this level; the crossing seems to be complete, no degeneration being visible on the same side. Between the upper and lower limits of the decussation many fibers seem to have disappeared since the degeneration in the anterior pyramid is denser and occupies a more extensive area than in the crossed dorsal column tract. These have presumably terminated in the gray matter of the bulb in this region.

In the first cervical segment the crossed pyramidal tract reaches its largest size. It lies in the column of Burdach of the opposite side, in contact with the posterior horn and gray commissure. It is somewhat triangular in outline, its ventromesial angle extending to the middle line and meeting its fellow of the homolateral side (fig. 4). All the fibers of the tract have decussated and there is no evidence of any degeneration in the

crossed lateral or direct ventral columns as is the case in the Canadian porcupine.

Sections through the second cervical segment (fig. 5) show a considerable change in the area occupied by the fibers of the tract. It is crescent-shaped; the dorsal border is concave; the mesial border lies against the posterior medium septum, oc-

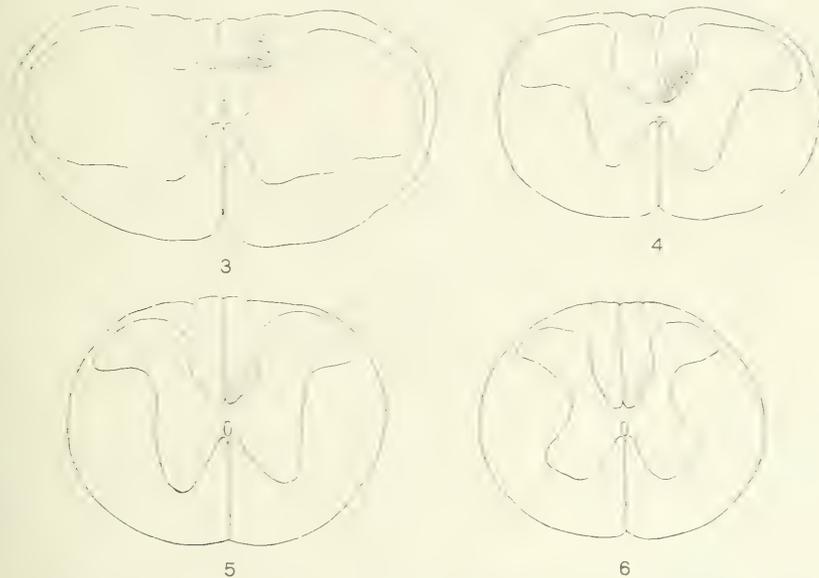


Fig. 3 Transverse section, medulla oblongata through lower (caudal) extremity of pyramidal decussation.  $\times 10$ .

Fig. 4 Transverse section, first cervical segment of spinal cord.  $\times 10$ .

Fig. 5 Transverse section, second cervical segment.  $\times 10$ .

Fig. 6 Transverse section, fifth cervical segment.  $\times 10$ .

cupying about one-fourth of the distance between the posterior gray commissure and the free margin of the section. The degeneration is less dense than in the first cervical segment indicating a distinct diminution in the number of fibers.

In the third, fourth and fifth cervical segments (fig. 6) the general appearance of the tract changes little, but there is a progressive falling off in the number of fibers which it contains.

The degeneration seems to be densest near the gray matter, the fibers becoming more and more scattered towards the dorsal border of the area.

Between the fifth cervical and first thoracic segments (fig. 7) a still further diminution in the number of fibers is evident. In the latter segment the tract, considerably reduced in size, occupies an oval area which is no longer in contact with the posterior median septum except at its ventro-mesial extremity.

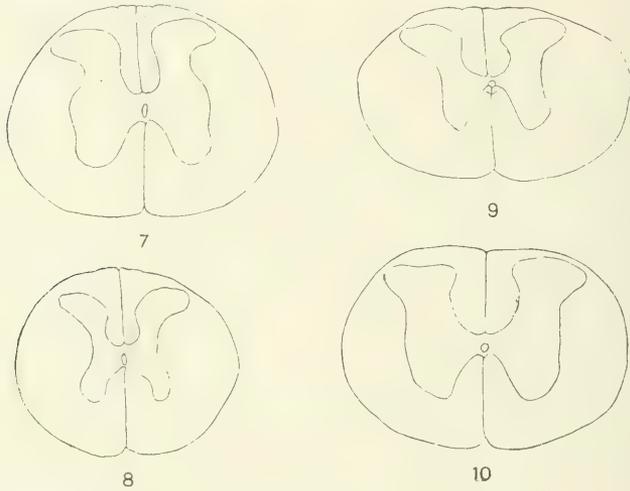


Fig. 7 Transverse section, first thoracic segment.  $\times 10$ .  
 Fig. 8 Transverse section, eighth thoracic segment.  $\times 10$ .  
 Fig. 9 Transverse section, first lumbar segment.  $\times 10$ .  
 Fig. 10 Transverse section, fourth lumbar segment.  $\times 10$ .

In the eighth thoracic segment the area of degeneration is still more restricted (fig. 8). It has now withdrawn from the middle line and lies in the recess formed by the narrowing of the neck of the posterior horn. Tracing it caudalwards it is found to occupy the same relative position in the succeeding segments, becoming more and more reduced in size until the fourth lumbar segment is reached, where it is represented by a very small number of scattered fibers lying against the neck of the posterior horn (figs. 9-10). Beyond this level it cannot be followed.

It is interesting to compare the above results, obtained by the Marchi method, where the medullated fibers alone are stained, with those of Linowiecki, in the same animal (guinea-pig), who used the pyridine-silver method which brings out the non-medullated fibers. According to his description: "In the seventh cervical segment the pyramidal tract is located in the ventral part of the posterior funiculus. . . . The fibers of the tract are more densely grouped ventrally and laterally near the grey substance and this gives the cross section of the two tracts somewhat the form of the letter V." (Compare with figures 6 and 7.)

At the level of the eighth thoracic segment, by the pyridine-silver method, the tracts are crescentic in outline and much diminished in size; they are still further reduced at the twelfth thoracic segment where they consist of two compact groups of axons which have become separated at the posterior median septum. Proceeding caudalwards they become less distinct and at the level of the second lumbar segment the groups tend to move posteriorly and to separate from each other. From here on they narrow markedly and fade in color until at the level of the fifth lumbar segment they consist of two narrow strips, one on each side of the posterior median septum, which are hardly visible.

It will thus be seen that the descriptions of the position and outline of the pyramidal tract, as brought out by the two methods, are in close agreement. This would indicate that the mixture of medullated and non-medullated fibers, of which the tract appears to be made up, is more or less uniform throughout its entire course in the spinal cord.

In the fifth lumbar segment, according to Linowiecki, "the tracts consist of two narrow strips on each side of the posterior median septum,"<sup>1</sup> but he does not say whether they are in contact at the septum or separated from each other. At the level of the fourth lumbar segment almost the same words might

<sup>1</sup> Taken as it stands, this sentence would seem to indicate that the tract is represented by *two* narrow strips on each side. What the author does mean, probably, is that there are two narrow strips, *one* on each side.

be used to describe the tract, as brought out by the degeneration method, if it be added that the narrow strip lies close to the mesial aspect of the gray matter forming the neck of the posterior horn (fig. 10).

#### SUMMARY

The course of the pyramidal tract in the guinea-pig, from the beginning of the decussation in the medulla oblongata caudalwards, as brought out by the method of secondary degeneration, with Marchi staining, is as follows:

The decussation begins about 1 mm. below the level of the calamus scriptorius and ends near the junction of the medulla with the spinal cord. All the fibers cross, between these limits, and most pass on into the funiculus cuneatus where they turn caudalwards into the spinal cord but many end in the gray matter of the bulb in this region. As this dorsal column tract is followed downwards, from segment to segment of the cord, its outline changes considerably (see figures) and there is a progressive diminution in the number of fibers which it contains, but this loss of fibers is most marked in the upper cervical and lower thoracic regions.

The tract cannot be traced farther than the fourth lumbar segment, where it is represented by a very few degenerated fibers lying close to the gray matter of the posterior horn.

According to Ranson the pyramidal tract consists of a mixture of medullated and non-medullated fibers, the former of which, while undergoing degeneration, may be stained by the Marchi method, the latter by the pyridine-silver method. The description of the spinal portion of the tract in the guinea-pig given by Linowiecki, who used the pyridine-silver method, is in close agreement with what I have found by the degeneration method; this would appear to point to the fact that the mixture of the two varieties of fibers, within the tract, is fairly uniform throughout its course.

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A STUDY OF THE AFFERENT FIBERS OF THE BODY  
WALL AND OF THE HIND LEGS TO THE CERE-  
BELLUM OF THE DOG BY THE METHOD  
OF DEGENERATION

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SEVEN FIGURES

This work was begun under the stimulus of the work of Bolk, who based an hypothesis of a localization of a series of coördinating centers in the cerebellum upon studies in comparative anatomy. Bolk has simplified the nomenclature of the parts of the cerebellum and, using his terms, sums up the localization in the cerebellum as follows:

The lobus anterior cerebelli contains the coördinating centers for the groups of muscles of the head, namely those of the eyes and tongue, the muscles of mastication and muscles of expression beside those of the larynx and pharynx; the lobulus simplex contains the coördinating centers for the neck musculature; the upper part of the lobulus medianus posterior contains the unpaired coördinating centers for the right and left extremities; the lobuli ansiformes and paramediani contain the paired centers for the two extremities, while the rest of the cerebellum has the coördinating centers for the trunk musculature ('07, p. 170).

In general, Bolk thinks that the coördinating centers for symmetrical muscles which act together are in the vermis, while the centers for those which act independently are in the hemispheres.

This hypothesis has been borne out by the experimental work of Rynberk ('04) from Luciani's laboratory, for example, by obtaining special movements of the neck muscles after a unilateral extirpation of the lobulus simplex. These results suggest further work on the end station of the fibers of the different regions of the body by the method of degeneration, though

it is of course clear that the tracing of the afferent fibers of each region to their end station does not unravel the nature of a coordinating center in the cerebellum. The results of tracing the fibers of the different regions of the cord to the cerebellum indicate that the fibers of each of the regions of the body sends fibers to almost the entire vermis.

The most recent and the most extensive work on determining the distribution of spinal fibers in the cerebellum is that of Sir Victor Horsley in 1909. In this article he gives a complete analysis of the literature of the work on the fasciculus spino-cerebellares ventralis and dorsalis. As far as the point of the distribution of the fibers to the cerebellum is concerned, the main results are as follows: In 1890 Auerbach stated that the fasciculus dorsalis (Flechsig) ended in the dorsal—that is to say, in the cephalic—part of the superior vermis, and the fasciculus ventralis (Gower's) in the ventral part. In 1892 Mott reversed this statement by showing that the fasciculus dorsalis ends in the vermis, caudal to the end station of the fasciculus ventralis, which enters the cerebellum farther cerebralwards by way of the brachium conjunctivum or superior cerebellar peduncle. This point is well shown in the well-known diagram of his figure 1, page 219.

Collier and Buzzard in 1903, in an analysis of human material, confirmed Mott's view of the relative position of the end station of the dorsal and ventral cerebellar tracts; that is, that the fasciculus spino-cerebellaris dorsalis ends in the inferior vermis, though they find that some of the fibers end in the nucleus dentatus and in the nuclei of the roof. The fasciculus spino-cerebellaris ventralis they trace by way of the superior cerebellar peduncle to the superior vermis but in small part also to the lateral hemispheres.

Sir Victor Horsley divided the cord in a general way into four regions: the first, from the first to the fourth cervical segment, representing movements of the head and neck; the second, from the fifth cervical to the first thoracic segment, representing movements of the arm; the third, from the second thoracic to the second lumbar, representing movements of the body; and the

fourth, from the third lumbar to the second sacral, representing movements of the leg. He made the lesion cover the fibers representing a given region, by destroying the cells of origin for the tract rather than the fiber tract itself. Indeed, his purpose was to determine which cells of the cervical and lumbar regions of the cord are homologous with the nucleus dorsalis. The lesion for the first or upper cervical region was in the gray matter of the cord, taking in the cells in the homologous position to the nucleus dorsalis and the cells of the middle region of the gray matter. Within the cerebellum the fibers passed to all the vermis except the most anterior part of the lobus anterior, namely, the lingula, and the most posterior part of the lobulus medianus posterior, namely, the uvula and the nodulus. Thus the end station for the afferent fibers of the neck is not limited to the lobulus simplex but includes almost the entire anterior lobe and most of the median posterior lobe as well.

The fibers of the second region, representing fibers from the arm, he found to pass forward mainly on the same side but in part on the opposite side. Within the cord they run both in the dorsal and in the ventral cerebellar tracts. Within the cerebellum they end in the lobulus centralis, the ventral half of the culmen and the ventral half of the pyramidalis; or in Bolk's terminology, in the lobus anterior, in all the lobulus simplex and in most of the lobulus medianus posterior. Horsley did not study the fibers of the third of the body regions, but the fibers from the leg region he found ended in exactly the same parts of the cerebellum as those of the arm region.

Thus from the literature it is clear that the spino-cerebellar fibers end in the vermis; that the fasciculus spino-cerebellaris ventralis (Gower's tract) ends in the more cerebral part of the vermis, while those of the fasciculus spino-cerebellaris dorsalis end in the more caudal part of the vermis. The fibers representing the four regions of the body—namely, the neck, the arms, the body and the legs—pass through both cerebellar tracts and are distributed to all parts of the vermis except the most anterior and the most posterior folia. These results are confirmed in the experiments herein reported.

As far as the symptoms of lesions of the cerebellar tracts are concerned, our results also agree with those of Horsley, who found that there was no loss of efferent (purposeful) movement in the muscles involved; that all the motor effects were transitory and probably due to interference with the anterior cornus, and that there was ataxia and clumsiness of movement. These results are practically the same as those of Bing.

#### TECHNIQUE AND METHOD OF INVESTIGATION

Three dogs, each about the size of an ordinary fox terrier, were used for the purpose of our study, and in all cases the technique of operation and preparation of material was identical. In Dog 1, experiments with regard to various sensations were carried out during the period between the operation and the killing of the animal for histological study. As these experiments were not of a sufficiently satisfactory nature, they were omitted in Dogs 2 and 3, but are recorded in connection with Dog 1 for the sake of completeness. The artificial lesion in the cords of the dogs was made as follows: An incision was made in the median line of the back, extending between the scapulae down toward the lower dorsal region, for a distance of about 10 cm. Very little hemorrhage took place and this was quickly stopped. Laminectomy of the 4th, 5th and 6th dorsal vertebrae was performed and the cord in its dura exposed. An aneurism needle was placed under the cord very gently and the cord in its dura was lifted slightly and rotated a little toward the left. A very superficial slit was then made with a narrow scalpel, through the dura and into the substance of the cord at right angles to its long axis, and on the right side of the animal, in an effort to cut the fasciculus spino-cerebellaris dorsalis (Flechsigs) and possibly the fasciculus spino-cerebellaris ventralis (Gowers); the cord was then slipped back. There was no hemorrhage in any case during the cutting of the cord.

The wound was closed in tightly by sutures of silk through the muscles, fascia, subcutaneous tissue and skin. All the dogs made prompt recoveries, but in Dog 2 the skin layer of the

wound was opened by the dog's scratching on his cage. The skin separated, but the wound was kept swabbed out with iodine so that the lower layers did not open and were not involved in the superficial infection. The dog improved steadily and granulations formed rapidly over the wound.

The operations were performed by Dr. Goetsch, of the Johns Hopkins Hospital, on Dog 1; by Dr. Hunnicutt, of the Johns Hopkins Hospital, on Dogs 2 and 3, under strict aseptic precautions, the total time of anesthesia being from an hour and a quarter to two hours; ether was used as an anesthetic.

The dogs were kept alive for periods of ten days to three and one-half weeks, during which time observations were made on them in order to ascertain the nature of the symptoms caused by the lesion. The wounds of the operations healed perfectly within a few days. The symptoms observed were recorded each day, and in brief were as follows:

*Dog 1.* April 26, 1910, the day after the operation, 9.00 A.M. No apparent disability except in use of hind legs; dog sits at rear of cage, with left leg extended and raised at an angle of  $35^{\circ}$  to  $45^{\circ}$ ; right hind leg somewhat flexed and lying on floor. When called and coaxed by snapping the fingers, the dog responds by wagging tail, and by slight efforts at movement, but does not actually change position. Head, fore legs and fore feet, are moved in a perfectly coördinated and intelligent way.

April 27, 1910, 9.30 A.M. Dog still sits in about the same position as on previous day, but the left hind leg, instead of being raised, is more nearly or quite touching the floor. When called, the dog crawls to the door of the cage, locomotion being accomplished mainly by the use of the fore legs, the animal remaining in the sitting posture throughout. The hind legs are both more or less flexed, and during locomotion perfectly definite, although almost ineffectual, movements are made by them; the toes of the left hind leg are occasionally flexed. Movements of all parts of body except hind legs seem perfectly normal.

April 28, 1910. When taken out of cage to-day, the dog moves along with its hip against the wall for support, the hind legs not working much better than the day before. Once it sat down and scratched with the right hind leg, just posterior to the right fore leg.

April 29, 1910. When taken out on the grass, the dog took several steps in a normal manner on all four legs, but finally the hind legs weakened, spread apart, and collapsed, throwing the rear half of the dog's body first to one side and then to the other. The dog was seen to scratch again in the same manner as yesterday, only the

left hind leg was used. Hot and cold water was applied to all four legs, by means of dipping the latter into a beaker of water (during these tests the dog was blindfolded). Following are the results:

Temperature of 3° to 0°C. Causes no effect on any of feet.  
 Temperature of 20° to 37°, 47°, 57° Causes no effect on any of feet.  
 Temperature of 67° Causes both hind legs to be withdrawn from the water 20 seconds after time of immersion; both front legs were withdrawn 2 to 3 seconds after immersion; pinching toes with forceps causes prompt withdrawal of all four legs.

April 30, 1910. This morning the dog shook the fore part of the body, also got up on all four legs and stood for some time.

Temperature of 37° Gives no effect on any of feet.  
 Temperature of 47° No effect on hind legs; right front leg was withdrawn when the tips of toes came in contact with the water; left fore leg not tried.  
 Temperature of 57° No effect on hind legs; right fore leg was withdrawn on being touched to the water; no effect on left fore leg.  
 Temperature of 58° All legs withdrawn from 6 to 12 seconds after time of immersion, fore legs a little sooner than hind legs; toes of hind legs were shaken in the water before withdrawal.  
 Temperature of 65° Same as 58°. Tail withdrawn 6 seconds after immersion in water of 56° temperature.

May 2, 1910. Dog showed marked improvement in walking more normally. Hind legs used most of the time, but with an unsteady, swaying motion from side to side.

Temperature of 37° No effect on hind feet; front feet were withdrawn upon contact with water. Note: When the fore feet were held in the water, no signs of discomfort were apparent.  
 Temperature of 43° Same as at 37°.  
 Temperature of 53° Same as at 37°, except that discomfort was evidenced when fore feet were held in the water.  
 Temperature of 57° Left hind foot withdrawn in 5 seconds; right hind foot withdrawn in 15 seconds; fore feet both withdrawn upon contact.

May 3, 1910. Improved general condition was noted to-day, with better use of hind legs, although the drunken, swaying gait was still marked.

- Temperature of 28° No effect on hind feet; fore feet withdrawn upon contact with the water.
- Temperature of 35° Hind feet withdrawn after 15 seconds; fore feet withdrawn upon contact.
- Temperature of 47° Right hind foot withdrawn upon contact; left hind foot not withdrawn at all; both fore feet withdrawn upon contact; no effect on tail.

May 4, 1910. Temperature observations.

- Temperature of 28° Hind feet at first withdrawn upon contact, but subsequently allowed to remain in water; fore feet withdrawn upon contact and not subsequently allowed to remain.
- Temperature of 37° No effect on hind feet; fore feet withdrawn upon contact, but allowed to remain upon subsequent immersion.
- Temperature of 47° All feet withdrawn almost immediately; when beaker containing no water was touched to the hind feet there was no withdrawal nor other noticeable effect; same beaker to fore foot caused withdrawal of latter; right fore foot was not withdrawn.

May 7, 1910.

- Temperature of 42°, No effect on hind feet; fore feet withdrawn upon contact.
- Temperature of 45°, 47° Same as 42°.
- Temperature of 55° Hind feet withdrawn after 9 seconds; fore feet withdrawn upon contact.

May 10, 1910. Again a marked improvement in the use of the hind legs was noted. Dog was very lively, running and jumping around, but there was still lack of coördination in the hind legs.

May 11, 1910. Dog was livelier than on previous day; ran about and sprang upon the observer in puppy-like fashion, playing around with very little departure from normal movements. However, the same uncertain gait of the hind legs was noticed when the dog would stop jumping and either walked or ran slowly away.

May 13, 1910. Knee jerks of hind legs present and equal on both sides.

May 14, 1910. Dog killed; brain and cord removed.

*Dog 2.* December 7, 1912. Operation, 10 A.M.

December 7, 1912. Dog conscious and sitting up at 2.30 P.M.

December 8, 1912. Sitting up; can move back legs.

December 9, 1912. Makes attempt at locomotion with hind legs.

December 10, 1912. Can stand up on all fours.

December 11, 1912. Walks a little, with a typical, extreme ataxic gait (back feet sometimes interfering with each other) and often falls to one side or the other.

December 13, 1912. In getting into his box, there seems to be more uncertainty of his right than of his left hind leg: dog walks more today, same gait but some improvements.

December 14, 1912. 4 P.M. walks about with considerable ease; follows one around the room, comes when called, etc. His hind legs, however, are very ataxic, the right being noticeably more so than the left.

December 15, 1912. Walks about; shows much improvement in gait.

December 16, 1912. Gait much improved; ataxia in right hind leg still shown.

December 17-20, 1912. Gait steadily improving; also marked gain in weight.

December 20, 1912. Dog killed; brain and cord removed.

*Dog 3.* December 14, 1912. Operation, 10.30 A.M.

December 14, 1912. 4 P.M.; dog gets up on all fours and walks about the room; his hind legs being very ataxic, but his recovery in general being quicker and his ability to walk coming much sooner than was the case in either Dogs 1 or 2.

December 15, 1912. Dog walks about the room; ataxia of right hind leg.

December 16, 1912. Walks about; ataxia of right hind leg perfectly distinct.

December 17-20, 1912. Improvement rapid and more complete than in Dogs 1 and 2; runs and jumps about the room.

January 8, 1913. By this time no ataxia can be noticed: dog's gait and actions are apparently normal in every way.

January 10, 1913. Dog killed; brain and cord removed.

At periods, varying from 10 days to  $3\frac{1}{2}$  weeks from the dates of operation, as noted above, the dogs were anesthetized with chloroform. The right femoral vein was opened, after which a cannula was inserted into the left common carotid artery and through the latter a liter and a half of 10 per cent formalin solution was injected into the animals.

After waiting half an hour in order that as much hardening as possible might take place, the brain and cord were removed carefully. The cord was cut into three approximately equal lengths and put at once into a vessel containing a 10 per cent solution of formalin. The material was left in this solution until various parts of it were wanted for study. The first blocks

were cut out of the cord three days after it had been put into the formalin and the other blocks were taken subsequently for study throughout the following year; all the blocks were from 5 to 10 mm. thick. The part containing the medulla and pons, with the cerebellum, was cut into four blocks which were numbered as is shown in figure 1. As will be seen, the first and second blocks contain, in Bolk's nomenclature, the lobulus medianus posterior of the cerebellum; the third block contains the lobulus simplex and the caudal part of the lobus anterior; the first block contains the rest of the lobus anterior. The blocks were all treated in the same manner; they were stained *en bloque* by a modified Marchi method; they were placed for from 5 to 7 days in the following solution:

Osmic acid	1 part
NaIO <sub>3</sub>	3 parts
Distilled water	300 parts

The blocks were embedded in celloidin and all the sections showed an excellent staining of the degenerated myelene.

#### EVIDENCES OF DEGENERATION IN THE SECTIONS

The study of the sections of the first dog showed above the lesion degenerated fibers scattered throughout the section on both sides. In the upper cervical region, as shown in figure 2, there is a very abundant, scattered, bi-lateral degeneration of fibers, covering the entire area of the fasciculus spino-cerebellaris dorsalis and ventralis. It is difficult to understand why there is so extensive a degeneration on the left side, inasmuch as the cord was cut only on the right; but as has been seen, the symptoms involved both sides and there is an almost symmetrical degeneration.

Sections through the first block containing the cerebellum, as shown in figure 3, have a concentration of the degenerated fibers in the corpus restiforme and along the lateral margins of the medulla, with a second concentration in the tractus spino-cerebellaris ventralis, especially of the right side. In the cerebellum the degeneration is confined to a band across the vermis

in its ventral third. This degeneration does not appear until the cephalic end of the first block is approached.

As one follows the sections farther cerebralward in the second block, as seen in figures 4 and 5, there is the same concentration of degenerated fibers in the corpus restiforme and along the right margin, while the fibers on the left side are less numerous but have the same general pattern. Within the cerebellum the degenerated fibers are found throughout the second block, that is, the lobulus medianus posterior. For the most part, the degeneration is confined to the vermis but in figure 5 can be seen a few fibers in the edge of the hemispheres.

From here on the course of the fibers of the fasciculus spino-cerebellaris dorsalis can be followed easily in their position in the inferior cerebellar peduncle. In the third block, as seen in figure 6, the fibers of the corpus restiforme can be seen in the folia of the vermis of the lobulus simplex. The cephalic limit of the ending of the fasciculus spino-cerebellaris dorsalis within the cerebellum is reached in the caudal half of the third block, as shown in figure 6. Above this level, namely, in the lobus anterior, only the fibers of the fasciculus spino-cerebellaris dorsalis are to be found (fig. 7).

It will thus be seen that we have traced those fibers of the fasciculus spino-cerebellaris dorsalis (Flechsig), which represent the legs and possibly the lower body wall, from their situation in the cord, up through the corpus restiforme of the medulla into the vermis cerebelli, in the caudal half of which they were distributed. In the most caudal part of the vermis their distribution is confined to one or two laminae, but farther cerebralward the distribution is very diffuse throughout all the laminae.

A part of the sensory fibers from the legs and lower body wall pass to the cerebellum through the fasciculus spino-cerebellaris ventralis (Gowers). These fibers occupy the antero-lateral margin of the cord, being more scattered than those of the dorsal tract of Flechsig. The ventral position of the tract is plain in figures 4, 5 and 6 for the region of the medulla. In the pons the

ventral fibers shift to their more dorsal position in the lateral margin of the brachium conjunctivum, as seen in figure 7. Throughout the cephalic half of the third block and a part of the fourth block—that is, in the lobus anterior—the fibers of the fasciculus spino-cerebellaris ventralis are distributed throughout the folia of the vermis, as seen in figure 7.

It has thus been made clear that the fibers from the legs and lower body wall pass to the vermis of the cerebellum and are distributed throughout the vermis, with the exception of the most anterior and the most posterior folia. A part of these fibers pass through the dorsal cerebellar tract and the inferior cerebellar peduncle to the caudal half of the vermis, while the fibers of the ventral tract pass through the superior cerebellar peduncle to the cephalic half of the vermis.

In the second dog the lesion was in the sixth segment and sections at the level of the operation showed that the entire dorsal cerebellar tract, and a part, if not all, of the ventral tract, were cut. Both above and below the lesion, degenerated fibers were very numerous all through the ventral half of the white columns and along the dorso-lateral margins. There were a few scattered degenerated fibers in the fasciculi gracilis and cuneatus. Above the lesion the degeneration was nearly identical with that found in the first dog. The degeneration of the cerebellar tracts was again double, though the lesion was confined to one side. There were fewer degenerated fibers on the un-operated side than in the first dog. In the distribution of the degenerated fibers in the cerebellum there was a little farther extension of the fibers into the lateral hemispheres. In the third dog the results were practically the same as in the other two. There was the same bilateral degeneration, less extensive on the un-operated side; the distribution of the fibers in the cerebellum also showed a slightly greater extension into the lateral hemispheres than is shown in the figures from the first dog.

## CONCLUSIONS

1. The only symptoms caused by a lesion of the spino-cerebellar tracts in the dog are those referable to a loss of muscle sense and tone. The symptoms were bilateral in all three experiments and there was almost complete recovery in three weeks.

2. These symptoms, in a lesion of the tracts at the level of the sixth thoracic spinal nerve root, are confined to the hind legs and possibly the lower portions of the trunk.

3. The fasciculus spino-cerebellaris dorsalis, so far as its distribution in the cerebellar cortex is concerned, is confined to the caudal half of the vermis, and to the medial portion of the lateral hemispheres.

4. The distribution of fasciculus spino-cerebellaris ventralis in the cerebellar cortex is confined to the cephalic half of the vermis.

5. There is no definite cerebellar center for association regarding the hind legs.

6. The cerebellar tracts are represented by crossed, as well as by direct, fibers in the dog.

My very earnest thanks are due to Dr. Florence R. Sabin, whose coöperation and help alone have made this paper possible; and I also wish to express my thanks and appreciation to Drs. Emil Goetsch, Jacobson, and Hunnicutt, of the Johns Hopkins Hospital, for their invaluable help in operating on the dogs used.

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## PLATE 1

## EXPLANATION OF FIGURE

1 Dorsal view of the medulla and the cerebellum of a dog, to show the blocks into which the cerebellum was cut. The well marked groove between the lobus anterior and the lobulus simplex falls in the third block. The first and second blocks contain the lobulus medianus posterior; the third block includes lobulus simplex and a part of the lobus anterior, while the fourth block includes the rest of the lobus anterior.

2 Section of the upper cervical cord of Dog 1, to show the degeneration above the lesion.  $\times 8$ . The outlines of all the sections were made with an Efinger apparatus and the degenerated fibers filled in free hand. The right side of the sections is the right side of the animal.

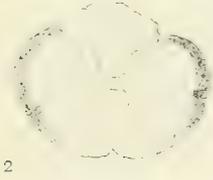
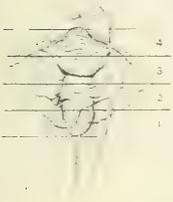
3 Section of the medulla and cerebellum of Dog 1, taken through the cephalic part of the first block as shown in figure 1.  $\times 8$ . It shows degenerated fibers on both sides, in the corpus restiforme and in the fasciculus spino-cerebellaris ventralis in the medulla. In the cerebellum it shows degenerated fibers in one folium of the middle region of the lobulus medianus posterior.

4 Section through the medulla and cerebellum of Dog 1, taken through the caudal part of the second block.  $\times 8$ . It shows the separation of the fibers of the corpus restiforme from those of the fasciculus spino-cerebellaris ventralis (Gowers).

5 Section through the medulla and cerebellum of Dog 1, taken through the cephalic end of the second block.  $\times 8$ . It shows degenerated fibers of the left fasciculus spino-cerebellaris dorsalis, entering the lobulus medianus posterior of the vermis.

6 Section through the medulla and cerebellum of Dog 1, taken through the caudal end of the third block.  $\times 8$ . The section is so near the line shown on figure 1 that the section of the pons is incomplete; it shows the lobulus simplex.

7 Section through the pons and cerebellum of Dog 1, taken through the cephalic end of the third block.  $\times 8$ . It is above the level at which the corpus restiforme enters the cerebellum and shows the fasciculus spino-cerebellaris ventralis in the edge of the brachium conjunctivum and the fibers of the same tract in the lobus anterior of the vermis.





# SOME NEW RECEPTACLES FOR CADAVERS AND GROSS PREPARATIONS

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EIGHT FIGURES

## RECEPTACLES FOR CADAVERS

Institutions which do not possess cold storage facilities usually keep cadavers in tanks of various kinds. These may be of metal (galvanized iron), wood with a metal lining (lead, zinc or copper), or of concrete. The fluids used are such, however, that the galvanized tank rusts through in a comparatively short time, since in most cases it cannot be protected by paint, on account of the solvent power of the alcohol and carbolic acid used. The stock is usually thin, necessitating the use of angle irons or planks in order to prevent bulging. Also on account of this weakness, if the tank contains material, it may not be moved without injury to the bottom. The lined tanks, which must be soldered at the corners, likewise eventually leak, the solution then making its way into the spaces between the lining metal and the wooden support. The lining is rarely smooth, considerable dirt accumulating, therefore, in cracks and uneven places. On account of these factors, the tank becomes foul and undesirable in a well-kept laboratory. Concrete tanks properly built and lined with cement are satisfactory, excepting that they cannot be moved and that they take up a large amount of space.

After some experience with various types of receptacles, the tank described below was designed for the Anatomical Laboratories at Pittsburgh. It has now been in use for more than a year and has given perfect satisfaction. Essentially it consists of a box built of two-inch cypress with half-inch bolts running in the wood, horizontally through the bottom and vertically in the sides and ends. The individual pieces of wood are fitted together as shown in figure 1. By this method of construction a solid and exceedingly strong box is obtained. If any leakage occurs through excessive drying, it is necessary only to tighten the nuts on the bolts. The case is further strengthened by two angle irons (*d*, figs. 2 and 4) running lengthwise along the bottom at the corners. Through these pass the horizontal bolts (*a*, figs. 2, 3 and 4), also the vertical bolts (*c*, fig. 3). The ends of

all other bolts pass through bars of iron one-half inch by two inches; *e*, figure 2, for the horizontal bolts, *b*, figure 2; *f*, *g* and *h*, figures 2 and 3, for the vertical bolts *c*. These strengthen the case and prevent heads and nuts from being drawn into the wood through its swelling or the tightening of the nuts. Two strips of oak one inch by six inches (*i*, figs. 2 and 3) are placed lengthwise under the tank in order to permit the floor underneath to be cleaned. These are removable so that they may be eventually replaced. It will be noted that they do not come in contact at any point with the wood forming the floor of the tank, thus preventing its rotting. The top is hinged and consists of one-inch cypress, properly battened. A gate valve for emptying the tank is desirable. Hot oil should be applied to the raw wood unless it is to be painted.

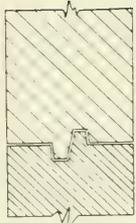
While this tank is moderately heavy, it can be easily moved when empty, or even when partially full, as it is not injured by the use of levers. Even when full of solution there is no bulging of the sides. Since all parts are tightly fitted, it may be used for the storage of cadavers in alcohol fumes. In fact, such a tank has been thus used here for nearly a year, the material keeping perfectly. Such a receptacle, six feet, six inches long by two feet, ten inches wide and two feet, eight inches high, inside measurements, will hold fifteen cadavers of average size. It is practically indestructible and should last indefinitely. The first cost is sixty dollars.

#### RECEPTACLES FOR GROSS PREPARATIONS

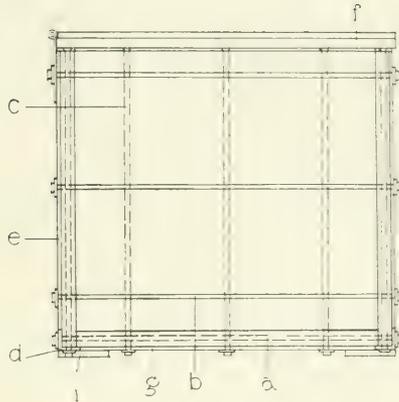
Large gross preparations, particularly dissections, are ordinarily kept in tanks similar in structure to those mentioned for the storage of cadavers, although often smaller in size. Such tanks are subject to the same disadvantages as when used for cadavers. In addition, they are usually unsightly in appearance and therefore undesirable in laboratories and museums. Glass jars of sufficient size for large human preparations are very expensive and easily broken; earthen crocks are likewise very fragile. It is not feasible, moreover, to use either of these for large preparations, such as longitudinal sections. The receptacles described below are designed to serve as museum cases for large specimens and as storage receptacles for material which it is desired to have constantly available for students or members of the instructing staff.

These cases are constructed on essentially the same plan as the cadaver tanks. Lighter material, however, may be used in their construction, since the cubic contents is considerably less. The cypress used is somewhat thinner, the bolts are three-eighths inch instead of one-half inch, while the angle irons and iron bars are omitted entirely, washers being used instead. The tank proper (*j*, fig. 6) is placed on a removable base (*k*, fig. 6) in order to make it of the proper height for use. The top, which is largely of glass, is sloped forward in order to give a good view of the contents when the case

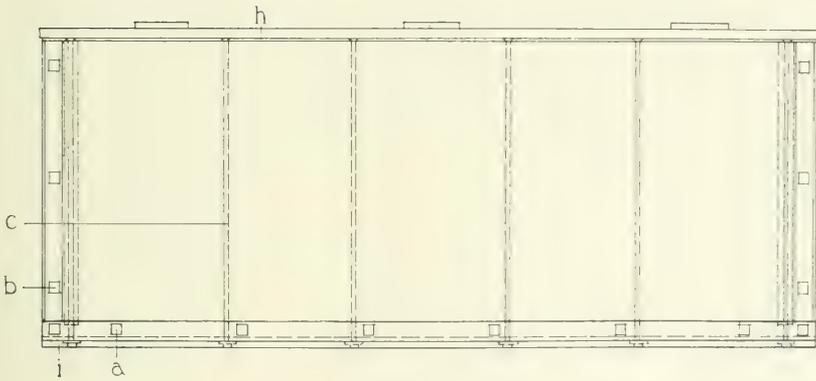
for Cadavers



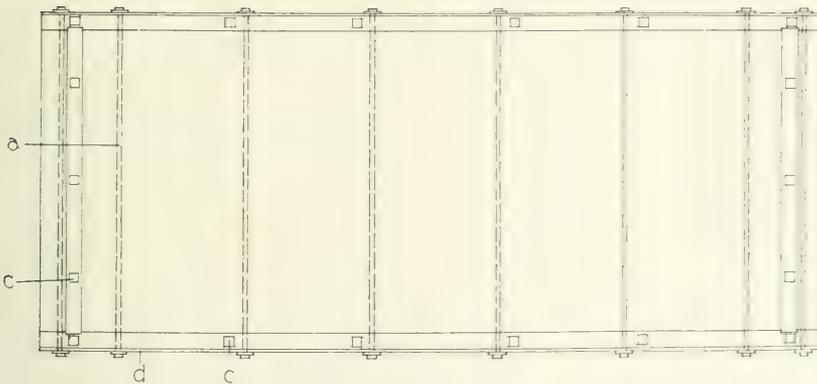
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2.

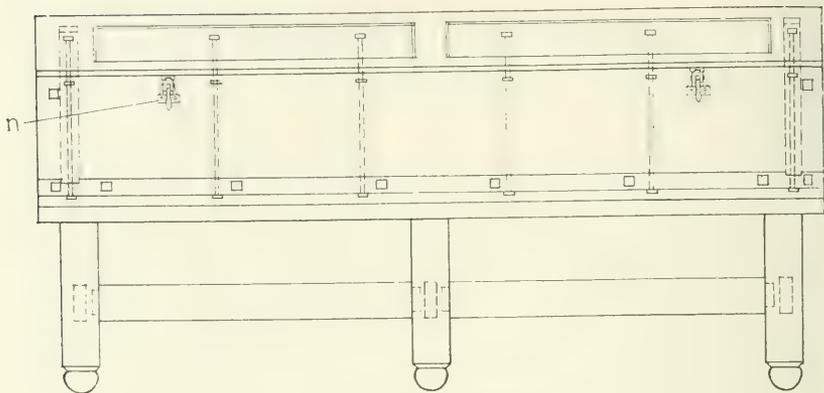


3 Side Elevation.

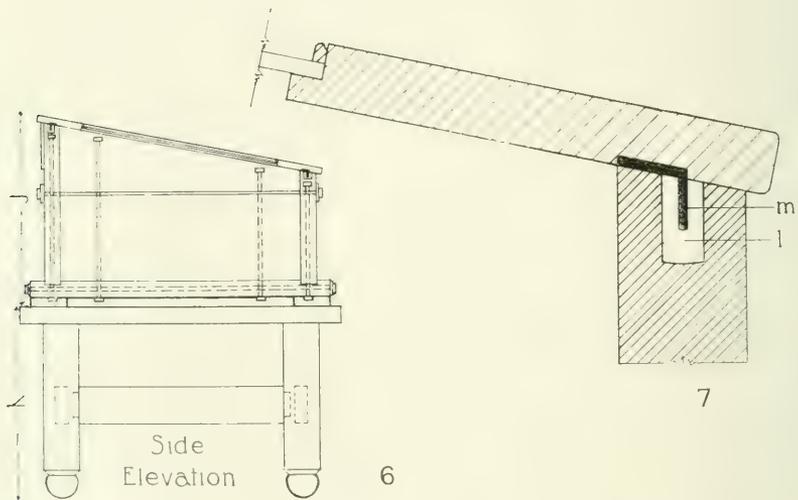


4 Plan of base

For Gross Preparations.



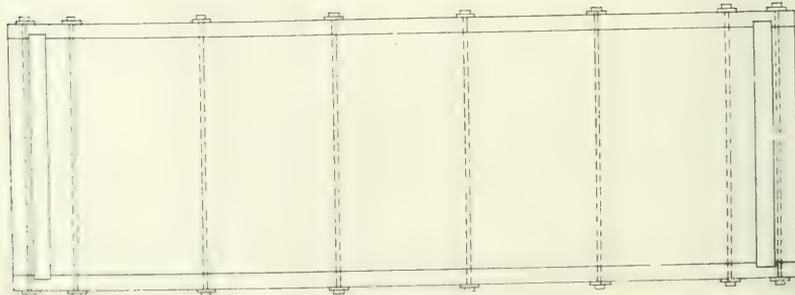
5. Front Elevation



Side  
Elevation

6

7



8. Plan of base.

is used for museum preparations. The groove (*l*, fig. 7) runs entirely around the case; into it projects one arm of an angle iron (*m*). When the groove is filled with cotton the case is made practically air-tight so that there is almost no evaporation where it is desired to keep material in alcohol fumes. The catches used are Corbin tool box locks number 1217 (*n*, fig. 5). By the use of catches of this type, it is possible always to draw the cover tight and likewise lock the case if desired.

At Pittsburgh, we have placed cases of this type in the dissecting-room, where we have found them very useful for longitudinal sections of cadavers and for large gross dissections kept as museum preparations. One case is used for the best dissections made each year by the class, which are thus available the succeeding year as demonstration preparations. Cases of this type, two feet two and a half inches wide, eight feet long, one foot high in front, one foot, six inches in the rear, inside measurements, with a base, may be secured finished for forty-two dollars each.

Acknowledgments should be made to Mr. E. B. Lee, architect, Pittsburgh, for the preliminary sketches. It should also be stated that the idea of using bolts in the manner indicated above was first suggested to me by some small wooden cases which I saw some years ago in the Anatomical Laboratories at the University of Pennsylvania.

## INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with  
Vol. 18, price per volume, \$7.50; foreign, \$8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price  
per volume, \$5.00; foreign, \$5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, begin-  
ning with Vol. 25, price per volume, \$7.50; foreign, \$8.00.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY  
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EMBRYONIC BODIES COMPLETELY ISOLATED  
FROM YOLK-SAC BLASTODERM

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TEN FIGURES

Recent experimental work points to the possibility of a final solution of the problem of the origin of intra-embryonic vascular endothelium. Methods of procedure affording results to which there can be no doubt of interpretation are especially desirable.

So far there have been developed two methods by which yolk-sac 'Angioblast' may be kept out of communication with intra-embryonic vessels: mechanical separation of the vessels of these two regions, and exposure of the developing embryo to anesthetics. The former method was employed to the extent of partial separation by Gräper,<sup>1</sup> Hahn,<sup>2</sup> and Miller and McWhorter.<sup>3</sup> These observers have obtained endothelium on both sides of chick embryos in which one side was severed from extra-embryonic blastoderm. The second method has been perfected by Stockard<sup>4</sup> who has, in cases of arrested development, been able to secure intra-embryonic endothelium quite independent of that in the yolk-sac.

The intra-embryonic vessels in the experiments of Miller and McWhorter were in part non-continuous and somewhat diminutive in size; this is perhaps due to the fact that heart-pressure is necessary for the normal growth of endothelium even after the latter has been established. The work of these two ob-

<sup>1</sup> Gräper, L., *Archiv für Entw. mech.*, Bd. 24, 1907.

<sup>2</sup> Hahn, H., *Archiv für Entw. mech.*, Bd. 27, 1909.

<sup>3</sup> Miller, A. M., and McWhorter, J. E., *Anat. Rec.*, vol. 8, p. 91, 1914.

<sup>4</sup> Stockard, C. R., *Proc. Am. Assn. Anatomists*, *Anat. Rec.*, vol. 9, no. 1, 1915.

servers was submitted as proof of the local origin of intra-embryonic endothelium; previous to and following its publication, this work was objected to quite vigorously on the following grounds: the incision may not have been made sufficiently early or sufficiently close to the embryonic body; vessels may have grown into the injured side from the unoperated side or from either end. To the latter objection Miller has replied that this unusual growth would require a permeation of such solid structures as notochord and neural tube. A later examination of Miller's material by Bremer, as well as Miller's own careful reconstructions, failed to reveal such ingrowths.

While the work of Miller and McWhorter seems in itself to be quite conclusive, a confirmation of their results by more rigorous methods of experimentation may not be superfluous. A most feasible method of procedure seems to be that of complete separation of an embryonic body, or a portion thereof, from the extra-embryonic blastoderm prior to a possible 'invasion' by the so-called yolk-sac 'Angioblast.' If in such a meroplast there should develop legitimate vascular cavities possessed of good endothelium it is confessedly futile to argue further for the necessity of 'Angioblastic' origin of intra-embryonic endothelium.

The following experiments meet, I believe, the objections urged against the work of Miller and McWhorter, supplementing at the same time the work of Stockard.

About forty chick embryos corresponding to stages 4, 5, 6 and 7 of Kiebel's Normentafel (Zweites Heft) constitute so far the material studied. The operations consist in varying degrees of separation of the projecting head from the remainder of the embryonic body and the blastoderm.

One experiment which I shall designate as Type I consisted in the following incisions (fig. 1): longitudinal incisions lateral to, but close to the projecting head on each side, extending from points slightly posterior to the anterior intestinal portal to the opaque area anteriorly; a transverse incision through the embryonic body just posterior to the anterior intestinal portal. Following this the blastoderm was entirely removed except the

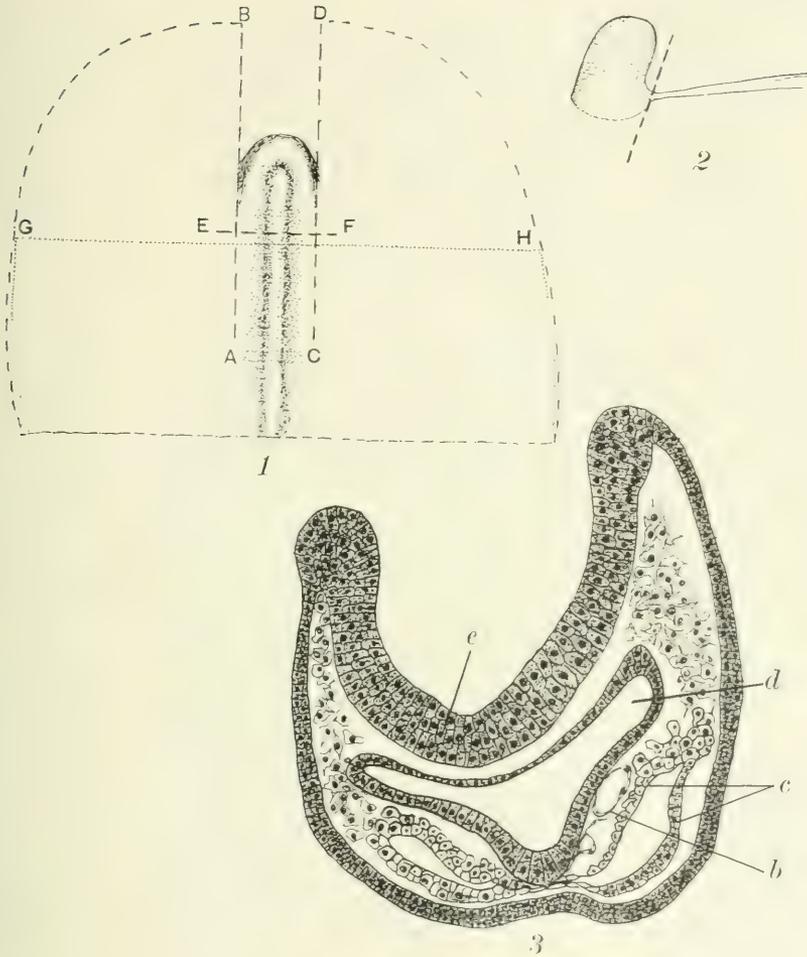


Fig. 1 Diagram to illustrate the blastodermal incisions in experiments of Types I and II. Broken lines represent the incisions in Type I. Dotted line *G* to *H* indicates the transverse incision in Type II in which incision *E* to *F* is omitted.

Fig. 2 Lateral view of the head-fragment of Type I pushed back to be severed from the proamnion at point where a broken line intersects.

Fig. 3 Section through the anterior portion of the forebrain of a head-meroplast, showing unusual head-coelom. Total incubation thirty-two hours. Operation at the time of the first intersomitic groove ( $\times 160$ ). Experiment, Type I, no. 19; *b*, anlage of ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

small strip of proamnion which suspended the head-fragment from the opaque area anteriorly. (In this way it was possible to section later the blastoderm and determine the status of vascular development in the extra-embryonic area and *to know certainly that the embryo had not yet been vascularized*). The head-fragment was then pulled backward and turned so that the proamnion could be snipped off close to the head-tissue proper (fig. 2), leaving a free meroplast which would sink to the bottom of the sub-germinal cavity. The egg was then sealed and incubated further.

Although many other methods were utilized, it will suffice for the present work to describe one more experiment—Type II. Longitudinal and transverse incisions were made as in Type I, except that the transverse incision extends entirely across the blastoderm. The blastoderm was removed to be sectioned while the head-fragment was left connected anteriorly with the opaque area by the small strip of proamnion. Thus the head-fold rested on a double membrane of ectoderm and entoderm which was also excised laterally but not anteriorly from the opaque area.

Complete separation of the posterior region proved to be quite unsatisfactory owing to the circumstance that the relief of normal surface tension induces abnormal conditions in the embryonic body. In the projecting head-region surface tension evidently does not enter so extensively into the mechanics of development. Furthermore a relatively small amount of injury in the head region serves to isolate completely an embryonic fragment in which development proceeds in a surprisingly normal manner.

In order to preclude the possibility of drawing conclusions from tissue which had already been 'invaded' by 'Angioblast,' the remainder of the blastoderm which had been removed at the time of operation was sectioned. The region of the embryonic body which would have first been vascularized was contained in the axial portion of such blastoderms. Before each incision the instruments were sterilized. These precautions together with that of complete isolation should render the

procedure sufficiently rigorous to satisfy all reasonable demands of experimental proof.

The tissue was fixed in a picro-acetic mixture; sections were stained in a modification of Mann's methyl blue-eosin stain.<sup>5</sup>

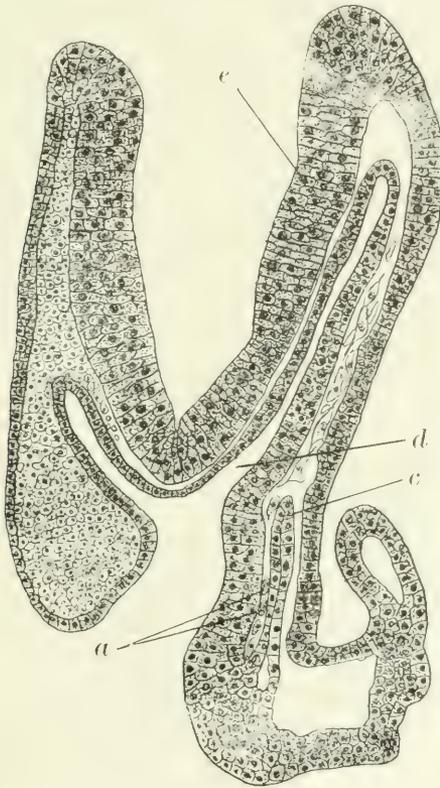


Fig. 4 Section through the forebrain of a head-meroplast, showing early stages in the formation of vasofactive cells. Total incubation, twenty-nine hours: Operation previous to the formation of the first intersomitic groove ( $\times 200$ ). Experiment, Type I, no. 24; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

which proved especially valuable in the differentiation of endothelium.

<sup>5</sup> Reagan, F. P., *Anat. Rec.*, vol. 8, no. 7, 1914.

When the head-fragments as above described had been incubated for a total period of from thirty to forty-eight hours and then sectioned, they were found to *possess blood vessels in varying degrees of development*. In general it may be said that regardless of the amount of incubation beyond a total period of thirty-three hours, differentiation never proceeded beyond the normal stages of differentiation at that age. After forty-eight hours some signs of degeneration made their appearance. It seems that the embryonic meroplast possesses an inherent capacity for differentiation which tides it over to the time when heart-pulsations would normally provide a means of tissue respiration. While differentiation proceeds always at the same rate and to the same extent, growth varies greatly. Meroplasts equally differentiated may vary greatly in size.

Practically the only unusual condition met with in these head-fragments is the presence of a head coelom (fig. 3), the origin, fate and significance of which will be considered later.

Between the base of the coelomic pouch and the pharyngeal entoderm rounded or cuboidal cells become proliferated. Their point of origin is in most instances between the base of the coelomic pouch and the pharyngeal entoderm (figs. 4, 5 and 7), where it is difficult to determine which of these two epithelia is of primary importance in such cell proliferation. Cells of this sort are undoubtedly proliferated to a certain extent by the pharyngeal wall and also by mesothelium. In neither of these latter cases do the cells originate by foldings and constrictions of cell aggregates, but singly or in a linear proliferation. This same region is found, in somewhat later stages (fig. 4) to be occupied by irregular stellate cells resembling mesenchyme cells. Their processes fuse forming a parenchyma-like complex which merges dorsally into the true interstitial mesenchyme.

Simultaneously with the accumulation of a plasma-like fluid which may be detected in this parenchyma by sections of its coagulum, the loose structure becomes transformed into a longitudinal tube of endothelium (figs. 6 and 7). The tubes thus formed, though far anterior to the heart-region, may simulate heart-formation in a remarkable manner. The coelomic pouches may meet to

possess a continuous cavity, a condition approached in figure 6. Since the pharynx in this region is already tubular it does not become constricted in this process, the endothelial tubes meeting ventral to it. There is no reason to believe (though such an inference is possible) that the endothelial tubes thus formed are new or abnormal formations: they occupy the position of normal ventral aortae.

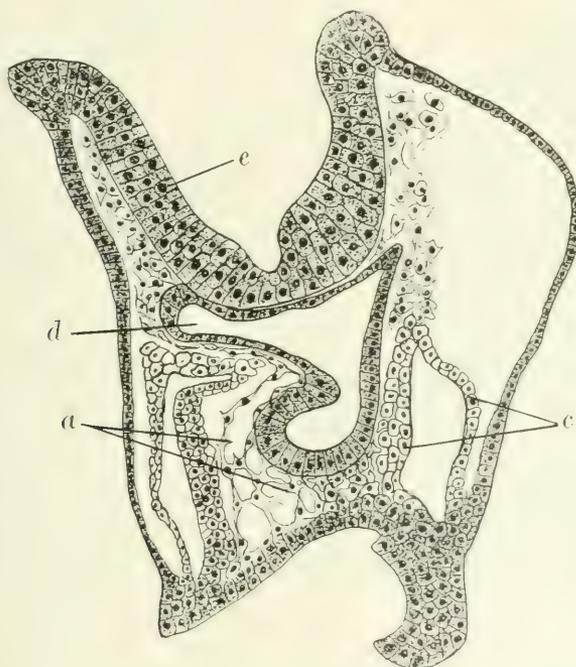


Fig. 5 Section through the forebrain of a head-meroplast showing a loose parenchyma in a position occupied by the isolated vasofactive cells of figure 4. Total incubation thirty hours. Operation at the time of the first intersomitic groove ( $\times 150$ ). Experiment, Type I, no. 18; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

The conditions so far described are found in embryonic fragments of Type I which were incubated not more than a total period of thirty-three hours. It will be well to consider some of the conditions found in a meroplast of Type II incubated for

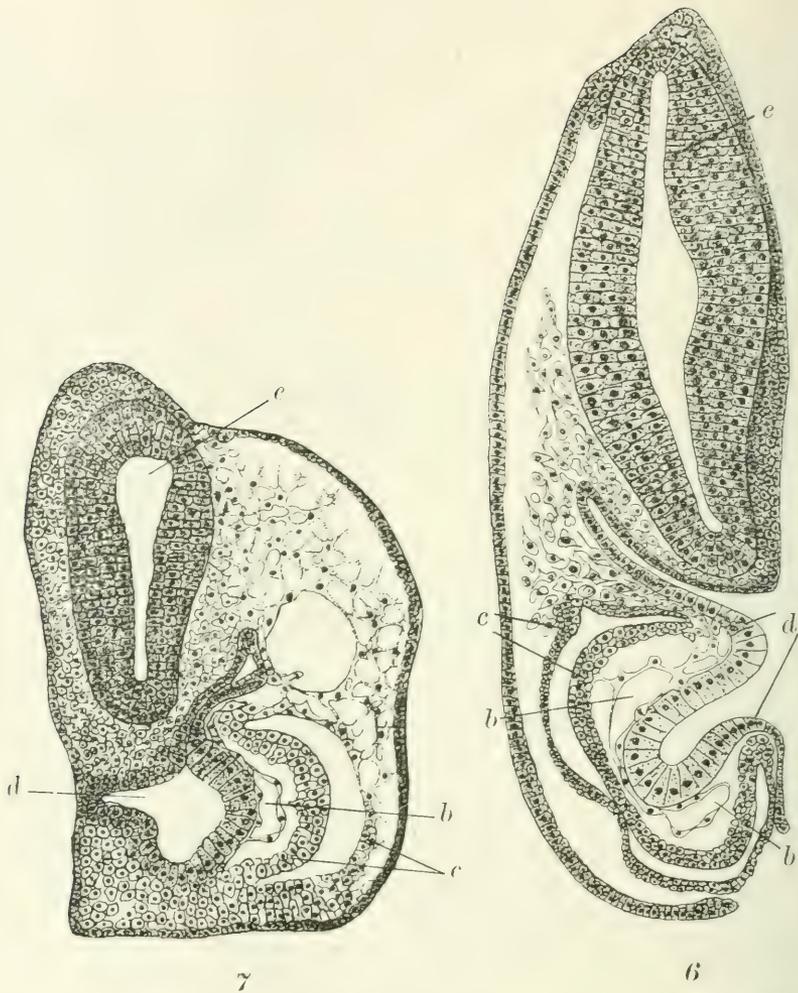


Fig. 6 Section through the forebrain of a head-meroplast showing anlagen of the ventral aortae as discrete vessels. The longitudinal incision on the right side of the head was relatively close to the neural fold. The cut edges of the pharyngeal entoderm have been pulled apart, the ventral tissues having swung to the left. Total incubation thirty-two hours. Operation at the time of the second intersomitic groove ( $\times 240$ ). Experiment, Type I, no. 31; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

Fig. 7 Section through the forebrain of a head-meroplast showing a well-defined ventral aorta. The right longitudinal incision was close to the neural fold. None of the excised head has regenerated; the mesenchyme is very compact near the cut surface the cells of which are somewhat epithelial. Total incubation thirty-three hours. Operation at the time of the first intersomitic groove ( $\times 175$ ). Type I, no. 17; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

a total period of forty-eight hours (figs. 8, 9 and 10). No attempt will be made at present to set forth the processes which intervene between the stages of thirty-two and forty-eight hours.

The proamnion (fig. 8), a region normally devoid of mesoderm, contains a rounded pouch which is continuous anteriorly with the extra-embryonic coelom. The cut edges of the ectodermal and entodermal layers of the proamnion have fused forming a blind sac around the enclosed coelomic pouch; likewise the cut

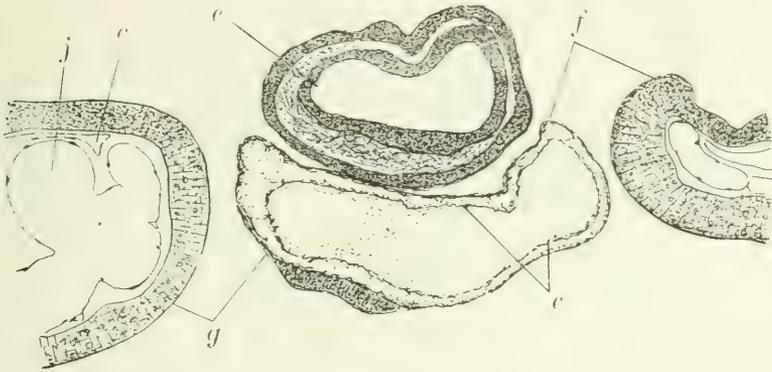


Fig. 8 Section through the forebrain of a head-meroplast showing a proamniotic sac containing a pouch of coelomic mesothelium. On the ventral side of the sac is a peculiar proliferation of entodermal cells very constantly appearing in experiments of this type, generally more symmetrically situated. Total incubation, forty-eight hours. Operation at the time of the second intersomitic groove ( $\times 55$ ). Experiment, Type II, no. 3. *c*, coelom; *e*, forebrain; *f*, ectoderm; *g*, entoderm; *j*, extra-embryonic vessels.

edges of the once continuous blastoderm have fused. On the ventral surface of the proamniotic sac will be noticed an entodermal thickening, in appearance not unlike an inverted neural groove. This structure is quite constant in experiments of this type. I would interpret it as a cell-complex representing potentially the floor of the fore-gut in case of normal infolding.

The transverse incision in this experiment was made some distance behind the site of the anterior intestinal portal, so that there projected behind the posterior extent of the proamniotic

sac a portion of the embryonic body bounded dorsally by ectoderm and ventrally by entoderm (fig. 9). The cut edges produced by the longitudinal incision have fused, the point of fusion being indicated by the apex of the projection on the left side in figure 9. In this figure we have a photograph of a 'projecting' head; the section passes through the anterior part of the mid-brain region, presenting a rather puzzling condition in that it is entirely devoid of fore-gut. Two large dorsal aortae are present: the larger one is located on the side containing the greater amount of mesenchyme; correlated also with the fact that the

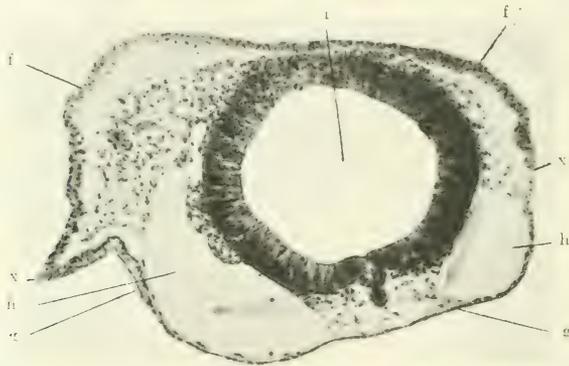


Fig. 9 Photograph of a section through the midbrain of the same meroplant as in figure 8, showing well developed dorsal aortae and the absence of a tubular pharynx in a tubular head. Fusion of entoderm and ectoderm at points indicated by *x* ( $\times 120$ ). *f*, ectoderm; *g*, entoderm; *h*, dorsal aorta; *i*, midbrain.

incision on this side was made at a greater distance from the median line. Both aortae are bounded by distinct and unmistakable endothelium. In the aortic cavities blood plasma has coagulated. No coelomic pouches are present in this section. Heart-formation has not taken place in this particular experimental case.

The phenomena under consideration are not to be regarded as regenerative changes. The head does not regenerate the yolk-sac, neither does the yolk-sac regenerate the head. In figures 6 and 7 it will even be seen that portions of the head itself were not regenerated. There is a genesis of the first order in

case of the development of endothelium. While it is not always possible to be sure of the extent to which experimental conditions portray a truly normal process, the results here presented, together with those produced by Stockard seem to afford positive evidence in favor of the local origin of blood vessels.

It is of interest to note the statement of Bremer (*Am. Jour. Anat.*, vol. 16, no. 4, p. 463) that mesothelial anlagen "might arise under abnormal conditions in positions where they are normally absent." The isolated cells proliferated from this



Fig. 10 Photograph of one of the first available sections of the blastoderm behind the incision *G* to *H* of Experiment Type II, no. 3, showing the freedom of the pellucid area from endothelium ( $\times 160$ ).

unusual mesothelium are, according to my observation, not comparable to the gross infoldings of cell-aggregates described by Bremer, though they seem to be vasofactive in nature. To designate certain of these proliferated cells as mesothelium would be as uncalled for as to designate others of undoubted entodermal origin as pharynx. While I do not question the ability of mesothelium to proliferate pre-vascular mesoderm (indistinguishable from mesenchyme), I do wish to question the justice with which Bremer would accredit mesothelial tissue with the production of the entire vascular tissue. An assignable reason for conferring this distinction on mesothelium might be the desire to maintain for endothelium a monogenetic origin—the first requisite to the specificity of a tissue.

The fact that endothelium exists in the sauropsidan yolk-

sac prior to the establishment of a coelom cannot be satisfactorily explained by a hypothetical "premesothelial stage of mesoderm" (Bremer, loc. cit., p. 463). It has been shown that 'Angioblast,' so far from being a daughter-tissue of premesothelial mesoderm, is really a parent-tissue of the latter; in isolated blood islands Rückert<sup>6</sup> has shown that cell groups proliferated from this early vascular tissue cleave to form slit-like cavities which unite later with other similarly formed cavities to contribute to the extra-embryonic coelom. Of logical necessity it follows that mesothelium and 'Angioblast' (in the sense of His) must have come from a common cell-complex.

Should we ever be so fortunate as to find the ultimate font and source of all vascular tissue there would be no objection to its designation as 'Angioblast,' so long as we bear in mind the original implications of the Angioblast Theory; the essentials of this theory have been outlined by Minot (Human Embryology, Keibel and Mall, vol. 2, pp. 498-99) as follows:

Comparative embryology teaches that the first bloodvessels appear on the yolk-sac collectively and at one time. They form a unit anlage which we call angioblast according to the suggestion of His. \* \* \* The angioblast probably maintains its complete independence throughout life. In other words it is probable that the endothelium of the blood vessels (and of lymph vessels) and the blood cells at every age are direct descendants of the primitive angioblast.

The fact that allantoic vessels may appear *prior to* those in the yolk-sac in early human development (Bremer, *ibid.*) is probably an expression of the tendency towards that sequence of local origin which is correlated with the functioning of the part vascularized. A different sequence is found in animals where the allantois functions relatively later.

When it is stated that endothelium differentiates from an 'indifferent' mesenchyme the specificity or non-specificity of the immediate source of the pre-vascular mesenchyme is not

<sup>6</sup> Rückert, J., Entwicklung der extra-embryonalen Gefäße der Vögel. Handbuch der Vergl. u. exp. Entw.-lehre, Bd. I, T. 1, 1906. Ueber die Abstammung der bluthaltigen Gefäßanlagen beim Huhn, und über die Entstehung des Randsinus beim Huhn und bei Torpedo, Sitzungsber. der Bay. Akad. Wiss., 1903.

brought into question; the statement merely means that in their earliest stages such cells are morphologically indistinguishable from other mesenchyme cells which may or may not be capable of like development. Whether each vasofactive cell behaves as it does in accordance with a definite teleology is at present entirely beside the question.

Like considerations apply to the study of 'indifferent' capillary plexuses, some of the meshes of which persist while others degenerate. That one should observe this process from time to time, and from the mere fact that the process takes place, be able to determine the extent to which the changes are due to heredity or mechanical influence is to be accepted with some caution; cell organization is excluded with great difficulty. In a regenerating jugular vein Clark<sup>7</sup> believes he has a case in which heredity plays no part. It may be stated that authorities on regeneration do not usually exclude considerations of heredity from the conclusions at which they arrive. Certain it is that the development of the vascular system furnishes an unproductive field for the solution of the problems of preformation and epigenesis.

In conclusion it is well to consider the following recently established facts which should share in defining our morphological interpretations. The yolk-sac is not necessarily the site of formation of the earliest blood vessels. Intra-embryonic vessels develop *in situ* when communication of extra-embryonic vessels with intra-embryonic tissues is prevented by chemical or mechanical means.

<sup>7</sup> Clark, E. R., Proc. Am. Assn. Anatomists, Anat. Rec., vol. 9, no. 1, 1915.

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## THE IDENTIFICATION OF TISSUES IN ARTIFICIAL CULTURES

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### TEN FIGURES

In spite of the numerous studies upon tissue culture the usefulness of the method is still limited greatly by uncertainty in classification of many cell types most vigorous in their growth and of commonest occurrence. The nerve cell, the thyroid parenchyma, kidney tubules, ectoderm and a few other tissues are easily identified. But the ever varying linear, reticular and epitheloid growths are of undetermined origin. Although they are the predominating form in cultures they have only been referred provisionally to the group of embryonic sustentative tissues.

The structure of the growths is of only secondary importance in determining their classification. While it has by no means been established that tissues take on a more embryonic character in the plasma it is true that with few exceptions they assume the form of cell strings, reticula or membranes. Thus even the tubules of embryonic kidney are described by Lewis and Lewis ('12 b) as growing out in the form of a loose mesh. Much can be accomplished in the way of classification by comparing the growths from various organs and drawing inferences based on their tissue composition. In this way the probability has already been established that many of the common growths are derived either from supporting tissues or endothelium. Yet the character of the growths is too variable and too little under control to arrive at a final classification by indirect methods. They must be traced directly to their source in the parent tissue. For this purpose sectioned cultures are necessary. Little direct

evidence of this character has been obtained because up to the present time whole preparations have been used to the almost complete exclusion of sectioned cultures.

In the present study sectioned and whole preparations were used to supplement one another in identifying the growths. Cultures were made from chick ventricle of ages ranging from four to eighteen days. Limb buds of from four- to seven-day embryos were used. A much smaller number of series were made from liver and intestine. The comparison of the growths from the younger and older organs disclosed certain differences dependent upon the histogenetic stage of the organs. These are considered briefly.

The cultures were made in plasma according to the procedure of Carrel and Burrows ('11). The description of this method has been given too frequently to require repetition in detail. The plasma was taken from young chickens varying in age from two weeks to four months. Most vigorous growths took place in clots from a mixture of two parts of plasma to one of distilled water.

The preparation of cultures for sectioning presents considerable difficulty due to the marked tendency to shrink shown by the plasma clot and the very watery cells of the growths. Experience shows that care in adapting the technique of fixation and imbedding to the peculiarities of this material is especially worth while.

A brief description of the various types of heart ventricle growth will be given before considering the evidences as to their classification. The cultures from embryos of more than five days' incubation are divisible into a number of regions, four of which have a concentric arrangement determined by the rounded surface of contact between plasma and tissue. These are not well-defined in preparations of four- and five-day tissue because of the flowing and distortion consequent on the fluidity of the early embryonic tissue. Figure 1 shows them diagrammatically in a section of a culture cut parallel to the cover-slip. The central zone *a* is made up of tissue that remains for the larger part inactive. There is no evidence for the migration of any of

its cells outward although it is not possible to prove that some few do not leave the region. The boundary of the inactive zone does not become well-defined until degeneration has begun and the tissue external to it has become modified by the migration outward of its cells. Degeneration is often found in eighteen- to thirty-six-hour cultures to be confined to a central area much smaller than finally occupied by the inactive zone. Evidently death takes place first at the center of the tissue because of its remoteness from the plasma. The limits of the dead region then extend gradually toward the surface of the tissue. The inactive zone shows pyknosis and chromatolysis of the nuclei

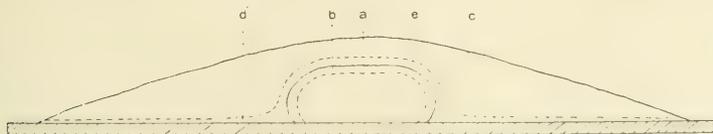


Fig. 1. Diagrammatic section of ventricle culture; *a*, inactive zone of implanted tissue; *b*, active zone of implanted tissue; *c*, region of reticular growth; *d*, cover-slip sheet; *e*, covering layer.

of heart-muscle cells. In two-week-old ventricle there is a clumping of the cytoplasm into large masses staining with basic as well as acid dyes. Epicardial and peritoneal endothelium have greater vitality than the heart-muscle. This also may be said for the endothelium of sinusoids except where the breaking down of the erythrocytes brings injury to the contiguous wall.

Surrounding the central zone except upon the cover-slip side, is a peripheral active region from which cell migration into the plasma takes place (fig. 1 *b*). It is never many cells thick and does not necessarily include all of the living tissue if the period of incubation of the culture has been short. In cultures of pulsating heart segments the cells contained in the active zone are put on the stretch at every systole. Fixation often causes the heart tissue to contract and thus preserve them in the condition of extension. Cell debris is usually present at the line of contact between tissue and plasma as a result of cutting the tissue from the parent mass.

Aside from a very fine, often degenerate reticulum, the remaining growths lying free in the plasma can best be described under two types, one fine and the other coarse. Neither of these give evidence of being separated by cell walls when stained with iron hematoxylin and erythrosin after Zenker or osmic acid fixations. There are all intermediate forms but many of the cultures are predominated by the one or the other type. Of the two kinds the finer is much more abundant. The nuclei in both varieties contain the one or two chromatic masses usually to be found in embryonic chick tissue. A measurement of the nuclei shows no constant difference in diameter from growths of heart-muscle, endothelium or reticular tissue. The cytoplasm of all cells in the plasma is watery and coagulates into a loose foam structure not resembling heart-muscle substance.

The finer mesh crosses the field at all angles. It differs from the coarse reticulum primarily in the much greater independence of its cells. It is made up of two intergrading cell forms of which one is elongated, slender and cylindrical while the other is polyhedral and usually triangular or quadrilateral in optical section. The ends of the first type and the angles of the other are drawn out into longer or shorter filaments. The cylinders may not be more than a micron in diameter although they are many micra in length. Their nuclei are forced to take on a rod shape by the limited diameter of the cells. The contact of the cells is at all times slight and often made only by the most delicate of filaments. The free ends at the periphery of the mesh send out fine pseudopodial processes. The two-cell forms intermingle freely in the reticulum.

The coarse mesh in all but the four-day cultures consists of bands 2 to 6  $\mu$  in width. They tend to flatten in the plane parallel to the cover-slip although they connect with each other at all levels. The growth has flowing outlines and forms loops which are characteristic in appearance and include spaces of more constant dimensions than found between the elements of the fine mesh. Nuclei are occasionally found side by side in the broader bands. The diameter of the narrower bands are no greater than usual for the fine mesh. In the nodes of the

coarser growth several nuclei occur all of which are in a plane parallel to the cover-slip. The triangular intersections of the strands  $2 \mu$  in diameter contain only one nucleus.

A sparse reticular growth of very fine texture often occurs in cultures of ventricle which have been injured in handling. By the use of dog plasma as a culture medium a similar growth is obtained. Both elongated and polyhedral cells are present in the fine mesh. The former are frequently drawn out into filaments not more than half a micron in diameter. The polyhedral cells also may be extended into such long threads at their angles that the central portion is much reduced in volume. The filaments are often tortuous and enlarged at successive points to give a bead-like appearance. The more normal part of this growth stains faintly. In other regions there is pyknosis and chromatolysis of the nuclei. The peculiarities of the growth are plainly an expression of decreased vitality and in many cases of actual cell death. In some instances it is the action of plasma from alien species that causes the injury. When the plasma is not responsible the growth comes from the tissues injured in cutting and handling. In this case toxic substances from the degenerating cells probably not only act upon the growth before it reaches the plasma but do harm by diffusing into the culture medium. Lambert ('12) describes a similar sparse and delicate growth from cultures of chick heart in rat plasma.

The cover-slip growth (fig. 1 d) is of almost as constant occurrence in vigorous cultures as the reticulum. It is so frequently in plain continuity with the latter that no doubt of the identity of the two is possible. Sections of many cultures made perpendicularly to the cover-slip show the reticulum grading into the cover-slip membrane and frequently stained whole mounts enable one to trace the mesh into the sheet growth. The continuity occurs most frequently close to the tissue where the growth is most crowded. It is often possible to make out a progressive flattening of the bands of the reticulum as they approach the cover-slip. Close to the tissue the cover-slip growth may be many cells in thickness. Tracing outward,

however, it soon flattens out into a single layer. The elements are often much flattened, especially at the border of the sheet and the nuclei may attain an area in this plane ten times as great as its usual cross section. The membrane growths agree with the reticular formation in the apparent absence of cell walls. While they have every appearance of a syncytial structure a final decision is impossible because a silver nitrate test for intercellular cement substance was not made. Aside from any differences as to cellular independence that may exist in various growths depending upon the greater or less development of cell walls it is certain that membranes show marked differences in this respect depending upon the extent of the spaces between the cells. The cover-slip growth associated with the finest mesh is made up of cells separated from each other by wide intervals of plasma and seldom intercommunicating by more than a few slender threads. The finer normal reticulum when flattened upon the cover-slip shows a frequent union by broad bands but intercellular communication may also be only by slender processes (fig. 6). In the membrane growth from the coarse reticulum broad attachments and multinuclear sheets occur.

The difference between the cover-slip growths corresponding to the coarse and fine mesh is especially noticeable at the border of the sheet. The former sends out cell bands which either project radially or form loops. The border of the membrane associated with the other mesh has a more finely broken border although its cells may also be connected with one another by their slender processes to form loops (fig. 6).

The third region of growth (fig. 1 c) of ventricle cultures is in the plasma next to the surface of the tissue. Burrows ('11) mentions its occurrence in early embryonic chick preparations but few others have referred to it. It is not confined to the earlier embryonic growths but is well developed from two weeks old ventricle. It has doubtless failed to attract attention because it can not be seen so well in whole preparations as in sections. It consists of flattened cells which are often piled upon one another to a great depth. Tangential sections from the surface of the implanted tissue opposite to the cover-slip

often show them to be as thin and sheet-like as at the border of cover-slip membranes. They are often elongated and rounded in cross section on the sides of the cultures. These dissimilarities of form are the result of the varying conditions of pressure in different regions of the culture brought about by the shrinkage of the plasma clot. The cells are in contact by slender filaments or less commonly by wide bands. Although frequently closely packed together a thin layer of plasma always separates them on most of their peripheries from neighboring cells. The transition of the layer into the reticulum and cover-slip sheet is usually gradual. As the reticulum is traced toward the tissue and into the covering sheet it is evident that even near to the tissue where the cells are the most flattened and in closest proximity they still form a compressed reticulum. Cell associations similar in make-up to the covering layer are often formed in relation to the free surface of the plasma or around droplets of serum contained within the clots.

The cultures from four-day ventricle give rise to an extremely heavy reticulum with bands often exceeding  $15\ \mu$  in width (fig. 5). Their massiveness will be appreciated when it is recalled that the coarse mesh of older heart cultures are at the most  $6\ \mu$  across. The appearance of the growth is also similar to the coarse mesh with its oval spaces. Thick masses apparently made up of the same tissues as the coarse mesh flatten out upon the cover-slip to form a sheet one cell in thickness at the periphery. Sometimes a finger-like form occurs in partial contact with the cover-slip and intermediate between membrane and reticulum. The great fluidity of both reticular and membrane growth is shown by their flowing outlines and lack of cell independence. A finer reticulum is also common in the four-day cultures which has nothing to distinguish it from the coarse form of the older tissue. The five-day cultures are intermediate in character between the growth from four-day and older heart. Figure 4 is from one of the finest reticular growths seen at this stage.

Before considering the evidence regarding the sources of the ventricle growth the tissues which compose it may be enumer-

ated. They include heart-muscle, nerve, endothelium and supporting tissue. Heart-muscle is, of course, the most abundant of these since it comprises the bulk of the myocardium. Nerve tissue is represented by a few neuroblasts. Endothelia include the endo- and peri-cardial layers and in the ventricle of the older embryos the walls of the sinusoids as well. The myocardium is separated on its inner and its outer surfaces from the endothelial covering by reticular layers. These consist of a mesh of formed substance upon whose strands are stretched cells which during embryonic development are becoming progressively more independent of their support. In the region of the atrial canal the sub-endocardial reticulum is continued into thickenings called endocardial cushions whose appearance is not unlike embryonic mesenchyme. Endothelium and reticulum approach the heart-muscle in abundance and like it are met on every cut surface.

In considering evidence for the growth of heart-muscle the cultures from six- to eighteen-day ventricle require consideration separate from that of four- and five-day tissue. Material from the former source gives no direct evidence for the participation of heart-muscle in the culture. Many hundreds of sections were searched for myofibrillae without success. Direct evidence for heart-muscle growth was obtained however in the sections of a culture from five-day heart which happened to include not only ventricle tissue but a portion of the atrial canal. The contents of the walls of the atrial canal was seen to be flowing out into a heavy band such as made up the coarser loop-like reticulum of the four-day ventricle cultures. The wall at this point is so thin and the process growing out from it relatively so large that the conclusion is unavoidable that the whole cell-mass was moving out into the growth. At six days muscle and reticulum make up about equal parts of the wall of the canal. At five days the tissue is in large part a primitive myocardial layer. Since there is every reason for supposing that the heart-muscle cells differentiate *in situ* the evidence is therefore very good that primitive heart-muscle cells take part in the growth. The appearance

of the coarse growths in unsectioned four-day cultures seems to justify their classification as primitive myocardium. Their strands broaden out in such a way at the base as to appear as a projection from the whole ventricle mass rather than from small portions of its surface. If primitive heart-muscle cells grow from five-day heart, it is, of course, probable that they are more frequently represented in growths of four-day ventricle. On the other hand, since the very heavy reticulum is rare in five-day cultures and entirely absent in growths of older tissue they probably do not grow from embryos more than five or six days old. These conclusions are in agreement with Burrows' ('11) observation of sparse growths of heart-muscle in a small part of his cultures of two-and-a-half-day chick heart.

A growth of nerve cells was not found in any ventricle culture. The neuraxones are so characteristic in appearance that they could hardly have escaped detection had they been present. Their failure to appear in the growth is doubtless to be explained by the smallness of their number and the consequent unlikelihood of their coming into contact with the plasma, for the responsiveness of neuroblasts to cultivation has been amply demonstrated by Harrison ('07), Burrows ('11), Lewis and Lewis ('12) and Ingebrigtsen ('13).

The abundant growths of the older embryonic ventricle and finer reticulum of the four-day organ which are not made up of heart-muscle tissue must evidently take their origin either from the endothelium of pericardium, endocardium or sinusoids if not from the reticulum. Few of the various studies of chick ventricle growths which have appeared are especially concerned with questions of identification. Lambert ('12) describes the mesh as of connective tissue origin and Burrows ('11) as mesenchyme. Lewis and Lewis ('12 b) think that it may be mesenchymal but consider the matter of its classification unsettled. In various growths of chick organs certain of these authors have suggested that flat polygonal cells may be of endothelial origin but have not traced their connection with the tissue. It is difficult to follow the growth either to endothelium or to reticulum. There

is confusion in the histological picture because of the cell débris which results from cutting the tissue. The collapse of the ventricular cavity and distortion of the tissue, especially in the younger ventricles, prevents contact with the plasma on surfaces sufficiently large to allow a proof of their continuity with the growth.

Cultures in which the pericardium and its reticulum are next to the plasma are better adapted for this purpose than sections through the endocardium covering the trabeculae of the spongy ventricle. Relatively large masses of pericardium may come in contact with the plasma while the endocardium is always intimately mingled with muscle. Figure 2 is from a photograph of a sectioned culture in which the active zone is made up of reticulum. The denser tissue internal to it is heart-muscle. Although the preparation was not killed until degenerative processes were well under way there is no difficulty in making out these tissues. The contrast between the two is seen much more clearly through the microscope than in the photograph because the muscle stains very intensely. A growth of fine mesh is plainly seen coming off from the reticulum. No traces of the pericardial endothelium which at first separated the reticulum from the plasma can be made out.

Figure 3 is from a photograph of a sectioned growth of the endocardial cushion of a thirteen-day heart. There has been no cutting with a knife. The free endocardial surface is in contact with the plasma. Because of the absence of the usual dead tissue resulting from cutting, strands from individual cells can be traced directly into the parent tissue. There is no room for doubt that the growth comes from the endocardial cushion. Just as the cushion is distinguishable from the reticulum by the presence of only one type of cell, so the elements of its growth are of a single form. They resemble the polyhedral cells of the fine reticulum.

There was no ventricle growth in which a connection could be traced with endothelium. The difficulties in the way of finding a region favorable for this purpose are too great to warrant concluding that endothelium does not take part. Indeed,

there is considerable reason to think that it does grow into the plasma. In the two cultures which have just been described the tissues concerned which are separated by endothelium from the plasma could not have grown into it had not the covering sheets ceased to bar their way. Inasmuch as no remnants of dead endothelial cells are to be seen, they have in all probability migrated into the plasma. Of the two types of ventricle reticulum the coarser has the greater similarity to endothelium. The finer mesh has already been shown to have its origin in part at least from sub-pericardial reticulum. It must not be forgotten in this connection that since the very fine degenerate mesh grades into the normal fine mesh and this again into the coarser variety, the claim is possible that since the first transition is due to differences of the plasma the change from the fine to the coarse type has a like explanation. Such an interpretation is not in agreement, however, with the conditions found in the heart or other cultures. The so-called normal fine mesh and the coarse variety are vigorous and abundant in growth. Their cells give every indication by structure and staining reactions of being healthy tissue. The very fine growth without question stands apart from these as a type modified by its struggle with an unfavorable environment. If the coarse mesh comes from endothelium and the fine mesh from reticulum the inter-mixture of the two may well result from their close association in the ventricle itself.

Lewis and Lewis describe two types of cover-slip membrane for chick ventricle cultures. One of these which they find to be syncytial is said to develop from nearly all embryonic chick organs. They think that it arises from mesenchyme or connective tissue. Their figures correspond closely with the cover-slip membranes associated with the fine mesh. They also occasionally get from heart a non-syncytial membrane with pigment deposits around the nuclei. This variety was not encountered in my preparations.

## LIMB-BUDS

It is of special interest to study limb-bud and ventricle cultures together because the growth of pre-muscle cells can be compared with primitive heart-muscle. The degree of similarity of the mesenchymal growths from limb-bud and of ventricle reticulum also has significance in view of the uncertainty as to whether reticulum makes its origin from endothelium or directly from mesenchyme.

The five-day-old embryonic limb-bud consists of ectoderm and closely-packed mesenchyme in which the axial scleretogenous tissue is just beginning to differentiate as an especially dense region. In the surrounding zone the pre-muscle cells can be distinguished by their elongation parallel to the axis of the bud. The vascular system is represented by sinusoids. Nerve fibers have not yet extended into the bud. The ectoderm consists of a layer two cells in thickness. At seven days a difference can be made out in the form and staining qualities of the sub-dermal and the scleretogenous tissues. The pre-muscle cells are markedly elongated. Sinusoids now form an extensive system and nerve fibers have migrated in.

The difference in appearance of the cells of scleretogenous pre-muscle and ectodermal regions combined with their separation into independent zones render the limb-bud favorable material for tracing the growth to its parent tissue. The manner of growth of the limb tissue differed greatly from heart, due to the influence of ectoderm and mesenchyme. The former tissue invariably retains its continuity as a sheet and when it grows vigorously can limit the distribution of other tissues to the region internal to it. The mesenchyme often flows out *en masse* taking with it sinusoids and pre-muscle cells (fig. 8).

The ectoderm can easily be traced from tissue to growth either in sections or whole preparations. Its presence in the plasma is clearly in part due to a creeping of the edge of the sheet out into the plasma. The portion retaining its contact is often stretched into a thin sheet. In the plasma the ectoderm may form masses several cells in thickness. Single cells or

small groups occasionally project from the border of the sheet but there is always a considerable cellular contact.

The various mesodermal elements of the seven-day limb-bud are already sufficiently differentiated to give rise to a number of distinct growths.

Scleretogenous tissue can be identified in the plasma of many cultures close to regions of its contact with the plasma. Sometimes there is a migration of an entire region of the scleretogenous axis out into the plasma but the cells show no power of orientation and soon die. In most cultures the scleretogenous tissue is internal to mesenchyme and ectoderm and for this reason unable to reach the plasma quickly. Making due allowance for this handicap in position the vitality of the tissue from the seven-day embryo still appears to be of a low order.

The pre-muscle cells can be easily traced out into the plasma in many stained whole mounts because of their spindle form in the organ and their still greater elongation in the plasma (fig. 8). They form long strings which seldom branch. They are often long enough to reach the confines of the plasma drop and be deflected parallel to its surface. When in contact with the cover-slip the growth still maintains its elongated form thus differing from any other membrane growth (fig. 9). It is of interest that both primitive heart and skeletal muscle retain sufficient plasticity to appear in the plasma.

Another mesenchymal limb-bud growth is made up of large masses of cells formed, as in the case of the scleretogenous tissue, by the loosening up of the intercellular bonds of entire regions and a flowing out upon the cover slip (fig. 8). The cells are piled upon one another without a definite arrangement and at the outer border of the mass lie scattered upon the cover-slip in a much more irregular manner than in the membrane growths of heart reticulum. In figure 8 the growth is plainly seen to be intermingled with spindle shaped pre-muscle cells. This identifies it as the little differentiated mesenchyme surrounding the scleretogenous and the pre-muscle cells. From among mesenchymal cells of cut surface a reticulum often ex-

tends into the plasma which is apparently also mesenchymal in origin. The membrane associated with it is similar to the usual cover-slip sheet described with the fine mesh of the ventricle. The view that it is mesenchymal is strengthened by the fact that similar although somewhat more flowing and embryonic growths occupy the chief place in cultures from five-day embryos. If the reticulum as well as the more massive growth is from mesenchyme there is need of an explanation for the development of two such different types from the same tissue. A possible clue is to be found in the fact that the reticulum only occurs in connection with the free cut surfaces while mesenchymal cell-masses are associated with the cultures in which a scant plasma drop has flattened out the border of the implanted tissue by its shrinkage. Together with the flattening of these cultures there is always an extension of the ectodermal sheet out toward the periphery of the drop thus greatly limiting the plasma accessible to the mesenchyme and remaining tissues.

If it be correct to regard the reticular growth of the limb-bud as of mesenchymal origin, then heart reticulum and mesenchyme are closely similar in their growths and as far as this evidence goes are closely related. If, on the contrary, it should be shown later that mesenchyme gives rise only to the massive growth the arguments from tissue cultures would be in support of the origin of reticulum from endothelium.

#### LIVER AND INTESTINES

A few series of cultures from five- and eight-day intestine as well as from five- and ten-day liver were prepared as a basis for comparison of the growths.

A fine reticulum can be clearly traced in many sections to the mesenchyme of the intestine. Growths which arise from surfaces of the intestine with portions of the peritoneum intact often contain trabeculae of greater diameter than found where the growth is plainly of mesenchymal origin. It is not possible to trace the broader bands definitely to the peritoneum because the mesenchyme is always found to be taking part in the growth where the continuity of the peritoneum is lost. It is very prob-

able, however, that they arise from the peritoneum just as similar growths from heart ventricle also probably are of endothelial origin. Whole mounts of the intestine sometimes show a flattening down of a part of the organ, accompanied by the flowing out of the mesenchyme of the region upon the cover-slip just as seen in limb cultures. It is usually possible in these preparations to trace the peritoneum out as an unbroken sheet into the plasma. Lewis and Lewis ('12 b) describe and figure this type of growth and look upon it as peritoneal. The reticular growth which has just been described as present in sectioned cultures does not occur where there is such a flattening but is confined to free surfaces of the tissue.

Liver cells do not take part in growths from the ten-day organ. A coarse and a fine mesh occur with about equal frequency. Figure 7 shows the cover-slip growth associated with the fine mesh. The coarse form appears in many sections to come from the peritoneum. Elsewhere there is no proof of a deeper origin. Figure 10 showing the fine mesh growth is from a ten-day embryo and is fixed after twenty-four hours of incubation. No peritoneum is present in the culture. The growth can not come from connective tissue septa except possibly at a few restricted regions. In ten-day chick livers a reticulum is already present, as can be ascertained in part of the implanted tissue where degenerating liver cells have dropped out of a section. Endothelium is also present in large amounts in the form of sinusoids. The occurrence of mesenchyme in the embryonic liver has not been proven. It is therefore not possible to determine the source of the growth from the interior of the organ.

#### SUMMARY

*Reticulum.* The common reticulum with its corresponding membrane-growth is traceable to sub-pericardial reticulum in six-day ventricle.

*Endothelium.* There is indirect evidence for a coarse reticular growth from the peritoneum of liver (five- and ten-day) and intestine (five- and ten-day) and from the endocardium and

pericardium of ventricle (five- to fourteen-day). An unbroken sheet is found to arise from intestinal peritoneum under certain conditions (six-day).

*Mesenchyme.* Sectioned cultures of intestine (six-day) gave rise to a fine mesh similar to that of ventricle reticulum. Mesenchyme is sometimes given off from limb-buds (five- and ten-day) in the form of disorganized masses of cells. Under other conditions reticulum and a corresponding membrane growth apparently also take their origin from limb-bud mesenchyme.

*Heart-muscle.* In one sectioned five-day culture the contents of the wall of the atrial canal is found to be moving out into a strand of a very coarse reticular growth, such as is common in cultures from four-day ventricle. Primitive myocardium of four- and five-day heart therefore apparently grows out as a coarse mesh but it is unlikely that heart-muscle of older ventricle has this power.

*Endocardial cushion of ventricle.* A growth of polyhedral cells resembling those from sub-pericardial reticulum was traced in sections to the endocardial cushion (thirteen-day).

*Scleretogenous tissue of limb-bud.* The scleretogenous tissue can move out into the plasma for a short distance but has little vitality (seven-day).

*Ectoderm of limb-bud.* There is an extension of the ectoderm out into the plasma but this is due, in part at least, to a creeping of the original layer, as is shown by its marked thinning on the surface of the limb bud (five- and ten-day).

*Pre-muscle tissue of limb-bud.* The spindle-shaped pre-muscle cells of seven-day limb buds give a characteristic linear growth in the plasma and upon the cover-slip.

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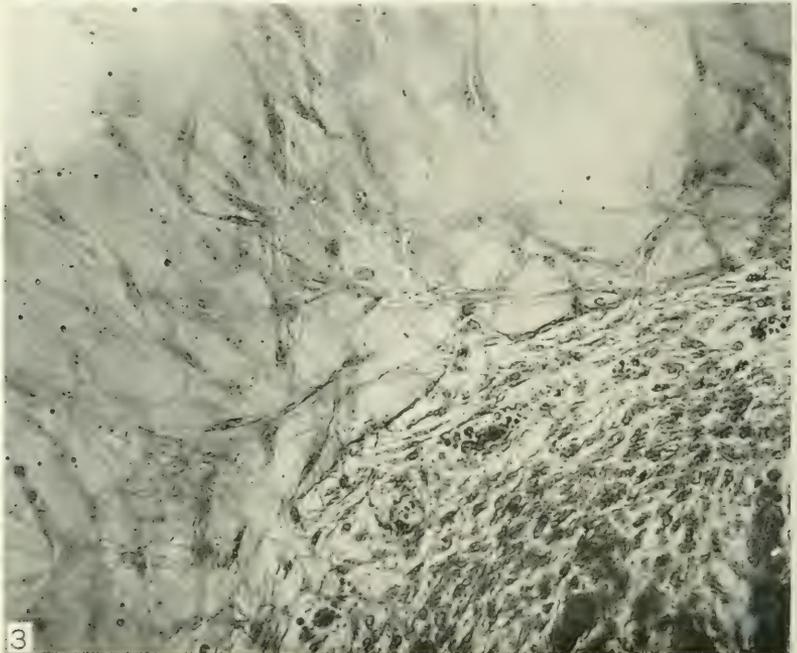
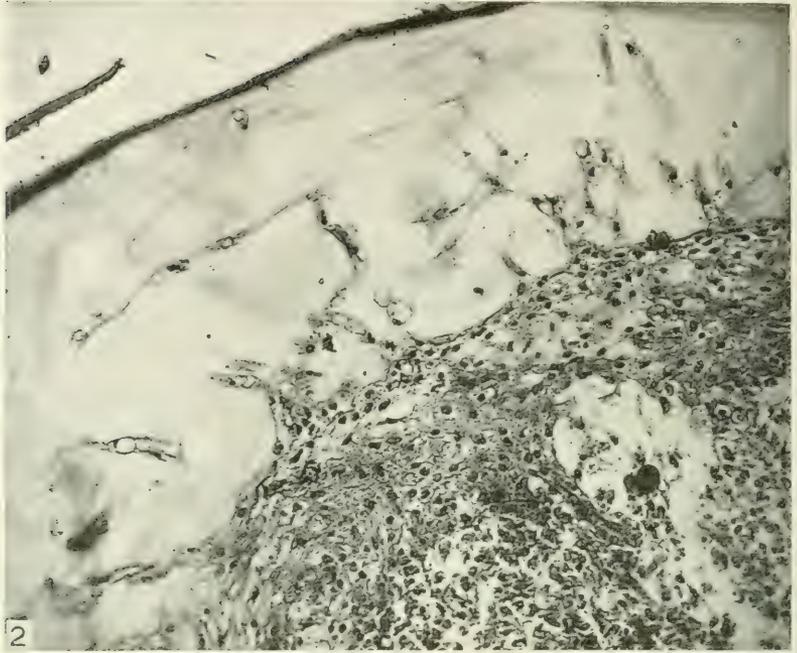


Fig. 2 Section of ten-day ventricle showing fine reticular growth arising from the reticulum. The more internally situated denser tissue is heart-muscle.  $\times 600$ .

Fig. 3 Section of thirteen-day ventricle; the fine reticular growth is coming from an endocardial cushion.  $\times 570$ .



Fig. 4. Fine reticulum of five-day heart; stained whole mounts.  $\times 250$ .

Fig. 5. Coarse reticulum of four-day heart; stained whole mounts.  $\times 270$ .



Fig. 6 Cover-slip growth associated with fine reticulum; nine-day ventricle; stained whole mounts.  $\times 530$ .

Fig. 7 Cover-slip growth associated with fine mesh of five-day liver; stained whole mount.  $\times 350$ .

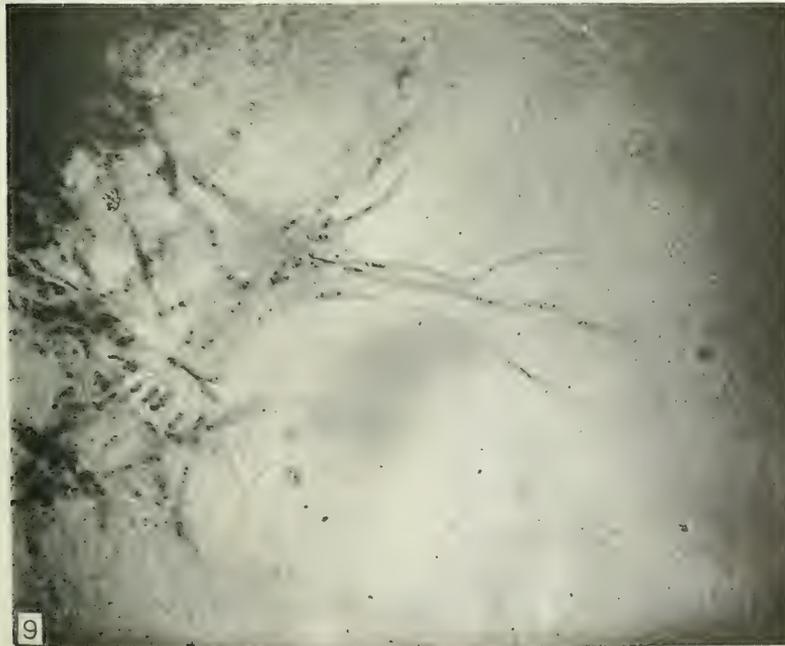
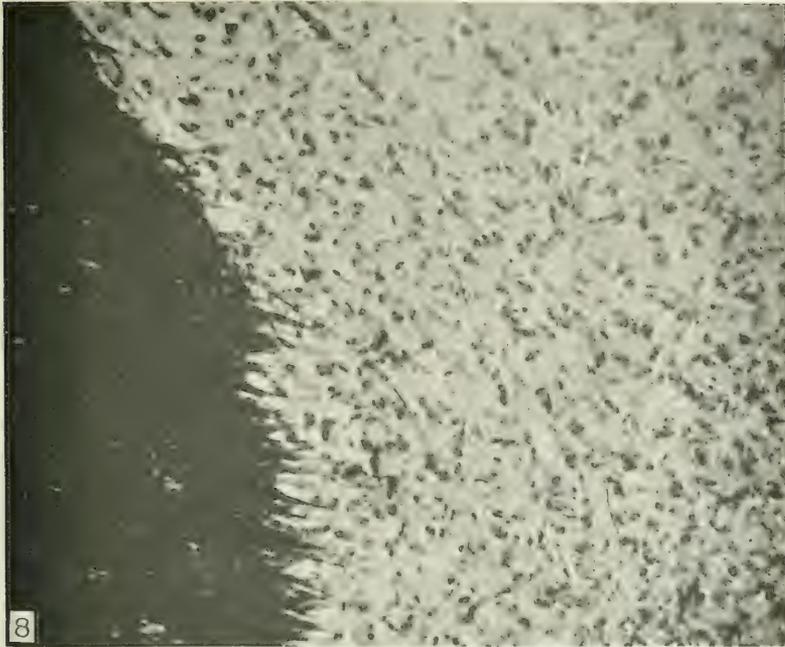


Fig. 8 Mesenchymal growth containing a few pre-muscle cells; spindle-shaped pre-muscle cells are also seen projecting from the edge of the implanted tissue; stained whole mount.  $\times 510$ .

Fig. 9 Cover-slip growth of pre-muscle cells from seven-day limb bud.  $\times 410$ .



Fig. 10 Section of culture from ten-day liver, showing fine reticular growth. The cellular *débris* in the outer zone of the implanted tissue is made up of the remains of dead liver and sustentative cells. The inner zone shown at the right of the figure contains still uninjured liver cells.  $\times 590$ .

# THE ACTION OF ULTRA-VIOLET RAYS UPON THE FROG'S EGG

## I. THE ARTIFICIAL PRODUCTION OF SPINA BIFIDA

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SIXTEEN FIGURES

While the method of the experimental embryological investigation detailed in the succeeding pages is of importance because of the constancy with which the condition of spina bifida may be produced in the embryo, the real value of the method and of its results lies in the interpretation which is thereby rendered possible of the physiological value of the several component portions of the fertilized ovum. The really fundamental problems to be determined by investigations of this nature are, first, whether the fertilized ovum is to be regarded as a composite structure made up of various system- or organ-anlagen, or the chemical progenitors or 'ferments' of such, distributed in definite and, perhaps, constant positions throughout the cytoplasm. Or, second, is the ovum to be considered in the sense of a unicellular organism, differing in no great respect from the physiological structure of unicellular organisms in general but possessing that specific potential to elaborate 'ferments' and pro-anlagen at successive genetic stages, and, ultimately the anlagen of the later developmental stages? In the former instance it is presumed that the embryonic parts are pre-localized in the cytoplasm of the ovum and make their appearance, in the words of Lankester, as "a sequel of a differentiation already established and not visible." In the second assumption the embryonic parts are unrepresented in the ovum, the regions of the cytoplasm being

then, so far as the future embryo is concerned, of equipotential value. It appeared to the author that a means by which a limited area of cytoplasm could be destroyed and yet left in its original relations to surrounding parts would afford a solution to this question. Accordingly, recourse was made to ultra-violet rays of such a degree of intensity as to cause the disorganization of the cytoplasm in from one to thirty seconds and of such a degree of concentration as to influence limited surface areas. Acting upon the suggestions made by Prof. E. H. Merritt, an apparatus was constructed which met these requirements fully.<sup>1</sup>

This apparatus consisted of a large induction coil actuated by a 110-volt direct current reduced by an unknown resistance. The potential, moreover, was raised by means of several Leyden jars shunted between the electrode wires. The terminals were made of iron, and were spaced about 5.0 mm. The eggs used for the purpose were those of the various forms of frogs occurring in the neighborhood of Ithaca, New York. These were obtained early in the morning, as soon after laying as was possible. At the time at which they were influenced they were in the undivided stage. Development was allowed to progress in the laboratory in some instances, and the eggs influenced at several later developmental stages, but no egg further along in its cycle than about the 64-cell stage was used. Furthermore, care was taken to reject such eggs as were collected late in the laying season for that particular species of frog, and particularly those located near the center of the egg bunches, specifically to avoid dealing with those possessing a tendency towards abnormal development. In preparation for exposure to the rays, the eggs were freed from their jelly, which had been found impervious to the light, and placed under a perforated tinfoil diaphragm. The perforations differed in size in different experiments. After the egg had been rotated so that a predetermined part had been brought directly under the center of a circular perforation in the dia-

<sup>1</sup> At this point I desire to express my indebtedness to Professor Merritt for helpful suggestions and to the Department of Physics of the University for the use of the apparatus with which the experiments mentioned in this paper were conducted.

phragm, both were then brought under the electrodes of the apparatus and the circuit closed.

While in the numerous experiments conducted, the various portions of the white and of the black hemisphere and of the equator of the frog's eggs were influenced in order, the author decided to limit the scope of this present communication to those effects produced by the rays when influencing the white hemisphere and the equator of the egg. Indeed, in addition to the significance of the findings of the investigation in the interpretation of the larger problem of ovum structure, the immediate purpose in presenting this paper is to establish the fact that the condition of spina bifida may be produced at will by this method.

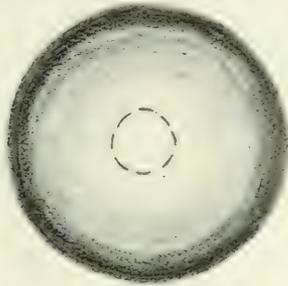


Figure 1

The aperture in the diaphragm used for this experiment was 0.4 mm. in diameter. The eggs averaged 1.7 mm. in diameter. Consequently but a small surface area proportionately of the total area of the egg was influenced. The latter amounted to 425.0 sq. mm. whereas but about 5.0 sq. mm. of this surface could be influenced by the rays. The relative sizes of these areas is brought out more clearly by reference to figure 1, which represents by a broken-line circle the portion of the surface area of the egg sphere illuminated. Further, it was found that the depth of penetration of the 0.4 mm. pencil of rays depended upon the length of exposure to the light. Uniform exposures of 30

seconds were employed in this series. A section through an egg so influenced is shown in figure 2, in which the depth to which the rays had penetrated is represented by the shaded portion on the right of the sketch. In this instance the rays passed in the plane of the section and at right angles to a tangent at the center of the surface of the affected area. The direct results of the illumination were corroborative of those previously observed by other investigators using violet rays, such as granulation of the chromatin and certain degenerative changes noted in the cytoplasm. No attempt was made, however, to study this aspect of the influence of the rays. It was noted in extremely long exposures of from 1 to 10 minutes, that masses of protoplasm



Figure 2



Figure 3

were in some instances extruded upon the egg surface, retaining, however, a slender connection with the main mass of the egg. In instances of such exovation, the egg died early after having made but little developmental progress. Such an exovate is shown in figure 3. It is of importance to note the fact demonstrated by the sketch that the mass of the exovate was approximately equal to that of the influenced area of the egg (compare with figure 2). The most plausible inference to be drawn from this phenomenon, in the terms of the interpretation of the ovum as an organism, seems to be that of an effort on the part of the ovum to rid itself of the chemically altered or dead protoplasm which can only act as a hindrance to its further developmental progress.

Reference to various series of experiments selected at random bring out the value of the method in the constancy of production of the condition of spina bifida. In one series of thirty-one 16-cell eggs used at the beginning of the experiments and exposed to the 0.4 mm. ray for 30 seconds each—various regions of the equator and of the white hemisphere being influenced—twenty-one developed abnormally, and but ten normally. Of the abnormal embryos, eight presented the condition of spina bifida. In another and later series of fifteen eggs in the 4-cell stage, influenced in the same manner, none developed normally. Most of these died during the early stages. Four, however, lived to swimming forms with two tails. Later in the spring, after the technique had been still further perfected and the eggs of the green frog were available, from which it was possible to remove the enveloping jelly more readily and more completely, the percentage of spina bifida embryos rose. In one set of five undivided eggs influenced in a similar manner, one died about twelve hours after the experiment, having made no developmental progress, and the four others grew to swimming forms presenting the condition of spina bifida, each having two tails. This last instance is merely representative of the high percentage of these forms of malformation obtained when the white hemisphere is influenced by the ultra-violet rays.

As has been mentioned above, the most effectual barrier to the penetration of the rays was the investing jelly. When this was completely removed an exposure of 10 seconds was sufficient to influence the egg. The presence of a very thin layer, however, completely blocked the passage of the rays even during an exposure of as much as 10 minutes. The author attributes most, if not all, of the irregularities in percentage production of spina bifida embryos to the presence of this jelly. The later results of the experiments were sufficiently assuring to warrant the conclusion that, when it could be positively known that the rays under the above conditions had actually penetrated the ovum in the regions above mentioned, the condition of spina bifida could be invariably brought about in the developing embryo. Taking these difficulties into consideration, however, the per-

centage production of the condition ranged between 85 and 90 per cent of the total number of eggs used.

An observation that recurred repeatedly was to the effect that the developmental period required by the eggs was lengthened as a result of the rays' influence. Under laboratory conditions ordinarily from 3 to 4 days were sufficient for the appearance

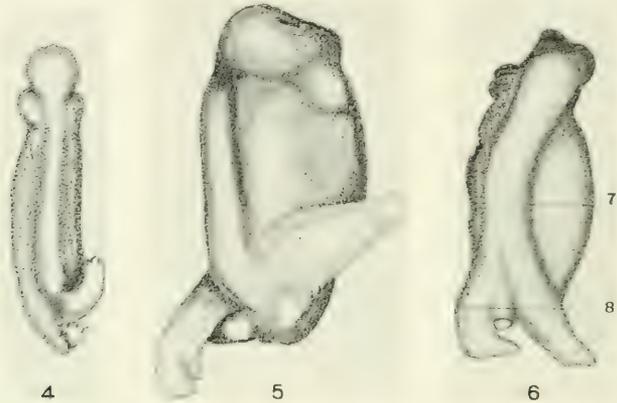


Fig. 4 A specimen of cauda bifida demonstrating the asymmetry of the tails. In this egg the rays had struck a portion of yolk farthest removed from the equator. The tails are provided, as is shown, with peculiar toe-like processes on their free extremities.

Fig. 5 In this tadpole the right tail encountered the body axis at an acute angle directed anteriorly. Two yolk plugs are to be seen, and the same splitting of the extremity of the left tail as was noted in figure 4.

Fig. 6 The lines 7 and 8 on this cauda bifida tadpole indicate the level of the cross-sections shown in the respective figures. In figure 7 the notochord lies ventral to the well differentiated neural tube. In figure 8 the asymmetry of the halved neural tube is shown, more particularly on the right side. Ventral to this lies the notochord, all traces of which are absent from the left half of the sketch. This right neural tube-half lay in the more actively used tail.

of the free-swimming tadpole-forms of the green frog; in the case of the experimented eggs, however, 5 or 6 days were required and in some instances 8 days. Furthermore, it was noted that during the 12 hours immediately ensuing upon the experiment the eggs seemed to have entered into a condition of temporary suspension of development, later resuming that process but with greatly lengthened tempo.

The further observation was made that free-swimming forms, such as are represented in figures 4, 5, and 6, seemed to be able to move about by the use of either tail, but that the swimming movements were more vigorous in one than in the other. This is an interesting fact in connection with the results obtained by study of the microscopical sections of the same specimens. In these it was learned that in the more favored of the two tails the neural tube was greater in diameter and extended a longer distance towards the tip of the tail. In some of the specimens,



Figure 7

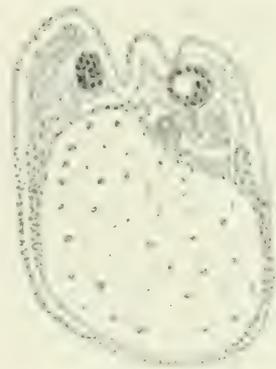


Figure 8

as is to be seen in figure 8, the notochord was limited to one tail. Figures 7 and 8 are cross-sections of the tadpole represented by figure 6. The right was the more active of the two tails during life. In figure 7 the neural tube, cut just cephalic to its bifurcation, is seen to be well differentiated, with the notochord lying ventral and adjacent to it. The section of figure 8 was taken immediately caudal to the bifurcation and shows the notochord confined to one (the right) tail.

Several specimens presented a peculiar relation of one tail to the longitudinal axis of the body; such are figured in 5 and 9. In the latter figure the main axis of one tail joined that of the trunk at almost right angles, whereas the other tail coincided fairly well with the main body axis. In figure 5 the right tail

met the body axis at an acute angle, looking forward. Such tails were, of course, useless from the functional standpoint; but their importance cannot be overestimated in furnishing exaggerated examples of the asymmetry of some of the types of spina bifida, such as were observed above in the cross-sections.

The study of the serial cross-sections demonstrated, furthermore, as these were followed in order caudally, that just posterior to the level of bifurcation of the neural tube each half tube des-

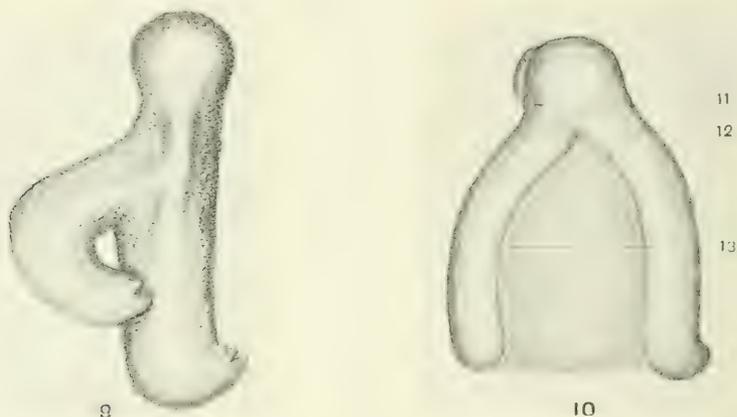


Fig. 9 This figure illustrates a marked instance of asymmetry with a division of the cord well anterior on the embryo. Here again the extremity of each tail is broken up into toe-like processes.

Fig. 10 In this embryo the rays had encountered an area well up on the equator in the median plane; hence, the bifurcation of the neural cord immediately posterior to the optic anlagen. The lines 11, 12 and 13 indicate the levels of the respective cross-sections illustrated by the succeeding figures.

tinued for each tail presented an asymmetrical outline, the lateral wall being considerably thicker than the median. This is shown in part by the right neural tube in figure 8. As the series was followed farther caudally, however, a readjustment of the tube cells was observable, each half now becoming either a solid rod or a tube entirely symmetrical so far as the thickness of its walls was concerned; (see also figures 12 and 13).

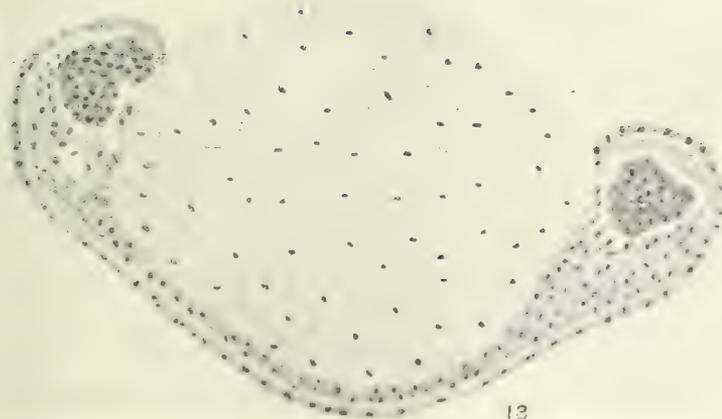
The neural tube was caused to bifurcate at various levels, dependent upon the portion of the hemisphere influenced.



11



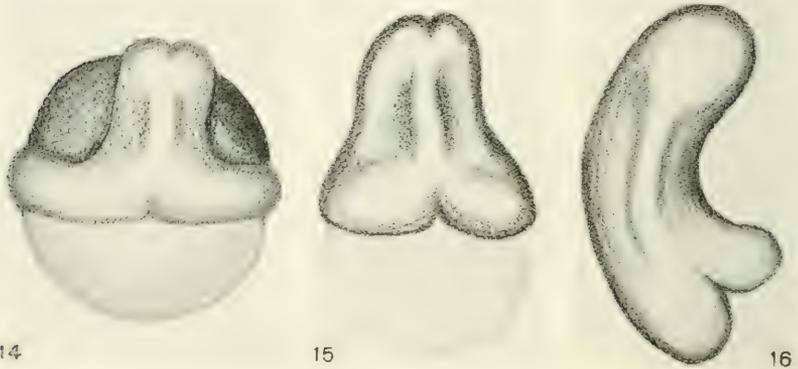
12



13

Figs. 11, 12, 13 In figure 12 the marked asymmetry of the neural tube halves immediately posterior to the level of bifurcation of the cord is shown. The neuroblasts become adjusted farther posteriorly, however (fig. 13) to form a more symmetrical tube or column of cells.

Where the rays struck close to or on the equator in the median plane of the egg the subsequent bifurcation was noted well forward towards the head region; figure 10 well demonstrates this fact. In figure 9 the point encountered was much lower down on the hemisphere, while in figures 6 and 4 it was still farther posterior. Such are really instances of cauda bifida. Another fact of the utmost significance was gained from studies of the histological sections. Since it was definitely ascertained that the rays had killed the portion of the egg illuminated, in no specimen was there to be observed, however, a deficiency or falling out of a portion of the neural tube, as one would expect



if the proanlagen or anlagen had been encountered by the rays. Notwithstanding the possibility of post-generation, with a replacement of the anlagen thus rendered inactive, the conclusion might be justified, tentatively at least, that the proanlagen are not located in the early stages of division of the ovum either in the yolk hemisphere or along the equator, but are confined wholly to a region well up on the black hemisphere. Before referring in detail to the results of other investigations dealing with spina bifida it will be well to emphasize some general considerations which must be taken into account in connection with the production of abnormalities.

It is taken for granted that there are many linking factors associating the developmental processes concerned in the pro-

duction of spina bifida with those of other forms of malformation occurring in nature and produced experimentally. Accordingly, one cannot well study the one without taking into consideration those general fundamental physico-chemical factors of development underlying both, upon the disturbance of which the production of anomalous conditions is dependent. The fact is well supported that the processes of differentiation in the tadpole, as in some other forms, appears to be dependent upon a series of complex and progressive chemical reactions which are of the nature of oxidations. In the later stages of development we are dealing with an organism whose chemical constitution differs considerably if not completely from that of the undivided ovum. The results of such reactions and chemical changes become apparent in the differentiation of the various anlagen. Eggs placed in pure water free from chemical compounds which might enter into the chemical structure of the organism, but supplied with a liberal amount of oxygen, can and do undergo the several processes of differentiation, finally hatching and swimming about. From that time on, however, when growth of the differentiated parts is initiated the organism requires a supply of various chemical substances for its appropriation. In this connection, it is significant that the great majority of defects produced experimentally are referable to changes in the differentiative stages of the embryo and only-secondarily to defective growth phenomena.

Bearing in mind, then, the chemical modifications occurring during the differentiating cycle of the embryo, it is fair to assume that at certain specific times in the genesis of the anlagen their chemical composition is such as to render them more ready participants in chemical reaction with the chemical agent employed. Results so obtained cannot be cited without reservation as applying to the state of the undivided ovum, consequently much of the chemical work conducted along this line is subject to considerable qualification and limited in the insight which it furnishes into the constitution, chemical or physiological, of the ovum. Before the full truth can be established on this point, it must be demonstrated that the toxic action of the chemicals employed was exerted upon the egg during the undivided state

and not afterwards; or, in other words, upon the chemical 'ferments,' or proanlagen, and not upon the anlagen cells when they have attained what might be termed a condition of chemical completeness. Only by this means can we decide between the two conceptions of the ovum; as an organism elaborating its organ anlagen at succeeding developmental periods, or as a composite, mosaic-like structure.

While the artificial production of defects has been known for a long while to be possible, but very few have succeeded in advancing any plausible explanations covering the instance of spina bifida embryos. There are two aspects to the problem of the artificial production of spina bifida which are brought out in a review of the literature dealing with this subject. The first consideration is whether we are dealing with the action of an external agent upon some specific substance in the egg; and the second, whether the nature of this reaction is specific, referable to the agent alone, which possibly reacts upon the egg as a whole.

Morgan in 1894 and O. Hertwig in the year following were both successful in the production by chemical means of a large percentage of embryos showing the defect of spina bifida. That 0.625 per cent solutions of sodium chloride should produce so high as 50 per cent of this form of malformation was an argument in favor of a definite specific chemical or physical property of the compound. Prior to this date observers had recorded only occasional instances of this defect and had failed to give a convincing indication of the nature of the upset in the physico-chemical factors concerned. Roux was the first to call attention to the occurrence of spina bifida among frog's eggs, owing, apparently, to conditions found in nature. Panum recorded 38 instances of spina bifida in chicks, among 404 monsters produced. He, with Dareste and Féré, obtained monsters of various kinds by the employment of variations in temperature (as did Hertwig), by varnishing the egg shells, by shifting the long axis of the egg to the vertical, by traumatic injuries, shaking, magnetism, electrical means, various gases, vapors of lavender and by injecting different toxins and chemicals, such as turpentine, an-

iseed, absinthe, and cloves into the white of the egg. Their inability to associate any given deformity with a known and controllable cause led to a failure in the analysis of the normal developmental factors of the embryo. Richter found three instances of spina bifida among several hundred hen's eggs upon which he had experimented. Spemann, however, produced two-tailed embryos by simply tying a ligature between the two blastomeres, demonstrating the bilaterality of the anlagen but throwing no light on the nature or antero-posterior extent of the organ-building substances. Fol, Rauber, Born, and O. Hertwig attributed the duplicity to double fertilization. This explanation was too compromising regarding the anterior portion of the embryo, and later was found to be unnecessary. Godlewski's experiments with reduced pressure, and Herbst's with lithium salts, Morgan's with the centrifuge, Samossa's with atmospheres of nitrogen and of hydrogen, and Wilson's with Ringer's solution and with sodium chloride, furnish additional evidence of the diversity of ways by which this abnormal condition may be produced.

In this connection, it is interesting to note that Mall has reported 12 instances of spina bifida among 163 pathological human embryos, attributing as a possible cause of the condition, faulty implantation of the embryo. Analysed still further, however, by analogy to the conditions found among lower vertebrates, it seems possible that the human ovum, too, requires but little else than a good supply of oxygen for its differentiation during the early stages of development. At this time the causal forces are operative for the production of spina bifida. Undoubtedly, a deficiency in the supply of oxygen could be brought about by the imperfect imbedding of the ovum in the uterine mucosa. Bearing this fact in mind in connection with the features of differentiation of the ovum given on page 375, it seems superfluous to seek an explanation of the condition in man through the action of chemical substances or of altered temperature. Though the possibility of the direct or indirect dependence of the processes of oxidation upon the action of the latter agents must be

admitted, reasoning from conditions as we find them in the frog, a sufficient and probably a more primal cause, at least, is referable to faulty oxidation.

Guthrie produced these defects by the use of strychnine, caffeine, and nicotine, as had Hertwig, but with concentration far below that of 0.625 sodium chloride. Jenkinson, however, tested out this question of the osmotic pressure of the salt solutions by employing a great variety of isotonic solutions of various salts, such as chlorides, bromides, iodides, nitrates, and sulphates of ammonium, lithium, sodium, potassium, calcium, barium, strontium, and magnesium, and, in addition, solutions of cane sugar, dextrose, urea, and gum arabic. He obtained spina bifida with especial success in his sodium chloride and sodium nitrate solutions. His conclusions are best given in his own words: "There is very little room for doubt, that the malformations in question may be due to some property of a salt other than its osmotic pressure." Bataillon had previously come to the conclusion that malformations were not specific to the means employed. Gurwitsch's belief was that halogens affected the position and development of the blastopore and of the brain, sodium chloride acting upon both, and sodium bromide upon the brain alone, whereas lithium chloride seemed selective on archenteron and blastopore.

It would appear, considering the production of this malformation by the diverse methods outlined above in connection with that detailed by this paper, that we were justified in concluding that in the question of specificity of reaction in the production of spina bifida the weight of argument at present refers the causative forces more particularly to an upset of a specific substance in the egg, rather than a specific action of the agent. It is in the conception of the changes which occur in the chemical composition of the ovum during its differentiation, as previously outlined, that we find support for this statement. It follows that the composition of any particular proanlage or anlage may be such at different stages of its chemical elaboration as to possess a marked affinity for widely varying chemical reagents. The

developmental end-product of the reactions so brought about would be the same, e.g., spina bifida, notwithstanding the wide diversity of character of the chemical reagents employed.

Furthermore, since we cannot deny, we must take into account the possibility of a two-fold manner of production of spina bifida; the one, owing to an upset in the contents of the cells of the unpigmented hemisphere whose yolk is intended for the nutrition or elaboration of the other component, viz., the proanlagen or chemical ferments restricted to the cells of the pigmented hemisphere. For the other, we can assume the possibility of an interference in the function of these proanlagen as the result of the chemical reactions experimentally induced, apart from an upset referable to the composition of the nutritive yolk particles. The author's work, however, points out clearly that in the white hemisphere alone are resident sufficient causes for the production of the malformation, so that, while the possibility of an involvement of the proanlagen exists, the weight of experimental evidence points to the yolk hemisphere as the more vulnerable of the two. Jenkinson observed, for instance, that as the result of chemical action the yolk cells were primarily affected, and Godlewski, employing reduced pressure, came to the same conclusion. The disturbing influences of insufficient aeration and cold, as ascertained by Morgan and others, were noted first in the yolk cells, and to this same region Morgan attributes the causative factors in the results of the centrifuge, while Hertwig drew the same conclusions from studies of overripe eggs.

The production of an area of altered protoplasm, which serves as a mechanical check to the approximation of the lips of the blastopore during the backward progression of the latter, emphasizes very naturally the importance of synchronized tempo in the two processes directly concerned with the elaboration of the neural cord. Under normal conditions the differentiation of the neural anlagen (consequent upon the backward migration of the proanlagen) occurs apparently synchronously with the backward migration of the blastopore and fusion of its dorsal lips. These two processes are approximately coöperative in point of

time, i.e., the anlagen of each half-tube become progressively differentiated in a backward direction at about the time when the half of the dorsal lip in which it is localized meets and fuses with its corresponding fellow of the opposite side. The two processes are not causally dependent upon each other, however, since differentiation takes place in the experimented eggs at about its former rate but now along the equator and not, as usual, parallel to the median plane.

The absence of the proanagen and anagen of the egg along the equator in the earliest stages of development of the ovum is sufficiently attested for by the ultra-violet method. Incidentally, it should be remarked that the restriction of these proanagen at all times to the pigmented hemisphere seems to the author's mind a very significant fact. In this connection, it should be stated that ultimately the yolk mass is wholly drawn into the body of the embryo. Even though by this later process the neural tube halves may be approximated, subsequent fusion does not take place, however, since each half tube has postgenerated into a whole tube.

The conclusions reached by this method of experimentation upon the fertilized ovum, are, therefore; first, that the killing of a small localized area of the yolk hemisphere or of the region of the equator of the frog's egg produces invariably the condition of spina bifida in the embryo; and second, that the neural tube proanagen, or formative substances, do not lie either in the yolk hemisphere or along the equator of the frog's egg, but are wholly restricted to the pigmented half of the egg. These proanagen attain their definitive positions by a process of backward migration, the rate of which is synchronous with that of the backward progression of the dorsal lip of the blastopore. The action of the ultra-violet rays in destroying a small localized area of the yolk hemisphere or equator results from mechanical causes in an upset of the synchronism of the two factors, i.e., differentiation of the neural anlagen and approximation of the lips. The former proceeds at its normal tempo, while the latter is retarded. Consequently, the former, always restricted to the pigmented hemi-

sphere, come to lie along the equator and are later carried towards the median plane by the subsequent approximation of the lips, but the half tubes, having already differentiated into whole tubes, do not subsequently fuse.

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## ON THE PRESENCE OF INTERSTITIAL CELLS IN THE CHICKEN'S TESTIS

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THREE FIGURES

Since there is a difference of opinion as to whether interstitial cells are present in the testis of the domestic cock, and because of the obvious bearing of the question upon the theory which attributes to these cells an important influence upon secondary sex characters, it has seemed worth while to investigate the matter. To illustrate the difference of opinion I shall abstract briefly two very contradictory reports on the subject.

Alice M. Boring<sup>1</sup> reports observations on testes of roosters from one day to twelve months of age. In the young as well as in the older testes she fails to differentiate any cells of the intertubular tissue from ordinary connective tissue cells. The variation in size, shape and character of the nuclei is attributed to mechanical conditions of pressure. The fat observed in the intertubular tissue was not found inside of the cell bodies, hence it was thought to be brought there by the circulation and deposited. Her conclusion is that there are no interstitial cells present at any time.

In the summary of the work done by J. des Cilleuls,<sup>2</sup> it is stated that the external differentiation of the rooster from the pullet begins to be apparent at about the thirteenth day; and that at this time interstitial cells first make their appearance in the testis. Des Cilleuls says the interstitial cells and cock characters increase *pari passu* and the cock characters are accentuated

<sup>1</sup> Alice M. Boring. The interstitial cells and the supposed internal secretion of the chicken testis. *Biological Bulletin*, vol. 23, no. 3, August, 1912.

<sup>2</sup> J. des Cilleuls. Interstitial testicular cells and secondary sex-character. Summary in *Journal of the Royal Microscopical Society*, December, 1912.

while the seminal tubes still remain in an embryonic condition, until after the sixtieth day. The explanation offered is that the internal secretion of the interstitial cells serves as a stimulus for the development of the secondary sex characters.

This report is made after the study of testes from cocks three, five-and-a-half, nine and eighteen months old. The tissue was removed immediately after killing the fowl and fixed in the following solutions: formalin, Zenker's, Bouin's and Ciaccio's fixative:

5% potassium bichromate .....	20 cc.
formalin.....	4 cc.
acetic acid.....	1 cc.

Fix in the above forty-eight hours, then in 3 per cent potassium bichromate one week. Sections were stained chiefly with hematoxylin, and congo red, iron hematoxylin, and Mallory's connective tissue stain. The Ciaccio fixative and Mallory's stain gave the best results for the study of the intertubular tissue, although the other preparations showed up fairly well.

For the study of fat I used frozen sections of tissue fixed in formalin. These were stained with Sudan III and hematoxylin.

Microscopic examination of the sections from the eighteen months testis shows the seminiferous tubules in a state of active spermatogenesis. The intertubular tissue is small in amount and compact, allowing the tubules to lie close together. Where three or more tubules come in juxtaposition small triangular or irregular areas are formed. In most of these areas there is a small blood vessel surrounded by connective tissue which contains both spindle- and oval-shaped nuclei. In other areas there are, in addition to the above structures, typical interstitial cells also, as shown in figure 1. The nuclei are round or oval in outline, rather rich in chromatin and contain an evident nucleolus. The cytoplasm is granular and in certain areas, especially around the nucleus, it is condensed, while near the periphery of the cell it is much less condensed or even vacuolated.

Sections from a five-and-a-half months' testis show the tubules in an inactive state, without spermatozoa, and the intertubular connective tissue slightly greater in amount than in the preceding chicken. The intertubular areas are somewhat larger, but

in other respects the appearances are quite similar to those observed in the cock of eighteen months.

In the sections of the three months' testis, the tubules are much smaller and lined with Sertoli cells, imbedded in which are numerous young sex cells. Relative to the tubules the intertubular spaces are far larger than in any of the older testes. The connective tissue with its spindle-shaped nuclei is readily differentiated from the interstitial cells. The former surrounds the tubules very closely, while the interstitial cells are usually located in the irregular areas formed by three or more tubules

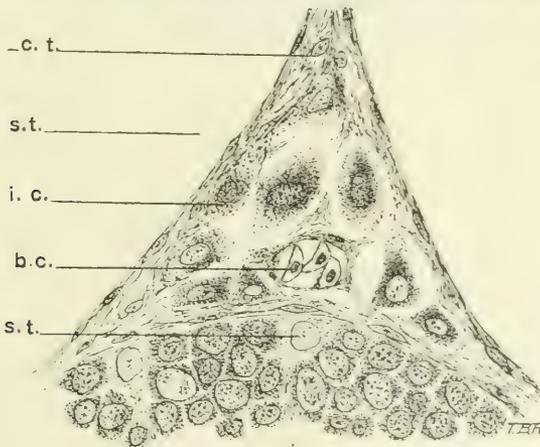


Fig. 1 *C. t.*, connective tissue; *s. t.*, seminiferous tubule; *i. c.*, interstitial cell; *b. c.*, blood cell.

coming close together. In most of these areas there are several interstitial cells; often they form large groups (fig. 2). The cell boundaries are more distinct than in any of the older testes and the cytoplasm is very much more vacuolated. Indeed some of the cell bodies appear almost clear, containing only the nucleus and a small amount of granular cytoplasm.

On examination of the sections stained for fat the interstitial cells in the three months' testis appear to be almost completely filled with fatty material (fig. 3). Thus the vacuolated appearance of the cells in figure 2 is explained. Most of the fat is within the interstitial cells, though there is a good deal free in the intertubular tissue and also a very small amount in the tub-

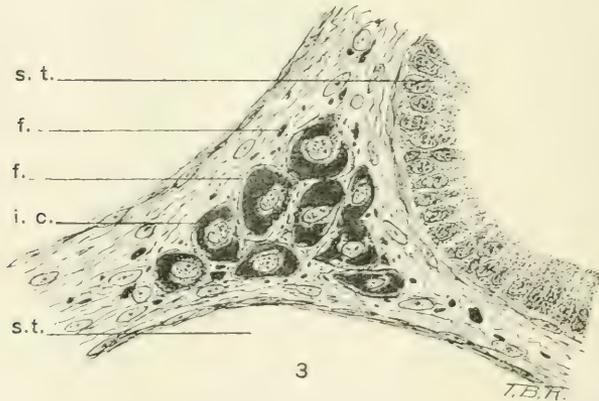
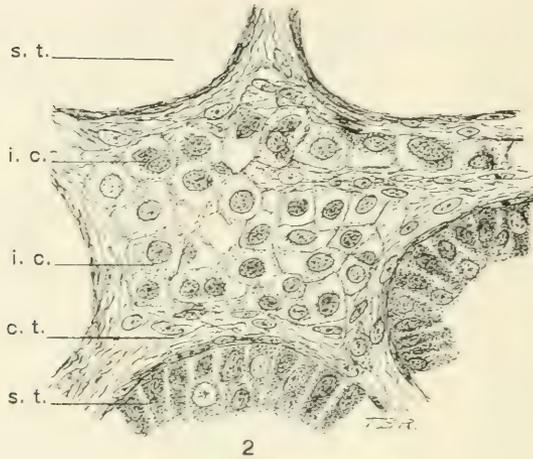


Fig. 2 *S. t.*, seminiferous tubule; *i. c.*, interstitial cell; *c. t.*, connective tissue.  
 Fig. 3 *S. t.*, seminiferous tubule; *f.*, fat; *i. c.*, interstitial cell.

ules. In the older testes the fat is very much less in amount and appears as very small particles both in and outside of the interstitial cells. No attempt was made to determine the nature of the fatty material; as it was not rendered insoluble by Ciaccio's fixative, it probably does not consist of phosphatid lipoids to any large extent.

The primary object of this short study was merely to determine the presence or absence of interstitial cells in the testis of the domestic cock; there can be no doubt but that they are present in all the stages examined.

## A SIMPLE METHOD OF BRAIN DISSECTION

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FIVE FIGURES

Every instructor realizes how hard it is to make clear to students the deep or inner structures of the brain. It is difficult to give them a lucid description of even the simpler and more superficial parts, but when it comes to explaining the intricate mechanism, it is an almost hopeless task.

Efforts have been made to disclose the regions, parts, tracts, and nuclear masses by means of a series of cross sections or fiber tract dissections. To all but those well trained in technique and familiar with the general make-up of the brain, these methods are confusing and difficult. Tract dissection necessitates a general understanding of how and where a tract runs, and a series of cross sections presents to a student a mass of labyrinthian vagaries. Most students remember an important structure in a cross section series as it appears in a few well-defined segments, but do not have a clear mental picture or distinct understanding of its extent and relationship. With the following method a student, being guided by a few easily located landmarks, can get the greatest degree of clearness and satisfaction from his work, and have the least amount of cutting and mutilating of tissue.

The procedure is as follows: Using one-half of the brain, clear away all pia mater from the regions to be cut; sylvian fissure, central fissure (Rolandi), post central fissure, superior frontal sulcus, and about the uncus and temporal pole. This is important to make the field of operation perfectly clear and prevent blocking the knife. It is also important to use a long scalpel, the blade of which should be about 7 or 8 cm. long and not more than 1 cm. in width; 0.5 cm. is still better. Place the hemisphere with frontal region upward or toward the student and depress the temporal pole sufficiently clearly to expose the uncus. Now cut across the upper part of this convolution, going from within outward and slightly downward, extending the cut about 2 cm. lateralward and the same backward (fig. 1). Make further depression of the temporal lobe there by widening the sylvian fissure, and cut at nearly right angle to the first incision along the lower border of the island (fig. 1). When this cut is extended 2 or 3 cm. directly backward, the tip of a cavity can be exposed, the anterior extremity of the inferior horn of the lateral ventricle.

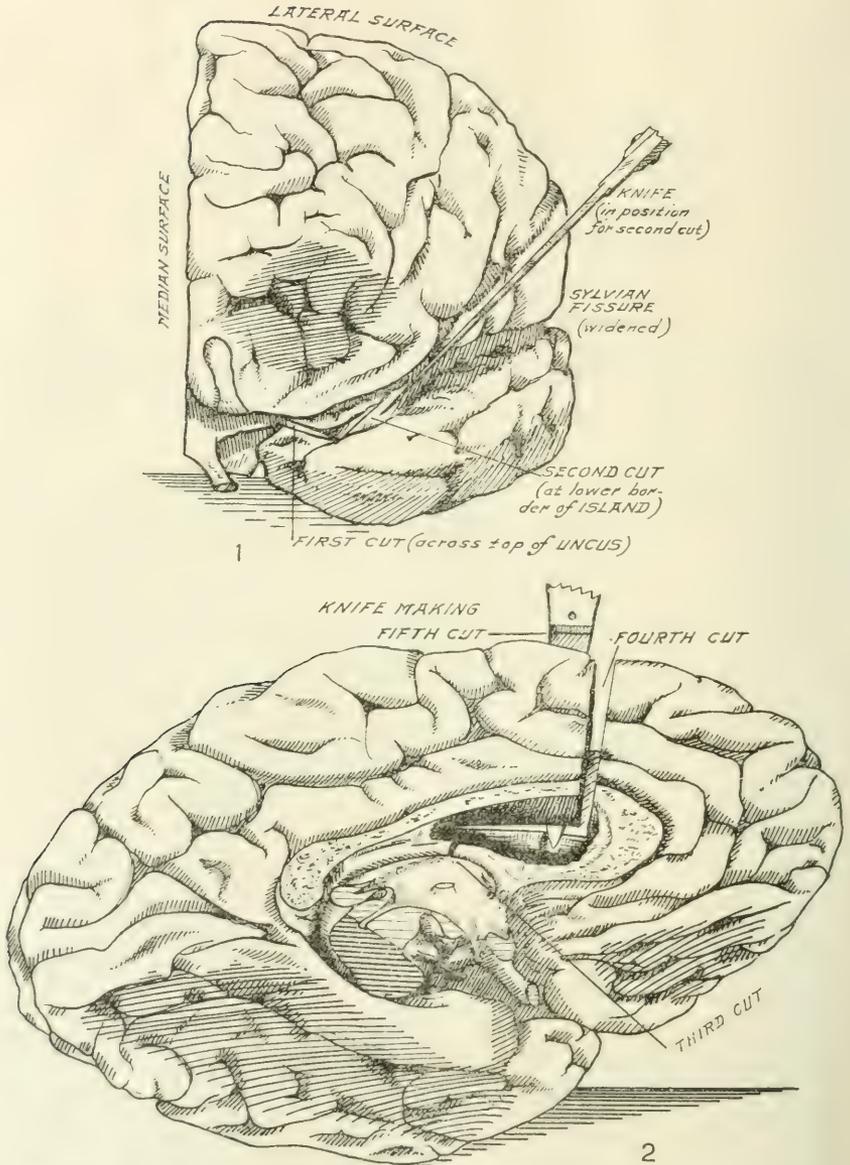


Fig. 1 Showing hemisphere in position, frontal pole forward and temporal pole depressed to make first and second cuts.

Fig. 2 Showing median surface with third and fourth cuts made and knife in position making fifth cut.

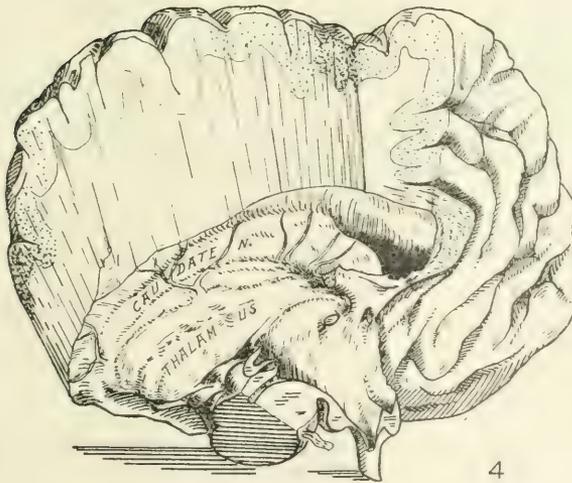
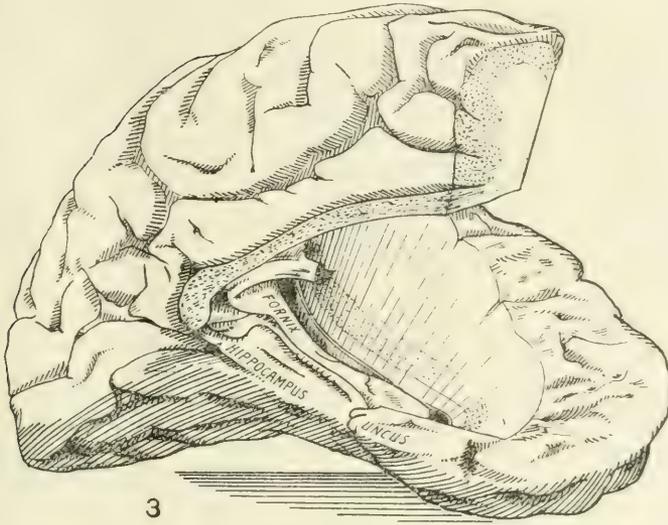


Fig. 3 Showing 'removable' portion, with hippocampus, fornix, and inferior horn of lateral ventricle.

Fig. 4 Showing 'basal' portion with lateral ventricle and its floor and lateral boundary structure in clear view.

Going now to the median surface of the hemisphere, cut the fornix just back of the foramen of Monroe (fig. 2). Make another cut into the surface of the hemisphere, beginning the incision at a point on the under surface of the corpus callosum just back of where it makes its turn downward, and terminating it on the superior median border a little anterior to the beginning point or directly above the tip of the corpus callosum (genu), thus making an oblique cut. The depth of the incision should extend as far as the superior frontal sulcus on the lateral surface or about 2 cm. from the superior median border, and to the lateral-most extent of the lateral ventricle or just above the caudate nucleus (figs. 2, 5). Now turn the knife at right angles to this plane

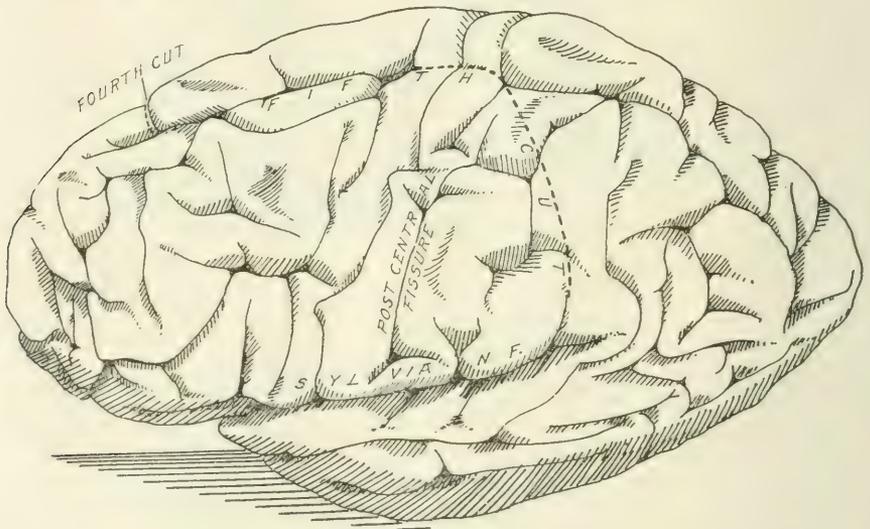


Fig. 5 Showing lateral surface and dotted line marking, on the surface, the course of the shoulder of the knife in making the fifth cut.

and make the following incision with the shoulder of the knife following the superior frontal sulcus or an arbitrary line at about this position, and the point following the lateral-most boundary of the ventricle, cut slowly and gradually backward, all the while elevating the excised portion to give clear view of the field (figs. 2, 5). When the knife comes to the post central fissure, let it swing in a sharp curve downward and backward to the posterior end of the sylvian fissure, reaching this point by following the upper terminal arm of the fissure (fig. 5). While making this curve, the knife should be held with the handle tilted backward so that the point will be a little anterior to the shoulder. This will insure the point cutting through the ventricle at its lateral-most boundary where that cavity turns downward. When

the shoulder comes to the sylvian fissure, lift the handle to about 60° with the horizontal plane, thus forcing the point downward, at the same time turning the sharp edge forward.

Now make a little more bold depression of the temporal lobe through the sylvian fissure, and continue the incision forward along the lower border of the island to meet the original cut at the uncus, all the while holding the knife at about 60° with the horizontal plane.

The incisions have now been completed. With the median surface facing upward, grasp the frontal lobe in the right hand and the occipital lobe in the left hand (this is for the left hemisphere; if the right hemisphere is used, the hands will be reversed), and carefully separate the two portions to about 3 cm. This will stretch out the choroid membrane which can be easily followed almost throughout its entire extent of attachment. When this is carefully studied, the removable portion containing the hippocampal lobe with its fornix can be entirely withdrawn, and a clear view of the lateral ventricle will be had (fig. 3). The complete separation of the two parts will rupture the choroid membrane, but the ragged edges will still give a clear view of the line of its attachment. On the basal portion, in plain view, will be the structures forming the floor and lateral boundary of the lateral ventricle, caudate nucleus, taenia semicircularis, thalamus, etc. The optic tract, geniculate bodies, quadrigeminal bodies, and pes pedunculus can also be easily seen (fig. 4). The two segments can be easily, quickly, and repeatedly separated with no harm whatever to continuity of tissue. When the sections are in place, the cuts are scarcely perceptible; when removed, there is the greatest amount of exposure of hidden structures. A special advantage in this method is that specimens too soft for fiber tract dissection or cross sectioning, or hardened after being mashed or pressed out of shape, can still be used with a good degree of satisfaction when cut as outlined above. There is to be offered this last and most important point, that the removable segment comes off from the basal portion in approximately the same course the hemisphere pursued in its early stages of development. Following this course of development as displayed by such a method of removing part of the hemisphere, it is much easier for the student to see how the velum interpositum was at one time a part of the wall, the roof portion of the forebrain, of the neural tube, and its presence in the fully developed specimen, attached to the sharp edge of the fornix on the one side and the taenia semicircularis and thalamus on the other, makes a closed cavity of the lateral ventricle and its horn.

This method is not to be used for complete work; the nuclear masses and fiber tracts demand deeper dissection. But using the method on one hemisphere and cross section on the other, the student will have far greater and more gratifying results, and will have good material, easily kept, for future reference.

Finally, I wish to express my appreciation to Dr. Bremer and Mr. Miller for their kindness in reviewing this paper and making valuable suggestions.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

THE ANATOMY OF THE DOMESTIC ANIMALS. By Septimus Sisson, S.B., V.S., Professor of Comparative Anatomy, Ohio State University, College of Veterinary Medicine. Second edition entirely reset. Octavo of 930 pages, 724 illustrations. Philadelphia and London: W. B. Saunders Company, 1914. Cloth, \$7.00 net; Half Morocco, \$8.50 net.

Preface to first edition. The lack of a modern and well-illustrated book on the structure of the principal domestic animals has been acutely felt for a long time by teachers, students, and practitioners of veterinary medicine. The work here offered is the expression of a desire to close this gap in our literature.

The study of frozen sections and of material which has been hardened by intravascular injection of formalin has profoundly modified our views concerning the natural shape of many of the viscera and has rendered possible much greater precision in topographic statements. The experience of the author during the last ten years, in which almost all of the material used for dissection and for frozen sections in the anatomical laboratory of this University has been hardened with formalin, has demonstrated that many of the current descriptions of the organs in animals contain the same sort of errors as those which prevailed in regard to similar structures in man previous to the adoption of modern methods of preparation.

While the method of treatment of the subject is essentially systematic, topography is not by any means neglected either in text or illustrations; it is hoped that this will render the book of value to the student in his clinical courses and to the practitioner. Embryological and histological data have been almost entirely excluded, since it was desired to offer a text-book of convenient size for the student and a work of ready reference for the practitioner. \* \* \*

Preface to second edition. This book supersedes the author's Text-book of Veterinary Anatomy. A comparison of the two will show the new title to be justified by the extent and character of the changes which have been made.

Continued observations of well-hardened material and frozen sections have led to a considerable number of modifications of statement. It is scarcely necessary to say that the recent literature, so far as available, has been utilized.

Many changes in nomenclature have been made. Most of the synonyms have been dropped or relegated to foot-notes. Exceedingly few new names have been introduced. Nearly all eponyms have been eliminated, on the ground that they are not designative and are usually incorrect historically. The changes made in this respect are in conformity with the report of the Committee on Revision of Anatomical Nomenclature which was adopted by the American Veterinary Medical Association two years ago. Progress in the direction of a simplified and uniform nomenclature is much impeded by the archaic terminology which persists to a large extent in clinical literature and instruction. \* \* \*

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## THE RÔLES OF NUCLEUS AND CYTOPLASM IN MELANIN ELABORATION

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ONE FIGURE

The more recent work on the formation of melanin seeks to derive this pigment from chromatin elements. In 1889 Mertsching attributed the formation of melanin to the breaking down of the cell and especially to the destruction of the nucleus. Jarisch ('92) makes the statement, "Das Oberhautpigment entwickelt sich aus einer Kernsubstanz, dem Chromatin, oder einem diesem chemisch oder wenigstens räumlich nahe stehenden Körper." Rössle ('04), in the discussion of pigment formation in melanosarcomas, recognizes five stages in the process, based on the appearance of the nucleus. He believes the melanin granules to be small particles of chromatin which are extruded from the nucleus, impoverishing the latter in the process.

Aurel von Szily ('11) has made a notable contribution to the theory of pigment formation from chromatic elements. He worked on the elaboration of melanin in developing eyes of a variety of vertebrates and in melanotic tumors of the human eye. According to his results, the melanin granules arise as colorless rod-like bodies (Pigmentträger) extruded from the nucleus, being derived directly from chromatic elements. These Pigmentträger are typical for species and locality of production. They also correspond exactly with the size and form of the melanin particles met with in that species and location. After being freed from the nucleus and wandering to a more or less peripheral position in the cell, the colorless Pigmentträger become colored probably by the action of cell ferments. The pigmentation of the Pigmentträger begins at one end of it and proceeds to the other. The origin of the Pigmentträger from the chromatin and their transition into the cell cytoplasm

may be followed step by step. The nucleus which gives rise to them may be either productive or degenerative. In the former case, no impoverishment of the nucleus takes place; in the latter, pigment formation is accompanied by marked degenerative changes in the nucleus.

In 1910 Harrison presented evidence of a new type upon the question of pigment formation. In his paper on nerve growth *in vitro*, he mentioned and figured certain cells which became pigmented during the life of the cultures. To quote, Harrison found that "the pigment first arose as a round mass of granules lying just to one side of the nucleus. This (mass) gradually increased in size and then the pigment granules became scattered through the cytoplasm" ('10, p. 812).

In 1912, while working on the reactions to light of embryonic connective tissue melanophores, material for a careful study of the actual elaboration of the melanin granules themselves was found in the developing connective tissue and epithelial cells of embryos of *Rana pipiens*. For this study, Harrison plasma cultures, living embryos and serial sections of carefully fixed embryos were used. The development of the melanin was followed in embryos varying in length from 3 to 10 mm.

Though the melanophoric cells found in the epidermis of older frog larvae are certainly mesodermal in origin, many ectodermal cells of young embryos elaborate this pigment within themselves. The pigment, however, after existing for a time in the cells, gradually disappears. That connective tissue cells elaborate melanin has long been an established fact. The mode of its development in these two types of cells is the same.

*Plasma cultures.* Small pieces of mesenchyme and epithelium from *Rana pipiens* embryos 3 to 4 mm. long were implanted in the plasma of frogs of varying species. Seventeen primary cultures were made. These lived in good condition over periods varying from three to forty days. In the case of the older cultures, the plasma was changed at frequent intervals. From these primary cultures, secondary were made, to the number of sixteen, by removing fragments of tissue and reimplanting in new plasma. In all, thirty-three cultures were studied.

At first (fig. 1, A) the cells were clear and translucent, without

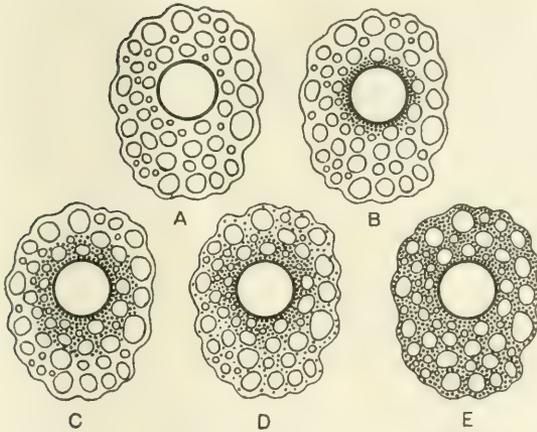


Fig. 1 Diagrammatic sketch to show the different stages of melanin elaboration. The heavily outlined circle in the center represents the nucleus of the cell, the smaller ovals represent oil droplets. The cell is represented in median section.

pigment granules, but contained fat droplets scattered throughout the cytoplasm and a more or less centrally placed nucleus. Then a few small, spherical, brownish granules became visible in the cytoplasm of the cell immediately adjacent to the nucleus, appearing simultaneously, or nearly so, on all sides of it. Their number gradually increased until a well defined hollow sphere surrounded the nucleus (fig. 1, B). On their first appearance, these melanin granules presented all the characteristics which are normal for them. No positive evidence of any increase in size of the individual particles of pigment was obtained. Owing, however, to their minuteness, an accurate determination of any such change presents almost insurmountable difficulties.

No granules made their appearance at any time inside the nuclear membrane nor in the cytoplasm of the cell away from the nucleus. The region of production was limited to that zone of the cytoplasm which is in contact with the nucleus. Nor was there any sign of the presence of colorless granules, which, by a process of pigmentation, could be transformed into the particles of melanin. The most careful search for any morphological structure within the cell that might serve as a *Pigmentträger* in von Szily's sense, was unavailing.

With each succeeding day, the number of melanin granules

increased. During the earlier stages of this process, they remained concentrated in the center of the cell, the periphery of the cloud of densely packed granules becoming larger and further away from the nucleus (fig. 1, C). Then the granules began to spread throughout the cell. This process was gradual and was accomplished by the slow separation of the individual elements of the dense cloud about the nucleus. At first, but a few granules, widely separated from one another, detached themselves from the central mass and wandered among the oil droplets nearest at hand. As this process of distribution, accompanied by further production of new granules around the nucleus, continued, more and more of the pigment particles spread toward the periphery (fig. 1, D), in such a manner that the number of granules per unit area steadily decreased from within outward. The periphery of the cell always contained fewer granules than the center until the final stage of melanin elaboration was reached (fig. 1, E). At this time, the cytoplasm of the cell was filled, one might say, to 'saturation.' Through this cloud of granules, the fat droplets and the nucleus were visible as clear, translucent areas, absolutely free from pigment. The entire process here described is completed in a period of eight to fourteen days, on an average.

*Living embryos.* By carefully dissecting out scraps of mesenchyme and epidermis from embryos of different ages and mounting them in plasma or isotonic saline (0.4 per cent), the different stages of melanin elaboration may be observed. That the steps thus seen are not continuous, but isolated from one another, is true. This objection to the method is obviated, however, by the check provided by the cultures. Every step in the elaboration of melanin observed in the cultures is exactly duplicated in the normal body. The light brown granules of melanin are found, at first, only in the region of the nucleus and then spread through the cytoplasm of the cell. They are colored on their first appearance, a fact which seems clearly to do away with the colorless Pigmentträger idea.

*Serial sections.* Like the study of fresh material from living embryos, that of serial sections serves principally as a check upon the cultures. A description of the findings here would

be but a repetition of facts already stated, with one important exception. The most minute examination of series fixed in all stages of melanin formation fails to show the slightest change in character and content or the least sign of degeneration or depletion in the nucleus. Nor can any evidence be found to show that the nucleus plays a part in the formation of melanin by a process of extrusion of any of its elements into the cytoplasm. All the granules of pigment are found in the cytoplasm near the nucleus, but they have no visible, structural connection with the nucleus or any of its contents.

Certain very important objections to the chromatin idea of the origin of melanin are evident from the results set forth above.

The nuclei of the pigment-forming cells suffer no depletion during the process. Though von Szily claims that certain nuclei which he terms 'active,' develop pigment without loss to their content, the actual depletion of many others was also seen by him. Rössle describes minutely many changes which occur in the nucleus and states that after the extrusion of chromatin to form melanin, the nucleus is bladderlike, with a reduced amount of chromatin.

No colorless anlagen for the melanin granules are to be found in the form of 'Pigmentträger' (v. Szily, '11) or 'Pigmentbildner' (Fischel, '96). That a chemical anlage in the form of a chromogen is present is almost certain in view of the work of Bertrand ('08) but that the melanogen exists in the frog as a definite morphological structure, which, without any other change than in its coloration, becomes the pigment granule itself, may be denied.

The process of pigmentation of a melanophore in the frog begins in the area nearest the nucleus and spreads from that point throughout the cell, that is to say, it progresses from the center of the cell to the periphery. This is in direct opposition to the observations of von Szily, who states that the pigmentation of the colorless Pigmentträger takes place gradually while they are wandering about in the cytoplasm. Indeed, his figure 4 (plate 4) shows the process going on irregularly throughout the cell and figure 8 of the same plate illustrates a process directly the reverse of that noted in this paper, namely, the appearance

of pigmented granules at the periphery of the cell before they are present near the nucleus.

The last, and probably the most important, objection to the supposed chromatin origin of melanin granules is the fact that no process of extrusion of chromatin nor any of the steps of such a process are to be observed. The pigment granules appear near the nucleus, in fact, in almost direct apposition to the nucleus, but no evidence was found in this work which even suggests a morphological relationship to either the nucleus or its contents.

It may safely be concluded that, in the normal ontogenetic origin of melanin in the frog, the chromatin plays no direct rôle. On the contrary, all the evidence obtained goes to demonstrate that the melanin granules are formed in the cytoplasm, from elements already present in solution in it, through some action of the nucleus.

Bertrand ('96) isolated an enzyme in plants (*Russula* and *Dahlia*) which, by its oxidizing action on tyrosin, was named tyrosinase. Von Fürth and Schneider ('01) found this same ferment in the haemolymph of Lepidopteran larvae and noted its occurrence in many animal forms. The action of this enzyme on tyrosin gives a melanin and von Fürth suggested,

. . . dass die physiologische Bildung melaninartiger Pigmente in den tierischen Geweben auf das Zusammenwirken von zweierlei Fermenten zurückzuführen sei: durch ein autolytisches Ferment könnte ein aromatischer Komplex aus dem Eiweismaterial abgespalten und dieser sodann durch eine Tyrosinase in ein Melanin übergeführt werden. (1901, p. 242).<sup>1</sup>

The remarkable results of Bertrand's ('08) more recent work demonstrate the manner in which tyrosin and its derivatives may form the various types of the melanins usually met with in the animal body. He determined that many substances may be transformed into melanin by the oxidizing action of tyrosinase, each giving a characteristic color. During oxidation, a play of colors results, the earlier stages of the process giving lighter colors than the more advanced. The essential constitu-

<sup>1</sup> It is not within the province of this paper to review in detail the literature on the melanins. The reader is referred to the excellent *Sammelreferat* of von Fürth ('04).

ent seems to be a benzene ring with an hydroxyl radicle. Tyrosin itself gives a black pigment, while paraoxyphenylacetic and paraoxyphenylpropionic acids give browns. It should be remembered, however, that the individual granules of 'black' melanin are brown; those of 'brown' melanins, yellowish in color.<sup>2</sup>

Gessard ('03) has given the strongest evidence yet adduced for the actual formation of melanin in the animal body from tyrosin. Working on melanotic tumors in horses, he determined the presence of free tyrosin and adds: "La tyrosine est donc le chromogène dont l'oxidation par la tyrosinase détermine la formation du pigment noir commun à divers produits physiologiques et pathologiques de l'économie animal." (p. 1088).

The recent work on protein digestion demonstrates that amino-acids are absorbed, unchanged, by the blood stream from the alimentary canal and are distributed to the tissues (Folin, '14). Van Slyke and Meyer ('13) have shown that "the disappearance of intravenously injected amino-acids from the circulation is the result of neither their destruction, synthesis nor chemical incorporation into cell proteins. The acids are merely absorbed from the blood by the tissues, without undergoing any immediate chemical change." They have also demonstrated that there is a limit to the amount of amino-acids that may be absorbed by the tissues, so that a certain equilibrium exists between the blood and the tissues so far as the amino-acids are concerned. Further, Osborne and Mendel ('12) have shown that, while certain amino-acid groups will sustain life if fed as an exclusive diet, "on the other hand it is clear that when certain amino-acid groups are lacking, nutritive equilibrium is impossible. The cyclic derivatives, *tyrosin* and tryptophane, appear to be included here" (p. 326).

Several of the protein putrefaction products mentioned by Bertrand as sources of melanin, as paracresol, paraoxyphenylacetic acid and others, all derivatives of tyrosin, also are known to be absorbed as such by the organism. There can be but little doubt that sufficient quantities of melanin-forming substances occur normally in the body.

<sup>2</sup> H. Eppinger ('10) isolated a melanogen from the urine of patients suffering from melanosarcoma which turned black on oxidation. This he believes to be derived, not from a tyrosin base, but from tryptophane.

J. Loeb has repeatedly made the statement that oxidation is a prominent function of the nucleus in normal development and regeneration. Perhaps the most striking proof of this fact in specific tissues in the adult has been given by the work of R. S. Lillie ('02). By soaking thin slices of living tissues in solutions of substances which, colorless in the unoxidized condition, give brilliant color reactions on oxidation, he was able to determine the exact location in the individual cell where this reaction proceeds to the greatest extent. His findings "furnish, it is believed, conclusive evidence that in many tissues the nucleus is the chief agency in the intracellular activation of oxygen; and, further, that the active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm" ('02, p. 420).

The findings in the normal development of melanin in the embryonic frog furnish strong histological evidence that the nucleus of the cells elaborating this pigment provides something vitally necessary for its production. The melanin granules appear, not in haphazard manner throughout the cell, but in the cytoplasm immediately about the nucleus or, in Lillie's words, "at the surface of contact between nucleus and cytoplasm." Lillie's work seems to indicate clearly that the vitally necessary element for melanin elaboration provided by the nucleus is an oxidizer. Jaquet in 1892 demonstrated that the oxidizing action of the cell was not alone a property of living tissue, but was also evinced by broken-down cells which were no longer living. Nevertheless, the oxidases present in dead cells were originally elaborated by the nucleus. Lillie's work demonstrates this.

The particular form which the oxidizing action of the nucleus takes in melanin elaboration is that of an oxidase, perhaps of a type of tyrosinase. A host of investigators, following in the footsteps of Bertrand's ('96) original discovery of the presence of tyrosinase in plants, have isolated this enzyme in many animal forms and in such bodily positions as to serve normally for the manufacture of melanin.

The data derived from these various sources may be briefly summed up as follows:

1. Tyrosin, or its derivatives, acted upon by an oxidizing

agent, tyrosinase, gives a melanin. (Bertrand, '96 and '08; von Fürth and Schneider, '01, etc.)

2. Free tyrosin was discovered by Gessard ('03) in horses with melanotic tumors and it is now a well known fact that derivatives of tyrosin are absorbed by the animal body.

3. Lillie ('02) gave definite proof of the rôle played by the nucleus as a producer of oxygen or of an oxidase.

4. The normal presence of tyrosinase discovered in many parts of the body by Gessard ('01, '02, '03,) Przibram ('01),<sup>3</sup> Dewitz ('02), Durham ('04), Weindl ('07), etc.

When the histological data presented in this paper are considered in connection with the facts just reviewed, it will be seen that they are in full accord with one another. While no evidence has been obtained from this work that tyrosin is present in the cells under consideration, it is shown that the base from which the melanin granules are formed probably exists in a soluble condition in the cytoplasm. The rôle of the cytoplasm, then, is that of a carrier of the chromogen. That the nucleus plays an all important rôle is evident.

#### CONCLUSIONS

It is felt that the evidence here brought forward demonstrates conclusively the following points:

1. That the theory of the origin of melanin from chromatin elements extruded from the nucleus into the cytoplasm is untenable, at least in the frog.

2. That, however, the nucleus plays an essential part in pigment formation by some activity which greatly resembles an oxidizing action.

3. That melanin is formed in the cytoplasm of the cell at the point of known greatest efficiency of the nucleus as an oxidizing agent.

The following general conclusion from these facts seems justified: that, in the cells of embryo frogs, melanin is formed from some substance (probably tyrosin or its derivatives) in solution in the cytoplasm when acted upon by the nucleus (perhaps an oxidase reaction).

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<sup>3</sup> Evidence given by von Fürth and Schneider ('01, p. 241).

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ON THE NORMAL SEX RATIO AND THE SIZE OF  
THE LITTER IN THE ALBINO RAT (*MUS*  
*NORVEGICUS ALBINUS*)

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*From the Wistar Institute of Anatomy and Biology*

ONE FIGURE

Literature dealing with the early development of the albino rat contains references to but two papers that give information regarding the normal sex ratio and litter size in this animal (Cuénot '99; King '11). Marked differences in the results of these two sets of investigations, which were made on relatively small numbers of individuals, render it necessary that a large series of observations should be recorded in order to furnish adequate standards by which one can judge the effects of experiments aiming to modify the sex ratio or to alter the fertility of the albino rat. To supply the material for such standards the data given in the present paper were collected.

All of the records given are of litters cast by stock albino rats kept in the animal colony of The Wistar Institute. During the period when the data were being collected (1911-1914) all of the animals used for breeding were subjected to similar environmental conditions, and they all were fed on a mixed diet that experience has shown is necessary if rats are to be kept in good condition for any length of time.

THE NORMAL SEX RATIO IN THE ALBINO RAT

Practically all of the data were obtained by examining litters at or very shortly after their birth, since the sexes can readily be distinguished at this time as Jackson ('12) has shown. The removal of the young rats from the nest entails some risk that the mother will not care for them after they are replaced, but it is necessary that the records be taken at this time if one wishes

an accurate determination of the sex ratio or of the litter size. Not infrequently litters contain one or more stillborn young which are usually eaten by the mother within a few hours after their birth. Often, too, some individuals in the litter, particularly if the litter is large, will be killed by the mother when they are several days old, or if one or more of the young rats in a large litter are constitutionally weak they will die from lack of nourishment, being unable to cope with their stronger brothers in their efforts to obtain food.

No attempt was made to obtain the sex records for all of the litters of stock albino rats that were born in the colony during the years 1911-1913. The data that were collected during this period have been grouped together, according to the months when the litters were cast, and are given in table 1.

TABLE 1

*Showing the sex ratios and the average number of young in litters of stock albino rats born during 1911-1913. Data arranged according to the months when the litters were cast*

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	28	194	103	91	113.2	6.9
February.....	18	123	65	58	112.1	6.8
March.....	32	236	113	123	92.2	7.3
April.....	16	101	47	54	87.0	6.3
May.....	21	135	69	66	104.5	6.4
June.....	27	194	108	86	125.6	7.1
July.....	22	160	87	73	119.2	7.2
August.....	12	77	40	37	108.1	6.4
September.....	11	80	38	42	90.5	7.2
October.....	46	316	159	157	101.3	6.8
November.....	16	117	65	52	125.0	7.3
December.....	26	195	102	93	109.7	7.5
	275	1928	996	932	106.9	7.01

One fact clearly brought out in the above table is that there is no restricted breeding season for the albino rat. Litters are cast during every month of the year, but, as the records for many thousands of litters show, relatively more litters are pro-

duced in the spring than during other seasons of the year. In table 1 the sex ratios for the different groups of litters do not show a very great range of variation considering the small number of litters involved. The highest sex ratio is that for the 27 litters cast during the month of June; the lowest sex ratio is found in the litters of the April group. For the entire series of 275 litters the sex ratio is 106.9 males to 100 females.

During the year 1914 an attempt was made to obtain the sex data for as many as possible of the litters of stock albino rats born in the colony. The cages containing the breeding animals were examined nearly every day throughout the year and practically all of the litters cast were recorded. The data obtained, arranged according to the months when the litters were cast, are given in table 2.

TABLE 2

*Showing the sex ratios and the average number of young in litters of stock albino rats born during 1914. Data arranged according to the months when the litters were cast*

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	57	407	202	205	98.5	7.1
February.....	56	410	197	213	92.5	7.3
March.....	58	432	210	222	94.6	7.4
April.....	51	367	199	168	118.4	7.1
May.....	60	430	217	213	101.9	7.1
June.....	101	744	387	357	108.4	7.3
July.....	116	821	430	391	109.9	7.0
August.....	109	776	438	338	129.6	7.1
September.....	111	751	377	374	100.8	6.7
October.....	31	187	104	83	125.3	6.0
November.....	33	188	99	89	111.2	5.7
December.....	31	178	96	82	117.1	5.7
	814	5691	2956	2735	108.1	6.99

Although the number of records taken during the year 1914 is about three times greater than that collected during the period 1911-1913, the range of variation in the sex ratios of the litters cast during the various months is only slightly greater than that given in table 1. The lowest sex ratio in this series

of records is found among the litters cast in February; the highest sex ratio occurs in the litters born in August. The sex ratio of the 814 litters examined during the entire year is 108.1 males to 100 females. This sex ratio is remarkably close to that found in the 275 litters previously recorded (table 1).

A summary for all of the data collected is given in table 3. In order to give equal value to the two sets of records the sex ratios in this table, and also the averages for the size of the litters cast in the various months, represent the arithmetical mean of the records as given in table 1 and in table 2; they have not been computed in any instance on a litter basis.

TABLE 3  
*A combination of the data given in table 1 and in table 2*

MONTHS	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
January.....	85	601	305	296	105.7	7.0
February.....	74	533	262	271	102.3	7.0
March.....	90	668	323	345	93.4	7.3
April.....	67	468	246	222	102.7	6.7
May.....	81	565	286	279	103.2	6.7
June.....	128	938	495	443	116.9	7.2
July.....	138	981	517	464	114.6	7.1
August.....	121	853	478	375	118.8	6.7
September.....	122	831	415	416	95.6	6.9
October.....	77	503	263	240	113.3	6.4
November.....	49	305	164	141	118.1	6.5
December.....	57	373	198	175	113.4	6.6
	1089	7619	3952	3667	107.5	7.0

As arranged in table 3, the data show that the sex ratios are somewhat higher in the litters cast during the latter part of the year than in those cast in the early part of the year. With the exception of the record for March the sex ratios for the litter groups from January to May show a variation of less than three points; and the sex ratios for the litters cast from June to December, omitting the record for September, vary less than four points. The pronounced drop in the sex ratio for the litters produced during September is found in both sets of records, and at present there is no satisfactory explanation for it.

In the total of 1089 litters examined there were 3952 males and 3667 females, giving a sex ratio for the series of 107.5 males to 100 females. This sex ratio is somewhat higher than that given by Cuénot, who found in 30 litters of albino rats a sex ratio of 105.6 males to 100 females, but it is practically the same as that given by King ('11) for 80 litters of albino rats (107.3 males to 100 females). The sex ratio found among adult rats is doubtless considerably lower than that given above, as growth experiments with the albino rat at present under way seem to indicate that female rats, as a general thing, live longer than male rats and show somewhat less susceptibility to disease at all stages of their growth.

It would be futile to make a comparison between the sex ratios of the various litter groups owing to the inequality in the number of litters recorded for the different months. For the purpose of a somewhat closer analysis than that given above, the two sets of records have been grouped in table 4 according to the season of the year when the litters were cast. The averages given for the two sets of records were obtained in the same manner as were the averages in table 3.

TABLE 4

*Showing the data for sex ratios and size of the litters in the albino rat arranged according to the season of the year when the litters were cast*

SEASONS	1911-1913			1914			1911-1914		
	NUMBER OF LITTERS	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER	NUMBER OF LITTERS	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER	
March to May....	69	94.2	6.8	169	103.8	7.2	99.0	7.0	
June to August....	61	119.9	7.0	326	115.6	7.1	117.7	7.05	
September to November.....	73	104.4	7.0	175	106.2	6.4	105.3	6.7	
December to February.....	72	111.6	7.1	144	99.0	6.9	105.3	7.0	
	275	106.9	7.01	814	108.1	6.99	107.5	7.0	

There is a very striking agreement between the corresponding sex ratios for the two sets of records, as is shown in table 4. In each case the sex ratio for the litters cast in the spring

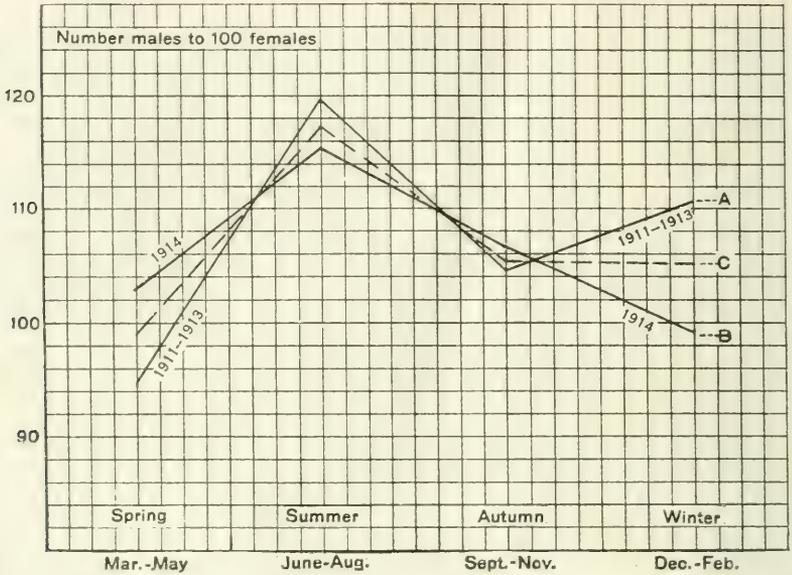


Fig. 1 Graphs showing variations in the sex ratio of the albino rat at different seasons of the year. A, graph constructed from data for litters cast during 1911-1913; B, graph constructed from data for litters cast during the year 1914; C, graph constructed from the averages for the two sets of data.

is considerably below the normal sex ratio of 107 males to 100 females; the average for the two groups giving a sex ratio of only 99.0 males to 100 females. Each set of data shows likewise a sharp rise in the sex ratios of the litters born during the summer months and then a drop to below the normal ratio for the litters born in the fall. The two sets of records for the litters cast in the winter months do not, for some reason, show the same agreement as those for the litters produced in other seasons of the year, as in one case the sex ratio is somewhat above the normal and in the other case it is below the normal.

Figure 1 shows graphs, constructed from the data given in table 4, which bring out very clearly the changes in the sex ratio that are found to occur among rats born at different seasons of the year.

Judging from observations and from the records for several thousand litters cast in our colony during the past six years,

the rat breeds more readily in the spring than in any other season of the year, and there is a second, less pronounced, period of sexual activity in the early fall. The lowest points in the graphs shown in figure 1 are found to coincide with the period in which the greatest sexual activity occurs. Lacking adequate means for heat regulation the rat suffers greatly from heat during the summer months, and for years the highest mortality among the animals in our colony has occurred in July and in August while relatively fewer litters are produced at this time than at other seasons of the year. It is during the hot weather when the breeding animals are not in the best physical condition that the litters produced show the highest sex ratio, as is indicated by the graphs in figure 1.

The seasonal variation in the sex ratios that is shown by these records cannot be ascribed to environmental conditions other than temperature, since the routine of caring for the animals in our colony is the same throughout the year and there is no change in the character of the food.

That the sex ratios in various mammals seem to show a pronounced variation at different seasons of the year has long been known. From the large body of statistics examined by Düsing ('83) it appears that relatively more boys are born during the winter than during the summer months. Table 5, compiled from data collected by Wilckens ('86) and by Heape ('08), shows the apparent seasonal variation in the sex ratio that occurs in the young of various kinds of domestic animals.

TABLE 5

*Showing seasonal variations in the sex ratios of some domestic animals. Data collected by Wilckens and by Heape*

ANIMALS	NUMBER INDIVIDUALS	NUMBER MALES TO 100 FEMALES		
		Birth in warm mos.	Birth in cold mos.	Birth during entire year
Horses.....	16,091	96.6	97.3	97.0
Cattle.....	4,900	114.1	103.0	107.3
Sheep.....	6,751	102.9	94.0	97.4
Swine.....	2,357*	115.0	109.3	111.8
Greyhounds.....	17,838	116.3	122.1	118.5

Except in the dog, and in the horse where these statistics are at variance with those collected by Schlechter ('84), the sex ratios as given in table 5 are relatively high among the animals born in the warm months and correspondingly low where the births occurred during the cold months. To be available for analysis by any current theory of sex-determination, however, these records would have to be arranged according to the time when conception occurred, since it seems most probable that sex is determined at or before the time of the fertilization of the ovum and cannot be altered by the nutritive or other environmental conditions to which the embryo is subjected. The gestation period in the rat is so short, only 21 days, that the time of conception and the time of birth may be said to take place in the same season of the year. Since the gestation periods in the various animals for which sex ratios are given in table 5 vary so greatly, the sex records cannot be arranged on any basis except that of the time of birth, and they are of value, therefore, merely as indicating that there is apparently a seasonal variation in the sex ratio of other animals as well as in that of the albino rat.

If it can be shown by a sufficiently large body of statistics that the sex ratio in various animals changes in a definite direction with the time of year at which conception occurs it will indicate that some metabolic process occurs in one or the other or in both of the parent organisms at stated periods which tends to swing the sex ratio in one direction rather than in the other. Assuming that sex is determined by the chromatin constitution of the spermatozoan that fertilizes the egg, we must add to this theory the probability that some form of chemical attraction or repulsion exists between each ovum and one kind of spermatozoan in order to account for the constantly increasing mass of evidence that under changed environmental conditions sex ratios in various animals can be altered in a definite direction. Chance, therefore, cannot play as important a rôle in the process of sex-determination as some investigators have maintained, and *any* egg is not fertilized by *any* spermatozoan that happens to come in contact with it. The laws of chance, according to our

present conception, are not subject to periodic changes in their action, and while they offer a very attractive explanation for the existence of an equality of the sex in certain species, they utterly fail to explain sex ratios that vary in a definite direction, whether as the result of seasonal changes or as the outcome of experimental attempts to modify the sex ratio.

#### THE EFFECTS OF THE AGE OF THE MOTHER ON THE SEX RATIO OF HER YOUNG

It has been stated by many investigators that the age of the mother has a pronounced influence in determining the sex of her young. According to a considerable body of statistics collected by Punnett ('03) the sex ratio among the first children in a family is 140 boys to 100 girls. This ratio falls to 117 boys to 100 girls for the second births among the children of these same mothers, and it then declines steadily until, at the ninth birth, the chances for the two sexes are about even. In a compilation of birth statistics for the first born of women of various ages, Bidder ('78) found that the sex ratio was 122.2 boys to 100 girls when the mothers were under 19 years of age; this ratio falls to 104.6 boys to 100 girls for the children of women between 20-30 years of age and it then rises to 131 boys to 100 girls when the first conception occurs after the woman has reached 40 years of age. Conditions closely paralleling these for man are found in the horse according to Wilckens, but this investigator states that heifers predominate among the first offspring of cattle.

Data given by Copeman and Parsons ('04) from their in-breeding experiments with mice show the relation between the age of the mother and the sex of the offspring as given in table 6. Normally there is about an equal proportion of the sexes in mice as is shown by the investigations of Schultze ('03) and of Welden ('06).

The sex records for the mouse, as given in table 6, agree with those for man and for the horse in that they show that the sex ratio in the young is at its lowest point when the mother is at

the height of her reproductive powers. Schultze, on the other hand, states that young female mice tend to produce a slight excess of females among their young, and he concludes that the age of the mother has no effect whatever on the sex of her offspring.

TABLE 6

*Showing the effects of the age of the mother on the sex ratio of mice. Data collected by Copeman and Parsons*

AGE OF FEMALE AT CONCEPTION	NUMBER OF LITTERS	NUMBER OF MALES TO 100 FEMALES
2 mos.....	21	103.7
3-5 mos.....	27	96.5
6 mos.....	21	123.3

For comparison with the records given by Copeman and Parsons and by others we have the sex data for 75 litters cast by 21 stock albino rats. These data, arranged according to the location of the litter in the litter series, are given in table 7.

TABLE 7

*Showing the sex ratios and average number of young in 75 litters of stock albino rats. Data arranged according to the position of the litters in the litter series*

LITTER SERIES	NUMBER OF LITTERS	NUMBER OF INDIVIDUALS	MALES	FEMALES	NUMBER MALES TO 100 FEMALES	AVERAGE NO. YOUNG PER LITTER
1.....	21	131	72	59	122.0	6.2
2.....	21	162	85	77	110.4	7.7
3.....	18	127	64	63	101.6	7.0
4.....	15	96	41	55	74.5	6.4
	75	516	262	254	103.1	6.8

At the time that the first litter was cast each of the 21 females was about three months old. As shown in table 7, the sex ratio in the young rats belonging to the first litters is 122.0 males to 100 females. For the individuals in the second litters the sex ratio drops to 110.4 males to 100 females, and it goes down to 101.6 males to 100 females for the rats belonging to the third litters. At the time that the females cast their fourth litters the majority of them were seven to nine months old.

The female albino rat, if she is in good physical condition, will continue to bear young until she is about fifteen months old. The third and the fourth litters of an albino female, therefore, are usually cast during the period when the female is at the height of her reproductive power. In the above table the sex ratio for the fourth litters is much lower than that for the first three litters, being only 74.5 males to 100 females.

The records given in table 7 are, of course, too few to furnish evidence from which very definite conclusions can be drawn. As far as they go, however, these records indicate that the sex ratio among the first offspring of very young females is higher than that found among the offspring of the same females at a period of life when they are at the height of their reproductive power. The results, therefore, are in agreement with those obtained by Punnett, by Bidder and by Copeman and Parsons. In what way the age of the mother can affect the sex of her offspring is not known as yet. The fact that female rats at the height of their sexual activity in the spring and fall and also at the zenith of their reproductive power tend to produce relatively more female than male young would seem to indicate that the physical condition of the female, either as the result of age or of environment, produces changes of metabolism that tend to affect the sex of the young. It is possible that anabolic processes predominating in the female at certain periods might affect the ova in such a way as to cause them to be more easily fertilized by a female-producing than by a male-producing spermatozoan. In very young females, on the other hand, and in females not in good physical condition, katabolic processes that would give the male-producing spermatozoa an advantage over the female-producing spermatozoa in the fertilization of the ova, might be assumed to occur. Until, however, our knowledge of the mechanism of sex determination rests on a more secure foundation than it does at the present time, it seems useless to offer even tentative suggestions as to the manner in which this mechanism can be influenced.

## THE RELATION BETWEEN THE SIZE OF A LITTER AND THE SEX OF ITS MEMBERS

Evidence for man as to whether one sex or the other tends to predominate in large families is conflicting. According to Nichols ('07), it has been shown by several investigators, particularly by Geissler ('89), that in large families there is a greater proportion of sons than in small families. Geissler's statistics show that in 159,042 families containing more than seven children the sex ratio was 106.8 boys to 100 girls, while in 839,719 families having from two to seven children each there were only 105.8 boys to 100 girls. From the statistics of a very much smaller number of families, Punnett ('03) comes to the opposite conclusion that girls tend to predominate more in large families than in small ones.

Copeman and Parsons's breeding experiments with mice show that the percentage of males is slightly less in large litters (containing more than 6 young) than it is in small litters. Welden, on the contrary, states that in a given generation of mice there seems to be a positive tendency for large litters to contain more males than females.

The sex data for 1089 litters of albino rats have been arranged on the basis of litter size in order to ascertain if, in this animal, there is any relation between the sex of the individuals and the size of the litters to which they belong. For the purpose of this analysis the litters have been arbitrarily divided into three groups: large litters containing nine or more young; medium litters with six to eight young; small litters having five or less members. The records collected during the year 1914 are sufficiently numerous to warrant their separation into groups according to the months when the litters were cast: the data obtained during 1911-1913, being too few to be divided in a similar way, have been grouped together. The results of this arrangement of data are given in table 8.

As shown in table 8, the results obtained by this analysis are so conflicting that no definite conclusions can be drawn from them. The data for the year 1914, arranged according to the months when the litters were cast, show that the highest sex

TABLE 8

*Showing the sex ratio in different sized litters of albino rats. Data collected during 1914 arranged according to the months when the litters were cast*

MONTHS	9 OR MORE YOUNG		6 TO 8 YOUNG		5 OR LESS YOUNG	
	Number litters	Number males to 100 females	Number litters	Number males to 100 females	Number litters	Number males to 100 females
January.....	14	108.7	27	93.1	16	91.7
February.....	19	98.9	25	87.6	12	87.5
March.....	24	87.1	22	98.7	12	125.0
April.....	14	112.3	24	144.3	13	75.8
May.....	23	104.6	19	85.9	18	126.5
June.....	34	100.6	42	109.9	25	131.3
July.....	36	97.7	56	120.6	24	116.7
August.....	29	129.9	57	134.1	23	111.4
September.....	28	100.0	46	98.1	37	107.8
October.....	1	125.0	18	119.3	12	140.9
November.....	3	93.8	15	123.4	15	100.0
December.....	3	130.8	15	106.3	13	133.3
	228	103.7	366	110.7	220	111.7
Data for 1911-1913.....	65	109.9	142	110.5	68	90.2
Total.....	293	106.8	508	110.6	288	100.7

ratio occurs in the members of the largest litters in only two cases, while in six cases it is found in the individuals comprising the smallest litters. In the records for the entire year the highest sex ratio, 111.7 males to 100 females, occurs in the individuals composing the smallest litters; the lowest sex ratio, 103.7 males to 100 females, being found in the rats belonging to the largest litters. The records for 1911-1913, on the other hand, give the highest sex ratio, 110.5 males to 100 females, in the individuals belonging to litters of medium size; the records for the small litters show a sex ratio of only 90.2 males to 100 females. For the entire series of data, litters of medium size show the highest sex ratio, 110.6 males to 100 females, and the lowest sex ratio occurs in the individuals of the small litters.

The lack of uniformity in the results of this arrangement of data indicate that apparently there is no well defined relation between litter size and sex in the albino rat.

## THE NORMAL SIZE OF THE LITTER IN ALBINO RATS

Available data concerning litter size in the rat indicate that the average number of young in a litter varies considerably in different species. Miller ('11) finds for the common gray rat (*Mus norvegicus*) that there is a range of 7 to 12 young in the litter and that, on the average, a litter contains 10.5 young. Data recorded by Lantz ('10) give 8.1 as the average number of young in a large series of pregnant females of this species killed in India. Litters of the black rat (*Mus rattus*) are apparently much smaller than those of the gray rat. Lantz states that 5.2 young is the average for the litters of this species. This average is practically the same as that given by Lloyd ('09).

But few observations have been recorded regarding litter size in the albino rat. Crampe ('84) states that the average size of a litter of albino rats is 5.6 young, which is exactly the result obtained by one of us (King '11) from an examination of 80 litters of stock albino rats. Cuénot records 8.5 as the average number of young in 30 litters of albino rats, but this is undoubtedly a higher average than would be found in a larger series of litters.

In addition to the sex ratios tables 1-4 give the average number of young in the various litters examined during the years 1911-1914. The records for the period from 1911-1913, as given in table 1, show that there is very little variation in litter size in the various groups of litters cast during the different months of the year; the range being from 6.3 young, the average size of the litters cast during April, to 7.5 young, the average of the litters produced during December. The largest litter examined contained 14 young, the smallest contained only two individuals. For the series of 275 litters the average size of the litter was 7.01 young.

A similar analysis of the data collected during the year 1914, as given in table 2, shows for the entire series of 814 litters an average of 6.99 young per litter, which is remarkably close to the average for the litters examined in 1911-1913. While the records for 1914, as a whole, show a great uniformity in the

average size of the litters cast in the various months, there seems to be a tendency for the litters cast during the first part of the year to be slightly larger than those produced during the latter half of the year. A similar tendency, however, is not noted in the records of table 1, so that it can have little, if any, significance.

Records for the entire series of 1089 litters give 7.0 young as the average number of individuals in a litter. According to these observations, therefore, the size of a litter of albino rats is, on the average, greater than that of the black rat, but it is smaller than that in the gray rat of which it is the domesticated variety.

The data for litter size, arranged according to the season of the year when the litters were cast, are given in table 4. A marked uniformity in the various series of records is again evident. In the final averages the litters cast during the fall of the year show a relatively small size. This result probably has little, if any, meaning, since it is due entirely to the low average size of many of the litters cast during the fall of 1914. Records for the litters cast in corresponding months of the years 1911-1913 give 7.0 young as the average number of individuals per litter. It is evident, from these results, that there is no pronounced seasonal variation in the size of the litters at all comparable to the evident change that occurs in the sex ratio at stated periods in the year. Seasonal changes in the sex ratio are independent of litter size just as the normal sex ratio is independent of litter size.

Crampe ('83) states that the first litter of an albino rat is not as large as the second and that the second litter is an index of the size of subsequent litters. The first part of this statement can be corroborated by our records, but the latter part of it needs to be modified. A large second litter gives no indication whatever as to the size of the following litters, as the records for litters from many hundreds of females collected by one of us shows. In many cases a large second litter is followed by an unusually small litter, and there are marked individual differences in females regarding the size of the litters they pro-

duce. Some females never have over five or six young in a litter: other females invariably cast litters containing eight or more young.

The average size of 75 litters cast by 21 stock albino rats is given, with other data, in table 7. In these records the average size of the first litter is found to be considerably less than that of the second, while the second of the four litters is the largest of the group, containing an average of 7.7 young per litter. In this particular series of records the average size of the third litters is considerably below that for the second litters, but in a larger series of data it would probably be found that the third litter is nearly, if not equal, to the second in size. The fourth litters are, as shown in table 7, only a little larger than the first, as a rule.

For the entire series of 75 litters the sex ratio is below normal, and the average size of the litters is somewhat small, being only 6.8 young per litter. The number of young in a given litter is dependent to a marked extent on the age and physical condition of the female (King '15), and it is not improbable, as previously stated, that these factors also have an effect on metabolic processes that play an important rôle in determining the sex of the embryo.

#### SUMMARY

1. Albino rats breed throughout the entire year, but the periods of greatest sexual activity are in the spring and autumn.
2. The sex ratio in the 1089 litters of albino rats examined was 107.5 males to 100 females.
3. There is, apparently, a seasonal variation in the sex ratio of the albino rat. Litters cast in the spring and early fall show a relatively low sex ratio; those cast in summer have a much higher sex ratio (fig. 1).
4. Data for 75 litters produced by 21 albino females indicate that the sex ratio among the first offspring of young females is higher than that found among the offspring of the same females when they are at the height of their reproductive power.

5. There is apparently no relation between the size of a litter of albino rats and the sex of its members.

6. The 1089 litters examined contained an average of 7.0 young per litter. Litters of albino rats, therefore, are smaller than those of the gray rat and larger than the litters of the black rat.

7. There is no pronounced seasonal variation in the litter size comparable to the seasonal variation noted in the sex ratios.

8. As a rule the first of an albino female's four litters is the smallest; the second and the third litters are the largest; the fourth litter is a little larger than the first.

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## AN INSTANCE OF ACIDOPHILIC CHROMOSOMES AND CHROMATIN PARTICLES

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ONE PLATE (TWELVE FIGURES)

Studying the cytological literature, I have been unable to find a record of acid staining chromosomes in a normally dividing cell. In an investigation of sections of petromyzon larvae numerous mesenchyma and blood cells are seen which contain nuclei staining a uniform and brilliant red. These cells are scattered among other cells having nuclei of exactly the same structure, yet staining the usual deep blue color with the hematoxylin eosin stain. After studying these cells more closely, I found that the cells with the red nuclei were able to undergo mitotic division in the same manner as the cells with blue nuclei, the chromosomes in such cases staining red rather than the characteristic deep blue or black seen in the neighboring cells.

Such cells have been found as free blood cells in the blood vessels and also as mesenchyma cells in the pharyngeal and head regions of the larvae. Wherever found, aside from the peculiar property the nuclei show in the absorption of the acid dye, these cells are exactly similar to others in the region in which the nuclei stain characteristically with the basic dye (hematoxylin). The accompanying plate shows the two types of cells in the resting condition and in different stages of mitosis.

It is of interest that these cells have been found only in the 5 mm. larvae of my collection. These particular 5 mm. larvae were procured at Naples by Professor Stockard in the spring of 1910. They were fixed at the time of collection in picroacetic and preserved in 80 per cent alcohol. I received them

in the fall of 1914, sectioned and stained them in hematoxylin and eosin. The remaining specimens of my collection, which comprise developmental stages ranging from the segmentation sphere up to an including a transforming larva and the adult, have been kindly supplied to me by Professor Gage. They were collected from the waters in the neighborhood of Ithaca, N. Y. Unfortunately, I do not have any 5 mm. larvae of the American species so am unable to say whether these cells are peculiar to the European species and to this age of larvae. Several specimens of these 5 mm. larvae show cells with nuclei taking the acid stain. They are not equally numerous nor do they take the stain equally well in all cases. In one specimen, for instance, such cells are difficult to find while in others they are distinct and plentiful. Embryos in which these cells are prominent may show more than half of the blood cells containing nuclei in which the chromatin has absorbed the acid dye.

Cells have been found in which both kinds of chromatin are present. Figure 7, represents such a cell in the resting state. Two lumps of chromatin in the central part of the nucleus have taken the basic stain while the chromatin at the periphery is stained with the acid dye. Heidenhain ('07) has shown a chromatolytic nucleus which somewhat resembles this, but in which the acid-staining chromatin was in the central part of the nucleus while the basic-staining chromatin was at the periphery. Figures 5 and 6 represent cells with two kinds of chromatin in the process of mitosis. Figure 6 shows that a small part of the acid-staining chromatin has apparently been taken over with the basic-staining group. Figure 5 represents the separation of the two kinds of chromosomes in the daughter nuclei which appears to have been complete. The great majority of these cells with acid-staining chromatin, however, are pure in regard to their staining reaction. Figures 1, 4, 8, 9 and 10 show nuclei containing no granule or any other part which absorbs the basic dye.

Stockard ('06) confirmed the observations of Schniewind-Theis ('97) in which it was shown that some nuclei in the deeper layers of actively secreting nectar glands of *Vicia faba* take the plasma

stain. In the living gland some rows of cells have a blue while others have a red coloration. By introducing alkaline and acid fluids to sections of the living gland, Stockard found that these cells responded to the fluids in the same way that litmus does to alkalies and acids. This experiment shows quite conclusively that the chemical reaction of the glandular plant cell is not constant during various physiological phases. The staining reaction also indicates that the nuclei apparently respond to the stain according to their physiological state. The stain used was Auerbach's (methyl green and acid fuchsin) which gives a delicate differentiation of the acid and basic qualities. It was determined in these investigations that materials were formed by the nucleus and passed out into the cytoplasm, the cytoplasm in such cases finally accumulating enough of the nuclear products to stain with the nuclear dye. Further, when the secreting activities of the nucleus had apparently been spent the nucleus stained with the plasma stain. These reactions occurred only in vegetative cells, the dividing cell always contained chromatin which stained in the normal way with the basic dye. In these studies the tissues had been carefully fixed with neutral fluids so as to preserve the chemical reaction of the living cell. The fixation used in my specimens, as was pointed out above, was an acid fluid, yet the differences in the reactions of the cells and portions of some nuclei were sufficient to maintain their character and to respond to the ordinary hematoxylin-eosin stain in the peculiar ways shown in plate 1.

The presence in the same section of the lamprey larva of cells with acidophilic nuclei together with cells which stain in the normal way, and the fact that both kinds of chromatin are present in a single cell, make it difficult to give any other interpretation of these reactions than that they represent the result of physiological changes which occurred during life.

As far as I can ascertain this is the first account of a case where the chromosomes in a dividing cell have definitely taken the acid stain.

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## PLATE 1

## EXPLANATION OF FIGURES

All figures were drawn with the aid of the camera lucida to the same scale of magnification (1/12 oil immersion objective, compensating ocular No.12). Higgs' carmine and true blue inks were used in reproducing the colors of the stained specimens.

Figures 1, 2 and 5 are mesenchyma cells; all other figures represent blood cells.





# THE CONNECTING SYSTEMS OF THE REPTILE HEART

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EIGHT FIGURES (TWO PLATES)

## THE SINO-VENTRICULAR CONNECTION

A sino-ventricular bundle, or dorsal ligament, has already been described by me in the hearts of *Lacerta agilis*, and *L. viridis*, of *Clemmys lutaria* and *Chelopus insculptus*. By observing this ligament in living hearts under the binocular microscope, and from the study of transverse, frontal and sagittal sections, it was seen to be a band of connective tissue, containing nerves and blood vessels, running between the sinus and the ventricle well over to the right side of the heart. (Laurens '13 a and '13 b). In my first paper it was further shown that this ligament had no significance for the coordination of the heart beat. This band of tissue had previously been described and experimented with by several investigators, and a discussion of their various views as to its structure and physiological importance will be found in my papers and in a recent publication by Mangold ('14).

Mackenzie ('13) has recently described in the heart of the salempenter, a South American lizard, a "sinu-auricular bundle" of specialised muscle, connecting the sinus with the specialised tissue lying in the floor of the auricle. Although from my earlier studies of the lizard and tortoise hearts it was certain that such a bundle did not exist in the hearts of the reptiles examined by me, this publication of Mackenzie's induced me again to go over my preparations, to which had been added in the meantime sections of the heart of the fence lizard (*Sceloporus undulatus*) and of the spotted tortoise (*Chelopus guttatus*). A part of this later material was fixed and stained by the same methods as were earlier used ('13 b)—fixation in strong Flem-

ming or in concentrated corrosive sublimate, and staining with iron hematoxylin and picric acid fuchsin. The remainder consisted of sections of hearts treated with methylen blue according to various methods, by Cajal's double impregnation method, as given by Hofmann ('02), and by the silver reduction method, as given by Meikeljohn ('13).

From this further study of these lizard and tortoise hearts no doubt has been thrown on the truth of the statement that the dorsal ligament is here a sino-ventricular bundle. But from Mackenzie's descriptions and from his figures it can also not be doubted that in the heart of the salempenter conditions are different. As he has himself pointed out, the conditions in this respect shown by the hearts of different reptiles are not the same and various stages can be recognized. We shall see that the conditions found in the hearts of the reptiles listed above represent still another stage in addition to those which he has described.

According to Mackenzie (p. 129) the "sinu-auricular-ring" of the fish heart is represented in the heart of the salempenter "by a bundle or leash of fibres which lies in the groove between the left venous valve and the spatium intersepto-valvulare." The sinu-auricular bundle "courses round the posterior and under aspect of the sinus venosus just where the left duct of Cuvier enters the sinus and runs . . . a short distance as a free bundle to become continuous with the specialised tissue lying in the floor of the auricle, this tissue becoming in turn continuous with the auricular canal." In the heart of the crocodile (p. 130) the "sinu-auricular muscle" is "present at the base of the left venous valve at the junction of the sinus with the spatium. There is no direct continuity between the sinu-auricular bundle and the auriculo-ventricular bundle in the crocodile. The interruption takes place in the region of the sinus septum where the left duct of Cuvier enters the sinus." Later (p. 135) he goes on to say:

The sinu-auricular nodal tissue appears to become lost in this septum. It would appear that there are reptiles which in respect of this point exhibit an intermediate stage between the lizard (salempenter) and the crocodile. An example of this is the iguana, in which the sinu-auricular bundle is interrupted by a cord of fibrous tissue with iso-

lated muscle fibres and large nerve trunks. This cord occupies a corresponding position to the continuous muscle structure in salempenter and in front appears again as a short isolated muscle bundle which in turn becomes continuous with the muscle of the auricular canal.

The conditions found in the lizard and tortoise hearts listed above represent another intermediate stage between that found in the iguana and that found in the crocodile, as described by Mackenzie. In these hearts there is no "isolated muscle bundle" which becomes continuous with the auricular canal. At the left venous valve, near its upper portion, there is, in the fibrous tissue a large group of nerve cells, which represents the endings of a branch of the right vagus nerve. The nerve fibers connected with these nerve cells can be followed for quite a distance along the right vein, as far as it is present in the sections, being connected with other large ganglia here and there along the vein. From this portion of the left valve there runs a band of connective tissue, a fold of the pericardium, which bending under the left vein becomes free from the dorsal surface. From this point it is continued downward as a free band, superficially over the dorsal surface of the right auricle to the ventricle, over the anterior dorsal surface of which it spreads, being wider at its point of attachment than elsewhere. Sometimes the bundle, before it reaches the ventricle, divides into two, or even three, parts, and often under these circumstances a fine branch can be seen bending still further to the right and running in the auricular-ventricular groove to the ventral side.

In the bundle there are numerous blood vessels and large nerve trunks with several groups of nerve cells. Sometimes these nerve cells are single and scattered, but there are also many large ganglia. By studying Mackenzie's figures of sagittal sections (plate 2, figs. 1-3) one sees on the dorsal surface of the hearts a mass of tissue which extends from the sinus region to the anterior dorsal portion of the ventricle. This tissue Mackenzie has not labelled, but it has a position very similar to the continuous sino-ventricular bundle in the hearts of the lizards and tortoises which I have studied. From figure 3 of this same plate of Mackenzie's, however, it is seen that the "sinu-auricu-

lar bundle" does go over directly into the auricular funnel musculature, the latter being, according to his representation, on the dorsal side a continuation of the "sinu-auricular bundle." A glance at the figures which are presented with this article will show that this is not the case in the animals with which we are dealing. The figures are untouched photographs of sagittal sections of the heart of the tortoise *Chelopus guttatus*. The hearts of the other tortoises and of the lizards show the same conditions. Drawings of the lizard heart have already been given (Laurens '13 b) and for that reason the tortoise is selected for the illustrations here.

After reaching the ventricle, the sino-ventricular bundle spreads out over its anterior dorsal surface. A portion of it runs to the back of the ventricle, while another portion goes down into the space between the funnel musculature and the inner wall of the ventricle. This space is filled with connective tissue containing blood vessels, nerves and ganglia (Laurens '13 b, fig. 4), and the portion of the sino-ventricular bundle which goes down into this space becomes continuous with this connective tissue, which is also, of course, a portion of the pericardium. The nerves in the sino-ventricular bundle, two of which are shown in figure 6, are also distributed, some of them to the anterior dorsal surface of the ventricle, and some to the connective tissue filled space between the funnel musculature and the inner wall of the ventricle. The latter innervate the auriculo-ventricular funnel and also supply the inner wall of the ventricle. Quite often the nerves in the sino-ventricular bundle are insignificant, and even entirely lacking, a fact which was also noted by Gaskell.

There is no muscle tissue, either continuous or isolated, in the sino-ventricular bundle. At its beginning (sinus end) and ending (ventricular end) a few isolated striated muscle fibers can sometimes be seen in the connective tissue (as in the sections from which figures 3 and 4 are taken). But it is clear that these muscle fibers have been pulled into this position by the knife tearing them away from the walls of the sinus and of the ventricle. In its free course there is only fibrous tissue in the bundle, in which nerve fibers, ganglion cells and blood vessels are found.

As the sino-ventricular bundle is followed in transverse and sagittal sections it is seen to be nothing more than a portion of the pericardium which is folded off as a free band to run between the sinus and the ventricle. In some places it is even connected with the pericardium proper over the right auricle by fine strands (fig. 5). By studying the figures, which represent sections in order from left to right, the manner in which the sino-ventricular bundle is folded off from the continuous pericardium can be made out. Figures 1 and 2 are sections to the left of the median line, and the dorsal ligament does not show at all. In figure 2, however, one of the large nerve trunks is seen running from the sinus, under the pericardium on the dorsal side of the auricle, to the ventricle across the auriculo-ventricular groove (Laurens '13 b and Dogiel and Archangelsky '06) to end in the auriculo-ventricular funnel musculature, after it has gone through the connective tissue in the space between the funnel and the ventricle. In figure 3 we see the beginning of the free portion of the sino-ventricular bundle in an out-folding of the pericardium on the anterior dorsal surface of the ventricle, and opposite to it a corresponding outfolding on the sinus wall. In figure 4 these folds have advanced further and in figure 5 they have met to form the continuous band of connective tissue, which can here be followed up along the sinus until bending over to the right it disappears from the section. Figure 6 gives another view of the sino-ventricular bundle further to the right. In this section a large nerve trunk is seen in the ligament coming from the sinus and going down into the connective tissue filling the space between the funnel and the ventricle. It also shows to the extreme right a portion of a smaller nerve going over to the outer wall of the ventricle. Figures 7 and 8 serve to illustrate the appearance of the ligament further over to the right hand side of the heart. From a study of these figures it will be clear, I believe, that the dorsal ligament is simply a fold of the pericardium, which, retaining its connection with the sinus and with the ventricle, runs free over the dorsal surface of the right auricle from the sinus to the ventricle, and is therefore strictly a sino-ventricular bundle.

## THE SINO-AURICULAR CONNECTION

The connection between the sinus and the right auricle is a direct muscular one in the reptile hearts described in this paper. Gaskell pointed out the fact that this was the case in the tortoise with which he was working, though the details concerning the manner in which the connection was actually brought about do not hold here. Külbs and Lange ('10) also describe a direct muscular connection between the sinus and the right auricle in the lizard. But in the heart of the salempenter (Mackenzie) the ring of specialized muscle with numerous nerve cells and fibers at the 'sinu-auricular junction' in the fish is represented by a "bundle or leash of fibers which lies in the groove between the left venous valve and the spatium intersepto-valvulare." In the reptile hearts that I have examined there is a complete muscular ring. Nerve cells, in larger and smaller ganglia, and nerve fibers are all around this ring in the connective tissue. The musculature of the sinus goes over into that of the right auricle in much the same way that the musculature of the auriculo-ventricular funnel goes over into that of the ventricle. At the junction of the sinus with the auricle there are the two valves which completely close the oval shaped opening which runs obliquely from the upper right hand side to the lower left, as Mackenzie shows in his figure on plate 3. At the right, or lower, valve, the musculature of the sinus goes over into that of the auricle at the free edge, the two kinds of musculature being here continuous. At the left, or upper, valve the conditions are somewhat different. In its upper portion the valve is a continuation of the wall of that portion of the sinus, and the musculature of the sinus joins directly with that of the auricle along the valve (fig. 7). But the extreme lower portion of the left valve, which, in sagittal sections taken from the left to the right comes first into view, is formed from a portion of the auricular septum, being really a continuation of it (fig. 3), and the wall of the sinus here goes directly over into this portion of the septum, the left valve being here separated from the wall of the auricle by a layer of fibrous tissue, a condition particularly clearly shown in transverse sections.

## THE AURICULO-VENTRICULAR CONNECTION

Attention may be here again called to this connection in the hearts of lizards and tortoises, since, from Mackenzie's description, there appear to be slight differences between the conditions found in the salempenter and those found in other lizards and in tortoises. In the salempenter, the auricular canal, according to Mackenzie, is specialized, an assumption also made by Külbs and Lange ('10) for the lizard (*L. viridis* and *L. muralis*) and by Külbs ('12 and '13) for the lizard and tortoise. It has already been pointed out (Laurens '13 b) that the musculature of the auriculo-ventricular funnel is not very different from the musculature of other portions of the auricles. The fibers and nuclei are similar to those of the auricles, though there is more sarcoplasm and fewer fibrillae. However, the funnel musculature is richly supplied with nerves and contains numerous capillaries, and in between its fibers, which are arranged circularly, there is a considerable amount of connective tissue. The striation of the fibers is distinct but fine, and is quite similar to that of the auricles. The striation of the ventricular fibers is coarser and the nuclei are much elongated and narrower than are those of the auricles and of the funnel.

The function of the auriculo-ventricular funnel in co-ordinating the contractions of the auricles and of the ventricle was very carefully worked out in the lizards, *L. viridis* and *L. agilis*, and in the tortoise, *Clemmys lutaria*. From this work (Laurens '13 a) it is evident that there is here a physiological differentiation in that certain portions of the funnel are more efficient than others in allowing the passage of the contraction wave from the auricles to the ventricle, and furthermore, in preserving the co-ordination of ventricular with auricular beat, when other portions of the connection between these parts of the heart are cut away. The portions showing this greater efficiency are the right and left sides of the funnel. Later (Laurens '13 b) it was shown that this physiological specialization had an anatomical basis in that, at these two portions, there was a more intimate connection between the funnel musculature and the musculature of the ventricle.

In the salemcenter (Mackenzie, p. 129) the auricular canal is described as "a tube invaginated into the ventricle, becoming at its lower end continuous with the ventricular musculature in the region of the papillary muscles to which the auriculo-ventricular valves are attached. This invagination does not of course occur at that part of the orifice where the auricular canal is continued on to the bulbus musculature." In all the lizard and tortoise hearts studied by me the invagination does take place around the whole circumference of the orifice, the funnel being only broken through at its entrance into the ventricle, by the bulbus with the musculature of which the funnel musculature becomes continuous. The direct continuity between the musculature of the funnel and that of the ventricle does not, of course, occur at this place.

Mangold ('14) points out that on the dorsal side the funnel musculature in the salemcenter, as described by Mackenzie, extends further into the cavity of the ventricle, before the fusion of the two kinds of musculature takes place, than it does in the hearts of the reptiles which were described by me, where it very soon becomes broken through by its fusion with the ventricle. This difference, however, is I think, very slight. By comparing Mackenzie's figures with mine it will readily be seen that the length of the ventricle of the salemcenter is relatively, when compared with its dorso-ventral thickness, less than that of the lizards and of the tortoises here described. Moreover, that the attachment of the auriculo-ventricular valves as represented in the salemcenter is much nearer the apex, and that the auriculo-ventricular funnel extends further into the cavity of the ventricle, than in the other lizards and tortoises. From a glance at figure 4 (Laurens '13 b) it will be apparent that on the dorsal side the funnel musculature is continued, although quite thin, almost to the attachment of the auriculo-ventricular valves to the papillary muscles, and the figures presented with the present article show this quite plainly. On the right and left sides, however, the funnel musculature is continued further into the ventricle, before the fusion between the two kinds of musculature finally takes place, the connection at these parts being

therefore more intimate than at other portions. Mackenzie does not mention whether the final continuity between the auriculo-ventricular funnel and the ventricle takes place at all portions at the same level.

There is one other matter concerning the auriculo-ventricular connection, and that is its innervation. Nerve fibers can be seen extending downward from the sinus in the pericardium and can be followed across the auriculo-ventricular groove. For the past two years I have been studying the innervation of the reptile heart, and particularly of the auriculo-ventricular funnel muscle, by means of the special methods mentioned earlier. Although perfect results have not yet been obtained, it has been seen that the auriculo-ventricular funnel is richly supplied with nerves, in the form of a net-work of fine fibers, which come to it from branches of nerves descending along the back of the auricles in the way described earlier by me ('13 b), and by Dogiel and Archangelsky ('06). Nerve fibers and cells are also found on the inside of the auricles, running along the inner edge of the walls and along the septum, and which come into the heart along or near the entrance of the pulmonary vein and of the left duct of Cuvier. These nerves also give off branches which run down between the auriculo-ventricular valves and the inner side of the funnel to finally become distributed to the latter. In the funnel musculature itself, nerve cells are scarce, only a few scattered ones being found here and there. But in the connective tissue of the groove, and of the space between the funnel and the inner wall of the ventricle, ganglia are numerous, particularly on the dorsal side, though in sections of some hearts the number of ganglia found on the ventral side, especially near the bulbus, and on the right and left sides of the funnel is also quite large.

The nerves which run over the dorsal surface of the auricles and of the auriculo-ventricular groove to be continued into the connective tissue between the funnel and the ventricle can also be easily seen in sagittal sections of material fixed in Flemming and in corrosive sublimate. In figure 2 one of these nerves is shown running down to finally become distributed to the auriculo-ventricular funnel musculature (see also fig. 6).

## THE PROBABLE FUNCTION AND FATE OF THE SINO-VENTRICULAR BUNDLE

The sino-ventricular bundle, contrary to the view of Imchanitzky ('09), has nothing to do with the coördination of ventricular and auricular contraction (Gaskell '84 and Laurens '13 a). Furthermore it has no function in bringing about a possible sino-ventricular rhythm. In the salempenter, however, according to Mackenzie, 'the sinu-auricular bundle' is made up of specialized muscle. In support of which statement, we have no physiological evidence.

The contraction wave begins in the walls of the sinus and spreads to the auricles along the sino-auricular junction, and from there along the auriculo-ventricular funnel to the ventricle. Can the 'sinu-auricular bundle' in the salempenter be a pathway for impulses passing direct from the sinus to the auriculo-ventricular connection and to the ventricle, and if so, what is the nature of these impulses? That they have anything to do with coördination can hardly be claimed owing to the physiological evidence against such an assumption. Gaskell (p. 83) showed that when the peripheral end of the 'coronary nerve' was stimulated that the rate of beat of the heart was not changed, although when the central end was stimulated a decided slowing of auricular rate took place. By the stimulation of either end of the nerve the force of the auricular contractions was diminished (p. 92). Further, Gaskell showed (p. 85) that, when the connection between the sinus and the auricles is severed so that the 'coronary nerve' remains intact and as the only connection between the auricle and ventricle and the body of the animal and an independent auriculo-ventricular rhythm is set up, stimulation of the right vagus brings about a decrease in the force of the auricular contractions alone. In one experiment, out of several, Gaskell obtained an inhibition of this independent auriculo-ventricular rhythm, when the right vagus nerve was stimulated. His explanation of this was that "when the coronary nerve happens to contain fibers which supply the particular muscles which originate the independent rhythm,

then stimulation of the vagus can inhibit that rhythm." Moreover, as Gaskell also pointed out, the 'coronary nerve' is only one of several nerve trunks, branches of the vagus nerves, which pass from the sinus to the ventricle and to the auriculo-ventricular funnel musculature, and it is therefore but natural that the auriculo-ventricular rhythm should be controlled by it when it happens to innervate the muscles which originate this rhythm, just as the other branches of the vagus nerves do.

The 'coronary nerve,' when the vagus is stimulated, is also no more able to conduct an impulse to the ventricle by which its force of contraction can be diminished, than are any of the other nerve trunks passing from the sinus to the ventricle (Gaskell, p. 91). It has also another function in common with the other branches of the vagus nerves in that it carries impulses, upon stimulation of the vagus which improve the conduction power of a strip between the auricle and the ventricle during a condition of partial block, though it may also have a function occasionally in increasing such a block by diminishing the conducting power of the connecting strip (Gaskell, p. 96).

The 'coronary nerve' is not always present, and is often very insignificant. From what has just been said it apparently has no particular function that the other nerve trunks do not have, and we are forced to the conclusion that its function is relatively unimportant, or perhaps better, no more important than that of the other branches of the vagus nerves.

From physiological evidence then, the effect of the 'coronary nerve,' like the other branches of the vagus nerves, are on the auricles, and it may be assumed to have an additional regulatory effect when, for any reason, the rhythm producing power of the auriculo-ventricular funnel is brought into prominence above that of the sinus. As we have seen, some of the nerve trunks which run in the sino-ventricular bundle go into the connective tissue between the funnel and the ventricle, which is a condition that was not realised at the time of my earlier publication. It is highly probable that the nerves of the 'sino-auricular bundle' of the salemphenter have the same function as the 'coro-

nary nerve' in the heart of the tortoise described by Gaskell. But what the function of the 'specialized muscle' of this bundle is, must remain for the present an unanswered question.

As to the probable fate of the sino-ventricular bundle, Mackenzie has given the steps leading up to the condition found in the reptile hearts described in the present article. In the salamenter there is a "sinu-auricular bundle of specialised muscle;" in the iguana the bundle is interrupted by a cord of fibrous tissue, a short isolated muscle bundle, which is continuous with the muscle of the auricular canal, being all that is left. In the hearts of the lizards and tortoises here described, the sino-auricular junction is a ring of muscle completely surrounding the opening of the sinus into the right auricle, and running from this region there is a band of connective tissue which goes to the anterior dorsal surface of the ventricle. In this band of connective tissue there are large nerve trunks and blood vessels some of which go to the ventricle, others into the connective tissue between the funnel and inner wall of the ventricle to become distributed to the musculature of both. In the crocodile, all that there is of the 'sinu-auricular muscle' according to Mackenzie, is at the base of the left venous valve at the junction of the sinus with the spatium.

Mackenzie speculates on the probable fate of the fibrous cord and of the distal isolated muscle bundle found in the iguana. He considers it "not improbable that it becomes incorporated in the auricular floor, and that it is represented in the higher reptilian and mammalian hearts by a bundle of fibrous tissue which runs in the basal wall of the auricle at the line of attachment of the septum between the region of the coronary sinus and the septum fibrosum." As we have seen, however, in the hearts of other lizards and tortoises; the 'distal muscular bundle' has disappeared, while the 'fibrous cord' is still present as a sino-ventricular bundle.

In this connection attention may be called to the recent publication of Stanley Kent in which it has been shown that in man "the auriculo-ventricular bundle is not the only path by which the functional connection between the auricle and ven-

tricle may be established," (Kent '14 a). In a series of papers Kent describes and figures a specialized tissue on the right lateral aspect of the heart, as a neuro-muscular connection between the auricle and the ventricle, with large nerve trunks in the fat and connective tissue of the groove, and also in the muscular tissue. He also shows ('14 c) that, when all other connections between the auricles and ventricles are severed, contractions of the auricles are still carried over to the ventricles. This connection, according to Kent ('14 a), may constitute a "local reflex arc which may perhaps exhibit only an occasional activity," and "which may be capable of controlling . . . coördination when the bundle is no longer perfect."

Whether the 'sinu-auricular bundle' of the salemperter, and the sino-ventricular bundle of *Lacerta agilis*, *L. viridis*, *Sceloporus undulatus*, and of *Clemmys lutaria*, *Chelopus inculptus* and *C. guttatus* have anything in common with this bundle described by Kent is of course a question for the future.

#### SUMMARY

1. The dorsal ligament in the hearts of *Lacerta viridis*, *L. agilis*, *Sceloporus undulatus*, and of *Clemmys lutaria*, *Chelopus inculptus* and *C. guttatus* is a sino-ventricular bundle of connective tissue, a fold of the pericardium, which, extending from the sino-auricular junction near the upper portion of the left venous valve, runs under the left vein and is continued as a free bundle to the ventricle on the anterior dorsal surface of which it ends; there becoming continuous again with the pericardium. In this bundle there are large nerve trunks and blood vessels. Some of the nerves go to the dorsal surface of the ventricle, others to the auriculo-ventricular funnel, after they have gone down into the connective tissue filling the space between the musculature of the auriculo-ventricular funnel and the inner wall of the ventricle, and still others to the inner wall of the ventricle.

2. The sino-auricular junction is in the form of an oval-shaped muscular ring. At the right valve the musculature of

the sinus and that of the auricle is continuous. At the left valve in its upper portion the musculature of the two chambers is also continuous, but in its lower portion the musculature of the sinus goes over into that of the auricular septum which forms this portion of the valve, there being here a layer of connective tissue between the left valve and the wall of the auricle.

3. The auriculo-ventricular junction has in cross section the form of a ring; in sagittal section it is in the form of a funnel which extends down into the orifice of the ventricle, with the musculature of which it gradually becomes continuous. On the right ventral side the ring is interrupted by the bulbus with the walls of which it also becomes continuous. The fusion between the funnel and ventricle musculature takes place at the lower level of the auriculo-ventricular valves near their attachment to the papillary muscles. The dorsal portion of the funnel is the first to become continuous with the ventricle, while the right and left sides are the last.

4. The auriculo-ventricular funnel is richly innervated by branches of the right and left vagus nerves which, coursing along the dorsal surface of the auricles, under the pericardium, cross the auriculo-ventricular groove, in the connective tissue of which there are many ganglion cells, go down into the connective tissue filled space between the funnel and the inner wall of the ventricle, and finally end in the funnel musculature and in the musculature of the ventricle. There are also a few nerves on the ventral side, and a few which course along the inner walls of the auricles and the auricular septum to end in the musculature of the funnel.

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## EXPLANATION OF PLATES

All the figures are untouched photographs of sagittal sections of the heart of the tortoise *Chelopus guttatus*. They are arranged in order from the left to the right hand side of the heart. In all of them the auriculo-ventricular funnel is shown invaginated into the cavity of the ventricle to become continuous with its musculature near the attachment of the auriculo-ventricular valves.

### PLATE 1

#### EXPLANATION OF FIGURES

1 A section through the left auricle passing through the right side of the opening of the pulmonary vein into the left auricle; a portion of the auricular septum is seen.

2 A section to the right of the opening of the pulmonary vein. On the dorsal side a nerve trunk can be seen running in the pericardium to end in the musculature of the auricular-ventricular funnel.

3 A section to the left of the opening of the sinus into the right auricle. On the dorsal side the beginnings of the folding of the pericardium, which from the sino-ventricular bundle, are shown.

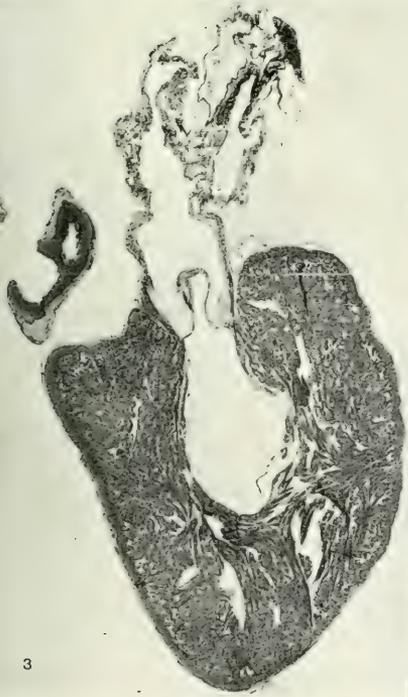
4 A section just to the left of the opening of the sinus; the folds of the pericardium have advanced further; the two venous valves are seen.



1



2



3



4

## PLATE 2

### EXPLANATION OF FIGURES

5 A section through the opening of the sinus into the right auricle. The sino-ventricular bundle is here seen extending as a free band over the auricle from the sinus to the anterior dorsal surface of the ventricle. Just above and to the right of where it joins the sinus there is a group of nerve cells and fibers, and further anterior and to the right, another group.

6 A section further to the right than figure 5. In the sino-ventricular bundle two nerve trunks can be seen; a stronger one running down into the connective tissue of the space between the funnel and the inner wall of the ventricle, and a smaller one, to the right, going to the outer surface of the ventricle.

7 and 8 Sections far over to the right of the auriculo-ventricular connection to show the fold of the pericardium which forms the sino-ventricular bundle becoming continuous with the pericardium over the sinus, the auricle, and the ventricle.





# THE ADULT ANATOMY OF THE LYMPHATIC SYSTEM IN THE COMMON RAT (*EPIMYS NORVEGICUS*)<sup>1</sup>

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## FOUR FIGURES

In the fall of 1914 the writer undertook the study of the origin and development of the lymphatic system in the common rat. Before the work had proceeded very far it was evident that a knowledge of the adult anatomy would be of no small amount of help in guiding and interpreting the work on the origin and development of this system. So the plan was changed to a study of the adult anatomy of the lymphatic system. This paper gives the chief results of the work.

Fifty rats have been examined in the course of the study. The lymphatic system was injected from the soles of the feet, the tip of the tongue, the lips, the walls of the intestines, the spleen, the lumbar, thoracic, inguinal, axial, intestinal and cervical lymph nodes. The first nineteen specimens were injected with India ink, using the hypodermic syringe for pressure. In the next six specimens, a solution of soluble blue was used, with a glass cannula and pressure bulb apparatus. The remainder of the work was done with Berlin blue gelatin mass, a small crystal each of thymol and potassium iodide being added to preserve and lower the melting point of the mass. The supply of gelatin mass was kept in a warm water bath, and the cannula occasionally warmed. Injections could be made through a much smaller aperture and in a much more satisfactory way by this method.

<sup>1</sup> An abstract of a thesis presented to the Graduate Faculty of the State University of Iowa for the degree of Master of Science.

## RESULTS

Injections made through the soles of the feet, showed a variable number of superficial lymph vessels joining to form larger lymph vessels which followed the main course of the radial and ulnar veins, or dorsal and plantar branches of the saphenous vein, to the elbow or knee lymph node (1, 2, 3, 4, fig. 1). From the single node, 3 and 4, the femoral lymph vessel continued to the lumbar node (5-6). A branch is given off from this vessel at the juncture of the superficial epigastric vein and the femoral vein, which leads to the inguinal nodes (7-8).

Just below the posterior end of the rectum is a small single node (30) which receives the lymph from the caudal region and appendages. From this node a vessel leads to the left lumbar node (6), or to the left femoral lymph vessel, joining it at about the juncture of the iliac and inferior vena cava.

The lumbar nodes (5-6), which are normally double, lie just caudad of the ilio-lumbar veins, on either side of the vena cava. There is a great range of variation, however, in the position of these nodes, due, in the main, to the variability of the ilio-lumbar veins. If the veins are well caudad, the lymph nodes may be crowded back opposite to each other; or, if the veins are well

## FIGURE 1

- 1-2, right and left elbow nodes
- 3-4, right and left knee nodes
- 5-6, lumbar nodes
- 7-8, inguinal nodes
- 9-10, renal nodes
- 11, cisterna chyli
- 12, cisterna group of lymph nodes
- 13, intestinal node
- 14, thoracic duct
- 15 to 20, axial nodes
- 23-24, jugulo-subclavian taps
- 25, thoracic group
- 26, posterior cervical nodes
- 27, anterior cervical nodes
- 28, submaxillary nodes
- 29, tongue and lip plexus
- 30, caudal lymph node

## FIGURES 2, 3

- a*, renal lymph vessel
- ci*, cisterna chyli; *12*, cisterna group
- 13*, intestinal node; *5-6*, lumbar nodes
- 9-10*, renal nodes
- p.c.v.*, posterior vena cava
- i-l.*, ilio-lumbar vein

## FIGURE 4

- 1, spleen; 2, fundus lymph vessel
- 3, pyloric lymph vessel
- 4, intestinal lymph vessel
- 5, connection with portal vein
- 6, intestinal branch to cisterna chyli
- 7, appendix; 8, mesenteric nodes
- 9, intestinal vessel
- 10, mesenteric branches of lymph vessel
- 13, intestinal node

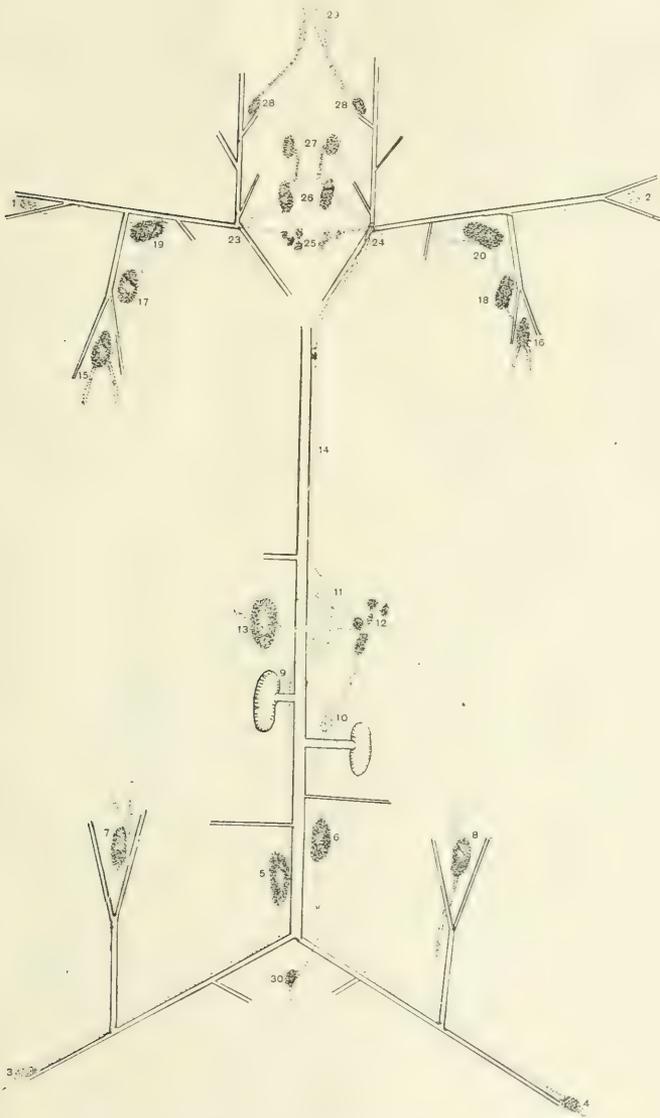


Fig. 1 Venous system shown diagrammatically in solid lines, the lymphatic system stippled. The lateral veins and lymph vessels are continuous, although not shown so in the figure. Lymph node 13, the intestinal node, so marked in all drawings.





Figs. 2, 3 Exceptional and type variations of the lumbar region

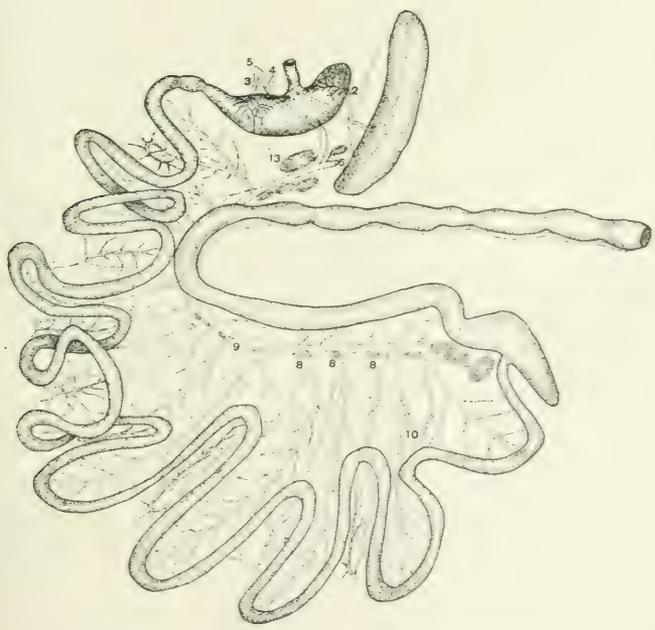


Fig. 4 The intestinal tract and lymphatics

forward, the left lumbar node may be a considerable distance in advance of the right node. In a few instances the double nodes were divided into two single nodes, which lie some distance apart, and in two specimens an additional double node was found at the bifurcation of the ilio-lumbar vein on the right side.

The lymph vessel leading from the right lymph node (5) is so variable that a definite description cannot be given. It usually joins the left lymph vessel in some way between the lumbar node and the cisterna chyli. Figure 2, D, E, F, shows some variations of a general type. Figure 3, A and B, shows two additional variations, which are more common, in the general plan, than any of the others. In only four specimens did the right lumbar lymph vessel lead to the right renal node (9) directly. In these cases a small lymph vessel tapped the renal vein from the renal node, and another vessel connected with the cisterna chyli (11), or the cisterna chyli group (12). In two specimens the right lumbar lymph vessel gave off a branch, which connected with the ilio-lumbar vein (fig. 3, C). This branch did not follow the veins to its tap, but went directly from the node across the ilio-psoas and psoas muscles to its juncture with the ilio-lumbar vein about 1 cm. from the vena cava.

On the left side the number of lumbar lymph vessels leading from the lumbar node (6) may vary from one to four, or form a network, depending somewhat on the mode of attachment with the right lymph vessel. However, all the vessels lead along the left side of the vena cava, in any case. If there is only one vessel, it will open into a single node (10), just anterior to the left renal vein, from which a branch is given to the renal vein and one to the group of single nodes (12) lying to the left of the cisterna chyli. If there be more than one lymph vessel leaving the lumbar node, some one of them will enter the renal node, the rest may join the cisterna group, the cisterna directly, the renal vein directly, or any combination thereof. The latter conditions are fewer than the single vessel method.

The number of nodes in the cisterna group (12) vary from one large one to six small ones. From this group one or more

vessels enter the cisterna chyli. Two special exceptions to this method are shown in A and B of figure 2. Drawing A shows both of the lumbar lymph vessels entering the cisterna chyli directly. The nodes of the cisterna group being closely collected about the periphery of the cisterna chyli, did not fill with the injection mass. The connection with the left renal vein was made from the intestinal lymph vessel (13) through a small branch vessel (*a*). The specimen from which drawing B was made, had no cisterna chyli, only a short vessel shunted off from the main thoracic duct marked its normal position (*ci*). The renal branch (*a*) came from the main lumbar lymph vessel. In both of these specimens the connection between the intestinal lymphatics and the portal vein was very prominent.

In addition to the connections with the cisterna chyli already mentioned, there is another lymph vessel received from the intestinal region past the intestinal node (13). The intestinal lymph does not pass through the intestinal node, but that the node is connected with the lymphatic system is shown by the fact that injections made from it fill the main lymph vessels. Also, the mass from the lumbar injections frequently pass into this node but not beyond it. From the cisterna chyli the thoracic duct (14) leads dorso-laterally along the superior vena cava and left innominate vein to its juncture with the venous system in the jugulo-subclavian district. Not a single specimen in the fifty rats showed the thoracic duct branching or entering the right jugulo-subclavian district, as pointed out by McClure and Silvester,<sup>2</sup> Silvester,<sup>3</sup> and Davis,<sup>4</sup> in other forms.

A double lymph node is found in the groin, the inguinal nodes (7-8), located in the bifurcations of the superficial epigastric vein, and so closely attached to the lower dermis that in removing the skin the node remains imbedded in the subcutaneous

<sup>2</sup> McClure and Silvester. A comparative study of the lymphatico-venous communications in adult mammals. *Anat. Rec.*, vol. 3, 1909.

<sup>3</sup> F. Silvester. On the presence of permanent communications between the lymphatic and venous system at the level of the renal vein in adult South American monkeys. *Am. Jour. Anat.*, vol. 12, 1912.

<sup>4</sup> Henry K. Davis. A statical study of the thoracic duct in man. *Am. Jour. Anat.*, vol. 17, 1915.

tissue. While this node is connected with the femoral lymph vessel, in only two injections did the mass run backward into it. The vessel leaving anteriorly from the inguinal node soon divides into two branches. These branches lead through the lower layers of the dermis to the axial region where they again join to enter a double node, which is usually found in the bifurcation of the lateral thoracic vein (15-16). From here a lymph vessel leads to another double node (17-18) located in the same general region only a little anteriorly; then to the third double node of this group (19-20) found by the intersection of the lateral thoracic and the axillary vein. This node also receives the lymph vessels from the elbow nodes (1-2). On the left side a lymph vessel leads from node 20 to the communication with the venous system, either through the thoracic duct tap (24), or through a separate tap in the immediate district. On the right side the communication is in the same relative position (23).

In the anterior part of the thorax, between the two innominate veins, are two groups of single nodes, the thoracic groups (25). Each group varies in the number of nodes it contains, from four to eight. When one node in the group is injected all the other nodes of that group fill up with the mass and show a vessel leading to the venous connection in the jugulo-subclavian district. The right group connects with the right district and the left with the left district. Injections from other parts of the lymphatic system do not show these nodes.

The tongue and lips have many small vessels which collect in larger main vessels on each side of the head. These vessels lead to the submaxillary lymph nodes (28), which lie at the branching of the external jugular vein into the anterior facial and transverse vein. From these nodes, vessels lead almost directly inward, dorsally, to single nodes lying on each side of the trachea. Further down the trachea, on each side, are two nodes lying in close proximity, one a very small single node and the other a large double node. A lymph vessel leads from the larger node to the jugulo-subclavian tap on its respective side.

From injections in the intestinal walls a great number of small vessels are shown collecting into larger and larger vessels until they finally join the great intestinal lymph vessel, which follows the large intestine from the appendix to the intestinal lymph node in the anterior part of the abdominal cavity. In the region of the appendix there are several lymph nodes, all of which are single. In some cases they are so united as to form one large compound node, 2 or 3 cm. long. There is usually a single node at the juncture of each mesenteric lymph vessel (8, fig. 4) with the main intestinal lymph vessel (9, fig. 4). Just a short way from the cisterna chyli is the large, single, intestinal node (13, figs. 1, 2, 4), which marks the branching of the large intestinal lymph vessel. From here one branch goes to the cisterna chyli (6) and the other to the portal vein (5). The injections made from the nodes in the region of the appendix did not always show the portal branch of the vessel, but there were a sufficient number of cases to demonstrate that such a connection is fairly common. There were a varying number of single nodes in the region of the large intestinal node which did not fill up with the mass from the intestinal injection. However, injections made from them showed that they were connected with the intestinal lymph vessels.

Injections made from the spleen (1, fig. 4) show four lymph vessels leaving at various places along the hilus, accompanied by the splenic veins. Near the cardiac end of the stomach the four splenic lymph vessels unite with a small lymph vessel from the fundus (2) to form the main splenic lymph vessel which now proceeds dorsally toward the pyloric end of the stomach, from which it receives another small lymph vessel (3). Almost immediately thereafter, the branch from the intestinal lymphatics, when present (4), and the splenic vessel unite to join the portal vein (5).

## REMARKS

*On the nodes.* There seem to be two types of nodes; (a) a single node in which the lymph enters at the periphery, passes through the body of the node to the hilus, where a lymph vessel is formed; (b) the double node, referred to above, in which two single nodes are bound together in the same capsule. Instances showing this double nature are seen when injections made distal<sup>5</sup> to a double node fill the posterior half of the node and pass on well into the vessels beyond before the anterior half of the node begins to fill; injections made in the posterior half of these nodes will not fill the anterior half for some time after the vessel leading from the node is filled; injections made in the anterior half seldom enters the posterior half; in some instances where normally a double node is found, two single nodes were found only slightly separated; and finally, dissections can usually be made, to show the double nature of the nodes. The significance of the double node has not yet been determined, but it is possible that the two parts perform separate functions. The posterior part of the node responds to the injection mass, just as the single nodes of the head, knee, elbow and caudal region do, while the anterior part responds as the intestinal and thoracic nodes. It is possible, therefore, that the posterior part and certain single nodes may act as filters primarily, while the anterior part and certain other single nodes may act as elaborators only.<sup>6</sup> A histological study is to follow.

*On the vessels.* No attempt was made in this study to determine the nature of the origin of the lymph vessels in the tissues. The injections do show, however, a definite tubed system beyond the origin, carrying lymph in only one direction and that toward the venous connection. The valves in the vessels are shown by the knotted appearance of those vessels filled with the mass. In general, the lymph vessels follow the blood vessels very closely the lumbar region being the main exception. The walls are

<sup>5</sup>The venous connection is considered the anterior end of the lymph vessel.

<sup>6</sup>Sabin, in Morris's "Human anatomy," Part II, page 702, recognizes one type of lymph node with two functions.

very thin and easily ruptured by mechanical devices, but are capable of considerable extention in situ without injury. Where a lymph vessel passes through a node it always enters at the periphery and leaves from the hilus.

*On the connections.* In addition to the venous connections pointed out by McClure,<sup>2</sup> Silvester,<sup>3</sup> and others, there appears to be two additional connections in the rat, the *portal vein connection and the ilio-lumbar connection*.

The portal vein connection receives the lymph from the spleen and stomach, and part of the intestinal lymph (fig. 4, 5).

The ilio-lumbar connection receives some lymph from the right lumbar lymph vessel (fig. 3, C).

The left jugulo-subclavian tap, studied by McClure and Silvester in several forms,<sup>2</sup> in the rat receives the lymph from the left side of the head and neck, the left thoracic group, the left fore limb, side and hind limb, the deep seated vessels of the right hind limb and lumbar region.

The right jugulo-subclavian tap<sup>2</sup> receives the lymph from the right side of the head and neck, the right thoracic group, the right fore limb and the superficial vessels of the right side and hind limb (fig. 1, 23).

The right renal vein communications, studied by Silvester,<sup>3</sup> when present in the rat, receives part of the lymph from the deep seated vessels of the right hind limb (fig. 1, 9).

The left renal vein communication,<sup>3</sup> receives part of the lymph from the hind quarters (fig. 1, 10).

The portal and ilio-lumbar vein connections have not been reported previously, to the knowledge of the writer.

*On general arrangement.* The lymphatic system of the trunk is sinistral in the rat. The irregular occurrence and the extremely variable connections of the right lumbar lymphatics, as contrasted with the comparatively stable left lumbar system, is evidence of this fact. In all of the appendages of the body the lymph vessels follow very closely the various branches of the venous system. When the lymphatic system is injected it is very easy to distinguish the arterial, venous and lymphatic systems, when they occur together.

In conclusion, I take this opportunity to thank Dr. Frank A. Stromsten for his many helpful suggestions in the course of this work, and for his human interest, which has made this work possible and a pleasure.

# SOME ANATOMICAL DEDUCTIONS FROM A PATHOLOGICAL TEMPORO-MANDIBULAR ARTICULATION

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THREE FIGURES

In a previous paper, giving some observations on the jaw-joint,<sup>1</sup> I stated my belief that ordinarily the jaw is opened solely by the contraction of the two external pterygoid muscles; that the apparent barrier to the forward movement of the condyle, offered by the articular eminence, is almost entirely removed in life by the presence of the meniscus, or interarticular fibrocartilage, which, by its peculiar shape and movements largely obliterates the working depth of the glenoid fossa; and that thus during this action the path described by the moving condyle is almost a straight, rather than a curved, line, the direction of which is nearly parallel to the plane of pull of the lower heads of the two external pterygoid muscles.

A few months ago, through the courtesy of Prof. F. W. Putnam, Director of the Peabody Museum at Harvard College, I was given access to its splendid collection of several thousand skulls. In the course of my work on this material I came across a particular specimen, offering very direct, corroborative testimony on the points just noted, which I had demonstrated in my 'physiological' model two years ago, as well as to hint at one or two facts not mentioned before.

This skull was that of an adult negro from Algoa Bay; it was in good condition, and was normal except for the bony surfaces

<sup>1</sup> Observations on the temporo-mandibular articulation. *Anat. Rec.*, vol. 7, no. 10, pp. 355-367.

at the right temporo-mandibular articulation, and for a very slight modification in the left joint as well. The teeth were practically all present, were well worn and equally so on both sides, indicating that the two sides were used interchangeably in chewing. At the left jaw-joint there was a slight roughening of the posterior part of the articular eminence, as if its covering of hyaline cartilage had been eroded at that point, but elsewhere the bony surfaces appeared normal.

At the right joint, however, very extensive changes had taken place. The condyle was elongated anteriorly to double the antero-posterior diameter of that on the other side. This made the altered condylar process about as long as it was wide, being roughly the size of a twenty-five-cent piece, though not circular in outline. It was made up of two flat surfaces, one sloping down and laterally, the other down and mesially, from a central line, running in a sagittal plane the whole length of the process. It thus resembled an inverted trough. A transverse section gave an obtuse angle of about 120 degrees, and a longitudinal, or antero-posterior, section was a straight line. Its shape was then very different from that of the normal condyle as found on the other side (fig. 1).

The opposing surface on the temporal bone presented an appearance equally altered from the normal. Over the region of the original articular eminence and the anterior half of the glenoid fossa was a rough surface, the reverse of that noted on the condyle, being a wide trough, with its two surfaces meeting also in a line running antero-posteriorly, the whole being somewhat longer in that direction than the corresponding condylar surface. Behind it was a part of the glenoid fossa, but evidently not utilized in any way as an articular surface (fig. 2).

Putting the jaw in its proper position, as determined by the perfect occlusion of the teeth, it was at once seen that the peculiar right condyle fitted exactly into the trough-like surface on the temporal bone, anterior to the remains of the old glenoid fossa, and that the left condyle was similarly advanced a short distance in front of its usual position. The motion on the left side, although restricted by the slightly advanced position of

the left condyle, was otherwise probably almost normal. On the right side the condyle moved in its socket, much as the carriage of a sliding microtome knife slides freely back and forth in its bearings, with no possible lateral deviation.

Obviously the right meniscus was either entirely lacking, or, if present, did not serve its ordinary function of adjusting the bones to each other, as shown by the shape of the articulating surfaces and the uniform thickness of the intervening space.

Nevertheless, as shown by the wear of the teeth, and the shape and relative size of the opposing surfaces of the enlarged condyle and its reverse, the former glenoid fossa and articular eminence, the condyle must have moved forward and backward during the various motions of the jaw very much as it did on the left side or as it does in a normal case. If there had been no forward and backward motion of the condyle, such as is necessary for proper trituration, the teeth could not have been so worn, and the articular surfaces would have had an entirely different shape. With only a pure hinge movement the jaw-joint would undoubtedly have been more like that of the Carnivora, a cylinder rotating about its transverse axis, fixed in a cylindrical fossa above.

It is easy to see how the muscles of mastication in moving the jaw during the activity of the osteo-arthritis, which was evidently the cause of this abnormality, while the diseased surfaces were still plastic, formed joint surfaces exactly adapted to their proper sort of motion and to no other. The meniscus apparently had to be sacrificed, due to the disease, and certain advantages pertaining to this mechanism were lost, such as the lessened degree of friction in this moving hinge-joint and the presence of an elastic cushion to take up the shock of sudden and hard biting. But a very satisfactory substitute for a normal temporo-mandibular articulation was thus manufactured, a substitute that gave the same kinds of motion, though less in amplitude, as ordinarily found, in opening the jaw and in trituration, and yet one that accomplished this without the presence of a glenoid fossa, articular eminence, functioning meniscus, or cylindrical condyle.

Due to the peculiar conditions obtaining in this unusual specimen we have here crystallized evidence of what kind of motion was going on in that joint, a motion which must have been similar to that of the other, practically normal, side. The evidence is much better than that offered in the ordinary case where the presence of an active meniscus makes more difficult an appreciation of the actual path of the advancing condyle. Here the path itself is molded for us, not in moving cartilage, but in the enduring material of bone.

Study of this 'ossified testimony' gives us the following: The path of the advancing condyle was in a straight line, as noted in the working model described in my previous paper. There is no glenoid fossa or articular eminence in this case. So in the ordinary skull these irregularities are nearly smoothed out by the presence of the meniscus; and the condyle, there also, moves in a nearly straight line (fig. 3).

If the lines of the two advancing condyles be joined by a plane, that plane is seen to be almost identical with the plane formed similarly by the lines of pull of the lower heads of the two external pterygoids. The latter plane makes a slight angle with the former, about five degrees above its level, just enough to enable the opening muscles, the two external pterygoids, to apply the condyle not too firmly against the skull above, rather than to pull it away from its bearings, keeping the joint surfaces in close contact ready for the instant action of the closing muscles to take effect at any stage of the process of opening. It is interesting to note, however, that in closing the mouth the muscle which retracts the condyle, the posterior fibers of the temporal, does so at not less than an angle of twenty-five degrees above the plane of the condylar path. Thus it always keeps the bony surfaces in very firm contact.

The path of the condylar advance lies in the plane of the temporal muscle, very naturally, it seems, as the strongest of all the closing muscles, and also as the only retracting muscle. Very likely it is this muscle that had most to do with the molding of the plastic bony surfaces during the active stage of the disease. The condylar path, in being parallel to the sagittal

plane, is thus parallel to the resultant of the combined pull of the two external pterygoids, the main muscles utilized in opening the mouth.

Examining the condyle of the diseased joint by an imaginary coronal section its two surfaces are seen to slant as follows: the slope running laterally from the ridge at its summit makes an angle of about twenty degrees with the horizontal, while the

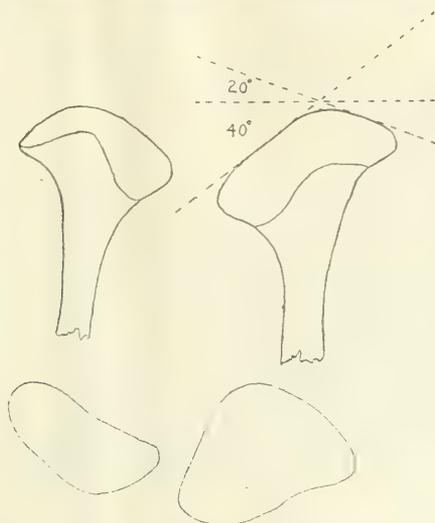


Fig. 1 Drawing of condyles of mandible. The upper figures show the condyles as seen from behind; the line below the upper part of the drawing gives the edge of the articulating surface. The lower figures show a view of the condyles from above. In each case the right hand figure represents a view of the pathological right side, showing its enlarged and altered articulating surface. The dotted lines show the approximate number of degrees of slope from the horizontal of each side of the joint surface.

surface running mesially from the ridge makes an angle of about forty degrees—being about twice as steep (fig. 1). The reason for this appears when we examine the planes in which the closing muscles act. The temporal muscle pulls practically vertically, not tending to dislocate the condyle either mesially or laterally. The masseter pulls laterally at an angle of twenty degrees from the vertical, tending to pull the condyle outward, the internal pterygoid tends to pull the condyle inward, as it acts at an angle

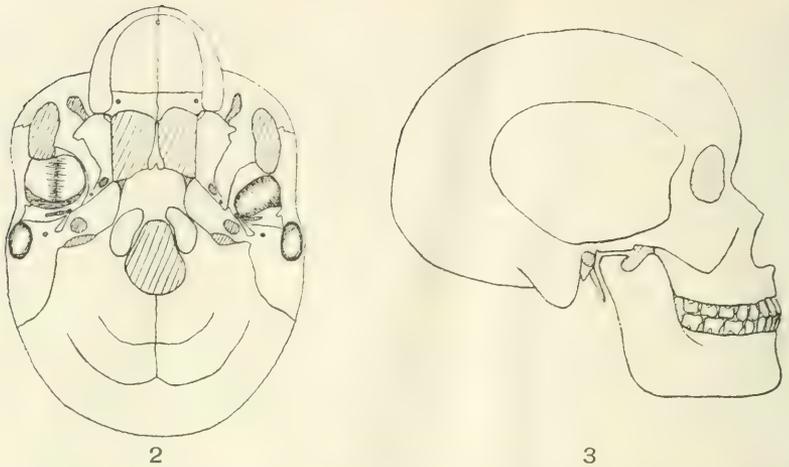


Fig. 2 Drawing of base of skull to show articulating surfaces for mandible. The broad oval area on the left of the figure is the pathological troughlike surface, articulating with the right condyle, shown in figure 1. The narrower oval of the opposite side of the skull is almost normal, although neither articulating surface extends as far back as the Glaserian fissure (shaded).

Fig. 3 Drawing of right side of skull. This shows the flattened articular surfaces of the pathological right jaw-joint, which allow the condyle to move forward and backward only in a straight line. The line of pull of the lower head of the external pterygoid muscle makes an angle of about five degrees with that of the joint surfaces, and is so directed as to pull the mandible against the skull during contraction.

of forty degrees to the other side of the sagittal plane. These lateral and mesial strains are exactly met, and in proper proportion, by the two sloping surfaces of the condyle as they are applied against the sides of the trough in which they run.

I further noticed that, although the surfaces of the condyle of an ordinary skull did not show these suggestive angles, if I placed an artificial meniscus above it—one molded in wax, fitting exactly that particular specimen—then the same significant slopes were seen in a transverse section of the condyle plus its meniscus.

While the study of this specimen perhaps adds little that is entirely new to our knowledge of the jaw-joint, it certainly gives very tangible proof of certain conditions, ordinarily not readily seen.

## LABORATORY AND TECHNICAL MISCELLANY

ARTHUR W. MEYER

*From the Division of Anatomy of the Stanford Medical School*

SIX FIGURES

### AN ODORLESS DISSECTING ROOM

To attain this desirable end we make use of the ordinary chemicals, namely: glycerine, carbolic, alcohol, formaline, and find that it is largely a matter of quality and proportion. Since the impurities in the cruder carbolic acid cling to, if not penetrate, the living as well as the dead, we have avoided its use altogether and substituted a much better grade of acid with a melting point of 35 to 38° C. Moreover, since carbolic acid of 33 per cent strength usually crystallizes especially in the viscera of the cadavers and in this strength always is an obstacle to efficient work because of its anesthetic effects, we reduce the proportion of carbolic acid to about 10 per cent. This reduction in quantity also compensates considerably for the increased cost of the better grade of acid used, avoids the strong odors of the impurities and numbing of the fingers during dissection.

In addition to the carbolic we use from 2 to 2½ per cent of formaline, 20 per cent alcohol, and 20 per cent commercial glycerine. Since the formaline effectively fixes the material a larger quantity of alcohol than needed to get the carbolic to dissolve and the glycerine to flow seems wholly unnecessary. It evaporates quickly upon exposure carrying odors with it and also materially increases the cost. The large quantity of glycerine used also adds considerably to the cost, but I know of nothing that can replace it.

The total quantity of preservative used per cadaver should, to be sure, vary with the condition and size of the cadaver. From twelve to twenty liters is what we use. This keeps the cost of the preservative per body down to about two to three dollars, which is a sum sufficiently small to be within the reach of all laboratories and especially of those that can afford lead-lined storage tanks. Although we use from 12 to 20 liters of preservatives per cadaver we depend on gravity for pressure.

All our anatomical material is kept in a saturated atmosphere, in ordinary wooden plank tanks each tank requiring only about a gallon of methyl alcohol per year in spite of the dry climate. A very weak solution (½ per cent) of formaline will, of course, accomplish the same result as the methyl alcohol and for some time I have immersed some cadavers

in a very weak solution of formaline and glycerine in water for experiment. These and other contemplated changes in our method I hope to know more about in the near future. I am sure that anatomists are aware of the disadvantage attending the use of formaline and methyl alcohol but with proper care in the dissecting room drying can nevertheless easily be prevented while the material is under dissection. We avoid the use of vaseline because of its messiness.

It is very seldom that we use a body within less than a half a year after preservation. Most of our subjects are a year old. It is true that the use of the formaline makes dissection somewhat more arduous because of the consequent toughness of fasciae, but the compensations resulting from its use are so many and its disinfectant qualities so desirable that I have not cared to omit it. Streeter found as much as 6 per cent unobjectionable. Our material also loses much color but that is not a serious disadvantage. The attempt to retain the color by the use of potassium nitrate or by the later use of alcohol during dissection has not been of sufficient success in our experience to warrant their continuation. Although the quantities of carbolic and formaline used are relatively small both are present in sufficient amounts to assure thorough disinfection and the bogey of infections still paraded in some manuals has never been encountered in my experience. We keep no surgical dressings on hand, for unless infected otherwise, all cuts sustained in the dissecting room heal promptly.

I realize, to be sure, that the condition of the cadavers when received should determine how much and to a certain extent also what kinds of preservatives are to be used. Everyone has his preferences but from what I have experienced in several laboratories and seen and smelled in others, I cannot hesitate in expressing my preference for a method which enables an instructor to be in the dissecting room for a whole day without subsequently revealing the fact on the street or in a banquet hall. Nor need students who have not changed their garb become a nuisance even to those near to them. The proper use of a good hand lotion will also accomplish much in this respect.

In one laboratory with which I was connected it was always necessary in the spring of the year, to scrape off the mold every morning before beginning dissections. Since mold grows in the presence of strong carbolic and formaline and as far as I have experienced in all climates, proper mechanical protection against contamination seems the most efficient and the simplest means of avoiding it. I would not imply, however, that the nature of the preservatives bears no relation to the growth of mold, for I am convinced that the use of arsenic is for this reason alone very objectionable.

No doubt, someone has suggested our climate as our best friend and most efficient helper. I gladly admit its beneficence but the same results can and also have been accomplished elsewhere by similar methods.

As preservative to moisten the material while dissection is in progress we use essentially the lotion used by Professor Mall for years—decades. But we cover the cadavers and tables with white oilcloth. It prevents

evaporation very well, looks neat, is cheap, can be discarded as soon as soiled and is therefore far preferable to oiled muslin used perennially.

Anatomists need not be reminded that in an absolute sense the above caption is, to be sure, a euphemism. Yet visitors to our laboratory both from at home and abroad have used that expression and lest there be skeptics I shall add that a medical visitor from Berlin, for example, repeated the words 'Geruchlos' and 'Sonderbar' on going through the laboratory. Likewise, an eminent English physiologist who was compelled to see the laboratory literally on the run, ejaculated as he hastily went through the dissecting room, "Well, I declare! Quite-unlike-the-old-dissecting room! Smells sweet!" Other instances could be given, but enough, and this much not as a toot from our own horn but merely for the benefit of the skeptic.

Architecturally our laboratories are not ideal; but we as others have hopes. Although the ceilings of our laboratory are sixteen feet high there is no ventilation except by means of a few three-foot-square sashes placed between six to nine feet above the floor. Steam heat is used. In spite of these things our laboratory is practically odorless. We don't deodorize the cadavers simply because we don't know how but we do not add to the natural odor through the use of preservatives from which there is less escape.

#### THE STAINING OF ELASTIC TISSUES

While using the various elastic tissue stains on the fetal vessels I noticed that sections stained in a watery solution of orcein and mounted in glycerine always showed a much more brilliant stain. In fact fine elastic fibers, which for some reason were scarcely noticeable by other methods, stood out very distinctly by the use of this method.

#### MORDANTING WITH IODINE FOR MALLORY'S CONNECTIVE TISSUE STAIN

For staining the reticulum of lymph and hemal nodes by Mallory's method a special fixation as recommended by him is desirable. That this is preferable is admitted but it was found that by a preliminary mordanting of sections in a very weak iodine solution, before staining, almost any kind of fixation would yield a fair result with Mallory's stain. The advantage of this procedure lies, to be sure, only in the fact that it is an emergency measure which often enables one to use this special stain on material fixed by other methods than the preferred.

#### MESH DISSECTING TABLE TOPS

The hypostatic accumulation of the preservative and of the body fluids in some bodies and especially in connection with the use of certain methods of preservation suggested the use of a wire-mesh, false table-top. Such a table-top also prevents the accumulation of fat and the preservative which is applied externally during dissection on the

table and keeps the cloth wrapping from becoming soaked and unsightly. Moreover, it makes the use of water for cleansing the material easy and agreeable.

Figure 1 shows the wire mesh top with the zinc binding. We use No. 14,  $2 \times 2$  galvanized iron wire mesh but would far prefer an aluminum mesh were it not so expensive and so flexible. Such a removable

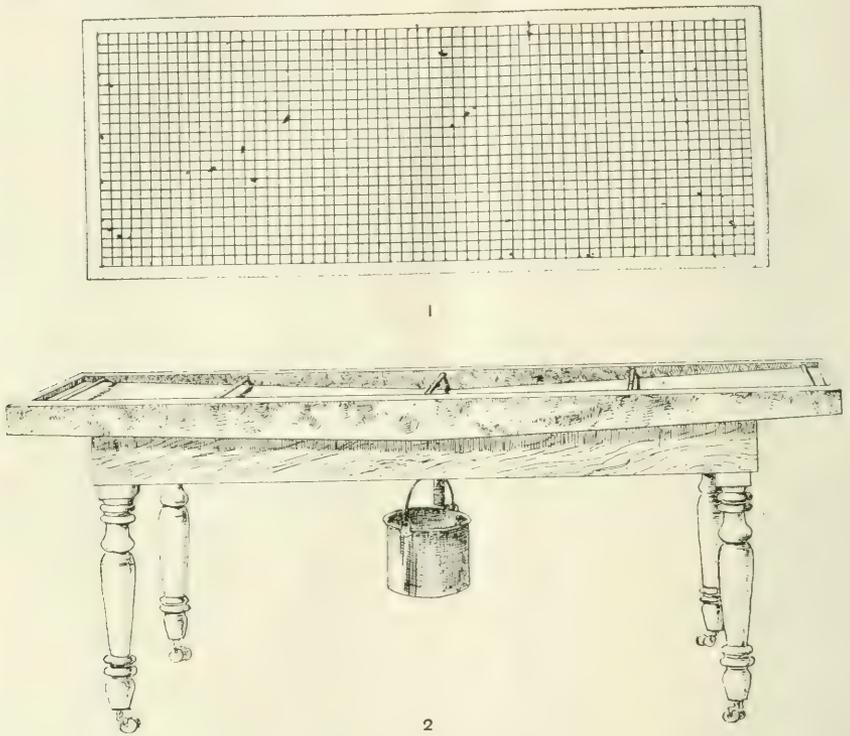


Fig. 1 Mesh table top

Fig. 2 Dissecting table ready for mesh top

mesh top can be used on any kind of table for, as shown in figure 2, it is merely supported by removable rods. Since this mesh and the zinc table tops can quickly be cleaned with a hose when desired without removing the body, the accumulation of small pieces of material can be prevented easily and the tables kept clean throughout the progress of the dissection. The meshes are about one centimeter square.

If desired, a cork stopper can be placed in the drainage pipe or a valve can be put on it so as to permit preservative or water to stand on the table beneath the mesh. This effectively prevents drying even when a body is used for a long time for demonstration purposes.

## MESH-BOTTOM AND FELT-SEALED SPECIMEN BOXES

One of the disagreeable features usually connected with the study of dissected and injected preparations and frozen sections is the fact that it is customary to keep them in boxes or jars containing a solution of carbolic acid. Indeed, in some laboratories such specimens are kept wholly immersed in a weak (5 to 10 per cent) solution of crude carbolic acid although as a matter of fact crude carbolic will dissolve in water only to the extent of about 3 per cent. Hence it is necessary to roll up the sleeves sometimes as far as the elbow, in order to remove the specimens for study. When removed the specimens also run and drip with the crude carbolic solution. In order to obviate these objections we have placed false bottoms in the usual galvanized iron boxes and avoided the use of crude carbolic. These false bottoms are made exactly like the mesh table tops except that a lighter grade of wire can be used. They lie upon triangular rests made of narrow strips of galvanized sheet-iron. These rests are about two inches high and run across the width of the boxes at such intervals as may be necessary properly to support the specimens (fig. 3).

The mesh is covered with oilcloth or muslin, and a piece of muslin, or cheesecloth, long enough to pass completely around in the box is put with its midpoint on the true bottom beneath the triangular rests, the free ends of the cloth extending up each side of the box. A small amount of a weak preservative containing but little or no alcohol is then poured into each box and the free ends of the muslin put over the specimens which lie on the false bottom. Capillarity keeps the cloth moist and the specimens can be removed at any time without the possibility of unnecessary soiling. They are always moist but never wet, dripping or macerated. Sections of frozen infants kept in such boxes for a period of four years are still in excellent condition in spite of frequent handling.

In order to prevent evaporation these boxes are provided with a gutter for the flange of the cover, as shown in figure 3. This gutter is lined with felt one inch thick, which can be fastened with a shellac or paraffine paint and which seals the boxes so effectively that suction is very evident when the lids are lifted. If desired, the gutters can also be filled with glycerine but this has never been necessary even in our long dry summers.

Coating the interior of the boxes with paraffine will delay corrosion from formaline-preserved material but in the course of years the boxes nevertheless get rather unsightly. Hence, I am at present arranging to have similar boxes made in white enamel. These will be permanent and far preferable esthetically.

## DRAWING AND BOOK STANDS

The need of convenient and stable individual book and drawing stands has been met variously in different laboratories. Although individual stands require more space and often make a room look

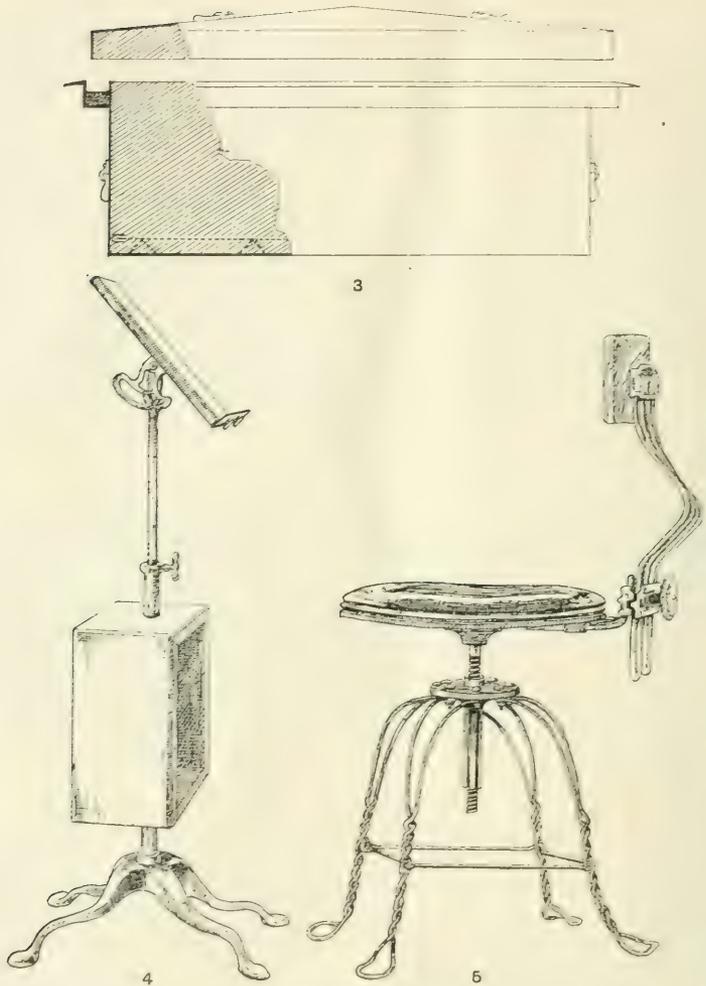


Fig. 3 End view of specimen box; triangle supports and mesh bottom in place.

Fig. 4 Drawing and book stand. Fig. 5 Adjustable wire dissecting stool.

somewhat disorderly yet ever since my student days I have been so thoroughly convinced of their advantages that I have gone to some trouble regarding the matter.

The stand shown in figure 4 has been in use in our laboratory for over five years. It is exceedingly stable and not one stand so far has

required repairs. The heavy base has four rather than three legs, for stability. The legs are not provided with castors, for the same reason, but castors can be added in a moment, for holes are provided. The book box, which measures  $12 \times 9\frac{1}{2} \times 6$  inches, is rotary on the stand. Hence the student can always easily reach the books. The oak drawing board, which cannot warp, is  $12 \times 6$  inches in size. It is adjustable for slant and height and the rod supporting it can also be rotated. It is provided with a metal retaining edge which can also be adjusted for height, and which can be dropped to the level of the board.



Fig. 6 Adjustable wire laboratory stool

The whole stand, which is easily dismantable, is finished in white enamel except the metal supporting rods, which are nickered, and the drawing board, which is finished in dark oak. The metal retaining edge is antique copper. This stand, which has answered our needs completely, can be obtained from Frank S. Betz, Hammond, Indiana, at prices from five to six dollars each, varying somewhat with the size of the order.

#### STOOLS FOR DISSECTING ROOMS AND HISTOLOGICAL LABORATORIES

While looking about for a durable and comfortable stool for the dissecting room, over half a decade since, I had the good fortune to be referred to the Chicago Wire Chair Company. I have not become a stockholder since. This company was then making a stool which answered our purposes provided we could be supplied with a longer screw assuring a greater range of adjustment. Through the courtesy of the manager of the company this was easily accomplished and we have equipped all our laboratories with the stools represented in figures 5 and 6. The latter is a stock model and can be purchased almost

anywhere but the former is modified to suit our needs. It is adjustable for a height of 18 to 26 inches and can easily be provided with metal ring foot-rests placed higher up than the rectangular rodbraces between the legs, if desired.

These stools, which are practically indestructible, are not at all expensive. Only one stool was damaged in six years and the parts that wear out or may break can easily be replaced.

The metal parts are finished in antique copper, although other finishes can be obtained. Both stools can be obtained with or without backs. From an experience of six years I am inclined to prefer both without backs, since an occasional careless student uses the back to knock a stool over or to tip it back while sitting on it, thus eventually loosening the screws in the oak top. The tops or seats are so built as practically to preclude warping. The address of the makers is La Salle Avenue and Ontario Street, Chicago. The only justification for this note and the accompanying illustrations lies in the frequent comments and inquiries of visitors and their durability and very reasonable cost.

#### LETHAL CHAMBER

The essential thing in the construction of this chamber for killing small animals (dogs, cats, etc.) with illuminating gas, is a galvanized sheetiron false bottom which rests and slides on galvanized sheetiron right-angled strips attached to the walls of the chamber. In order to protect the zinc binding of the mesh bottom from soiling, an apron of wood, or preferably of sheetiron, is fastened to all sides of the box above the border of the mesh. Beneath the mesh bottom is a galvanized iron pan which is easily removable.

The animals are placed in the chamber, the front of which is provided with a well-fitting door, and the gas turned on gradually. If this is properly done dogs seldom utter a sound. When the animal is dead the removable mesh bottom and pan can be hosed off, thus keeping the chamber clean. Soiling of the animals is also made much less likely by the use of the mesh false bottom.

#### ANIMAL CAGES WITH HINGED BOTTOMS

In handling dogs it is often inconvenient to lean far into the cages in order to reach the retreating animal. To obviate this difficulty a slightly raised and inclined board floor two feet wide can be provided on the near or door end of the cage. The rest of the floor can be made of a galvanized iron mesh which is hinged near the wooden floor on a galvanized iron rod, so that the farther end of the mesh floor of the cage can be raised by means of a rope or wire from the door end. This compels the animal to come forward to the door and makes inspection and removal very easy for both animal and caretaker.

## SECTIONAL PORTABLE LABORATORY CASES

It is often a great convenience to be able to move cases from one laboratory to another to meet some temporary need. Hence a special type of case with two sliding glass doors each in the base and top was designed. These cases can be built in one piece or the base and top can be built separately, and then fastened to each other by a few screws when placed in position.

Our cases are approximately 30 inches wide and 6 feet high, inside measure. The base is 2 feet deep but the top only 16 inches, leaving an offset of 8 inches which serves as a shelf. Since the doors are not hinged, anything standing on the offset need not be removed before the doors are opened and there is no danger of sweeping things off onto the floor when the doors are opened hastily. Directly beneath the offset are two drawers 5 inches deep, placed side by side. The central position of the drawers makes them very accessible. The size of the drawers and that of the unit itself can be made to suit personal preferences. The sliding doors can be provided with showcase locks or they can be locked with a metal peg (a sawed off 20-penny spike) and a hasp and padlock. The peg is placed in a hole which passes through the overlapping sashes in the middle and the hasp is brought over it and locked in place.

## DRAWING FROZEN SECTION

Few students of anatomy attain sufficient command over pen, pencil and brush to be able to make rapid satisfactory freehand drawings of frozen sections. Moreover, even if they had this command of drawing, the time consumed would be entirely too great when it is desired to make a considerable series of life-size drawings. We have obviated this difficulty by making rapid tracings on glass with India ink directly from the specimens, as is commonly done. These plates are then put on an inclined frame and illuminated from below by an electric light. By placing drawing paper over the ink tracing an accurate copy can quickly be made in pencil or ink and the details filled in from the specimen. The latter are handled on a wooden tray and are always turned over between two trays or a tray and a glass pane, to prevent damage. Pencil drawings can be made permanent in the customary way with shellac. The ease with which drawings can be made in this way encourages students to get as complete a series as possible for later reference, as well as for immediate use.



## NEUTRAL STAINS AS APPLIED TO THE GRANULES OF THE PANCREATIC ISLET CELLS

W. B. MARTIN

*From the Anatomical Laboratory of the Johns Hopkins Medical School*

Through the investigations of Bensley and his pupils<sup>1</sup> we are aware that two types of cytoplasmic granules are present in the cells composing the islets of Langerhans in the pancreas of most mammals. These granules are distinct from those found in normal pancreatic parenchyma cells and this enables us to identify islet tissue. Bensley's valuable contribution rests on the application of a neutral dye (e.g., Reinke's neutral gentian) to pancreatic tissue which has been fixed in a particular way. At the suggestion of Dr. H. M. Evans, therefore, this work was taken up with the view of determining the best method of preparation of the neutral dyes, the concentration of the staining solution necessary for the best results and further to investigate certain of the dyes allied to gentian violet and orange G, in the hope of obtaining a neutral stain more efficient than Reinke's neutral gentian.

Gentian violet is a mixture of two dyes of the triphenylmethane series, hexamethylpararosaniline and pentamethylpararosaniline. Therefore, when gentian violet is combined with orange G the resulting neutral stain is also a mixture of two dyes and it is this mixture that is known as neutral gentian. As the two components of gentian violet differ somewhat in their staining properties and as the relative proportion of these constituents vary in different samples of gentian violet on the market it would seem advantageous to substitute the pure hexa or penta compound for the mixture. This at once suggests the substitution of other dyes of the triphenylmethane group on the basic side of the reaction and also the replacement of orange G by other acid dyes of the azo series. This idea has been carried out and a number of neutral stains prepared. These dyes have been applied to pancreatic tissue fixed by the Bensley method. The result of the study of these neutral dyes is set forth below with a brief description of each dye.

The acid dyes and basic dyes combine in molecular proportion. In some cases the ratio is a simple one of 1 : 1 and in other cases the ratio may be 1 : 2 or 1 : 4. For example, ethyl violet combines with ponceau

<sup>1</sup>M. A. Lane, The cytological characters of the areas of Langerhans. *Am. Jour. Anat.*, vol. 7, 1907; R. R. Bensley, Studies on the pancreas of the guinea-pig. *Am. Jour. Anat.*, vol. 12, 1912.

4 G B in the ratio of 1 : 1, with orange G in the ratio of 2 : 1 and with trypan blue in the ratio of 4 : 1.

The method of preparation of the neutral dye is the same in each case. A concentrated aqueous solution of the acid stain is added to a similar solution of the basic substance. This should be done slowly and the mixture stirred thoroughly. The neutral point may be determined in the following manner: After each addition of the acid substance the mixture is stirred and a drop is taken on a glass rod and placed on a piece of ordinary filter paper. The neutralized portion of the mixture, being in the form of a precipitate, settles at once on the paper, while the liquid portion containing the unneutralized stain spreads in a circle around the deposit. By the color of the outer ring can be determined whether the solution contains an excess of the acid or the basic dye, and by the change in the degree of colorization one can readily perceive the approach of the neutral point. When this is reached the outer ring is entirely colorless. The end point is thus made as exact as in any other chemical reaction and the filtrate in such a case is either clear or only slightly colored. One thus avoids an excess of either stain and the residue is practically free from either of its single constituents. This is of practical importance, for the failure to free the neutral stain of one of its components may account for some of the difficulties that have been encountered in the use of neutral gentian. The residue obtained above is filtered, washed with distilled water and allowed to dry either in the air or in an oven at a low temperature.

In order to determine the concentration of the staining solution necessary to obtain the best results, solutions of varying strength were used, the staining time remaining fixed. A solution of known strength was made up in absolute alcohol and this was kept as a stock solution. From the stock, staining solutions in 20 per cent alcohol ranging in concentration from one of 8 mgm. of the solid dye in 50 cc. of alcohol to one of 0.25 mgm. of the dye in 50 cc. alcohol were prepared. The strength of these various staining solutions is given in table 1. Positive or negative results are indicated by the corresponding mathematical signs. To obtain the best results fairly dilute solutions should be used. A solution of neutral ethyl violet orange G containing approximately 2 mgm. of the crystal dye to 50 cc. of 20 per cent alcohol is satisfactory. A staining solution of neutral azo fuchsine should contain 0.5 to 1.0 mgm. of dye to the same amount of alcohol.

When brought together in molecular proportions these dyes react with the precipitation of a neutral dye. On drying, this is a dark green powder giving a deep purple violet color in alcohol:

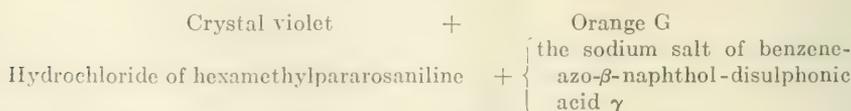


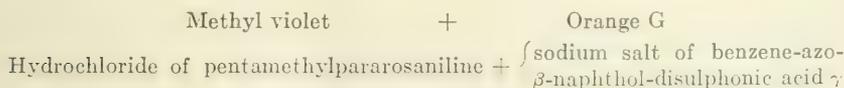
TABLE 1

Showing relative staining power of different neutral dyes. The numbers refer to Schultz's Farbstofftabellen, 1914

Milligrams of neutral dye added to 50 cc. of 20% alcohol for staining solution	8	4	2	1	0.5	0.25
Orange G (38) (A) + gentian violet	+	-				
+ penta methyl violet (515) (C.J.)	+	-	-			
+ hexa methyl violet (516) (C.J.)	+	+	-	-		
+ crystal violet (517) (B)	+	+	-	-		
+ ethyl violet (518) (B)	+	+	+	+	-	
+ victoria blue B (559) (B)	+	+	+	+	+	-
Ethyl violet + azo eosin (94) (B $\gamma$ )	-	-				
+ brilliant croceine 3B (227) (B $\gamma$ )	+	-	-			
+ croceine orange G* (B $\gamma$ )	+	+	+	-		
+ Ponceau 4 G. B. (37) (A)	+	+	-			
+ diphenyl brown (347) (Sch)	+	+	+	-		
+ diamine brown (344) (Sch)	+	+	+	+	+	-
+ azo fuchsine G (146) (B $\gamma$ )	+	+	+	+	+	+
+ trypan blue (391) (C)	+	+	+	+	+	+
+ heliotrope B** (Sch)	-	-	-			

\* From Fabrenfabrik of Elberteld Company. Although given by Schultz in his last edition (V) as identical with ponceau 4 G. B. the dye is undoubtedly different.

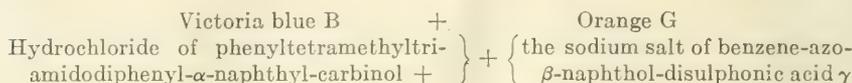
\*\* Not given in the last edition of Schultz under this name.



Hexa methyl violet, which is given in the table, has the same composition as crystal violet but is a purer product and gives slightly better results. Both of these dyes react with orange G in the same manner, giving a coarsely crystalline neutral dye of a green color and with a fine metallic luster. Sections of pancreatic tissue stained in any one of the above dyes present much the same picture, though the hexa methyl violet is decidedly superior to the penta methyl violet. In each case the zymogen granules of the parenchyma are stained a vivid heliotrope on a light orange background. The nuclei of all the cells are blue or violet, and in the islets of Langerhans the granules in A and B cells of Lane are differentially stained in the same manner as when stained in neutral gentian. This is to be expected, as gentian violet is a mixture of methyl violet and crystal violet and in staining power lies intermediate between the two:



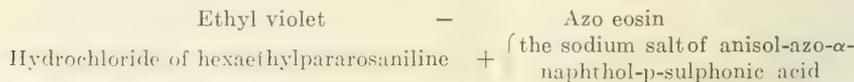
Ethyl violet and orange G react in the proportion of 2 : 1 in the usual way, giving rise to a neutral stain. This forms lustrous green crystals having a slightly bronze cast and gives a violet solution in alcohol. This stain is much superior to any of the above. While the general picture is the same, it is much more intense in its action and the three types of granules are more sharply differentiated. It has the advantage also of resisting the action of the dehydrating and differentiating agents better:



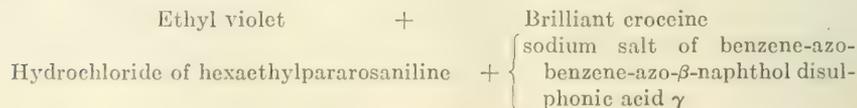
The neutral dye is obtained in the same way and forms lustrous crystals of a deep brownish-red color. The alcoholic solution is blue without the red cast of the stains so far mentioned.

Sections stained in this dye present a somewhat different picture. The zymogen granules are stained blue on a pale yellow background. The granules in the islet cells are stained a more intense blue and retain their color when differentiated from a dilute solution longer than the zymogen granules. The nuclei of all the cells are stained a beautiful blue green, the nucleolus and chromatin network standing out clearly against the rest of the nucleus. This dye will stain in high dilutions, but on account of the deficiency in color contrast between the granules and protoplasm is not as desirable a stain as the neutral ethyl violet.

The series of neutral stains so far given have been combinations of orange G with various basic dyes of the triphenylmethane series. Of these ethyl violet is the most valuable. In the following experiments this dye has been joined to a number of acid dyes of the azo series and the staining power of the resulting compound studied:



The neutral stain crystallizes, forming bronze green crystals, and gives a deep violet red solution in alcohol. It is, however, lacking in staining power and in concentration as high as 32 mgm. of dye to 50 cc. of 20 per cent alcohol stains the zymogen granules very faintly and the nuclei not at all:



Two parts of ethyl violet combine with one part of brilliant croceine. The neutral stain is a lustrous crystalline substance of a fine green

color forming a violet red solution in alcohol. The protoplasm of the cells stains a pale pink while the zymogen granules take a slightly darker shade. The granules in the islet cells are stained faintly and the two types can be made out but the differentiation is poor:

Ethyl violet + Croceine orange G

Fine bronze crystalline substance giving a violet red solution in alcohol. Sections stained in this dye are a bright yellow. The zymogen granules are a reddish brown. The granules of the islet cells of one type are stained a deep blue while the other type takes the same yellow color as the background. In higher dilutions the zymogen granules still take the stain but the islet granules are not stained. The nuclei are shown fairly well:

Ethyl violet + Ponceau 4 G. B.

Hydrochloride of hexaethylpararosaniline + { sodium salt of benzene-azo-β-naphthol-β-sulphonic acid

These dyes combine in the proportion of 1 : 1, forming a gummy residue which crystallizes out on standing and gives a deep red solution in alcohol. The staining action of this dye is very much like that of the neutral ethyl violet orange G compound except that it is dissolved out much more rapidly by differentiating agents and on this account is entirely unsatisfactory:

Ethyl violet + Diphenyl brown

Hydrochloride of hexaethylpararosaniline + { sodium salt of benzidin { salicylic acid monomethylamidonaphthol-sulphonic acid

Ethyl violet and diphenyl brown combine in the ratio 2 : 1 to form a neutral stain. This is a dull black amorphous powder giving a violet alcoholic solution. The zymogen granules appear heliotrope on an orange background. The granules in the islet cells take the stain but they are not sharply differentiated:

Ethyl violet + Diamine brown

Hydrochloride of hexaethylpararosaniline + { the sodium salt of benzidin { salicylic acid amidonaphtholsulphonic acid

Ethyl violet combines with diamine brown in the same proportion as with diphenyl brown to form a neutral stain. This is a dark brown powder giving a violet red solution in alcohol. Stained sections show dark red zymogen granules on a pink background. The nuclei stain exceedingly well. The granules however in the islet cells are not well differentiated and take a shade of red not very different from the background. The general picture resembles that seen in sections stained in neutral azo fuchsine except for the character of the islet granules.

Ethyl violet + Heliotrope B  
 Hydrochloride of hexaethylpararosanine + { the sodium salt of diansidine-  
 di-monoethylamidonaphthalene sulphonic acid

This is a dark brown amorphous powder giving a deep violet alcoholic solution. Zymogen granules are stained a light heliotrope and the protoplasm a faint pink. The granules in the islet are not well stained. This was not tried in concentrations higher than 8 mgm. of stain to 50 cc. of 20 per cent alcohol and since it offers a fairly good color contrast it might be useful in higher concentrations.

Ethyl violet + Trypan blue  
 Hydrochloride of hexaethylpararosanine + { the sodium salt of tolidine-  
 diamidonaphtholdisulphonic acid H

Four parts of ethyl violet are required to neutralize one part of trypan blue. The neutral dye is a deep green crystalline substance. The alcoholic solution is blue with a slight violet tint. This is a very intense stain and resists the action of alcohol and acetone. The zymogen granules are stained a deep purple blue against a light blue background. The nuclei stain fairly well. The differentiation of the granules in the islet cells is however poor. Both types appear to take the stain but the color contrast throughout is not sharp enough:

Ethyl violet + Azo fuchsine  
 Hydrochloride of hexaethylpararosanine + { the sodium salt of p-sulphoben-  
 zene-azo-dioxynaphthylene sulphonic acid

Ethyl violet and azo fuchsine combine in the ratio of 2:1. The dry neutral dye prepared in the same way as the above is a fine crystalline powder of a green color which gives a violet red solution in alcohol. Pancreatic tissue stained in this dye presents a very brilliant picture. The zymogen granules are a deep purple on a light pink background. The nuclei in all the cells are stained fairly well, the chromatin material being red. The two types of granules in the islet cells are stained differentially, one type taking a violet stain and the other a distinct red. This is a very intense stain and may be used in high dilutions.

It is resistant to the action of acetone and alcohol thus making possible a more careful differentiation than with any of the other stains used. Sections stained in it have little tendency to fade and preparations made nearly a year ago are as brilliant as when first made.

From a consideration of the group of dyes just described it is evident that two of them stand out as distinctly superior to any of the others as a stain for pancreatic tissue. These are the compounds formed by the union of ethyl violet with orange G and with azo fuchsine. These are both powerful dyes, staining cell granules very intensely. The color contrast between the different types of granules and between the granules and the cell protoplasm is very sharp. They are efficient in high dilutions and gross precipitation of stain on the tissue is avoided. Finally it may be said the granules and the nuclear chromatin retain these stains well in the presence of acetone and absolute alcohol, thus rendering differentiation easy.

On referring to the table given above it is seen that the neutral stains formed by combining orange G with different basic dyes vary in staining strength and that as the basic substance used becomes more complexed the staining power of the neutral dye increases. Thus, the hexamethyl violet gives a more efficient stain than the penta methyl violet, the hexa ethyl compound surpasses the hexa methyl combinations while victoria blue joined to orange G gives a neutral dye that will stain efficiently in higher dilutions than any of the others.

The dyes tested in the above study were not secured through dealers but in each instance from the firm concerned in its manufacture. I am indebted to Dr. Evans, who placed his collection at my disposal, and we wish to thank the following houses for coöperation both in the supply of dye samples and in the confirmation of the precise chemical make-up of the dyes used: Farbenfabrike of Elberfeld Company; the Badische Company; the Berline Aniline Works; Kalle and Company; Leopold Cassella & Co.; Carl Jäger; and Schoellkoff, Hartford & Hanna Co.

## INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with Vol. 18, price per volume, \$7.50; foreign, \$8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price per volume, \$5.00; foreign, \$5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, beginning with Vol. 25, price per volume, \$7.50; foreign, \$8.00.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

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PHILADELPHIA, PA.

# SPOLIA ANATOMICA ADDENDA I

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*From the Division of Anatomy of the Stanford Medical School*

TWENTY-SEVEN FIGURES

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## PERFORATE SPHENOIDAL SINUSES WITH SUBDURAL DIVERTICULA

Specimen *a* is from the body of a white man, twenty-eight years of age, who died of tuberculosis.

There are two sphenoidal sinuses, as usual, in this specimen; the right sinus extends several millimeters across the median line. The septum is located in almost a sagittal plane lying 2 to 3 mm. to the left. The form of the right sinus is oval with its long axis—which is practically parallel to the upper surface of the basilar process of the occipital bone—making an angle of

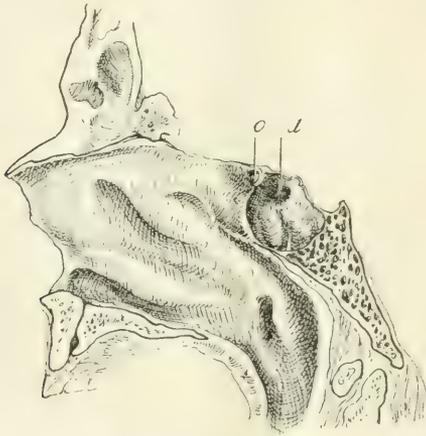


Fig. 1 Developmental defect in the lateral wall of the sphenoid and sphenoidal diverticulum; side view.

approximately forty-five degrees with the vertical. This diameter measures 25.5 mm., the vertical one 15 mm. and the transverse (right to left) 18 mm. The lining membranes of the sinuses are thin and they are not abnormally adherent anywhere. The roof of the right sinus is very thin, measuring only 0.35 mm. (by micrometer caliper) directly beneath the sella and a little to the left of the median line; its minimum thickness is only 0.5 mm.

Although the configuration of the left sinus is similar to that of the right it is much smaller, measuring only 11 mm. in a transverse (right to left) diameter and 22 mm. in the longest

oblique direction; it too is normal in appearance. The combined sinuses extended only about half way beneath the sella. The ostia are normal in size and position.

The nasal cavity is capacious but normal in appearance and the conchae, except the superior, are small. There are four conchae on the left side, the posterior ethmoidal cell opening into the supreme meatus.

At about the midpoint of the ventral (anterior) portion of the lateral wall of the right sinus immediately beneath its roof there is an oval defect (fig. 1*d*). The longest diameter of this oval defect measures 7 mm. and extends antero-laterally, lying almost in a horizontal plane and making forty-five degrees with

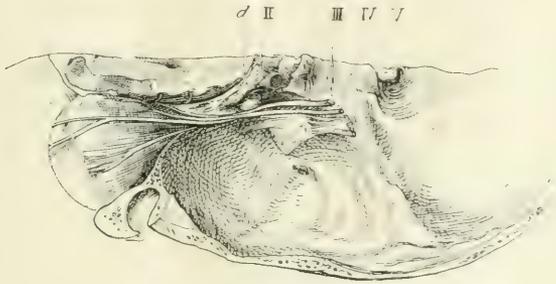


Fig. 2. The same as figure 1; cranial view.

a saggital plane. The short diameter measures only 4.5 mm. Through this opening a diverticulum of the sinal lining, which is 6 mm. long, protrudes into the subdural space. The wall of this diverticulum is very thin, nowhere adherent to the exceedingly thin (0.5 mm.) but regular margin of the defect and can be inverted with entire ease. It extends slightly forward and upward into a triangular space bounded by the optic nerve antero-medially, the carotid artery postero-medially and the reflection of the dura laterally (fig. 2). The dura, which surrounds the base of the diverticulum on all sides, does not envelop or cover the defect but merely comes into contact with the encephalic surface of the bony margin bounding the defect. Hence it is evident that the mucous diverticulum extended directly into

the subdural space and must have been in direct contact with the arachnoid.

There is no corresponding or other defect in the wall of the left sinus but the corresponding region is marked by a depression which lies in the base of the posterior root of the lesser wing. This root is absent on the right side. The anterior clinoid process on the left side joins with the middle, forming a complete foramen for the carotid artery. That on the right side unfortunately had been partly removed but the condition of the middle clinoid process, which is wholly intact, shows clearly that it was not joined to the anterior on this side, and the lateral wall with its dural reflection shows that the posterior root of the clinoid process was absent on this side.

The anterior and middle ethmoid cells and the frontal sinus were large but the posterior ethmoid cells were extremely small, being 3 to 4 mm. in size.

Specimen *b* is from the cadaver of a man thirty years old, who also died of tuberculosis.

The left sphenoidal sinus in this specimen is somewhat larger than the right and extends completely beneath the hypophys-eal fossa, being separated from the pons by an exceedingly thin bony wall only 0.37 mm. thick. The hypophyseal fossa is large and long, in a dorso-ventral direction. The dorsum sella is T-shaped in section, low (5 mm. high) and composed of a thin plate of bone which bears the large (by comparison) posterior clinoid processes. The floor of the sella formed the dorsal (posterior) half of the roof of the sinuses.

The ventral (anterior) half of the lateral wall of the left sinus contains a defect similar in position and character to that in the preceding specimen. This defect is oval also but somewhat larger than the preceding for it measures  $6.5 \times 4.5$  mm. The bony margin bounding it protudes slightly intra-cranially, forming a small cuff around the defect; this margin is only 0.25 mm. thick.

The diverticulum of mucous membrane which protrudes through this defect extends fully 3.5 mm. beyond the plane, extending across the dural reflections over the optic and oculo-

motor nerves; it is slightly enlarged distally. Although the lining membrane of the sinus is somewhat thicker than that of the preceding case, it is nowhere adherent and could be easily inverted. The relations of the diverticulum to the surrounding structures are exactly the same as in the previous specimen. It has a total length of 6 mm. and a width of 7 mm., in a line parallel to the optic nerve, and a width of 4.5 mm., in a cranio-caudal direction. Since a thin bony rim extends intracranially around the defect, for several millimeters the optic nerve lies more above than medial to the defect. This bony rim also covers the ventral knee of the carotid artery.

The dorso-ventral diameter of the left sinus is 29 mm. and it extends several millimeters beyond the median line, which it intersects somewhat obliquely, the ventral half of the septum lying a trifle to the left of the median line. This sinus does not extend beneath the hypophyseal fossa on the right side except at its most caudal portion, although laterally to the right it extends beneath the middle cerebral fossa.

Just as on the left side of the previous case, there is a depression in the lateral wall of the right sinus in a position corresponding to the defect on the left side. As before, this depression was covered by the posterior root and in part also by a bony extension from the anterior to the middle and to the posterior clinoid process, representing the ossified ligamenta interclinoidea not infrequently present. On the left side, on the contrary, there is no such extension from the anterior to the middle, but only from the middle to the posterior, clinoid process.

The finding of these defects in two subjects, the skull of neither of which was damaged by disease or injury, among only eight cadavers simultaneously under dissection, remotely suggests John Burroughs' dictum that "the number of birds one sees depends on the number one looks for," for I cannot believe that these instances are isolated cases, especially not after I have examined a small series of skulls with especial reference to the shape, form and position of the posterior root of the lesser wing and the osteology of the surrounding region. It is, for example, comparatively common to find a depression—i.e., an evagination

of the sphenoidal sinuses into the base of the posterior root—and in one cleaned remnant of a skull found in the laboratory one of the sphenoidal sinuses communicated with the cranial cavity at exactly the same place as in the preceding specimens. Although the lesser wing of the sphenoid had been removed in this specimen it was evident from the character of the margin of the defect in the lateral wall, and from the character of the wall itself, that it is not improbable that the defect was present in this specimen also before it was cleaned for only a very slender posterior root could have been present. However, the mere presence of a very slender posterior root, or perhaps even its absence, is not necessarily an indication that the sphenoidal sinus is not completely separated by bone from the cranial cavity. The absence of this root is probably of significance only if the sphenoidal sinus is as large or larger than normal, or perhaps still more accurately, only when there is a tendency to extend the sinus laterally in the region of the base of the root. Just why absorption should be especially active here at the junction of the pre- and basi-sphenoids, I do not know, and it is possible, of course, that tension exerted through the root in consequence of unequal growth after its fusion with the lateral wall may cause evagination of the sinus into the base of the root and its absorption.

The absence of the posterior root in both the above specimens must have left a weak point in the wall at this region. Since the reflections of the dura over the carotid artery, the second nerve, and over the third, fourth and sixth nerves in the adult lie at a higher intracranial level than the wall of the sinus, it is evident that the dura at one time must have been depressed over this region in order to clothe the portion of the lateral wall later occupied by the defect. In pre- and early post-natal life, to be sure, there could have been no such dural depression, but with the change in contour and in the relation of the different portions of the sphenoid bone and the extension of the air sinuses, with approaching maturity of the bone such a condition was bound to appear. Since in these cases the lateral wall of the sphenoid was not reinforced by the posterior root, as is normally

the case, it might be assumed that this region formed a point of least resistance to the developing and encroaching air sinus, were it not for the fact that other portions of the sinal wall are not reinforced and yet no perforations or defects result. That the increased intra-sphenoidal air pressure associated with such occasional phenomena as sneezing, coughing or forcible and obstructed expirations of any character, could be responsible for local atrophy of the bony wall and the underlying dura seems decidedly unlikely, although the form of the diverticula and the bony margin of the defects seems to suggest this. Indeed, no other explanation seems to fit the anatomical findings. But until we know more about the cause of the development of the air sinuses and the factors which control their development in the different directions, it seems quite futile to speculate on the genesis of these peculiar defects.

It seemed highly probable to me, at first sight, that the dura must have covered these diverticula, but that it did not do so except at the very beginning of their extension through the bone is beyond question. Indeed, the perforation of the dura by the diverticula is the most interesting and unexpected thing. Moreover, it would have seemed likely that the cerebrospinal fluid, the other meninges and the brain substance might have forced these diverticula of mucous membrane back into the sinuses, or rather prevented their protrusion. It would also seem as if one might have expected a diverticulum of the arachnoid, accompanied or unaccompanied by brain, to extend into the sinus as a result of the unopposed intracranial pressure. Moreover, since a defect was produced in the dura, it also seems as though the arachnoid should also have yielded to the same influences. Unfortunately, the brains had been removed from both these skulls, but the durae were undisturbed in these regions. The presence of the protruding diverticula is conclusive evidence, however, of the fact that practically no intracranial pressure was exerted upon them, for their extremities were entirely unattached to the durae and their inversion into the sinuses was prevented only by atmospheric pressure. Besides, had they been at all firmly attached to the arachnoid it is more than

likely that a tag of arachnoid would have remained attached to their distal extremities when the brains were removed.

The only investigators who mention any defects whatsoever in the lateral wall of the sphenoid are Zuckerkandl ('82), Spee ('96) and Onodi ('03). The first spoke of having noticed dehiscences and small defects in the lateral walls, and the latter of small defects in the region of the sulci carotici in a juvenile skull. Onodi, who examined 4000 entire and several hundred cut skulls, found vascular sulci and foramina in the lateral wall and also larger or elongated dehiscences in these vascular sulci. But neither he nor Gibson, who recently examined 85 specimens, found any defects comparable to those here reported. Onodi also emphasized the fact that such dehiscences as he found may result from traumata, pathological conditions and senile atrophy, as well as be artifacts or developmental anomalies.

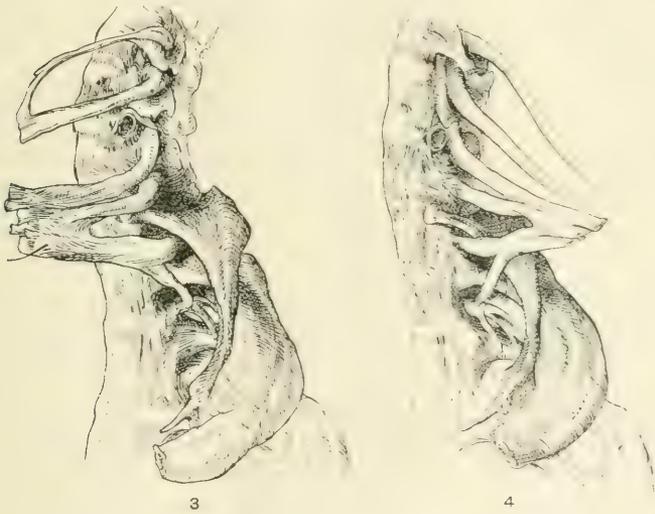
Since small defects in the lateral walls in the region of the carotid sulci—and in other places, for that matter—are seen not very infrequently in cleaned and dried skulls, it is evident that such skulls do not furnish proper evidence regarding the actual frequency of these abnormalities. If the walls of the sinuses are exceedingly thin they are easily injured when the dura is stripped and still more easily eroded in the cleaning. Hence, skulls in which the durae have not been removed can alone be regarded as furnishing reliable evidence.

Since the clinical significance of such defects in the lateral osseous wall, accompanied or unaccompanied by protrusions of the lining mucosa into the subdural space, must be evident to everyone emphasis on this matter is unnecessary.

#### CERVICAL RIB ASSOCIATED WITH EIGHT CERVICAL VERTEBRAE

The body from which this specimen was obtained was that of a female Swede 38 years old. There was nothing especially peculiar about the cervical rib which was present on the left side (fig. 3) except that it was well formed. It reached to within 2 cm. of the sternum and measured 7 cm. in length, along its concave border from head to tip, and the same dis-

tance along its convex dorsal border from the prominent articular tuberosity of the eighth cervical vertebra. The distal extremity of the rib was thickened and articulated with the upper surface of the first thoracic rib, about 2 cm. from the costo-chondral junction of the latter. A true articular capsule and an articular surface were present. The one on the first thoracic rib was about 3.5 mm. across. Both articular surfaces were covered by cartilage, and from the lateral surface or the distal extremity of the cervical rib a narrow but strong ligament, almost tendin-



Figs. 3-4 Cervical rib and brachial plexus.

ous in character, extended to the sternum. It ran parallel and directly cranial to the border of the first thoracic rib. Only slight mobility was possible between these two ribs, which mobility was greatly increased by section of the articular capsule at the distal extremity of the rib.

The head of the rib is small, the neck broad and flat, the tubercle comparatively large, and the shaft looks decidedly twisted because of the presence of a prominent groove formed

by the medial fasciculus of the brachial plexus as it crosses the shaft obliquely. The distal extremity of this rib is somewhat thickened.

The lower surface of the broad thin neck and the upper surface of the distal third of the shaft are grooved by the *ninth* cervical nerve; and the medial fasciculus of the brachial plexus and the cranial surface of the neck and the medial half of the body by the eighth nerve. The very thin and sharp medial margin of the broad neck lies between these two constituents of the brachial plexus, which join directly distal to this sharp border, 3 cm. distal from the head. This sharp medial margin also bears a slight impression from the subclavian artery. The communication from the first thoracic intercostal nerve did not cross this rib but ran along its lower border (the plexus was drawn cranially in figure 4) joining with the ninth nerve before the latter crossed the cervical rib to reach its upper surface, upon which it lay nearly to its extremity. If the subclavian vein also made a slight impression it was in common with the artery and not separate from it.

There is no separate extra rib on the right side, but a thin broad fibrous band extends from the prominent transverse process of the eighth vertebra to the mid-point of the first thoracic rib.

The cervical vertebrae are all normally formed; there is no sudden transition from the spinous process of the sixth to the seventh, or from the latter to the eighth, but merely a gradual increase in length of the spinous processes from the second to the eighth, and from the latter to the first thoracic. The extra vertebra had the character of a thoracic vertebra on both sides, although the two halves were not symmetrical. This also agrees with the statement of Bardeen ('00) who found in an examination of 59 spines that "There was some variation in the form of the vertebrae on the two sides of the body but in none of the bodies which we examined for the purposes of the present study was a given vertebra of different type on the left side of the body from what it was on the right side." The asymmetry was due to the presence of the rib, and hence of an articular facet on the

body and the transverse process on the left side, and the presence of a long transverse process with a foramen on the right. The dense band of fascia mentioned above arose from the extremity of this transverse process and extended to the upper border of the first right rib. The unusual length of the transverse process, as well as the contained foramen, confirm Todd's conclusion that all elongated transverse processes in this region are



Fig. 5 Cervical and thoracic curvatures in a spine with eight cervical vertebrae.

to be regarded as resulting from the attempted formation of rudimentary ribs.

The vertebral artery entered the transverse foramen of the fifth vertebrae on the right and that of the sixth on the left side.

As is usual, the cervical curvature as represented in figure 5 was markedly accentuated. As judged by the alignment of the

spines from the dorsum, there is no more than the usual amount of scoliosis, but there is a rather decided curvature to the right in the regions of the bodies of the 4th to 8th dorsal vertebrae. The maximum curvature is located opposite the body of the sixth cervical vertebra and the whole curvature is accentuated by the presence of flattening of the bodies of the 4th to 8th vertebrae on the left side. Since there is no such flattening on the right side, the convexity of the scoliotic curve is less evident than its concavity. The flattening of the bodies is so extensive that it can scarcely be attributed to the aorta alone. The bodies and the discs of the cervical vertebrae are only very slightly reduced in thickness and the cervical spine is hence longer than usual, especially if compared with individuals of corresponding stature. The increase in cervical curvature seemed to be compensated for, at least partly, by an accentuation of the dorsal curvature but no reduction in length compensatory to the cervical increase was evident; nor was there any reduction in the number of vertebrae in other regions. Twelve dorsal, five lumbar, five sacral and three coccygeal vertebrae were present.

The brachial plexus was normal in formation and distribution. It was not shifted cranially but caudally with the extra vertebra. This was the case of the cervical plexus also. Hence it is evident that in this case the presence of the plexus did not have any inhibitory effect upon the formation of an extra rib, as Patterson suggested. Nor was there any pre-fixing of the plexus, as emphasized especially by Jones ('13). The contribution from the first thoracic nerve was fully as large as normal, and this case then stands in striking contradiction of Jones' statement that

Just as varying grades of imperfection of development of the first thoracic rib are the outcomes of varying degrees of postfixation of the plexus, so the varying grades of perfection in the development of a cervical rib are the outcomes of varying degrees of prefixation of the plexus. And just as the postfixation may readjust itself with the rib elements at a lower level, so may the prefixation readjust itself at a higher level.

This generalization would also seem to be contradicted by the case reported by Frank ('14).

The very intimate relation of the ninth and first thoracic nerves to the cervical rib would seem to imply that considerable nervous disturbances probably resulted from pressure upon them, as emphasized by Goodhart ('09), Todd ('12), Thorburn ('05) and others.

Since such an excellent discussion and a summary of the literature on cervical ribs is given by Le Double ('12) and also by Streissler ('13) I shall only add that my own experience confirms Le Double's statement that variations in number of the cervical vertebrae are exceedingly rare. Nor does Streissler mention a case of supernumerary cervical vertebra accompanied by a cervical rib, although his summary covers 80 pages and includes 297 citations from the literature.

#### THE EFFECT OF UNILATERAL ABSENCE OF A VERTEBRA ON THE PRODUCTION OF SCOLIOSIS

*A priori*, one would be likely to conclude that the failure of development of the right or left half of a vertebra would result in a very evident, even if not in a marked, deformity of the spine. That this is not an inevitable result, however, was shown in the body of an adult male from which the specimen represented in figure 6 was taken. In this case only a slight deviation in the alignment of the spinous processes was noticeable after skin, fascia and some of the dorsal musculature had been removed, so that the true nature of the anomaly was not noticed until the thorax had been opened and the relations and the marked ventral scoliosis were revealed.

There were also a number of other abnormalities in this cadaver. The twelfth ribs were exceedingly small, as shown in figure 7, and the tenth and eleventh were long floating ribs. The xiphoid was long, curved, narrow, and completely ossified. The twelfth ribs, which measured 2 mm. in length, are symmetrical. Each is provided with a small articular facet 3 mm. in diameter on the body of the vertebra, and with a true articular capsule; they were slightly movable. Since the head and neck of the cadaver had been removed before the body was received

at the laboratory it is impossible to speak with certainty regarding the number of cervical vertebrae but even assuming that the normal number was present—according to Le Double six cervical vertebrae occurred in only 0.14 per cent of 1420 fetal, infantile

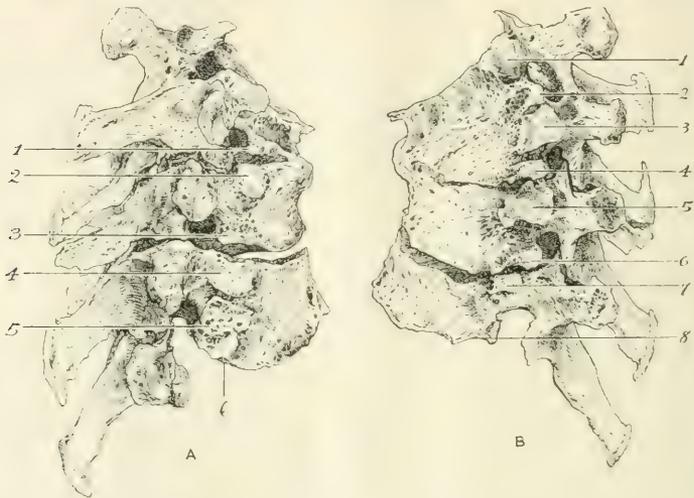


Fig. 6 Unilateral absence of half a vertebra and oblique fusion of the fourth to eighth thoracic vertebrae.

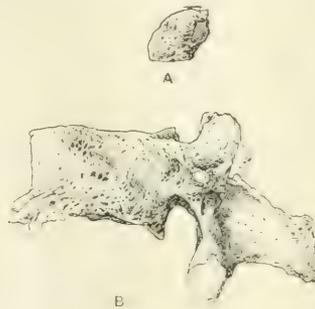


Fig. 7 Twelfth thoracic vertebra and rudimentary rib.

and adult vertebral columns reported in the literature—the total number of pre-sacral vertebrae is only twenty-two.

There are twelve vertebrae in the thoracic but only three in the lumbar region, the fourth lumbar being completely incor-

porated in the sacrum, which is composed of six vertebrae. The spinous process and the lamina of the first sacral are entirely distinct and the inferior intervertebral articular facets are preserved completely on the right, and almost completely on the left, side of the first sacral vertebra. But the sacrum differs in no essential respects from many similar sacra seen in every laboratory. As figure 7 shows, the thoracic vertebra—the twelfth—which bears the very small twelfth ribs has the characteristics of a lumbar rather than of a thoracic vertebra. This applies especially to the character of its spinous process and the direction of the surfaces of the superior articular facets. The transverse processes of the tenth and eleventh dorsal vertebrae bear no articular facets and have the characteristics of the normal eleventh and twelfth dorsal vertebrae. The corresponding ribs also have the characteristics of the normal eleventh and twelfth ribs. Hence if this, the twelfth dorsal numerically, be regarded as a lumbar vertebra in spite of the presence of rudimentary ribs, and the fifth lumbar be regarded as having fused with the sacrum, as is evidently the case, the normal number of five lumbar vertebrae is accounted for. This, however, leaves only eleven dorsal vertebrae represented by only eight distinct bony elements. The first three and the last four of these eleven dorsal vertebrae are separate bony elements quite normal in form and size. The eighth independent element is a bony complex represented in figure 6, and—as indicated by the number of ribs and transverse processes—should be composed of four vertebrae. There are five independent spinous processes, however, as shown in figure 6. On closer inspection, however, it will be seen that the second and third spinous processes are really only half processes of two different vertebrae each of which is represented by half a vertebra located on opposite sides of the body. The second spine belongs to a half vertebra on the left and the third spine of the complex belongs with a half right vertebra which is fused with the body of the succeeding vertebra. This relationship becomes clearer upon examination of the bodies. As seen in figure 6, *a* and *b*, showing right and left sideviews respectively, there are only three bodies in this complex. Moreover, the right portion

of the body of the first vertebra in the complex is reduced decidedly in thickness and the left portion is decidedly enlarged. The former bears only a small articular demi-facet and the latter a large and small demi-facet, and one large entire articular facet on its body. Hence it would seem that this portion of the complex represents a little less than one vertebra on the right and two vertebrae on the left side. The same thing is indicated by the presence of two transverse processes on the left and one on the right side and by the presence of a dorsal ridge, which clearly marks the line of fusion of the two lamina on the left side, and by a sulcus between the large middle articular facet on the left side of the body, which divides this large facet into two parts one of which resembles a demi-facet. The other no doubt represents a demi-facet also, although it looks like a whole facet and is large enough for one. Since a single rib—the fifth articulated here—this composite articular surface evidently represents only two demi-facets of adjoining vertebrae.

As viewed from the front, the upper surface of the body of the first vertebra in the complex slopes decidedly from left to right and although this great inequality in the thickness of the body was compensated for largely by difference in the thickness of the intervertebral disc and very slightly also by a slight inequality in the body of the third dorsal vertebra, it nevertheless caused a short but marked scoliosis, noticeable only ventrally.

A reference to figure 6, *a*, will also show that there was a shifting caudally of the lamina, transverse processes and pedicles on the right side, as a consequence of which the last lamina and transverse process, although normal in size and shape, are left without a pedicle. All the transverse processes, lamina and pedicles on the right have been shifted one vertebra caudally, and actually belong to the bodies one vertebra farther cranially. This asymmetrical bilateral error in segmentation also explains the presence of the two half spinous processes and is further confirmed by the presence of two half and one whole articular facets on the right side of the body of the last vertebra of the complex, as shown in figure 6. Hence it is evident that the right

half of the last composite vertebra, in this complex of four, articulated with three ribs, as did the left side of the body of the first. Since the lamina and intervertebral articular facets of the first two vertebrae are fused on the left side of the complex, only two normal articulations remain on this side, although three are preserved on the right. One of the articular facets on the left side is also on a pedicle.

The particular interest attaching to this error in segmentation lies, I take it, in the fact that the absence of one half segment was accompanied by the oblique fusion of dissimilar halves of succeeding segments, with consequent production of a floating lamina and transverse process, both of which have a normal form and size.

Aside from a few minor abnormalities present in this cadaver, the most interesting thing was the position and blood supply of the kidneys. The lower pole of the left kidney reached only to the level of the tenth ribs. This kidney was small ( $9.3 \times 4.7$  cm.) and received its main blood supply from a renal artery which arose at the level of the origin of the superior mesenteric. This artery bifurcated several centimeters from the medial border of the kidney, which it penetrated directly cranial to the pelvis. An additional large artery, which entered the medial surface about midway between the hilum and the lower pole, arose in common with the inferior mesenteric and a right renal 5 cm. cranial to the bifurcation of the aorta. Directly from the apex of the lower pole an accessory vein emerged and emptied into the vena cava.

The right kidney, which was considerably larger, measured  $11.7 +$  by  $5 +$  cm. Its lower pole reached the level of the lower border of the lumbar vertebrae, almost half of the renal mass lying caudal to the bifurcation of the aorta. The superior pole reached the middle of the second lumbar vertebra. This kidney had three renal arteries. The main renal took its origin from a trunk common to the inferior mesenteric and accessory left renal, and bifurcated about 2 cm. from the renal mass. A second renal artery arose from the lateral border of the right common iliac about 1 cm. below the bifurcation of the aorta. From here it took a slightly upward course, passed dorsal to the

kidney and bifurcated about 2 cm. before reaching the lateral renal border. One branch ended on the lateral border and the other on the ventral surface.

A third renal artery arose from a trunk common to the middle sacral and fifth lumbar arteries. This vessel emerged between the two common iliac arteries, then curved abruptly to the right, crossed the right common iliac ventrally and bifurcated at the border of the vena cava, the branches entering the ventral surface of the kidney 1 cm. lateral to the medial border.

Two large renal veins emptied directly into the vena cava. The largest, which was located farthest cranially, arose near the lateral border of the ventral surface between the lower and middle thirds by two trunks, which soon joined and emptied into the vena cava about 2 cm. cranial to the superior pole. The second vein arose by two branches, coming from the dorsal and ventral surfaces respectively, near the medial border of the superior pole.

The ureter arose mainly from a larger pelvis lying on the ventral surface between the upper and middle thirds and from three small accessory pelves located on the ventral surface of the middle and lower thirds. Each of these pelves had a ureter which joined the main ureter separately.

#### FUSION OF THREE CERVICAL VERTEBRAE

The specimen found in a skeleton of *Troglodytes savagei* is composed of the 3rd to 6th cervical vertebra, inclusive. As the illustration (fig. 8) shows, almost complete fusion has occurred. The three transverse processes on the left side, although very different in form, are quite separate, however. The articular processes are fused completely but the location of the individual processes can still be determined because of the presence of sutures in the inter-articular regions.

The extrusions of the right transverse processes of the third and fourth vertebrae are fused in such a way as to form an almost complete border through which the fifth nerve passed ventrally. The fusion of the transverse process of the third



is absent on this side but the base containing the transverse foramen is preserved. The bodies of these vertebra are fused so completely that the exact limits of the individual corpora can nowhere be detected.

The character of this splendidly preserved specimen shows clearly that the fusion did not result from injury or disease but is a simple case of embryonic fusion. The remaining cervical vertebra are normal in size and number, there being 13 dorsal, 3 lumbar, 2 sacral and 3 coccygeal vertebra. A marked dorsal inclination or retro-flexion of the dens epistropheus is present

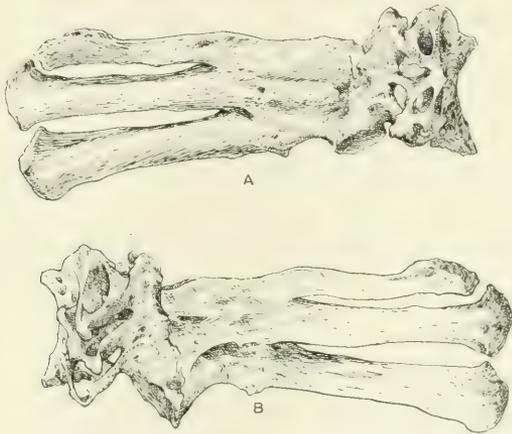


Fig. 8 Fused third to sixth cervical vertebrae of a gorilla.

and may be due to the lessened mobility of the cervical spine resulting from the fusion of the 3rd to 6th vertebra.

#### AN UNUSUAL THORACIC DUCT

The careful statistical study made by Davis '15 has supplied us with a better basis for grouping anomalous ducts and for judging the frequency of the different types. Since a case of a double duct, observed by me some years since, differs from those recorded by Davis, Svitzer, von Patruban, Wutzer & others, it seems worth while to give a short description of it. The skeleton and notes at hand.

A cisterna chyli formed by the confluence of three lymph vessels was present in the usual location. From it a large left thoracic duct extended cranially and emptied into the subclavian vein after being joined by the left cervical trunk. But in addition to the left thoracic duct a smaller duct ran to the right from the cisterna chyli and then extended cranially parallel to the left duct, continued directly with the left cervical and joined the left thoracic duct by a transverse branch in the bronchial region after receiving a large bronchial trunk. In addition to being double, this specimen of the thoracic duct was interesting in the fact that the right cervical trunk joined the right thoracic in the thorax in the retro-bronchial region and sent a transverse communicating branch, which joined the left thoracic duct after receiving a large bronchial trunk.

#### A LARGE PHRENICO-HEPATIC ARTERY

Although the occurrence of small hepatic branches from the phrenic arteries is mentioned in all anatomies as normal I have been unable to find a description of a large vessel such as noted here. The vessel in question, which was fully 2.5 mm. in caliber, arose from the right phrenic and entered the fossa ductus venosi near its cranial extremity. It then divided, sending one branch cranially into the left lobe the other branch running caudally along the fossa. A few centimeters from the point of bifurcation a second branch was given off to the left lobe at about the midpoint of the vessel but the main trunk extended onward to the porta hepatis, where it divided into three branches which entered the quadrate lobe. The phrenic itself arose directly from the aorta opposite the left renal artery and gave off a supra renal branch before reaching the diaphragm.

#### CORPORA LIBERA ABDOMINALIS VERA ET POTENTIALIA

In a former article I have reported the finding of true appendices epiploicae (not coli) and discussed their genesis and significance. Since then I have found two more cases of appendices and one case of corpus liberum.

One of these appendices was found in the ventral surface of the great omentum of a cat; it is shown in figure 9. Its actual length  $a-b$  was 8.5 mm. and the cylindrical vascular tip, which very closely simulated a hemal node, was 2 mm. long and 1.3 mm. in caliber. The vessels going to it were not visible in the gross.

In figure 10 a much larger fatty bovine omental appendage is represented. Its greatest width was 14.5 mm. and its length 22.2 mm. The capsule is thin and the whole appendage has the appearance of normal fat.



Fig. 9 Omental appendage from the cat; actual length,  $a-b$ , is 8.5 mm.; length of hemal nodule at tip is 2 mm. and its diameter is 1.3 mm.

Fig. 10 Omental appendage from a steer; actual size  $14.5 \times 22.2$  mm.

The third specimen was found in a small cavity on the internal surface of the ventral wall of the greater omentum of the body of an adult male. It is oval in form and measured  $2 \times 1$  cm. It is smooth and soft and was contained within a small sac about twice its size, which was located on the internal surface of the ventral reflection of the great omentum. It could easily be rolled between the fingers and the character of its surface, as well as its form, suggest that it had often been rolled about by the action of the peristaltic movements on the great omentum.

On section this body is found to have a well-defined connective tissue capsule, 0.5 mm. thick, which contains a partially degenerated fatty material. Although no histological examination

was made there is little likelihood that this body was other than a fatty omental appendage, such as that shown in figure 10. The fact that it was contained in a small omental bursa is likely accounted for by a slight inflammatory reaction which probably accompanied its detachment from the omentum. The mere process of torsion of the pedicle which precedes and accompanies separation would alone favor the exudation of a sufficient amount of serum to cause omental adhesions about the appendage. However, since these adhesions would be unlikely to form over the whole surface of the appendage, the continuous effects of peristalsis could later free it and convert it into a corpus liberum within its own small sac, instead of within the omental bursa.

Upon the genesis of a thickened connective tissue capsule from a very thin peritoneal layer I have no information to offer. That matter has been discussed often since Virchow called attention to the thick cartilaginous capsules which frequently surround loose bodies. However, since seeing the report of the extraordinary large free body found in the abdominal cavity by Campbell and Owen ('14) I am prompted to add that no evidence whatever for the idea that free bodies continue to grow in size after detachment was found in any of the cases which came under my observation. Nor can I say I should have expected to find any, for the evidences of degeneration present even in very small loose bodies, as well as the evidences obtained from tissue culture and from corpora libera articulationes—*corpora oryzoidea*, joint mice—speak very strongly against such a supposition. Moreover, it is well known that the presence of comparatively small loose bodies often necessitates operative interference and the larger the uncalcified body the greater the danger from degeneration if detachment has been sudden.

#### BIZARRE LYMPH FOLLICLES

During my investigations on hemal nodes, especially of the sheep, I was frequently impressed with the singular form of the lymph follicles in some lymph nodes. We are so in the habit of regarding the follicles as spherical bodies that a form such as

is represented in figure 11 seems odd indeed. Strangely enough, I never noticed any condensation of the reticulum surrounding these follicles, such as is commonly present in the spherical forms, nor could I relate their form with any peculiarity of the node or the surrounding parenchyma. They seem to result from a diffuse rather than a strictly localized proliferation of lymphocytes. I am aware, to be sure, that the least-resistance theory can also be applied here and it might perhaps offer a satisfactory explanation were it not so difficult to see why the

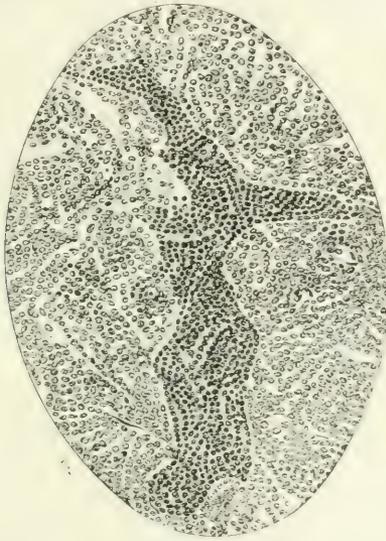


Fig. 11 A strangely-shaped lymph follicle from a lymph node of a sheep.

obstruction to growth in the various directions around an ordinary germinal center should be so nearly equal in an over-whelming number of cases of follicle formation.

The occurrence of a more or less laminated appearance due to an arrangement of the cells in more or less orderly concentric rows is also a fairly common occurrence in spherical follicles and not infrequently one meets with follicles which simulate Malpighian corpuscles very closely because of the presence of a central artery.

## INTERCALATED (?) HEMAL NODES

The hemal node shown in outline in figure 12 was found in the pre-vertebral fat of a sheep. It is a very small flattened node measuring only  $2.5 \times 3 \times 1.5$  mm., but is peculiar in being trans-fixed, as it were, by a vein. Although the specimen has not been examined microscopically, from what I know of hemal nodes I feel certain that it also has arterial relations and is therefore not intercalated in a vein, as the figure might suggest and as has been assumed by some. Indeed, I have never seen a hemal node solely intercalated in the course of veins, although I have seen several nodes which were completely crossed by both veins and arteries (figs. 10 and 11, "Hemolymph nodes of the sheep," Stanford University, 1913).



Fig. 12 Hemal node with two veins; actual size about 3 mm.

Since this node is so small it might seem that one of these vessels is a vein and the other a distended artery had not injections frequently shown that it is not extremely rare to find two veins leaving a single node in opposite directions. I am certain this is the case here.

## THE GENESIS OF SUPERNUMERARY SPLEENS

One of the theories of the genesis of some accessory spleens has long held that they not infrequently result from the isolation of small processes or lobules from the main mass. The main and

supernumerary spleens shown in figure 13 very likely had such an origin. The specimen taken from a cat is interesting in that it shows a small narrow splenic process extending out from the parent mass, with a vessel running from the latter to the small nodular supernumerary spleen lying several millimeters distant. The main spleen was entirely normal in size and appearance and one can scarcely doubt that in this case mechanical factors were responsible for the isolation of the small splenic nodule and that hence it represents the former distal extremity of the process and is consequently a true daughter spleen. Serial sections of the latter show a wholly normal and typical splenic structure.

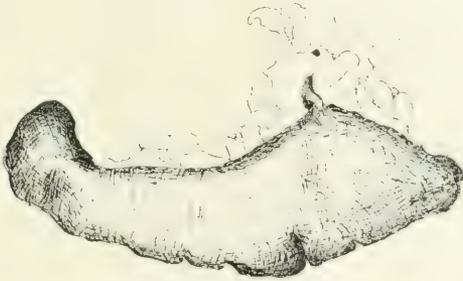


Fig. 13 Mother and daughter spleens from the cat; actual size of daughter spleen  $2 \times 1.5$  mm.

#### DEPLETED HEMAL NODES

##### 1. *Parathymic node of Ovis aries*

Some years since, while incidently interested in the occurrence of accessory parathyroid and parathymic glands in sheep, I observed that small hemal nodes were especially frequent near the parathymus glands (The parathymus glands of the sheep, *Anatomical Record*, volume 1, 1905). These nodes, which measured only a few millimeters in diameter, could not be distinguished from the *isolated* parathymus glands with the unaided eye, and were frequently found upon microscopic examination to contain very little lymphatic tissue. Some of them were indeed mere sacs of blood containing small islands or chains of

islands of lymphatic tissue. Sometimes, as in the case of the node a cross-section of which is shown in figure 14, the capsule was extremely thin. This particular node was taken from a young sheep only seven months old, but similar specimens were found in much younger and older animals.

2. *Paraprotid node from a newborn pig, Sus domestica*

My attention was called to this specimen (fig. 15) some years since by Professor Sabin, through whose courtesy I am enabled to refer to it here. The structure of this node, which measured only a fraction of a millimeter, is entirely comparable to that above, obtained from the sheep, except that it is still more depleted of lymphatic tissue. However a careful scrutiny of the wholly depleted areas of the node from the sheep, by means of high magnification, reveals only very slight differences. Indeed, except for the specific and age differences there may be said to be distinctions between these nodes.

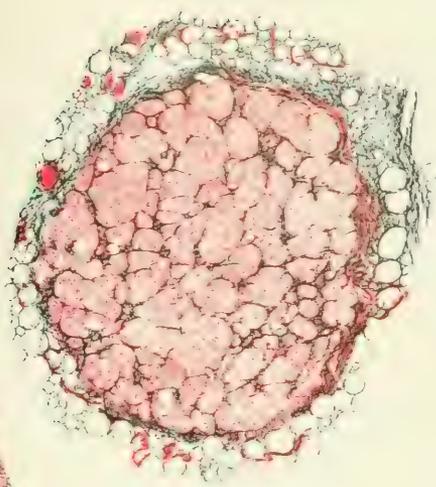
3. *Node from the gastro-hepatic omentum of a colt, Equus caballus*

This specimen was the only hemal node found during a careful inspection of the thoracic and abdominal cavities, of the cervical axillary and abdomino-inguinal regions of three approximately full-term colts, which material I owe to the courtesy of my colleague, Professor Jenkins. The node was  $5 \times 3 \times 2$  mm. in size, intensely dark red and lay among pink lymph nodes in the gastro-hepatic omentum. As figure 16 (giving the appearance of a portion under high magnification) shows, there is far greater depletion of the lymphatic parenchyma in this node than in that from the sheep. The node is in fact merely a sac of blood-cells like that from the parotid region of the pig.

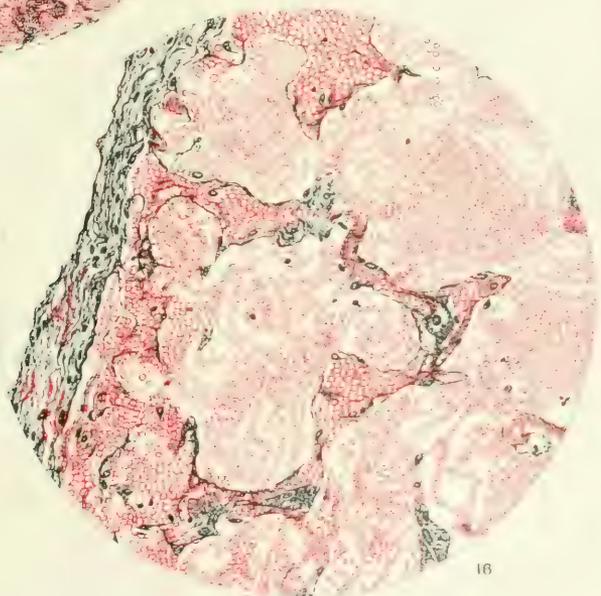
It seems decidedly interesting to me that nodes from such different species and regions, of such different sizes, and from animals of different ages, should have so similar a structure. A comparison of these with hemal and splenic nodes, represented and described elsewhere, will also show that small areas from



14



15



16

Fig. 14 Depleted hemal node from a lamb.  $\times 54$ .  
Fig. 15 Depleted hemal node from a newborn pig.  $\times 12$ .  
Fig. 16 Depleted hemal node from a colt.  $\times 275$ .

such haemal and splenic nodules from cats, dogs, sheep, bovines, goats, horses and pigs are found to have a very similar and wholly comparable structure. Indeed, were it not for specific cellular differences, we could with considerable justice speak of an identical structure.

The node from the sheep has a very thin capsule, while those from the pig and horse have relatively thick capsules, in some places at least. But since the variations in thickness of the capsules of hemal nodes and supernumerary spleens from the same animal are subject to wider fluctuations, this matter is evidently of no great significance in the differentiation of nodes. The node from the sheep also contains lacunae and vessels within the lymphatic tissue which contain disintegration products of erythrocytes, but these may be present merely because this node represents an earlier stage in a process of disappearance or appearance. Personally, I feel quite convinced that whether found in fetal or adult material, these are decadent nodes, although I am wholly open to conviction and do not urge my own conclusion.

The mention of splenic nodules in this connection is heterodox, no doubt, at least if the term splenic is to imply the origin rather than the characteristics of nodes. I would not expect a spleen to develop anywhere, but neither would I conclude that all spleen-like nodules had their origin in the splenic anlage or the main spleen itself and are hence daughter spleens. Moreover, until we are better informed upon the origin of hemal nodes and supernumerary spleens a definite conclusion would seem to be quite unjustifiable.

#### PANCREATIC SPLEENS IN RABBITS

The occurrence of spleen-like nodules in the pancreas of cats and rabbits was recorded elsewhere (Meyer '14). Some of these nodules are completely and others only partly imbedded in the pancreatic tissue and vary in size from those barely visible to the naked eye to others three or more millimeters in diameter. Some of these intra-pancreatic spleens have no capsule what-

soever and are surrounded by pancreatic tissue, except where in contact with the capsule of the spleen itself. Others have a distinct capsule of their own. Some of these nodules contain very small amounts of erythrocytes, while others at first sight look like mere hemorrhages. They may contain lymph follicles or typical Malpighian corpuscles or may be composed mainly of a diffuse mass of lymphocytes. There may be no trabeculae, or large ones may be present. Phagocytosis was never noticed and so far I have found these pancreatic splenic nodes in cats and rabbits only.

Figure 17 gives the appearance of one of the smaller splenic islands found in the pancreas of a rabbit. A capsule is absent in this portion; only one lymph follicle is present and islands of pancreatic alveoli surround and extend into the splenic island. The latter contains little supporting tissue and is composed almost exclusively of lymphocytes and erythrocytes, including capillaries and vessels.

A small section of another nodule is shown under higher magnification in figure 18. The definite capsule, with its serous envelope and a capillary near the periphery of the node, are very evident.

The fate of the uncapsulated splenic islands is a matter upon which I can offer no evidence, although it hardly seems that they can become and remain permanent constituents of the mature pancreas. Their presence to be sure, is easily accounted for from a developmental standpoint and it is possible that their apparent greater frequency in some animals is due to specific differences in the times, or rates even, of development of the spleen and pancreas. These cases of supernumerary spleens recall the case of Rokitansky of a spleen in the *head* and of Kolb in the tail of the pancreas. The extremely interesting cases of Sneath (13) of a scrotal spleen and of de Tyssieu (14) of an intra-hepatic spleen also suggest that supernumerary spleens may apparently occur anywhere in the peritoneal cavity, although I presume one would be justified in a certain amount of scepticism regarding the identity of apparently splenic structures.

## PIGMENTED 'HEMOLYMPH' NODES

As stated previously, the occurrence of pigment in hemal nodes is a comparatively rare thing. It is common, however, in hemorrhagic lymph nodes. Some of the latter are literally crammed full of light golden and brassy pigment and some darker pigment may also be present. Most of the pigment in hemal nodes is usually extra-cellular, although phagocytosis is very evident.

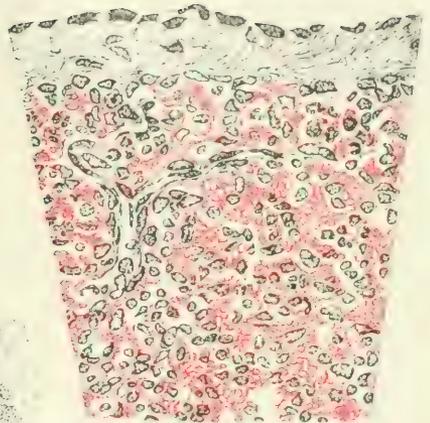
Figure 19 shows a highly magnified portion of a node, the identity of which may be open to question. This node, which was 4 to 5 mm. in size, was removed from the abdominal cavity of a sheep. It is characterized especially by the presence of sinuous masses of intensely black pigment which suggests India ink. In fact, upon cursory examination one might suppose this section to come from a hemal node which had been injected with India ink (cf. fig. 5, *The hemolymph nodes of the sheep*, Stanford University, 1914). But this is not an injected specimen at all, and the great mass of the black pigment granules in certain areas lie in capillaries in the lymphatic parenchyma. It is this gorging of the capillaries with black pigment granules which makes it simulate an injection. Nevertheless, large quantities of pigment granules are scattered about among the erythrocytes and accumulations of bright yellow pigment are also seen outside of the capillaries. Very little phagocytosis is visible in the sections of this node and the pigment gives one the impression that it is adherent to or even imbedded in the walls of the capillaries.

## 'MIXED' HEMOLYMPH NODES

The portion of a section of the node in figure 20 shows an intra-capillary arrangement of black pigment entirely comparable to that in the previous specimen. In this specimen, however, no yellowish pigment is present and the capillaries are much more perfectly outlined by the contained pigment. Since this node, which was taken from the axillary region of a lamb, was also injected, it is evident that very large quantities



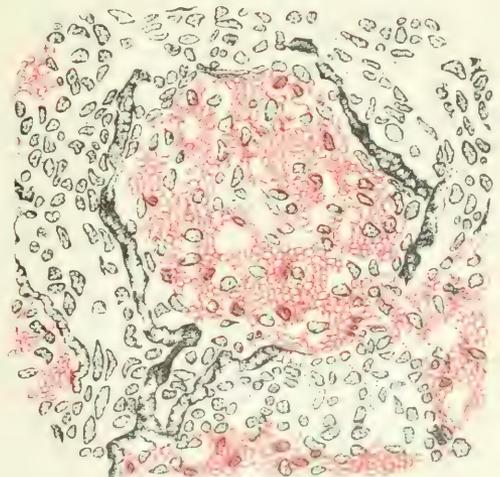
17



18



19



20

Fig. 17 Pancreatic accessory spleen from a rabbit. Camera lucida. 79

Fig. 18 Section of a pancreatic spleen from a rabbit.  $\times 410$ .

Fig. 19 Pigmented hemal (?) node.  $\times 515$ .

Fig. 20 Pigmented mixed node.  $\times 515$ .

of pigment must either have been formed within the node or been transported there. The capillaries are outlined in this manner by the black pigment throughout practically this whole large node. There are also loose pigment granules in abundance in the parenchyma but there is little evidence of phagocytosis. This node was obtained from an animal five and a half weeks old, which was killed by bleeding. Forty to fifty small hemal nodes were found in the abdominal cavity. None of these nodes were in connection with the mesenteric lymphatics although many of the lacteals which were engorged with chyle—the lamb had nursed before killing—ran about and over hemal nodes both within and at the root of the mesentery.

Many pigmented and pink nodes that were injected were found to be lymph nodes. But in the left subscapular region a large dark red node, 1 cm. in size and flattened latero-medially, was found. Except for the disposition of the vessels this node looked like a hemal node and seemed to be a mere sac of blood. It was located at the bifurcation of the subscapular vein lying between the dorsal and ventral branches. Since it was so large it was injected with India ink and as I had never found a true hemal node in this region the result of the injection did not wholly surprise me. The India ink quickly entered typical afferent lymphatic vessels, which ran to reddish nodes which lay along the course of the axillary vessels. The nearest of these axillary nodes was 4 to 5 cm. distant and the injection could be followed from node to node to the jugulo-subclavian junction. The result of the injection then would seem to indicate that this dark red node was, after all, a typical lymph node.

An anomalous result of the injection was the fact that some of the India ink easily and quickly found its way into the dorsal branch of the subscapular vein. Indeed, so much of the ink entered the axillary vein from here that some of it was later recovered from this vein by means of filter paper and identified as such. This ink had entered the axillary vein from the branch of the subscapular, which was connected with the node by a small vessel which clasped the node after the manner of the afferent lymphatics but which was filled with blood before

injection. The efferent vessel, which was also filled with blood, likewise arose by numerous branches which clasped the opposite sides of the node as they emerged from it so that the branches from these two vessels practically clasped the whole node from opposite sides.

Upon examining the right axillary and subscapular regions, a node apparently identical in all respects with that found on the left side was found in exactly the same location. Careful inspection showed that the large vessel leaving the node, which was also engorged with blood, had the typically beaded appearance of lymphatic vessels. Had it contained lymph instead of blood no one would have questioned its lymphatic character.

The short trunk (0.5 cm. long), which was formed by a number of clasping branches which emerged from the node and entered the dorsal branch of the subscapular vein, was filled with blood and also had the appearance of the short vessel in the previous specimen. This node was excised and imbedded in celloidin; figure 20 was made from a portion of a section.

The gross appearance of both nodes and the results of the injection of that on the left side seem to indicate that both these nodes were undoubtedly in communication with the subscapular vein by means of a short vessel, the branches—or radicles—of which embraced the node, and that they were also in communication with axillary and cervical lymph nodes by means of a vessel which had the form and characteristic of a lymph vessel.

That the India ink entered the subscapular vein without the least obstruction and without the least distension of the node is good evidence that the internal architecture of the node was not unduly disturbed. Those familiar with the history of the injection of the lymphatic system will no doubt be reminded immediately of the similar results obtained by the early experimenters—especially by those using mercury. Nevertheless, even the authority of such great names as those of Haller, Mascagin and Sömmering, not to mention Cruikshank, regarding lymphatico-venous communications, has been compelled in the course of time to yield to the higher authority of facts.

Even if the observations of Fohmann, Lauth and Panizza, on the occasional termination of the lymphatics in the femoral and iliac veins of birds, and the similar observations of J. Müller on amphibia and of Fohmann on the latter and on swine, remained unconfirmed, no one could disregard the recent observations of Baum, Huntington, McClure and Sylvester, made in connection with modern methods. Besides, there are the early anomalous cases in human cadavers of Conring, Duvernoy, Kaaw, Kulmus, Hebenstreit, Mertrud, and also the interesting observation of Wutzer, which was confirmed at the time by J. Müller.

Hence, it seems to me that one can hardly doubt that these two nodes really were connected with the subscapular veins in such a manner that the venous current was shunted through them and then passed through the efferent lymphatics, thus coloring the intermediate lymph vessels and nodes pink. Had there not been two nodes on opposite sides of the body, with wholly similar characteristics, one might have assumed that the point of the needle had damaged the internal architecture in such a way as to let the blood enter both a lymphatic space and a venous radicle. But even such an assumption does not explain the characteristics of the nodes before injection.

The specimen which was sectioned is completely filled with blood and lymphatic tissue. Lymph sinuses are nowhere visible, and erythrocytes and lymphocytes are intermingled except in those portions of the lymphatic parenchyma which are extended throughout the node like large trabecula, or in areas in which there are many follicles and very little blood.

Designating these nodes as 'lusus naturae' does not, to be sure, explain their presence, nor does the rare occurrence of such specimens justify one in regarding them as being representatives of a separate type of node. Nor are they the exceptions which prove the rule, although they help very materially in establishing the occurrence of atypical lymph and hemal nodes and in explaining occasional anomalous results obtained by injections of lymph nodes, as already emphasized.

## DIAPHRAGMATIC LYMPH NODE IN A RABBIT

This node, which measured  $4 \times 3 \times 2.5$  cm., was located on the central tendon of the diaphragm, ventral to the vena cava. It lay partly on the tendon and partly on the diaphragmatic musculature and projected into the abdominal cavity. It had the appearance of a lymph node but no lymph vessels were seen in its neighborhood. Upon microscopical examination it was found to be provided with a very evident but ill-defined capsule.

It is a very vascular node, contains no evident lymph sinuses but some coagulum which looks like lymph and also several degenerated areas of considerable size. Some of the parenchyma is quite definitely arranged in cords. Degenerated erythrocytes and a few pseudo-polykaryocytes are also present. Since the rabbit had been treated with goat serum it is of course possible that the areas of degeneration were partly or wholly, due to these injections. There was also an excess of serous fluid in the peritoneal cavity.

## INTRA-GLUTEAL BURSA

Two large subcutaneous bursae were found symmetrically placed in the gluteal region of the female cadaver. They were empty and measured 5 cm. in depth and 2 cm. in width. Both were located in the medial margins of the glutei maximi muscles, directly beyond the lateral margins of the ischio-rectal fossae. The skin over them was wholly normal in appearance and they were partly contained in the subcutaneous fat of these regions. The far larger portion of each, however, was contained in the glutei. These bursae were in no sense comparable in location to the ordinary subcutaneous bursae and could with entire justice be designated as intra-muscular. Their walls were very thin and smooth and contained no evidences whatever of having had an inflammatory origin. Since their genesis in this location would be a matter of pure surmise it seems futile to speculate.

## AN ANOMALOUS VAGO-SYMPATHETIC PLEXUS

Since comparatively few students do a sufficiently careful dissection adequately to reveal the sympathetic system, our knowledge of variations and developmental defects of the autonomic system lags far behind that of other systems. Furthermore, the descriptions in our textbooks are rather inadequate. In addition to greater skill and time on part of the student, a searching supervision on part of the instructor is also necessary to reveal the many variations. Besides, not everyone can be specially interested in the sympathetic system; I, for one, confess to having given it scant attention.

Both the right vagus and sympathetic are normal in the cervical region of this subject except that the vagus passes dorsal to the subclavian artery. The superior cervical ganglion is large and the middle and inferior ganglia are 1 cm. apart and are joined by two trunks. The lateral trunk of this joining loop gives off a branch 1 cm. long, which forms an annulus vertebralis instead of an annulus subclavii, as is not infrequently the case.<sup>1</sup> A fairly well-defined ganglion marks the point where the annulus vertebralis begins to form. From the latter several branches are given off and from the dorsal trunk of the annulus numerous branches extend to the common carotid, subclavian and innominate arteries. The two subdivisions of the sympathetic join directly after forming the annulus, and join the first thoracic ganglion. The latter is well-defined, although somewhat elongated, and the rest of the right sympathetic chain is normal.

About 1.5 cm. caudal to the inferior cervical ganglion of the sympathetic another somewhat stellate ganglion, about 4 mm. in size, is located. This ganglion was joined by the recurrent laryngeal branch of the vagus, which was rather small. The recurrent branch is not merely enclosed in the same sheath but definitely forms a ganglion with the sympathetic at the point of union. A somewhat larger branch than the pre-ganglionic branch of the vagus leaves this ganglion to form the inferior

<sup>1</sup> I have seen an annulus innominatus but once.

laryngeal nerve, which is accompanied by a tracheal and several esophageal branches.

Three parallel branches about 1 mm. in caliber leave the caudal portion of the ganglion and join with several similar though somewhat smaller branches, which arise from the caudal end of the dorsal arm of the annulus vertebralis to form the right pulmonary plexus; these branches are about 3 cm. long. The accompanying diagram, figure 21, illustrates these anatomical relations as found in the cadaver of a white female.

#### TRAJECTORIAL STRUCTURE IN THE SACRUM

Since it will always remain an impossibility mathematically to prove the existence of trajectorial structures in the spongiosa of

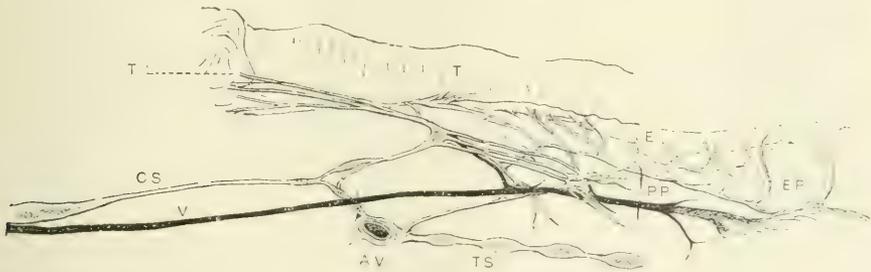


Fig. 21 An unusual vago-sympathetic plexus.

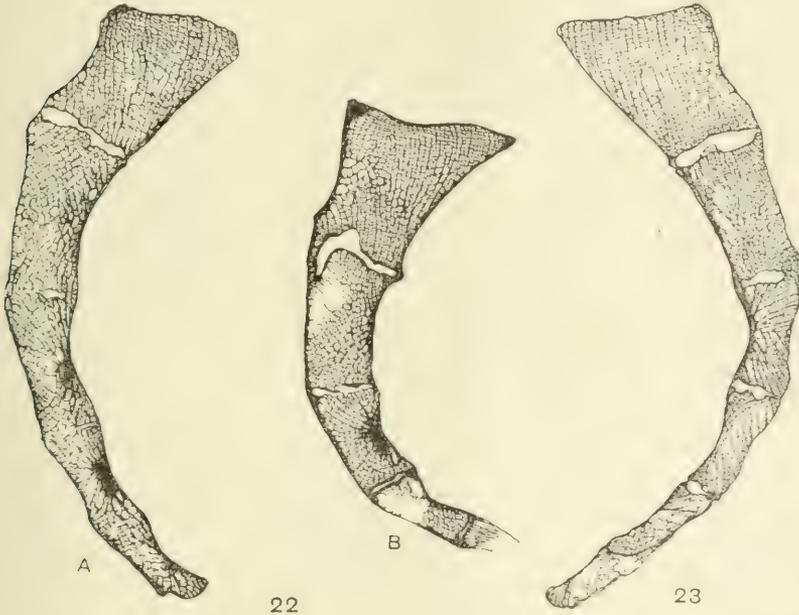
bones, their presence here and there must always remain a matter of opinion, even if not of conjecture. Although mathematical proof of the existence of such an architecture must remain impossible because of the indeterminable character and the complexity of the acting forces, a more careful examination will probably reveal the presence of such an architecture in some locations heretofore undescribed. Indeed, until the whole human skeleton has received as careful an examination as some of the bones, it is highly probable that we shall also remain ignorant of many decidedly interesting variations in the spongiosa architecture, even if not of the prevailing fundamental structural types.

During an inspection of a series of sections of laboratory remnants, my attention was attracted to some sagittal sections of sacra made in the ordinary dissecting-room routine. One of the things noticed was a prominent spur of compacta, which extended dorsally from a mid-point on the ventral surface of some of the bodies of the sacral vertebra (fig. 22). These spurs or bars, which reinforced the ventral portions of the individual sacral vertebra, were not infrequently porous and looked as though they were in a process of dorsal extension. The portions of the spurs which were not porous were extremely firm and hard, and in fact looked eburnated. None was ever found in the first sacral vertebra. In short sacra they seemed to be commonest in the bodies of the second and third, and in long sacra in the third and fourth, sacral vertebrae; they were never observed in straight sacra. Not infrequently the compacta was slightly thickened opposite the mid-points of these vertebrae and when the spurs were absent the thickening often was located at the bottom of a depression in the middle of the ventral surface.

But a more striking thing still was the existence of a very evident trabeculated disposition of the spongiosa in the third and fourth and less frequently also in the more distal sacral vertebrae. This architecture was very definite and very similar in all cases where it was evident. As indicated in figures 22 and 23, the main trabeculae were perpendicular to the ventral surface at its mid-point but there were also oblique trabeculae on either side. Other smaller and less evident trabeculae extended approximately at right angles to these.

Since such a disposition of the spongiosa was not present in all sacra examined, one is naturally tempted to speculate. But it is likely wise to defer any possible explanation until a more extensive survey can be made. It is clear, however, that the thickening of the ventral compacta, the occurrence of spurs and the peculiar disposition of the spongiosa here described, are all admirably adapted to resist the tendency of body weight in sitting and of muscle pull from breaking particularly the middle and also the lower sacral vertebrae by ventral flexion.

The illustrations are a faithful representation of the originals, though they show the characteristic architecture somewhat inadequately. From an examination of these drawings it will be evident how well-adapted such an architecture is to withstand the effect of the forces that must be active in every sacrum, even if they are not equal in magnitude or in direction.



Figs. 22-23 Sagittal sections of portion and entire human sacra, showing spurs and trajectories.

#### THE MOLDING EFFECT OF MUSCLE PRESSURE

Attention was called in another connection, to the effect of tendon pressures in affecting, even if not in determining, the relief of bones. The following three specimens of humeri—two of which I owe to the courtesy of my colleague, Professor Ophüls—seem to show the effects of muscle pressure and tension

in a striking way. In the left humerus, shown in figure 24 *a*, the whole greater tuberosity, which is decidedly enlarged by the deposit of much additional bone as a result of periostitis, is literally drawn out in the direction of pull of the spinatus and teres minor muscles. In fact, the whole bony mass looks as though it had been pulled in this direction while in a viscous condition.

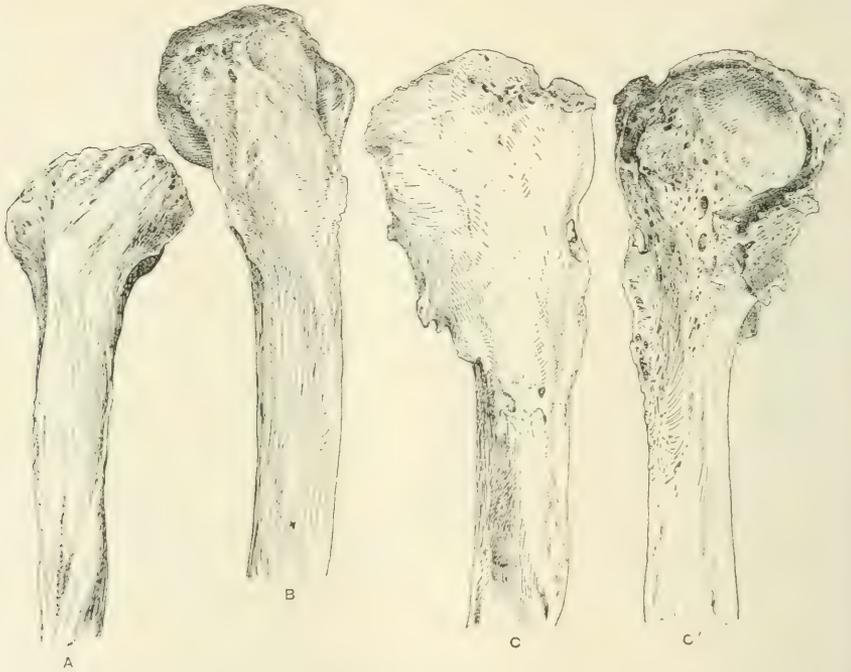


Fig. 24 Humeri showing the molding effect of muscle pull and pressure.

The lesser tuberosity and the rest of the bone are practically normal save for very slight evidences of arthritis. Aside from the slight arthritis there was nothing about the rest of the skeleton or the cadaver which suggested an explanation for this peculiarity of the greater tuberosity.

The other two humeri, shown in figure 24 *b* and *c*, were isolated specimens. The greater tuberosity of the right humerus, *b*, shows a peculiarity similar to *a*, but the deposit of new bone is

far greater in extent, and the proximal portion of the shaft of the humerus immediately distal to the head is bent medially. The humeral head and the whole lateral surface of the proximal part of the shaft are also flattened and new bone has been deposited along both tubercular ridges and on the medial side of the surgical neck; the rest of the bone is normal.

In addition to the dragging dorsally of the greater tuberosity, distinct flattening and molding of the lateral surface of the proximal portion of the shaft and of the new bone are plainly evident. This molded surface is also marked by very fine sulci, which suggest arterial impressions. Even delicate anastomoses of fine sulci are present and quite a complete network is evident under slight magnification with a reading-glass.

The tuberosities of the third specimen—the right humerus, *c* and *c'*—are in the normal location and the greater tuberosity was but slightly affected by the disease. The intertubercular sulcus is quite normal but is roofed over very largely by a thin layer of bone, which is part of the large thin mass of new bone deposited on the proximal portion of the humeral shaft. This thin mantle of new bone rises above the lesser tuberosity and hoods it and the humeral head. The outer surface of the new bone shows a canalization similar to that in the previous specimen, but it is more complete and shows sulci made by larger vessels. The molding effect of the deltoid on this new bone, is so unmistakable that a mere glance suffices to reveal it.

Since the lateral portion of the humeral head is capped by the new bone it could have articulated with a portion of the glenoid fossa only. The rest of the humeral shaft is practically normal.

An explanation of the observations recorded here seems quite impossible in the entire absence of a clinical history. Arthritic deposits rarely show such very evident molding and since the dragging dorsally of the greater tuberosity, especially in the first specimen, is not limited to the new bone, one is tempted to assume that these humeri must have been fairly plastic at some time. But the condition of the rest of the bones does not confirm such a supposition and I do not see how a greater plasticity could be confined to such a small area.

## FREELY ENDING CAPILLARIES IN HEMAL NODES

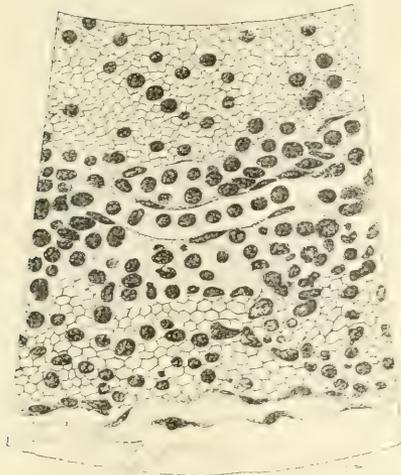
I have elsewhere expressed the opinion that capillaries in non-depleted hemal nodes communicate directly with the venous lacunae. The main reasons for coming to this conclusion were that the results of injections into the vena cava and aorta in lambs gave entirely comparable results and that capillaries could not be seen ending freely within the parenchyma of the nodes. In case of the injections the injected mass of ink could never be seen in a freely ending capillary and was always found in the venous lacunae.

In figure 25 a section of a capillary is shown, containing a number of leucocytes arranged in a row within the capillary and also several beyond the mouth of the capillary, similarly arranged. This drawing was made with an oil immersion lens and the specimen would seem to represent leucocytes either entering the free end of the capillary or passing through a gap in its wall.

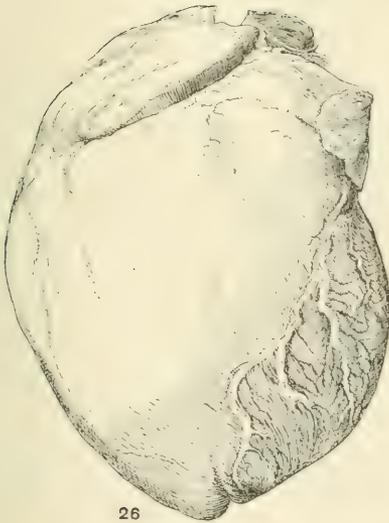
It is the only specimen I have found in a careful study of many many sections indeed and has been in my possession for some years. I report it here with some indifference since it is of questionable value. A somewhat similar arrangement of erythrocytes near a real gap in the wall of a venous lacuna, is a much more frequent thing, however, and this and other observations seem to suggest that such gaps in the walls of the lacunae occur normally in hemal nodes.

## HEARTS WITH BIFID APICES

As reported and fully set forth by Mall ('12) and the literature cited by him, hearts showing more or less of an indentation at the apex are not very rare. The two hearts shown in outline in figures 26 and 27 possess this characteristic to a varying extent, the bifurcation in that shown in figure 27, being very marked. This notch between the apices of the right and left ventricles was also much more evident at autopsy and is deeper than an outline of the exterior indicates. This heart, which was obtained from the body of a white man who had fasted absolutely for sixty days, was quite flabby and probably for



25



26



27

Fig. 25 Freely ending capillary in a hemal node of the sheep.  $\times 1050$ .  
 Figs. 26-27 Human hearts with bifid apices; half natural size.

this reason the tip of the right ventricle was turned laterally so that the gap between the two apices was wide. The specimen is otherwise normal. Since Mall ('12) explained this anomaly fully, further comment is unnecessary here.

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# EXPERIMENTAL STUDIES AIMING AT THE CONTROL OF DEFECTIVE AND MONSTROUS DEVELOPMENT<sup>1</sup>

A SURVEY OF RECORDED MONSTROSITIES WITH SPECIAL ATTENTION TO THE OPHTHALMIC DEFECTS

E. I. WERBER

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TWENTY-NINE FIGURES

## INTRODUCTION

The problem of the causes underlying defective development has recently received very exhaustive and illuminating treatment by Mall ('08). Basing his conclusions on his own study of 163 pathological human ova and on recent results of work in experimental embryology, he suggests that the human monsters are—with the exception of the hereditary 'merosomatous' terata—due to injurious influences of atypical environmental factors. He makes the specific suggestion—which seems justified in the light of evidence brought forth by him as well as by clinical data—that the monstrous development of some ova may be due to their inadequate nutrition owing to the imperfect implantation in a diseased uterus. It would seem that this hypothesis may hold good at least for some pathological embryos aborted during the first two months of pregnancy. It is obvious, however, that Mall's interpretation could not be extended to monstrous fetuses of the later months of pregnancy or to monsters after full-term birth; for an ovum suffering from lack

<sup>1</sup> This contribution is based on a paper read by title ("Is defective and monstrous development due to parental metabolic toxemia?") at the meeting of the American Association of Anatomists held at St. Louis, December 29, 30, 1914. *Anat. Rec.*, vol. 9, no. 1, pp. 133-137.

of nutrition could not well be imagined to live on to these later stages of development.

Some environmental factors must be looked for other than faulty implantation of the ovum, to account for the occurrence of such cases. The results of investigations in experimental embryology and teratology by Dareste ('91), Roux ('95), Hertwig ('96, '10), Féré ('94), Morgan ('02, '04) Stockard ('07, '09) and others, who obtained monstrous development of ova which had been subjected to the action of physical or chemical modifications of the environment, suggested to me that the human as well as the other mammalian monsters may be due to the physical or chemical action of some substances in the blood of one of the parents on either one of the sex cells or on the fertilized ovum, respectively.

Stockard ('09) has expressed the unsupported view that cyclopia in man may possibly be due to an excess of magnesium salts in the blood of the mother, and in later publications ('10 b), concluding from his experiments with alcohol solutions, he suggests that the cyclopean defect might perhaps be attributed to alcoholism of either one of the parents. It is rather evident that this latter assumption may be regarded as justified only for a negligibly small percentage of monsters. For such defects are not infrequently found in mammals as well as in other vertebrates where the possibility of alcoholism is entirely eliminated. The solution of this problem appeared to me to be elsewhere. Since the metabolism is the main source to which chemical modification of the body might be traced, I concluded that the toxic substances found in the blood of individuals afflicted with some metabolic disturbances might be the ones which could be made responsible for the origin of monstrous development.

#### MATERIAL AND METHODS

To test the validity of this hypothesis it would be necessary to produce experimentally in mammals such disturbances of metabolism as diabetes, nephritis, jaundice, etc., to mate thus diseased animals and eventually to study the heredity of such offspring as they may beget. Such experiments, however, are

difficult to perform in the absence of certain facilities, which are not usually accessible to the biologist. On the other hand, the spontaneous occurrence of these diseases in animals is too rare to permit of conclusive breeding experiments. It was necessary, therefore, to confine myself to a preliminary step in the investigation. This consisted in subjecting eggs of an oviparous vertebrate to the action of some toxic substances found in the urine under certain pathological conditions of metabolism.

The eggs of *Fundulus heteroclitus* were chosen, and after being fertilized they were exposed in early (1 to 2, 2 to 4, or 4 to 8, and 8 to 16, cells respectively) cleavage stages to the influence of solutions in sea-water of such substances as urea, butyric acid, lactic acid, acetone, sodium glycocholate, ammonium hydroxide, etc. Only with two of these substances, viz., butyric acid and acetone, have so far definite and positive results been obtained.

In the case of butyric acid, 10 cc. of a  $\frac{1}{12}$  to  $\frac{1}{14}$  gram molecular solution in 50 cc. of sea-water was found to be approximately the optimal solution, i.e., causing the greatest number of eggs to develop in a defective manner. If fertilized *Fundulus* eggs were left in this solution for fifteen to twenty hours and afterwards transferred to pure sea-water a great number of them gave rise to monsters. Similar results were obtained with solutions of acetone. Here the eggs were exposed in a number of dishes to the action of solutions of 20, 25, 30, 35, 40, 45 and 50 cc. of a gram molecular solution of acetone added to 50 cc. of sea-water. A varying relative number of deformed embryos was found in all dishes, increasing with the strength of the solution up to 40 cc. of acetone and decreasing in solutions still stronger, which caused an increase in the death-rate of the eggs. The length of exposure to the action of acetone was forty-eight hours in most and twenty-four and seventy-two hours respectively in some experiments. It was found that while long exposures increased the mortality of the eggs the difference in effect on the surviving eggs was very slight between exposures of twenty-four and those of forty-eight or even seventy-two hours. This points to the probability that it is mainly the initial effect

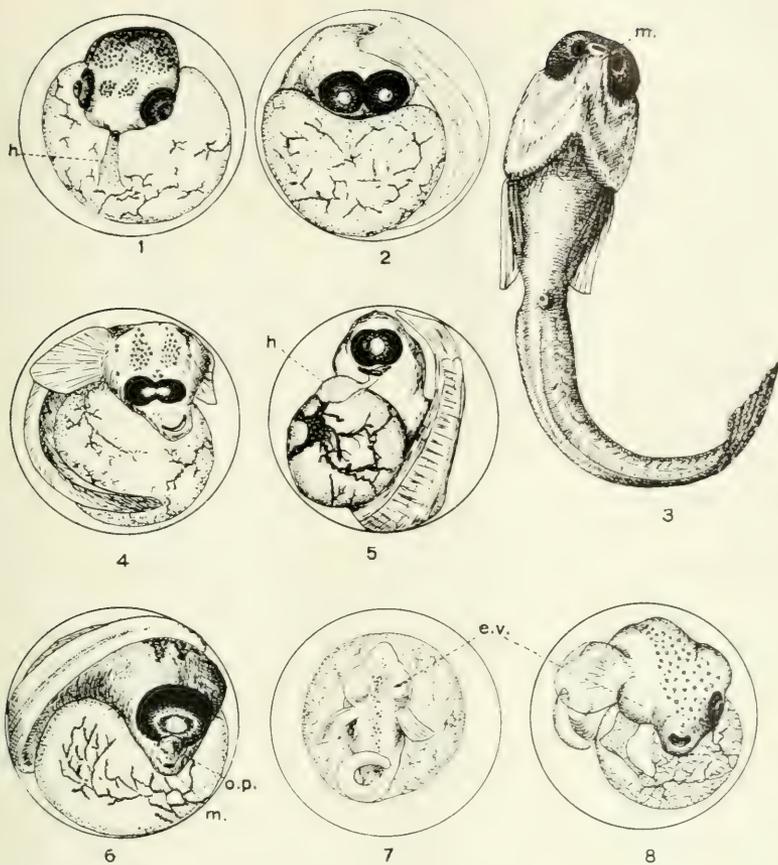
of the toxic solution on the ovum that causes it to develop in an atypical manner. The eggs were fixed and preserved after Child's sublimate-acetic-formaline method and were left in 4 per cent formaldehyde until the time of their imbedding. They were imbedded in celloidin-paraffin, chloroform being used as a clearing agent. Sections were cut 6 or 7 $\mu$  thick, stained with Delafield's hematoxyline and counterstained with erythrosin.

#### SURVEY OF THE MORPHOLOGICAL RESULTS

##### 1. *Deformities of the sense organs and the mouth*

The morphological results obtained in both series of experiments, with butyric acid and acetone, being very much alike, it will suffice to state that the deformities enumerated here were found to be common to both. Great numbers of cyclopean embryos were found in both these series of experiments. I have recorded in my observations the occurrence of transition from two normal eyes in the typical position in the head all the way down through the more or less closely approximated eyes or eyes of a double composition and true cyclopia to complete anophthalmia (figs. 1-7) as described by Stockard ('09) in his experiments with magnesium chloride and alcohol, and by Lewis ('09) in his pricking experiments. Other defects in the development of the eyes such as asymmetric monophthalmia (fig. 8) and microphthalmia (fig. 9) were also found to occur abundantly. In some embryos all that could be detected of the eyes were lenses only or a rudiment of the choroid coat with or without a rudimentary lens. Coloboma, by which is understood a patency of the embryonic fissures of some parts of the eye, was found in some cyclopean as well as in two eyed but otherwise defective embryos.

Stockard ('09) has also described a peculiar change in the form and position of the mouth of the cyclopean embryo. The mouth in such embryos has the appearance of a snout, a proboscis-like structure, and is pushed down below the cyclopean eye. This displacement into the ventrolateral position Stock-



Figs. 1-29 Sketches of monstrous *Fundulus* embryos.

Fig. 1 Normal *Fundulus* embryo, twelve days old, to show the position of the eyes; *h.*, heart.

Fig. 2 Synophthalmia bilentica, from  $\frac{1}{16}$  gram molecular butyric acid, eighteen days old.

Fig. 3 Synophthalmia bilentica, from  $\frac{1}{12}$  gram molecular butyric acid, twenty-eight days old, with 'proboscis'—mouth, *m.*

Fig. 4 Synophthalmia bilentica, from  $\frac{1}{12}$  gram molecular butyric acid, twenty-eight days old.

Fig. 5 Synophthalmia unilentica, from  $\frac{1}{12}$  gram molecular butyric acid, twenty-four days old.

Fig. 6 Cyclopean embryo, showing one unusually large median eye with fused olfactory pits, *o.p.*, and proboscis-like mouth, *m.* From  $\frac{1}{12}$  gram molecular butyric acid, eighteen days old.

Fig. 7 Anophthalmic embryo with club-tail and distended ear vesicles, from acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), thirteen days old.

Fig. 8 Asymmetrically monophthalmic embryo, with greatly distended ear vesicles, *e.v.*, one pectoral fin only and club-tail. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), thirty-two days old.

ard attributes to the circumstance that the cyclopean eye being frontally located has caused the mouth to move downward. This interpretation, however, seems to be insufficient in view of the fact that I have observed in my experiments this occurrence not only in cyclopean monsters (fig. 6) but also in some cases of synophthalmia (fig. 3), asymmetric monophthalmia (fig. 8) and in many cases of dorsal microphthalmia (fig. 9). In some asymmetrically monophthalmic embryos (fig. 29) the mouth, while being apparently normal in shape, occupied the position in which normally the lacking eye would have to be located.<sup>2</sup>

Abnormalities of the olfactory pits were also almost invariably found to occur in embryos exhibiting various degrees of median 'cyclopia' (fig. 6) as well as in asymmetrically monophthalmic embryos (fig. 20). They usually corresponded to the anomalies of the eyes of a given embryo, that is, were either blended into one median pit or exhibited various degrees of approximation or fusion respectively in the cyclopean embryos. In the asymmetrically monophthalmic embryos where the mouth had taken the position of the missing eye, the nasal pit of the side possessing the eye was usually found to be in the normal position, while the pit belonging to the side lacking the eye has sometimes been found to be located posterior to the mouth. This unilateral ectopia of the nasal pit in the monophthalmic embryo is probably secondary to the ectopia of its mouth. These changes in shape and position of the mouth as well as of the olfactory pits are apparently due to processes of regulation after an blastolytic destruction of a certain area at the anterior end of the early embryo's body.

In a great many embryos the auditory vesicles reached enormous size, which on microscopic examination seemed to be due

<sup>2</sup> In a previous note ("The influence of products of pathologic metabolism of the developing teleost ovum," *Biol. Bull.*, vol. 28, no. 1, pp. 51-57), it was stated (p. 54) that in some cases of asymmetric monophthalmia an open orbit was found on the side lacking the eye. This error was made owing to the transparency of the living specimens. It was the mouth in the exact position of the eye that was mistaken for an 'open orbit.' The error was found when the drawings of the living embryos were compared with the fixed specimens.

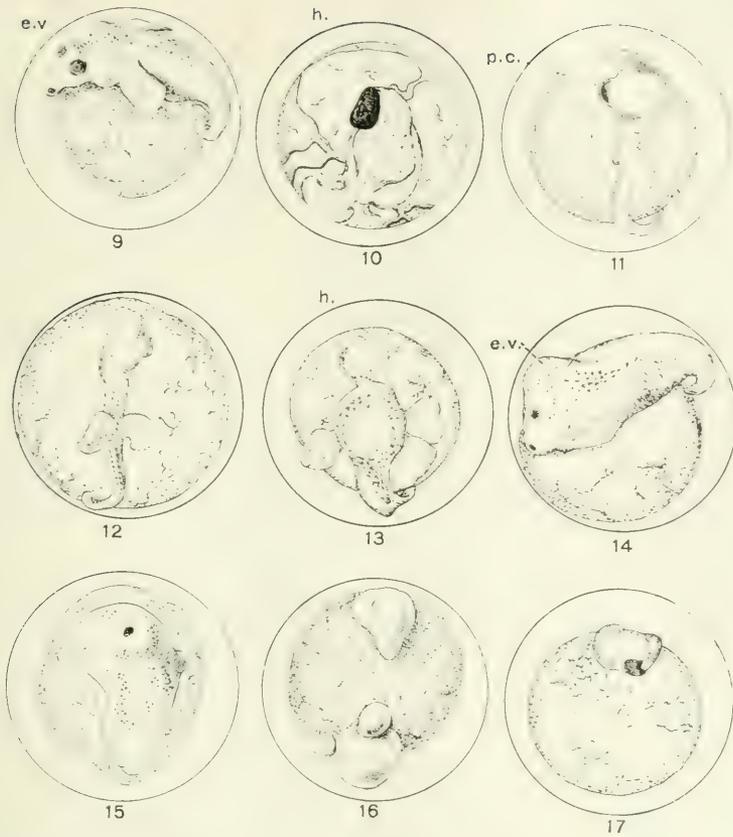


Fig. 9 Microphthalmic embryo (small eyes dorsally located), with distended ear vesicles, *e.v.*, From  $\frac{1}{16}$  butyric acid, twenty-nine days old.

Fig. 10 Greatly malformed cyclopean embryo with dorsally located eye, rudimentary pectoral fins and club-tail, from  $\frac{1}{12}$  gram molecular solution butyric acid, thirteen days old.

Fig. 11 Greatly malformed embryo from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water) with one rudimentary lateral eye, without fins, club-tail; *p.c.*, distended pericardial vesicle.

Fig. 12 Extremely malformed anophthalmic embryo from acetone solution (20 cc. gram. molec. sol. to 50 cc. sea-water), fourteen days old.

Fig. 13 Greatly deformed anophthalmic embryo, with head partly constricted off from the rest of the body. From acetone solution (30 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

Fig. 14 Extremely malformed, oedematous embryo, with one rudimentary lateral eye, distended ear vesicles, *e.v.*, with club-tail and without pectoral fins. From  $\frac{1}{2}$  gram molecular butyric acid, fourteen days old.

Fig. 15 Amorphous embryo from acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

Fig. 16 Egg with amorphous tissue fragments on yolk-sac, from acetone solution (30 cc. gram molec. sol. to 50 cc. sea-water), thirteen days old.

Fig. 17 Meroplastic embryo with rudimentary eyes, from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), twelve days old.

to an oedematous distension (figs. 7, 8, 9, 14). In some monophthalmic embryos which had hatched a similar observation was made to the one recorded by Stockard ('09) in his experiments. These embryos "swam in circles, often whirling around with great rapidity, much as Japanese waltzing mice do. Others swam in irregular spirals and only progressed in a straight direction with difficulty." In most of them I observed that when forced to swim in a straight forward direction they would immediately drop to the bottom of the dish, while they were able to swim for some distance along the wall of the finger-bowl in which they were kept. Stockard attributes this functional anomaly to "a defective muscular arrangement, the animal's body being slightly bent or twisted so that it is unable to straighten perfectly." From my own observations and Stockard's ('10 a) microscopic findings I am rather inclined to think that in these embryos—at least in my own experiments, if not also in those of Stockard's—the locomotor anomaly is due to defects in the semi-circular canals.

## 2. *General defects; 'meroplastic embryos'*

Besides the already referred to deformities of the sense organs there were found in both butyric acid and acetone solutions great numbers of embryos which developed in a much more defective manner, the malformation involving practically the entire bodies. Thus curiously deformed dwarfs with vestigial eyes or blind, or slender, elongate, greatly malformed embryos (fig. 11) often with waistlike constrictions (figs. 12 and 13) were of not infrequent occurrence. A considerable number of eggs were recorded with amorphous embryos (fig. 15) or only amorphous fragments of tissue (fig. 16) on the yolk-sac. The amorphous embryos would correspond in homology to those found in man and described by Mall (l.c.) and others. On microscopic examination of several amorphous embryos it was found that the nervous system, the notochord and the viscera while having developed, are highly abnormal and rudimentary in structure. The sections present pictures similar to those of

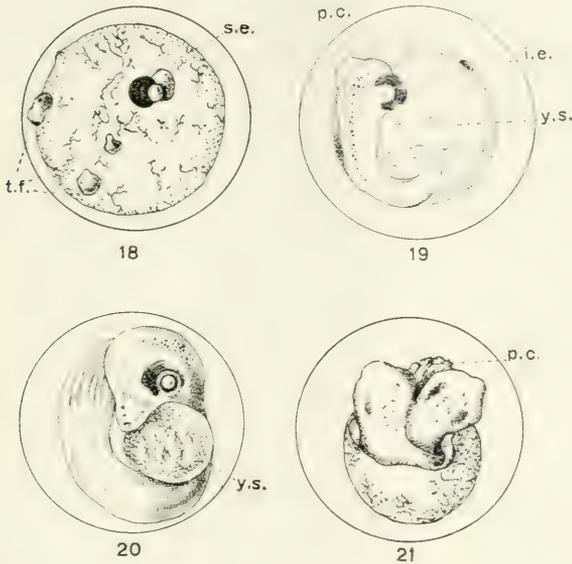


Fig. 18 Egg with 'solitary eye,' *s.e.*, and three very small clastolytic tissue fragments, *t.f.*, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 19 Asymmetrically monophthalmic embryo, with club-tail, without pectoral fins. On the yolk-sac at a distance from the embryo is seen an 'isolated eye,' *i.e.* From acetone solution (40 cc. gram molec. sol. to 50 cc. sea-water), 12 days old; *p.c.*, pericardial vesicle, *y.s.*, yolk-sac.

Fig. 20 Asymmetrically monophthalmic embryo with 'proboscis'—mouth, without pectoral fins. From  $\frac{1}{2}$  gram molecular butyric acid, twenty-eight days old.

Fig. 21 Duplicata anterior; both components are anophthalmic. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old.

sectioned amorphous fetuses of man. The amorphous fragments may be compared to the 'nodular forms' of Mall which he found in some aborted human ova.

By far the most numerous were found to be eggs in which only a part of the body (fig. 17) had developed—'meroplastic embryos' (Roux, *l.c.*). In these the hind parts of the bodies were missing to a greater or lesser extent, while the anterior parts were often extremely deformed, their shape being much distorted and only more or less rudimentary eyes being present. Many of them in

which an anterior half of the body had remained would belong to the class designated by Roux (l.c.) as 'hemibryones anteriores,' which he and subsequently other investigators found to develop from the frog's egg if one of its first two blastomeres was punctured with a hot needle.

The other cases of meroplastic development concern embryos in which either more than the anterior half of the body had developed, or less. They range all the way from those in which hardly much more than the tail was missing to those in which only a very small anterior part of the head (usually with one or sometimes with two vestigial eyes) was present.

### *3. Independent development of an eye from a fragment of the medullary plate*

By far the most curious and most significant of all the meroplastic ova recorded in these experiments were some in which all that was left of the embryo was a fragment of brain tissue with a solitary eye. The fragment of brain tissue was in some cases somewhat larger than the solitary eye to which it has given rise, while in others it was smaller. In one of these eggs (fig. 18) in which the solitary eye was rather defective—the chorioid fissure being patent—several small amorphous fragments of tissue could be observed at different points of the yolk-sac at a considerable distance from each other. Since I have observed such fragments of tissue on many other eggs in which either a defective embryo or nothing else besides the amorphous fragments had developed, I am inclined to think that such cases give us a clue as to what processes may be involved in bringing about the effects recorded in this work.

The 'solitary eyes' when observed in the living egg had the typical appearance of eyes and no doubt could be felt that the interpretation of these sporadic cases was correct. However, as no other similar case was known, it seemed rather improbable that a small fragment of the medullary plate would be able to go on developing independently so far as to give rise to such a complex organ as the eye. The possibility suggested itself that the 'solitary eye' of such an egg may be connected with an

embryo which had sunken in the yolk-sac leaving the eye on the surface. The main objection to this would, of course, be that such an egg would die in a very short time, for the embryo could not possibly receive a sufficient oxygen supply; and the death of such a sunken embryo would soon cause autolysis of the ovum. More convincing proof seemed to be furnished by the fact that the yolk-sacs of the eggs, owing to the method of fixation, were translucent, while the embryo had turned white and opaque. If an embryo had sunken into the yolk-sac, it could thus easily have been detected. But, in spite of very careful examination of the ova with solitary eyes, not a trace of an embryo could be seen within their yolk-sacs. Moreover, in order to establish this fact of the independent development of the eye on the firm basis of unmistakable evidence, I sectioned one of these eggs. I have purposely chosen the egg in which besides the solitary eye several (some three or four) very small fragments of tissue could be observed on the yolk-sac, thinking that the latter ones might possibly offer a basis for the interpretation of the morphogenesis of the solitary eye.

The microscopic examination (fig. 22) of the sectioned egg proved that my interpretation of the case as that of a 'solitary eye' was perfectly correct. In the sections it can be plainly seen that the blastoderm had overgrown the entire yolk-sac just as this takes place in normal development. But no embryo can be seen in the yolk of any of the sections. On the yolk-sac besides the solitary eye there can be seen on some sections the fragments of tissue mentioned above, one of which makes the impression of a defective spinal cord and when followed out in successive sections is seen to give rise to the eye in question. Some blood vessels have also developed in a very abnormal fashion and no heart is present. Of the other amorphous tissue fragments one proved to be another eye, although very rudimentary in structure. The nervous elements of this second eye are obviously greatly inhibited in development, neither a choroid nor an iris are present, but the cornea and the lens have developed to a degree permitting of safe identification. The distance between the position on the yolk-sac of this eye

rudiment and that of the well developed solitary eye being as much as  $262\mu$  it seems evident that a blastolytic fragmentation of the early embryonic material and a subsequent shifting of the fragments to distant parts of the yolk-sac's surface has occurred.

Two more eggs with solitary eyes were cut into sections and a microscopic examination again confirmed the correctness of their being interpreted as such.

Several eggs were also found with asymmetrically monophthalmic and otherwise malformed embryos in which a completely isolated tissue fragment with a well developed eye could be seen on the yolk-sac at a distance from the embryo which made its connection with the same appear impossible. One of these eggs was sectioned and the microscopic examination revealed the fact that the isolated eye was in no connection whatsoever with the embryo.

In view of these findings no doubt can now be felt that the 'solitary eye' (embryone absente) and the 'isolated eye' (embryone praesente) offer ample evidence of the fact that a very small fragment of the medullary plate may be able to develop independently of the rest of the embryo's anlage far enough to give rise to such a complex organ as the eye. *This is, as far as I am aware, the first case on record of the independent development ('self-differentiation,' Roux) of the eye.*

#### *4. Defects of the brain*

The destructive influence of butyric acid and acetone on the developing egg of *Fundulus* manifests itself also in the severe injuries sustained by the central nervous system. In teratophthalmic embryos the brain was in most cases found to be abnormal to a greater or lesser degree. The forebrain may often be unpaired while the mid- and hind-brain of the same embryo are bilateral in symmetry. The hemispheres of one brain may often differ considerably in size and shape as well as in other respects. Thus, e.g., there may be seen a striking developmental inhibition in one hemisphere where most of the

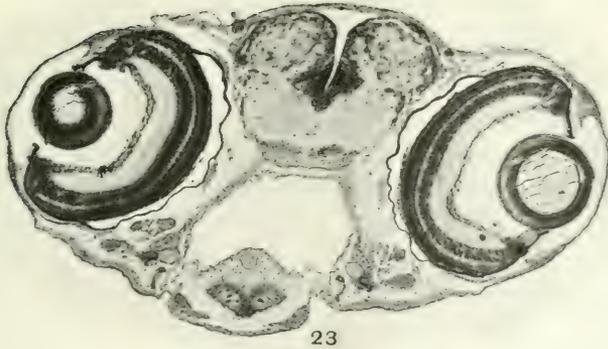
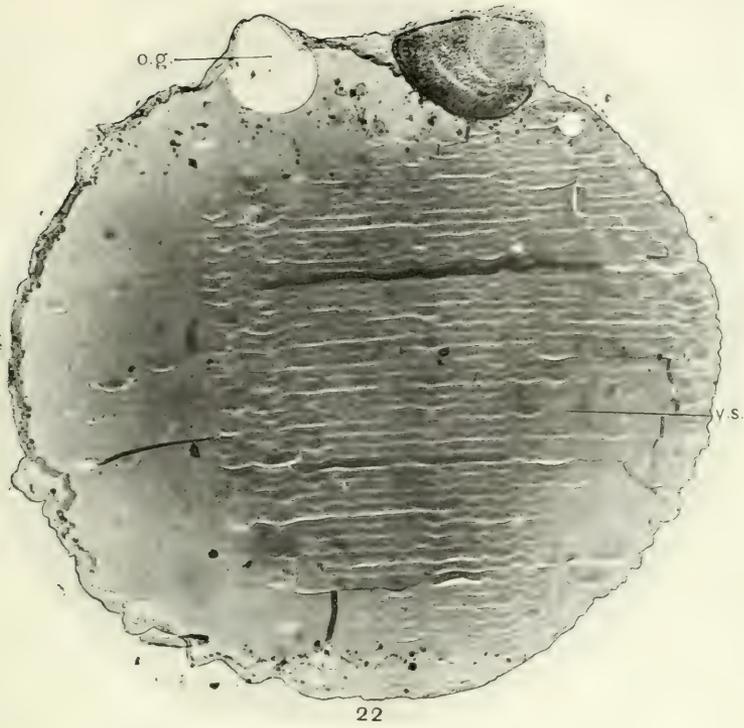


Fig. 22 Photomicrograph of section of egg with 'solitary eye,' (Cf. fig. 18); *y.s.*, yolk-sac, *o.g.*, oil globule space.  $\times 80$ .

Fig. 23 Photomicrograph of transverse section through the eye region of a normal *Fundulus* embryo, fourteen days old, one day after hatching.  $\times 80$ .

nervous elements had not proceeded beyond the neuroblast stage, while the other hemisphere may be made up of apparently well developed nerve cells and fibers. It was also often observed both in the brains and the spinal cords of teratophthalmic or otherwise defective embryos that wherever the neuroblasts failed to differentiate into nerve cells a great many large, clear and empty tissue spaces (figs. 26 and 27) were present. The probability suggests itself that these spaces in the living embryos may have been filled with body fluid. For, the heads of some defective embryos when observed in the living or even the preserved specimen were distended to a degree suggesting oedema. As was mentioned before, the same is true also for the ear vesicles. On microscopic examination it can invariably be seen that the usual size of the latter ones is due to (an apparently oedematous) distension of the semi-circular canals. A condition of hydrops is thus produced in the embryo which (since the fish brain has neither lateral ventricles nor a choroid plexus) might perhaps be considered as homologous with the internal hydrocephalus of man. Furthermore, the cranial (figs. 25 and 26) cavity may be unusually large, often containing some fibrin. Both this condition and the intracerebral oedema may often be present in the same embryo. Genetically these dropsical conditions probably are due to an arrest in the development of the blood or lymph vascular system.

##### *5. Defects of the blood vascular system*

There is a very wide range of variation in the deformities to which this system is subject. The heart is almost perfect in some embryos in which cyclopia is the only superficially noticeable defect. In more extremely malformed embryos, however, the heart may be only an exceedingly delicate, straight tube in some embryos, while it may be absent altogether in others. It is interesting to note in this connection that while acardia was frequently observed in eggs in which a whole although malformed embryo had developed, a heart, though usually more or less of a rudimentary structure, but functioning, could be

found in some eggs in which only meroplastic embryos or no embryo at all had developed.

While in some embryos the heart can be plainly seen to be connected with the great vessels of the embryo and indirectly with the larger vessels of the extraembryonic area, no such continuity exists in most of the deformed embryos which develop without a complete circulation, or, often without any circulation whatsoever. To J. Loeb ('93) belongs the credit for this remarkable discovery, which is now corroborated by Stockard's ('15) and my own findings. The blood vessels of the yolk-sac may sometimes be present in the form of irregular, dense, apparently continuous networks, or in some cyclopean embryos they may approach in pattern and size the normal blood vessels, while in cases of more extremely malformed embryos only blood islands may be seen scattered in the yolk-sac. Yet blood vessels are usually found in such cases in the embryo itself. It may well be said that only few embryos which develop in butyric acid and acetone solutions, while possessing blood vessels, have a circulation continuous with the extraembryonic area.

This accounts for the interesting fact that very often large lacunae filled with erythrocytes are seen in the bodies of some monstrous embryos as well as in the extraembryonic areas. These lacunae are very striking at the first glance at a living ovum on account of the bright red color of the erythrocytes. Another observation which was first recorded by Stockard ('15) and which I have also frequently made is that a large mesenchyme space, usually in the head region or sometimes in other parts of the body, may frequently contain many leucocytes of apparently the polymorphonuclear variety (fig. 24). The elements of the blood which come from different sources are thus seen to be isolated due to absence of a continuous system of circulation. The bearing of these data on the problems of vasculogenesis and haemogenesis is evident and I expect to discuss it elsewhere.

That these data may also have an important bearing on the genesis of some forms of hydrocephalus has already been pointed out above. It is easy to imagine that even in man a local in-

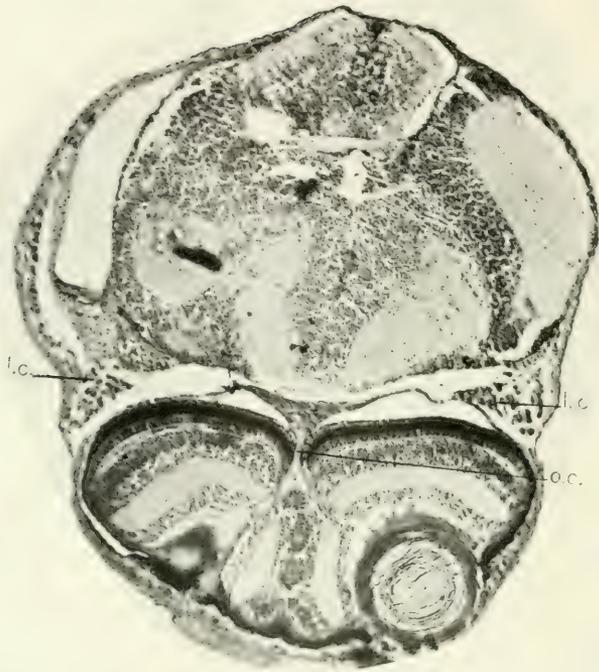


Fig. 24 Photomicrograph of a transverse section through the eye region of a monstrum synophthalmicum bilenticum (Cf. fig. 2); *l.c.*, leucocytes, *o.c.*, optic chiasma.  $\times 100$ .

hibition in the development of the blood and lymph vessels in the head region may result in accumulation of fluid in some parts of the head, even in the lateral ventricles of the brain due to lack of adequate drainage. Careful anatomical investigations of some well diagnosed cases of hydrocephalus may possibly bear out the validity of this assumption.

A case described by Smith and Birmingham ('89) may be of interest in this connection. They report a fetus aborted during the fifth month of pregnancy with general oedema due to complete absence of lymphatic vessels. On microscopic examination of the skin and subcutaneous tissue they found in the sections large spaces, "in some parts clear and empty, in others filled

with a colloid material, evidently coagulated lymph." From their appearance and position they concluded that they were dealing with greatly distended lymph spaces, the overdistension being the result of the absence of a lymphatic drainage system.

#### 6. *Deformities of the appendages*

One of the most frequently found malformations concerns the fins. Both the pectoral and caudal are very often rudimentary (figs. 7, 9 and 10) and diminutive in size. In some deformed embryos the pectoral fins or all fins may be absent altogether (figs. 11, 14, 19 and 20) and the tail in such embryos is club-shaped. I have also often recorded embryos in which only one pectoral fin was present (fig. 8) while only one embryo was found with three pectoral fins. One is reminded by these deformities of the fish's appendages of well known cases in the human being with which they seem homologous. The club-foot in the human fetus and congenital absence of upper limbs in man have often been found. Likewise cases of supernumerary upper limbs have been recorded in man as well as in other mammals.

The fact that such defects can be produced in the fish by chemical action suggests that in mammals they may be due to like causes.

#### 7. *Duplicities*

The tendency of butyric acid and acetone to produce twins seems to be only slight for I have observed only a few such cases. I have recorded only one case comparable to the 'Siamese twins' type of the human. In this egg two deformed embryos with a common heart had developed on opposite sides of the egg. Some other cases of twin formation found in these experiments belong to the type known as 'duplicitas anterior' (fig. 21) which Speeman ('04) produced experimentally by tying a ligature around the fissure between the first two blastomeres of the amphibian egg. Several cases of duplicity have also been recorded, the nature of which I expect to ascertain by microscopic examination.



A few illustrative examples will now be given, after which a discussion of the morphogenetic factors of teratophthalmia will be taken up.

Figure 5 represents a case of unilentic synophthalmia. The egg was subjected between the second and third cleavages,—i.e., about  $2\frac{1}{2}$  hours after insemination—to the action of 10 cc. of  $\frac{1}{10}$  gram molecular solution of butyric acid for twenty hours and then transferred to pure sea-water. Already on superficial examination the composite structure of the eye is easily recognized. It is seen that the approximation of the two eye components is so close as almost to give the appearance of perfect cyclopia. Transverse sections (fig. 26, p. 549) show that the eye is composed of two incomplete optic cups facing each other and enclosing a single lens of about the usual size. The cornea and iris are normally developed and the retinal layer is well differentiated. Two optic nerves are seen to pass out of the eye in a few loose bundles of fibers and, after having formed a chiasma, to enter the opposite sides of the brain.

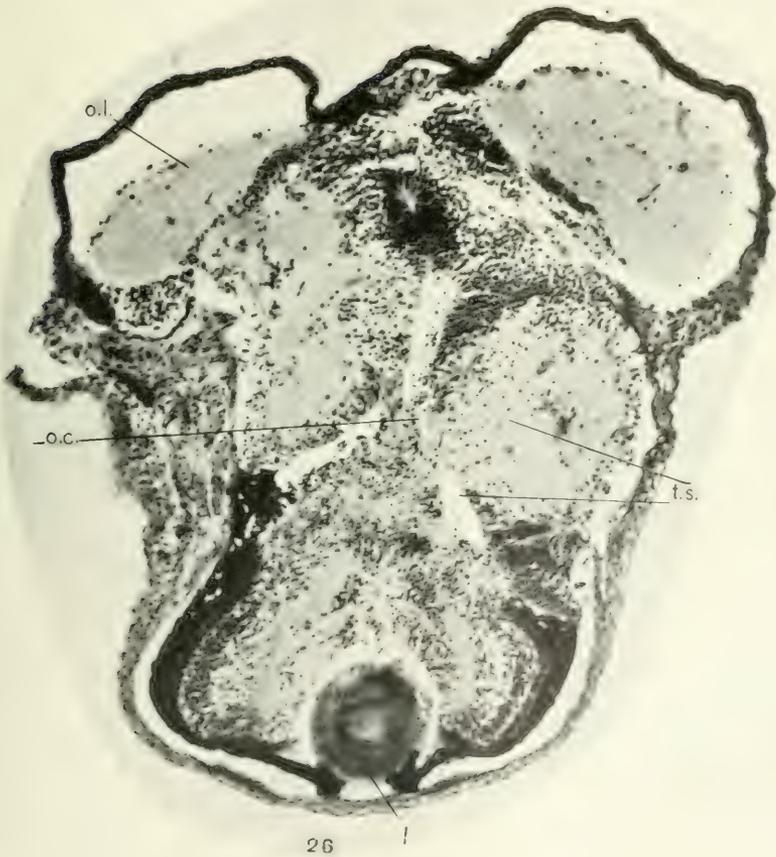
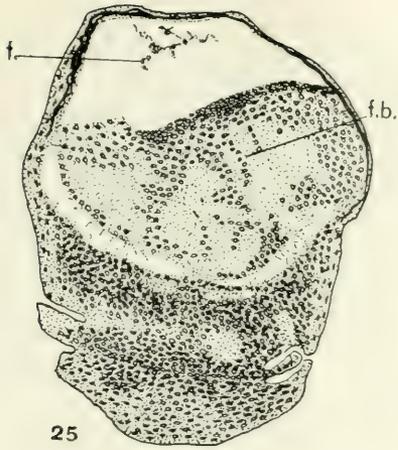
The incompleteness of the fused optic cups is probably due to the circumstance that at a very early stage of development a large part of the ophthalmoblastic material had been eliminated from development owing to the chemical alteration caused by the butyric acid. The injury sustained by the embryo must apparently have been the severest at the most anterior point of the main body axis, diminishing gradually posteriorwards. The following data seem to substantiate this interpretation. The forebrain is unpaired (fig. 25) and the rest of the brain is when followed in successive sections posteriorwards seen gradually to present more and more distinctly the condition of bilateral symmetry. The midbrain and hindbrain while being bilateral, exhibit, however, a certain other abnormality. The injury here was apparently mainly restricted to the blood and lymph vessels, the earliest anlagen of which seem to have been arrested in their development. This condition can be recognized by the great number of large, clear and empty spaces (fig. 26) in the tissues of the posterior parts of the brain, which in the living embryo have apparently been filled with fluid owing to

the existing imperfection in the circulation. A condition of oedema has thus apparently resulted from lack of drainage. No other abnormalities of these parts of the brain or any other part of the embryo can be seen, which makes it appear very probable that the anterior part of the embryo body is the most sensitive one and thus subject to the highest degree of injury.

In figure 2 is seen an eighteen days old embryo exhibiting the condition of 'synophthalmia bilentica.' This egg had been subjected to the action of 10 cc. of a  $\frac{1}{16}$  gram molecular solution of butyric acid in 50 cc. of sea-water for twenty hours, after which time it was transferred to pure sea water. In the specimen *in toto*, the eyes, while being very close together, could not be considered as fused. On microscopic examination, however, it was found that the eyes were fused more posteriorly (fig. 24, p. 544) and that the fusion is the more intimate the more posterior is the section examined. If all sections be examined it can be clearly seen that the highest degree of the injury sustained by the egg due to the treatment is in the most anterior part of the embryo's body, i.e., in the region of the eyes. The abnormalities found in this part of the body besides the fused eye involve also the olfactory pits, which are so closely approximated as to be partially fused, and the forebrain, which is unusually small in size and unpaired. The transverse sections of this embryo are somewhat oblique and the double eye being somewhat asymmetrically located in relation to the chief body axis, in the figure the lens of one eye can be seen to be sectioned about midway while of the other lens the most posterior part is cut. The two lenses, however, can be clearly made out in the figure. The choroid coats which are imperfectly developed and the well differentiated

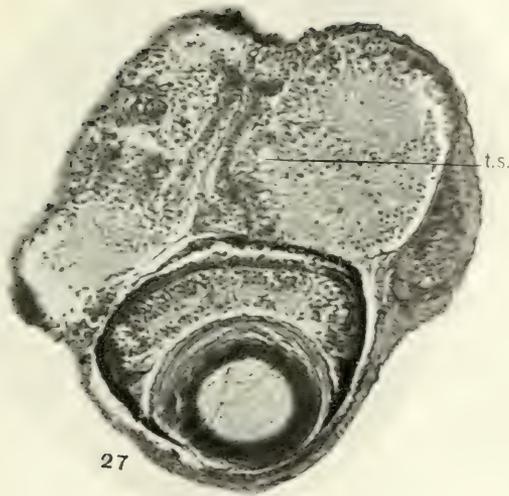
Fig. 25 Camera lucida drawing of a transverse section anterior to the eye region of a monstrem synophthalmicum unilenticum (Cf. fig. 5), showing unpaired forebrain, *f.b.*, greatly enlarged cranial cavity occupied by some fibrin, *f.*  $\times 140$ .

Fig. 26 Photomicrograph of a transverse section through the eye region of the same embryo as in figure 25, showing the two components of the synophthalmic eye, facing one another, with one lens, *l.* Many tissue spaces, *t.s.*, are seen in the brain. The cranial cavity in the region of the optic lobes, *o.l.*, is greatly distended, *o.c.*, optic chiasma.  $\times 160$ .

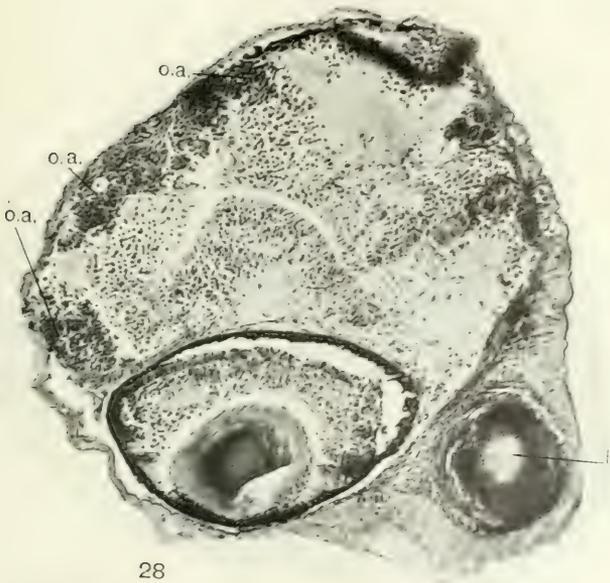


retinal layers are seen to be perfectly coalesced ventrally, while dorsally they arch inwards to leave an opening for the optic nerves. The latter come as separate bundles of fibers from each one of the eye components and fuse into one trunk just at the point of emerging from the double eye. This common optic nerve trunk is in other sections seen to enter only one hemisphere of the brain. The cavity between the sclera and the brain has widened out and is on both sides near the head integument occupied by large leucocytes of apparently the polymorphonuclear variety. These leucocytes are seen in all sections of the embryo densely filling spaces between the tissues or they may also be found more scattered in the mesenchyme. The blood vessels of this embryo as well as of its extraembryonic area are rather scarce, and the discontinuity of the latter ones being very striking, the suggestion is at hand that the circulation of the embryo was imperfect. This would account for the accumulation of white blood corpuscles in the mesenchyme as well as for the numerous apparently oedematous interstices which they often filled. Summarizing the abnormalities found in this embryo, it may well be said that the degree of injury sustained by it, being highest in the immediate region of the eyes, diminishes along the main body axis posteriorwards, the mid-brain and hindbrain, excepting the anomalies due to an inhibited circulation, being apparently normal in all other respects.

Of a much higher degree is the injury sustained by the case of perfect cyclopia with a supernumerary lens, cross sections of which are represented in figures 27 and 28. The egg had been subjected in the eight-cell stage to the action of 35 cc. of a gram molecular solution of acetone in 50 cc. of sea-water for forty-eight hours. The embryo when killed was twenty-seven days old. A single median eye is seen in a transverse section (fig. 27) which is smaller in size than a normal eye, the lens only being disproportionately large. All other structures of the eye, viz., cornea, iris, choroid coat and retina are present, the latter having differentiated in a defective manner. At a somewhat more posterior level of the brain, the following view is presented (fig. 28). Laterally from the eye on one side is seen a large well



27



28

Fig. 27 Photomicrograph of a transverse section through the eye region of a twenty-seven days old cyclopean embryo, one day after hatching, showing a small median, imperfectly differentiated, eye with a disproportionately large lens. The forebrain is very abnormal, and tissue spaces, *t.s.*, point to oedema. From acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water).  $\times 160$ .

\* † Fig. 28 Photomicrograph of a transverse section of the same embryo as in figure 27, at a more posterior level, showing supernumerary lens, *l.*, on one side and optic anlagen on the other side of the cyclopean eye.  $\times 160$ .

differentiated lens surrounded by an epithelial capsule and anteriorly and laterally by a cornea, which is found to be in continuation with the cornea of the cyclopean eye. On the other side of the eye between its lateral border and the head integument is to be seen a large patch of cells with deeply stained nuclei and a little higher upwards on the same side (o. a.) and bordering the integument two more such patches of tissue can be seen in close approximation. On careful examination it was found that these three patches of cells represent fragmentary optic anlagen. The one of these optic anlagen which borders the eye has even differentiated into a small retinal layer which is in about the same stage of differentiation as the same structure of the cyclopean eye. There is only one optic nerve present which in more posterior sections can be seen to enter as a trunk one of the hemispheres. The brain is much deformed and more so anteriorly, where its symmetry is obscured, than posteriorly, where its bilateral symmetry can be recognized without difficulty. Many large clear tissue spaces can be seen in the brain in almost all sections which in the living apparently represented persistent early embryonic vascular anlagen and were filled with body fluid owing to lack of drainage caused by an imperfect circulation.

This embryo I regard as one instance of the perfect cyclopean condition, for only one complete eye has developed. Such indications as are present of the other optic anlage give us a clue to the genesis of the single-eyed condition in this case. Although the injury sustained by the embryo is not localized and is of a high degree, it is highest in the most anterior portion of the body, diminishing gradually posteriorwards along the main body axis. The anterior portion of the very early embryonic anlage has apparently undergone great destructive alterations of a blastolytic nature, owing to which the ophthalmoblastic material of one side was fragmented and dispersed while the corresponding material of the other side has been injured less severely and has given rise to an imperfectly developed median eye. The material which would in the normal embryo eventually be represented by the interocular area seems to have, owing

to some regulatory processes, moved lateralwards, where it has given rise to an independent lens. To the same regulatory process is it probably due that the ophthalmoblastic material of the less injured side has been shifted medianwards to give rise to the cyclopean eye.

The case of 'asymmetric monophthalmia' which I shall now describe will, I believe, also point to unmistakable evidence that in teratophthalmic embryos the sustained injury is usually the severest at the anterior end of the body.

The egg had been subjected for forty-eight hours to treatment with acetone, 35 cc. of a gram molecular solution of which were added to 50 cc. of sea-water. The embryo was killed one day after hatching when it was sixteen days old. On examination *in toto* there was seen to be present only one eye in the usual lateral position while the mouth occupied exactly the position of the missing eye. In transverse sections (fig. 29) it is seen that the one eye present is well developed and apparently normal in structure. No indication of another eye or optic anlage can

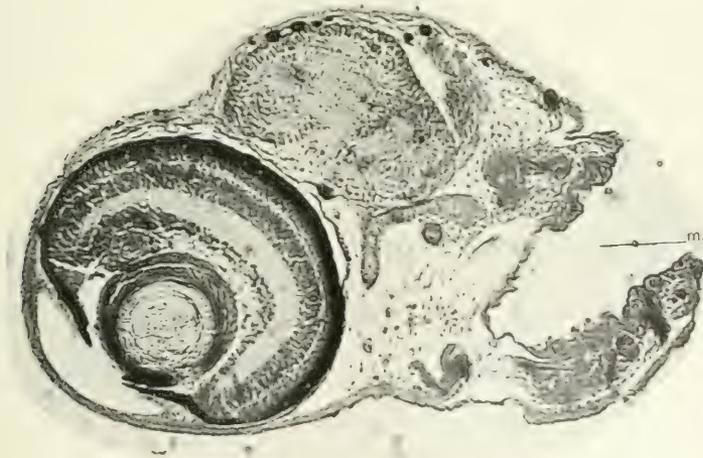


Fig. 29 Photomicrograph of a transverse section through the eye region of a monstrum monophthalmicum asymmetricum, from acetone solution (35 cc. gram molec. sol. to 50 cc. sea-water), sixteen days old, one day after hatching. The mouth, *m.*, occupies the position of the missing eye.  $\times 120$ .

be found anywhere in the sections and only one optic nerve is seen in the more posterior sections to pass out of the retina and terminate in the optic lobe of the opposite hemisphere. The brain is symmetric as far as its bilaterality is concerned, but is asymmetric in regard to the position occupied by the two hemispheres in relation to the main body axis. As can be seen in the figure, the hemisphere of the side where the eye has developed, is in its normal position while little can yet be seen of the other hemisphere, which has been shifted posteriorwards and comes to view in more posterior sections. The same posteriorward displacement has also affected the olfactory pit and the semi-circular canals of the side lacking the eye. There are no other abnormalities to be recorded for this embryo. The abnormalities found, however, justify the conclusion that the injury sustained by the early embryo was chiefly a unilateral one at the anterior end of the body. Owing to this injury the ophthalmoblastic material of one side has suffered complete destruction. On the same side, owing to subsequent processes of regulation, the mouth has arisen in what was to be the position of the eye and a posteriorward displacement of the brain hemisphere of the injured side has taken place. The sustained injury, while being lateral from the median axis of the body, was severest at its most anterior end where it has eliminated the material for an entire organ.

The cases described above and many more similar ones which have been studied led me to accept in the main the 'fusion theory' of cyclopia which has in recent years been advocated by Lewis ('09) and Speemann ('12) and to reject as untenable Huschke's ('32) view of the early single anlage of the eye which Stockard has quite recently ('13) adopted in his morphogenetic analysis of cyclopia.

Stockard, who was the first to produce experimental cyclopia in fish by chemical agents, has in his earlier work ('09) suggested that the developmental defect is due to the specific anaesthetic action of the chemicals (magnesium chloride, chloroform, ether, etc.) which he used. He thought that the giving off of the eye anlagen by the brain was inhibited often in an unequal manner

on both sides, thus giving rise by fusion of the inhibited anlagen to various transition stages between two normal eyes and cyclopia and anophthalmia. Recent investigations of McClelland ('12), however, who obtained similar results with a great variety of non-anesthetic substances, have caused Stockard ('13) to abandon his anesthetic theory of teratophthalmia. He now believes that the eye anlage in the medullary plate is single and median in position, that in normal development this single anlage eventually divides into two portions which move lateralwards, where they develop into the optic vesicles. If the embryo is subjected to the action of toxic chemicals this separation of the original single median optic anlage into two, may, he concludes, be inhibited to a greater or lesser degree and thus various degrees of the cyclopean defect may result. He even submitted the theory of the single optic anlage to an experimental test which, he believes, answered the query in the affirmative. It is unfortunate that Stockard's statements are not substantiated by better evidence than that which he brings forth, since he has failed to support his statements by illustrations of specimens *in toto* and in sections of the material which he regarded as permitting of such important conclusions. *Until this evidence is furnished, however, the theory of the single median optic anlage in the medullary plate would hardly seem to be acceptable.* On these grounds, we cannot agree with Stockard's conclusion on the morphogenesis of cyclopean defects.

The 'fusion theory' of cyclopia is, I believe, justified in the main, and has recently been ably supported by Speemann (l. c.), Mall (l. c.) and W. H. Lewis (l. c.). These authors assume a coalescence or fusion of two originally separate optic anlagen. Just how this fusion comes about may still be a matter of discussion. I think that Speemann's and Lewis' suggestions are very illuminating just on this point. Both these authors have produced all those conditions of one-eyedness which Stockard has obtained in his investigations, and which I have recorded in my experiments, a description of some of which is given above.

Both Speemann and Lewis have produced the teratophthalmic condition by mechanical injury of a small area at the ante-

rior end of the early embryo; and Lewis holds that the collapsing of the wound surfaces effected the approximation of the two optic anlagen. The degree of this approximation would depend upon the size of the fragment of interocular tissue which Speemann eliminated by constriction and Lewis by pricking. This, they conclude, would account for the various degrees of the cyclopean condition. Even asymmetric monophthalmia can in this way be accounted for, as it is easy to imagine that in the experiment the injury inflicted to the embryo may sometimes be somewhat lateral from the embryo's main body axis and that a complete eye anlage may thus be destroyed. The teratophthalmic condition would then, as Speemann points out, have to be regarded as a 'defect' (evidently meaning a mechanical defect) rather than a developmental inhibition.

My own conclusions are very similar to those arrived at by Speemann and by Lewis. I have, however, found it necessary to modify the fusion theory of cyclopia to conform to Stockard's and my own findings. The explanation offered by these authors is open to the criticism made by Stockard, namely, that "cyclopean eyes are rarely in size and extent equal to the sum of the two normal eyes combined." It is obvious that this objection is justified and that conclusions based on results obtained from mechanical experiments cannot be extended to cover the results of the chemical experiment or to account fully for the occurrence of the cyclopean defect in nature under unfavorable environmental conditions.

The following modification of the coalescence theory of cyclopia meets the objection raised by Stockard, covers the results of the chemical experiment and thus, I believe, may be extended to the morphogenesis of various cyclopean defects in nature, where the causal factor is undoubtedly a chemical one.

When the fertilized egg is subjected to the action of toxic substances it will sustain an injury, which—depending upon the stage of development, the substance used and the strength of its solution—may be a general one, i.e., involving the whole or a large part of the embryo's body, or a locally restricted one. The results of Stockard's and McClendon's work, as well as some

results of my own experiments, point to a blastolytic injury of a restricted area at the anterior end of the early embryo's body in the case of teratophthalmia. Child's<sup>3</sup> ('09, '12) important discovery of the 'axial gradients', according to which the anterior end of a flat-worm's body is the most sensitive one to the action of injurious substances would seem well to justify this assumption. In the early vertebrate embryo, before the organs have been differentiated, probably very similar physiological conditions obtain, and thus we may well assume that its anterior end is the point of least resistance. When the egg is acted upon by a toxic substance, a restricted area at the anterior end of the embryo's median body axis becomes so altered chemically as to be eliminated from further development or it may go on developing to a certain point beyond which it is chemically unable to proceed. This restricted area at the anterior end of the body axis is the region between the future optic anlagen or even the region of those anlagen. The size of the injured area at the anterior end is probably subject to considerable variation, and thus it may comprise the material which would normally correspond to the future interocular area and cause an approximation of the potential optic anlagen or it may extend even over the latter ones, thus eliminating parts of them, while the uninjured parts would coalesce after approximation and form any one of the various degrees of the synophthalmic condition. Or, finally, the injured area may comprise the whole of one potential optic anlage and little or no material of the future interocular area, thus causing the embryo to develop into a cyclopean monster if the uninjured optic anlage is shifted medianwards, or into an asymmetrically monophthalmic monster, if no such change in the position of the uninjured or less injured ophthalmoblastic material takes place.

<sup>3</sup> Child offers an interesting interpretation for this high degree of sensitivity. According to him the rate of the metabolic reactions seems to be the highest at the anterior end of the planarian's body, decreasing gradually along the main body axis in the direction away from the head. This theoretical postulate of the metabolic 'axial gradient' was substantiated by the rate at which various points along the body axis have given off CO<sub>2</sub>, the measurements having been made by Tashiro with his own biometer-method.

Here too, the size of the chemically injured area may vary and thus in some instances a small fragment of the injured potential anlage may survive and develop into a diminutive whole, but somewhat rudimentary, eye, or into an eye fragment with a well differentiated retinal layer and choroid coat.

However, it is necessary to assume that the injury which results in teratophthalmia is sustained at a very early stage of development. At that time the volume of the area which receives the injury is relatively small and such minute parts of the embryo as may represent the potential double anlagen of the eyes, are, since growth proceeds in a cubic proportion, relatively much nearer each other than they would be at a somewhat later stage, e.g., in the embryonic shield. This would account for the fact that the cyclopean eye may be very small in size, much smaller even than the normal eye. For, at this stage, the eliminated area may contain much more material than of one potential eye. The remaining ophthalmoblastic material may undergo some regulatory process to form eventually a single eye of corresponding size or a double, fused eye if enough of the ophthalmoblastic material of both sides survives, or finally if the injury sustained by that material be so extensive as to comprise all of it, the condition of anophthalmia may be the result. This conception of the morphogenesis of teratophthalmia, while recognizing the coalescence theory of cyclopia as justified, also meets the objection which Stockard raised to Speemann's and Lewis' generalizations, namely, that the cyclopean eye is often much smaller than the sum of two normal eyes combined. At the same time, it makes it appear unnecessary to resort to a theory whose probability is so questionable as that of the "single median optic anlage in the medullary plate" (Stockard).

\* \* \*

The mechanism involved in the action of butyric acid and acetone in bringing about the great variety of recorded effects will have to be taken up as one of the further steps of this investigation. At the present time, I can only state that there seems to occur in the eggs when subjected to the action of these solutions

an elimination of material of the blastomeres or of the germ-disc and probably also of the yolk-sac. This elimination of material may be due either to the precipitating or solvent effect respectively of the chemicals which were used in these experiments. Since many eggs were found with living but extremely malformed embryos, the yolk-sacs of which were ruptured allowing some yolk to escape, the suggestion is at hand that another factor—some force like a temporarily increased internal osmotic pressure—may by its action have brought about the fragmentation of the germ-substance. On examining many eggs in which only amorphous tissue fragments can be found and the eggs with 'solitary eyes' (embryone absente) or eggs with 'isolated eyes' (embryone praesente) one gains the impression that either the blastomeres or the germ-disc had been blastolytically fragmented owing probably to both physical and chemical factors. These tissue fragments sometimes appear at such great distances from each other, or even from a malformed embryo, that there seems to be no doubt left that they have moved out of their original position along the axis of the embryo. Having examined many thousands of these pathological ova I believe that I possess enough evidence to justify the assumption of blastolytic processes of a physical or chemical nature, such as fragmentation due to a temporarily increased osmotic pressure or to chemical alteration of some parts due to the solvent or precipitating effect respectively of the chemicals used in these experiments. Mall speaks (l. c.) of a similar process which he assumes for some pathological human ova, and which he terms 'dissociation of cells.' The effects of these apparently blastolytic processes vary enormously. For whatever parts survive them, may go on developing into a whole defective, or a meroplastic, embryo, or even into an isolated organ.

Just what brings about this great range of variation in the noted effects can at present hardly be more than conjectured. It would seem probable that the stage in which the egg is subjected to the action of the toxic solution may play an important part in that respect. For as Conklin ('12) has shown ". . . stages during kinesis are more susceptible to modification than stages

during interkinesis. Almost all persistent alterations of structure occur during cell division, few of those which occur during the resting period are permanent." However, this and similar other inquiries must be left to future investigation.

#### CONCLUSIONS AND SUMMARY

1. Starting from the assumption that monstrous development is due to parental metabolic toxemia, experiments were performed in which *Fundulus* eggs were subjected to the action of some substances occurring in the blood or urine respectively of man during metabolic disturbances.

2. With two of these substances, i.e., butyric acid and acetone, positive results have been obtained, a great variety of monsters having been produced which are analogous or homologous respectively to human and other mammalian monsters. The monstrosities concern the eyes (cyclopia, synophthalmia, monophthalmia asymmetrica and anophthalmia), the ear vesicles, the olfactory pits, the mouth, the central nervous system, the heart and blood vessels, the fins (unpaired fin, absence of pectoral fins or all fins, club-tail, etc.) and body form.

3. A condition of hydrops was found in many embryos, due apparently to blood vascular abnormalities which might be considered as homologous to some forms of hydrocephalus in man.

4. In many eggs parts of the embryonic material have suffered destruction, while the remaining parts have developed into anterior hemiembryos or other meroplastic embryos.

5. Eggs have been found in which one eye has developed from a small fragment of the medullary plate independently of an embryo (the 'solitary eye,' the 'isolated eye').

6. Regarding the mechanism of action of butyric acid and acetone the conclusion has been reached and some evidence offered that the various monstrosities are brought about by a process of blastolytic fragmentation due to some factors not yet ascertained.

7. Regarding the formation of the various degrees of the 'cyclopean' defect it is concluded that the fusion theory of Speeman

and Lewis is justified in the main. An additional assumption is made, namely, that the blastolytic process which eliminates parts of the potential interocular or ophthalmoblastic material takes place at a very early stage of development (i.e., before the formation of the embryonic shield).

8. The results obtained tend to justify the assumption that monstrous development may be due to metabolic toxæmia.

The experimental part of this work has been carried out at the Marine Biological Laboratory at Woods Hole, Mass., during the summer of 1914. It is a pleasure to me to acknowledge my indebtedness to the director, Dr. F. R. Lillie, for the facilities granted. I am also under great obligation to Professors Conklin and McClure and to Dr. E. N. Harvey, whom I have often taken occasion to consult on some points of the work. For many courtesies I am indebted to Professor Dahlgren and Mr. W. E. Hoy.

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# THE DEVELOPMENT OF THE LYMPHATIC SYSTEM IN THE LIGHT OF THE MORE RECENT INVESTI- GATIONS IN THE FIELD OF VASCULOGENESIS

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Does the endothelium of the lymphatic system arise, at any time or place, in a discontinuous manner and independently of that of the veins? As we shall see, the determination of this question constitutes a solution of the lymphatic problem.

The view that lymphatic endothelium spreads continuously and uninterrupted throughout the body of the embryo from the endothelium of the veins, is merely an extension, and application to the endothelium of the lymphatic system, of the well-known view held by His, that the endothelium of the intra-embryonic haemal vessels grows continuously and uninterrupted into the embryo from the yolk-sac angioblast. Such a method of origin necessarily implies that all intra-embryonic endothelium arises only from a pre-existing endothelium which takes its origin in the yolk-sac, and that in the body of the embryo a discontinuity of origin never occurs.

The view opposed to the 'ingrowth' or 'angioblast' theory of His has been closely associated with the names of Rückert and Mollier (1). This view consists in the claim that the endothelium of the intra-embryonic haemal vessels develops *in situ* in the body of the embryo, and that it is not derived from the yolk-sac angioblast.

Since the lymphatics merely represent a component part of a general vascular system, to which the haemal vessels also belong, the probability at least, is that, in the genesis of their endothelium, and in the establishment of a continuous system of vessels, the lymphatic and haemal vessels should follow a common genetic

plan. Let us consider, in the light of the more recent investigations in the field of the vascular system, what this plan may be.

It is not the purpose of the present paper to give a review of the investigations of those who have consistently maintained a local origin for the endothelium of the intra-embryonic haemal vessels. It is only necessary to refer to the more recent and excellent paper by Schulte in which such a review and critical analysis of their work is given.

The investigations of Schulte (2) on the mammalian embryo have shown in particular, that the yolk-sac angioblast cannot possibly aid in forming the endothelium of the umbilical veins; Schulte has also demonstrated in a most convincing manner, that the endothelium of other main intra-embryonic haemal vessels develops *in situ* from mesenchymal cells.

It should be clearly borne in mind that, until quite recently, the investigations which have dealt with the origin of intra-embryonic endothelium have not been experimental in character, but have been based largely upon a study of fixed material in which, however, a local and discontinuous origin of blood-vascular anlagen has been observed.

Let us now see how the view that the endothelium of the intra-embryonic blood-vascular system develops *in situ*, and does not grow into the embryo from the yolk-sac, has been borne out by experiment.

Two types of experiment have thus far been made to determine this question: (1) The partial separation of the embryo from the yolk-sac, or, the complete separation and isolation of a portion of the embryo from the rest of the embryo and from the yolk-sac, at a time prior to the possible invasion of the embryonic axis by the yolk-sac angioblast; (2) by observing the effects produced on the developing blood-vascular system in embryos which have been allowed to develop under the influence of anaesthetics or other chemical agents.

The experimental investigations of Hahn (3), and Miller and McWhorter (4) have shown, by effecting a separation on one side between the body of the chick embryo and the yolk-sac, before vessels have appeared in the area pellucida, that blood

vessels make their appearance in the body of the embryo in a typical manner on the operated side. These vessels differ from those on the unoperated side only in size and rate of development, differences which may be correlated with their reduced drainage area and the consequent diminished quantity of circulatory fluid.

These experiments of Hahn, and Miller and McWhorter have conclusively shown that the yolk-sac angioblast cannot have grown into the embryo on the operated side. In order to eliminate the possibility, however, that the vessels on the operated side may not have been formed *in situ*, but by an invasion of angioblast from the normal unoperated side, Reagan (5) has recently completed a set of experiments in my laboratory which conclusively disprove this contention. Instead of separating only one side of the embryo from the yolk-sac, Reagan has been able to develop the heads of chick embryos, which had been completely separated from the rest of the embryo and from the yolk-sac, and in which endothelial lined vascular channels of mesenchymal origin invariably appear. As in the case of the experiments of Miller and McWhorter, the operations were performed at a time before it would have been possible for the intra-embryonic tissue to have been invaded by yolk-sac angioblast.

Gräper (6), under the direction of C. Rabl, performed a set of experiments on chick embryos, somewhat similar to those of Hahn, and Miller and McWhorter, and, although he noted the presence of independent blood-islands in the body of the embryo, he was unable to interpret them as having been formed *in situ*.

Jacques Loeb (7) was the first to observe the effects produced by certain chemicals (NaCN) on the developing blood vessels in fish embryos. He was able to produce a condition in which a beating heart and blood were present, but no circulation: a condition which, as stated by Schulte, can hardly be reconciled with the doctrine that the vessels of the embryo have a primitive continuity of lumen with those of the yolk-sac, for it is inconceivable that in such circumstances, a beating heart could fail to effect a circulation.

The investigations of Stockard (8) supplement and coincide with those of Hahn, Miller and McWhorter, Reagan, and Loeb in a most decisive manner. Stockard has shown that, not only do anesthetics arrest the development of the intra-embryonic blood vessels in the embryos of *Fundulus*, at an early ontogenetic stage, but in such a manner that no doubt can now exist that, under normal conditions, these vessels are formed *in situ* by a conrescence of independent and discontinuous anlagen, and that their endothelium is derived directly from mesenchymal cells. It is interesting to note in this connection that Wenkebach (9) had already observed in the body and yolk-sac of the living fish embryo (*Belone longirostris*), that mesenchymal cells play an important rôle in the formation of vessels and sprouts. In their general features the observations of Wenkebach have been confirmed by Raffaele (10).

It is thus seen that experimentation bears out the observations made upon fixed and living material, that the intra-embryonic blood-vascular channels do not grow into the embryo from the yolk-sac, but are formed *in situ* by a conrescence of independent and discontinuous anlagen, whose endothelium is formed from intra-embryonic mesenchymal cells.

The vascular plexus formed in the extra-embryonic area of the vertebrate embryo, is as we know, at first represented by discontinuous, independent and circumscribed anlagen, the cells of which possess a local origin. Clefts or spaces, the future lumina of the plexus, soon make their appearance in a discontinuous manner amongst the cells of these anlagen, and it is by a conrescence of these vascular spaces that a continuous system of vascular lumina is finally formed. The cells which constitute the walls of these vascular spaces become transformed into the endothelium and, when blood-islands are present, the more centrally situated cells form the primary blood cells. It is interesting to note in this connection that McWhorter and Whipple (11) have recently been able to demonstrate and record photographically the conrescence of separate vascular anlagen in the area pellucida of the chick's blastoderm *in vitro*.

If we compare the development of the intra-embryonic blood-vascular channels, as determined by observation and experiment, with that of the plexus which arises on the yolk-sac, we find, in the genesis of their endothelium from mesenchyme, and in their formation by a conrescence of independent anlagen, that the intra- and extra-embryonic blood-vascular channels follow exactly the same genetic plan.

If one attempted to follow the development of these intra- or extra-embryonic blood-vascular channels by means of injections, it is evident that this method would reveal only the extent to which a continuous system of injectible lumina had been established at the time the injections were made. It would fail completely to reveal the facts which have been definitely determined by experiment, that the injectible lumina had been previously formed by a conrescence of independent and uninjectible vascular spaces, and that the endothelium which forms the walls of these lumina had been formed *in situ*, not from a pre-existing endothelium, but from mesenchymal cells.

Since we now know that the intra-embryonic blood vessels, like those in the yolk-sac, are formed by a conrescence of independent anlagen, and that their endothelium is formed *in situ* from mesenchymal cells, the question naturally confronts us as to the method by means of which these independent anlagen become connected with one another to form a system of vessels with continuous lumina, that extend throughout the body of the embryo.

There appear to be only three possible methods by means of which such connections could take place: (1) Either by means of a proliferation or migration of the cells of which the original independent anlagen are composed; (2) by a further local *in situ* differentiation into endothelium of the embryonic cells which intervene between the independent anlagen; or (3) by a combination of these two methods.

We all recognize the fact the endothelium, like other tissues of the body, is capable of growth after it has once been formed. In no other manner could we account for the increase in size

which blood vessels undergo in the embryo after they have attained their adult structure and form. It is also possible for anastomoses to be formed between different blood vessels by means of a growth or sprouting of their endothelial walls, so that, in some cases, an increase in their extent, through growth, may actually take place. It is therefore quite probable that growth may play a considerable rôle in establishing a condescence between the independent endothelial-lined anlagen of the blood-vascular system. From whatever standpoint it may be considered, however, the growth of an endothelium is a feature of secondary significance as regards the problem at hand, since the main question at issue does not concern the possibility that endothelium may or may not grow, but rather how the endothelium is formed that does the growing.

The distinction between the actual genesis of endothelium and the growth it may undergo after it has once been formed is naturally one that has been disregarded by those who maintain that intra-embryonic vascular endothelium is not directly a product of mesenchymal cells. A special specificity has therefore been attributed by the supporters of the 'angioblast' theory to the endothelium of the intra-embryonic vascular system, on the ground that it takes its origin only from the yolk-sac angioblast. In accordance with this view, it is by means of one continuous and uninterrupted growth of a pre-existing endothelium (yolk-sac angioblast) throughout the body of the embryo, that the endothelium of the blood-vascular and lymphatic systems is formed.

Since the 'angioblast' theory of His no longer holds, the question of the specificity of tissues is involved in the vascular problem only to the same extent as is the case for any other tissue in the body. Whether the mesenchymal cells of the embryo are in an embryonic or undifferentiated state, and capable of further differentiation into cells which form muscle, connective tissue, endothelium, etc., is entirely beside the question; provided we know that the product of these intra-embryonic mesenchymal cells actually forms endothelium and that the latter is not derived from the yolk-sac angioblast. Also, the question con-

cerning the origin of these mesenchymal cells, whether derived from entoderm, mesoderm or mesothelium, does not concern us here. The main point at issue is the establishment of the fact that the endothelium of the intra-embryonic haemal vessels is the product of a local *in situ* differentiation of certain cells in the embryo which have not been derived from the yolk-sac angioblast.

Let us now compare these conditions of the intra-embryonic blood-vascular system, as determined by sections and experiment, with those of those of the embryonic lymphatic system.

Our knowledge of the embryonic lymphatic system is gradually approaching a state where, in such forms as teleosts and amphibia, it may also be possible to determine by experiment how the lymphatic system is formed. A thorough knowledge of the lymphatic channels and the order of their appearance in the normal embryo would be quite essential, however, before experiment could be successfully applied. Since the anlagen of the lymphatics do not make their appearance in the embryo under normal conditions until after the veins have been established and have begun to function, it is quite possible, in cases of arrested development of the venous system, as demonstrated by Stockard in *Fundulus*, that development might never be successfully carried to the lymphatic stage. Be this as it may, until the problem has been tested by experiment, our knowledge and interpretation of lymphatic development must, for the present, be based upon the observation of fixed and of living material, and its comparison with the known developmental stages of the blood-vascular system, as observed in fixed and in living material, and as verified by experimental means. If it can be shown that the anlagen of the lymphatic system present exactly the same conditions in fixed and in living material, as those of the blood-vascular system, it is reasonable to infer that in their development the lymphatic and blood-vascular systems follow exactly the same genetic plan. *If one were to observe that in certain cases intra-embryonic blood vessels were formed in the living embryo by a sprouting or growth of a pre-existing endothelium, would he now be justified in claiming that all of the remaining*

*blood vessels of the embryo were formed in the same manner?* In view of the fact that we now know that intra-embryonic blood vessels are not all formed in this manner, it would seem that a similar interpretation might also apply to the lymphatics.

Whatever else the case may be, in view of the above-mentioned experimental investigations of Hahn, Miller and McWhorter, Reagan, and Stockard, it can now be definitely stated that the endothelium of the lymphatic system is neither directly nor indirectly a product of the yolk-sac angioblast. Such being the case, it must either arise *in situ*, like the endothelium of the intra-embryonic veins, from cells other than from a pre-existing endothelium; or, be a product entirely of the endothelium of the veins. If the former case be true, the endothelium of the lymphatic system should present exactly the same independent and discontinuous method of origin in the embryo as that of the extra- and intra-embryonic haemal vessels; and, if the development of the lymphatics were followed by the injection method, the same restrictions as regards the injectibility of its independent anlagen should also necessarily apply. On the other hand, if the lymphatic system is entirely a product of the endothelium of the veins, its origin from mesenchyme should naturally never occur. *As a matter of fact, since intra-embryonic vascular endothelium has been shown by experiment to be a local product of mesenchyme, there now remains no valid reason or significance in the claim, as regards its specificity, that lymphatic endothelium is solely a product of that of the veins.*

Let us examine the evidence at hand and see whether the endothelium of the lymphatics, like that of the haemal vessels, develops *in situ* in the mesenchyme, or whether it forms an exception to that of the haemal vessels, and sprouts continuously and uninterruptedly throughout the body of the embryo from an endothelium already formed.

It is not the purpose of the present discussion to give an historical review of the literature bearing upon the development of the lymphatic system but merely, on the basis of comparison, to call attention to the evidence in favor of the view that the lymphatics, like the haemal vessels, are formed by a concre-

cence of independent and discontinuous anlagen, and that their endothelium arises *in situ* from intra-embryonic mesenchymal cells.

A principal contention of Huntington and McClure (12) regarding the development of the lymphatic system has been that its anlagen arise independently and discontinuously in the embryo, and that its endothelium does not spread continuously and uninterruptedly throughout the body from the endothelium of the veins. We have repeatedly shown that the lumina of the lymphatics are formed by a conrescence of discontinuous and independent lymph vesicles or lymph spaces, and that the cells which constitute the walls of these spaces are derived *in situ* from mesenchyme and not from the endothelium of the veins. In the early stages of our investigations we laid especial stress upon a plan of development for the lymphatic system of mammals which we described under the name of the 'extraintimal' theory of lymphatic development, and which may be briefly described as follows: The development of the thoracic ducts (13) and mesenteric (14) lymphatics in the cat is correlated with the degeneration of certain venous channels, many of which are tributaries of the azygos division of the supracardinal veins (15). A series of independent lymph spaces arise discontinuously in the mesenchyme external to the intimal lining of these degenerating vessels and, as these lymph spaces gradually become conrescent to form continuous channels, the latter, following a line of least resistance, utilize the static line vacated by these degenerating veins. In this manner certain of the main lymph channels of the mammalian embryo follow the course of and finally occupy completely the territory formerly occupied by veins. This principle of extraintimal replacement of abandoned venous channels by lymphatics accounts for the sinistral drainage plan finally assumed by the thoracic duct system in the embryo of the cat. The cranial or azygos division of the left thoracic duct of the embryo persists as the main line of drainage in the adult, in correlation with a degeneration in the embryo of the left supracardinal (left azygos) and left postcardinal veins and the left duct of Cuvier.

It is evident and appears clearly in our earlier publications, that the fundamental plan of development followed by these replacing lymph channels does not depart from that followed by other channels, either in mammals or in any other vertebrates where the development of the lymphatics is unaccompanied by the replacement of degenerating veins. Where, as in the case of the trout, lymph channels do not develop along the course of degenerating veins, an extraintimal replacement of a degenerating vein by a lymphatic necessarily does not occur. *It is therefore plain that the extraintimal replacement, as described by us, possesses only a mechanical significance, and is merely an adaptation of a common plan of lymphatic genesis, through the concurrence of independent anlagen, to the local conditions which prevail only in certain districts of the mammalian embryo.*

The same general plan of development as outlined above by Huntington and McClure for the lymphatic system of the cat has also been found by Kampmeier (16) to occur in the embryo of the pig. His description of the independent and discontinuous anlagen of the thoracic ducts which he found in the injected pig embryo loaned him by Professor Sabin, needs no further comment.

F. T. Lewis (17) has described the presence of a chain of discontinuous 'lymphatic spaces' (endothelial-lined anlagen) in the rabbit embryo which lie along the azygos veins in the path of the future thoracic duct. He regards these anlagen, however, as having been detached from the veins. Concerning these multiple anlagen of Lewis, Sabin (18) has stated as follows:

Since these spaces are lined with a definite endothelium, they form a much more serious obstacle to the theory of growth of the lymphatics from the endothelium of the veins than the more indefinite spaces to be found in earlier embryos, and I cannot but think that if these multiple endothelial-lined isolated spaces do exist along the veins in later stages, they would form serious evidence against the theory of the origin of the lymphatics from the veins. Or at least if the lymphatics, in their growth, do pick up isolated endothelial-lined spaces, we shall again be left without a clue as to the origin of the lymphatic system.

It is significant to note that, although Sabin considers these isolated endothelial-lined anlagen of Lewis as having been

detached from the veins, she nevertheless now recognizes their existence in the pig embryo, and regards them as entering into the formation of the thoracic duct (19, 1911, p. 424).

The point I wish to emphasize in this connection is that Sabin now recognizes the fact that lymphatics may be formed by the concrescence of multiple and independent endothelial-lined anlagen and that she has thus far presented no valid evidence that these anlagen have been detached from the veins.

Sala (20) and more recently Miller (21) have shown that the thoracic ducts of the common fowl are formed by a concrescence of independent and discontinuous lymph spaces and that their endothelium is formed *in situ* from cells other than those which constitute the endothelium of the veins. Miller has further made the important discovery that groups of blood cells develop in the mesenchyme along the line of the thoracic ducts and that the latter subsequently convey these blood cells to the venous circulation. We therefore find that hematopoiesis may actually occur in connection with the development of certain lymph channels, a condition which Huntington (22) has also recently verified for certain lymphatics of mammals.

West (23), by a study of injected and uninjected embryos, has recently found that the posterior lymph heart of the common fowl develops in the mesenchyme and secondarily establishes a connection with the veins. He has also found that hematopoiesis occurs in the mesenchyme in relation to the independent anlagen of the lymph heart, and states that the blood cells thus formed are not to be confounded with those which may later back into the lymph heart from the veins (see E. L. Clark, *Anat. Rec.*, vol. 6).

Huntington (24), in a paper on the development of the lymphatic system in reptiles (chelonians, lacertilia), has shown that the systemic lymphatics develop in the mesenchyme independently of the endothelium of the veins. A particular feature of his investigation is that he was able to demonstrate that the periaortic lymph channel in *Chelydra serpentina* arises in the mesenchyme in an area entirely free of veins.

Stromsten's (25) investigations on the development of the lymphatic system in reptiles (chelonians) have led him to conclude that the lymphatics are formed by a condescence of independent and discontinuous lymph spaces and that lymphatic endothelium is formed *in situ* from mesenchymal cells. His observations were based largely upon a study of injected embryos, and showed that the injecta did not reach the independent anlagen of the lymphatics, prior to their condescence with one another to form a system of continuous lumina which had established a communication with the veins.

Fedorowicz (26) and more recently Kampmeier (27), have shown that the continuous lumina of certain lymphatics are formed in the amphibia (anura) by the condescence of originally independent and discontinuous lumina. They conclude, however, that the endothelial walls of these lumina have been derived from the endothelium of the veins. E. R. Clark (28) regards the lumina of the developing lymphatics in amphibia (in tail of larval amphibia) as always continuous and capable of injection, while Fedorowicz and Kampmeier describe them as discontinuous at the start.

The writer (29) has demonstrated the presence of discontinuous and independent lymph vesicles in the trout embryo, which cannot be injected from other lymphatic anlagen or from the veins. Many of these vesicles arise in the mesenchyme remote from the veins, and no connection can be observed between their endothelium and that of the veins or that of any other lymphatic anlagen. One of these independent lymph vesicles, the subocular lymph sac, can actually be observed in the living trout embryo. On account of the relatively large size of this vesicle it has proved a most favorable object for experimentation and for study in sections, not only in proof of the fact that it arises independently of the veins, but also that its endothelium is of mesenchymal origin.

Allen (30) has investigated the development of the lymphatic system in *Polistotrema* (*Bdellostoma*) *stouti* and speaks of the lymphatics of fishes as veno-lymphatics. He states: "I expect to show that the main factor in the construction of the veno-

lymphatic system is the same as was described for the caudal lymph hearts, namely, the formation and union of certain mesenchymal spaces."

Allen, independently of Miller, has also observed that an active hematopoiesis occurs in the mesenchyme in relation to the developing caudal lymph hearts of *Polistotrema*.

Except for differences of opinion regarding the origin of lymphatic endothelium, it may be observed that the above-mentioned investigators agree for the most part that the continuous lumina of the lymphatics, like those of the haemal vessels, are formed by a concrescence of independent and discontinuous anlagen.

If we disregard entirely the personal equation which may have influenced any or all of the above-mentioned investigators to interpret their findings in accordance with one or the other view, the fact still remains that the anlagen of the lymphatic system, as observed in sections of injected and uninjected embryos, have been found to be identical with those of the intra-embryonic blood-vascular system, which has been shown by sections and experiment to be formed by a concrescence of independent anlagen, and its endothelium to be formed *in situ* from mesenchymal cells. *Since we possess exactly the same kind of evidence in favor of the mesenchymal origin of lymphatic endothelium, as we formerly did for that of the intra-embryonic haemal channels, before experiment was applied, it therefore seems highly improbable that the endothelium of two sets of similarly appearing anlagen, belonging to the same general organ system and developing side by side, should differ in its mode of origin, rather than follow a common genetic plan.*

We know that the lymphatics, both in the embryo and in the adult, establish a permanent communication with the veins at typical points (31). The question therefore arises, what rôle, if any, may be played by the veins in establishing such communications with the independently formed lymphatics? In the development of the general vascular system which includes the arteries, veins and lymphatics, the end result desired is the formation of a connected system of vessels which subserve definite functions in the economy of the general vascular system.

If the general vascular system develops progressively in a uniform manner, by a conerescence of independently formed anlagen, and the lymphatics, as is the case, form the last link in completing the chain, it is evident that the same factors should account for the establishment of a connection between the veins and the independently formed lymphatics as between the independently formed anlagen of which the veins and lymphatics are originally composed. Such a connection could alone be established, either by a growth or sprouting of the endothelium of the veins; by means of a further *in situ* differentiation into endothelium of the mesenchymal cells which intervene between veins and the independent anlagen of the lymphatics; or, both of these factors might be involved. If a connection should be established by a sprouting of the endothelium of the veins, such sprouts would possess no significance beyond the fact which we all recognize, that all vascular endothelium is capable of growth after it has once been formed. The question, however, is not whether endothelium is capable of growth, but rather what are its limitations and how is the endothelium formed that does the growing. It is therefore evident, if, at the points at which the lymphatics establish permanent communications with the veins, venous endothelium should contribute to the formation of the lymphatics, it would play only a subsidiary rôle, and serve only as a means, in common with the endothelium of other independently formed vascular anlagen, of bringing two independently formed portions of the vascular system into communication with each other to form a continuous system of channels. Such veno-lymphatic connections have been hitherto described by Huntington and McClure (32) under the name of 'venolymphatics.' It is evident, however, that these 'venolymphatics' would not differ, in any sense, from other connections, where a similar growth of endothelium is concerned, when made for the purpose of establishing a connection between any of the other independently formed anlagen of the general vascular system.

To sum up: The development of the general vascular system—haemal and lymphatic vessels—is a uniform process, which consists in a local origin (genesis) of endothelium from mesenchy-

mal cells and a growth of endothelium after it has once been formed.

In view of what has been said above, it would therefore appear that the lymphatic problem, in its broadest sense, should not be interpreted in terms either of a venous or non-venous origin, but rather in terms of the uniform phases of genesis and growth which may characterize the establishment of vascular channels in general.

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## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

THE INVESTIGATION OF MIND IN ANIMALS, E. M. Smith, Moral Sciences Tripos, Cambridge, 194 pages, 9 illustrations, bibliography and index. Cambridge, England, at the University Press, 1915.

Preface. There are few people who cannot relate some apparently striking instance of animal intelligence; the majority of such cases, however, will not stand critical examination. The science which has for its object the systematic investigation of the brute mind is Animal Psychology, and it would seem that the methods of this youthful discipline are still unknown to many, even among those who profess an interest in animal conduct. It is, then, with the purpose of presenting a brief account of the modes of procedure employed by Animal Psychology, its aims, trend, and the general nature of the results hitherto obtained, that this little book has been written. In a work of this character discussion and controversy would have been out of place, so the treatment has been confined as far as possible to description and illustration; at the same time attention has been drawn to some of the chief difficulties inherent in the inquiry. A complete and exhaustive presentation of facts was, of course, out of the question, and much that is of interest and importance has had, inevitably, to be omitted; but it is to be hoped that the interested reader of leisure will refer to some, at least, of the original articles mentioned in the bibliography, nearly all of which will be found to contain further references.

Under the headings Protozoan behavior; retentiveness: habit-formation, associative memory and sensory discrimination, instinct, homing, imitation, and the evidence for intelligence and for ideas, the author gives a most readable and informing account of the newer work on Animal Behavior. The book is written as a sketch and that plan of treatment is followed perfectly—with no lapses into detail and with a happy exclusion of technicalities.

The author's purpose is to present a review of the investigations in the field of animal behavior and to point out the main views which are now current. Concrete illustrations of experimental results are given, conclusions are weighed and matters calling for further study indicated. The needs of both the layman and the behaviorist are met by this essay. H. H. D.

THE CLINICAL ANATOMY OF THE GASTRO-INTESTINAL TRACT, T. Wingate Todd, M.B., Ch.B., F.R.C.S. (Eng.), Henry Willson Payne Professor of Anatomy in the Western Reserve University, Cleveland, Ohio; late Lecturer in Anatomy, University of Manchester, 264 pages, 32 illustrations, Index of authors quoted and a subject Index, Manchester at the University Press. Longmans, Green & Co., London and New York, 1915. \$1.75.

# THE SOUND-TRANSMITTING APPARATUS IN NECTURUS

H. D. REED

*From the Zoological Laboratory, Cornell University*

SIX FIGURES

The sound-transmitting apparatus of *Necturus* having been the subject of so much investigation, further comment would seem unnecessary. The results gained from the study of this apparatus in other groups of urodeles, however, and the uniform conclusions of Kingsbury ('05) and Norris ('11) and the significance of Herrick's ('14) and Brunner's ('14) observations bearing upon the rank of *Necturus* among tailed amphibia, demand a further investigation of the subject in order to determine the precise origin of every portion of the apparatus. Further study seems especially important when coupling the evident neotitic status of this species with conclusions which accordingly might be drawn regarding the nature of the fenestral elements.

In *Necturus* the sound-transmitting apparatus is composed of a single plate that accurately fills the somewhat elliptical foramen vestibuli of the mature animal and is connected by a well-defined stilus with the suspensorium of the jaws. Any relation with other extraotic elements is wanting entirely.

In typical urodeles, as for example the *Amblystomidae*, this apparatus is a double structure (Kingsbury and Reed '08). It is composed of a cephalic piece, the columella, and a caudal portion, the operculum. Each element is distinct in its origin and they are, therefore, without morphologic relations. Of the two elements the columella is the first to appear and develops wholly outside and independent of the ear capsule, only secondarily coming to lie against the membrane of the fenestra. It is essentially a flat plate connected with the suspensorium by a stilus. The columella becomes definitive in larval life and is to

be considered as the more primitive of the fenestral elements. Upon the assumption of a terrestrial existence, the columella apparently becomes functionless and fuses wholly or in part with the ear capsule, while a new element, the operculum, becomes *cut out* from the walls of the capsule just caudad of the primary fenestra. The operculum bears a perilymphatic prominence from which the *M. opercularis* extends to the shoulder girdle. It is important to note that these elements are not only different in origin, but each possesses distinctive skeletal relations: the columella with the suspensorium, the operculum with the shoulder girdle.

As mentioned above, the sound-transmitting apparatus of *Necturus* is composed of a single plate which possesses the anatomical relations of the columella of the amblystomid forms. In the Plethodontidae the sound-transmitting apparatus is in the form of a single plate filling the fenestra, but unlike that of *Necturus*, the plate in this family possesses the anatomical relations of both columella and operculum; that is, the cephalic end is connected with the suspensorium of the jaws by a stilus, while the caudal end comes into relation with the shoulder girdle through the *M. opercularis*. Development shows that the morphology of the plate is in harmony with these conditions. It is a double structure; the columellar portion arises outside the ear capsule, while the opercular portion is formed from otic cells which unite during development with the columellar element, producing a single definitive plate, the components of which differ in their morphologic nature.

Conclusions appear to be uniformly in favor of a greater structural similarity between *Necturus* and the larvae of the plethodontids than with those of any other group. In view of this and what has been said above respecting the characteristics of the fenestral elements in various urodeles, it seemed advisable to examine as complete a series of embryos and larvae as it was possible to obtain. The following were accordingly studied:<sup>1</sup> embryos 11, 12, 15, 16, 17, 18, 19 and 20 mm. long, and larvae

<sup>1</sup> An important gap in the series was filled by the generous loan of preparations of the 44 mm. stage by Prof. H. H. Wilder.

21, 22, 23, 24, 25, 26, 35, 40, 44, 48 and 70 mm. in length, respectively. The chief difficulty in arriving at conclusions respecting the morphology of the fenestral elements is the determination of the exact origin of parts. For the embryonic stages specimens 1, or at most 3 mm., apart in length proved satisfactory. Different specimens of a given length vary with regard to internal conditions of development. By employing several specimens of the various lengths it is possible to obtain every step in the development between two given stages and one is thereby enabled to trace the origin of elements cell by cell.

It is very evident that embryos from 15 to 20 mm. in length are the important stages in determining the origin of the columella in this species. A careful study of these series substantiates in every respect the conclusions of Platt ('97) and Kingsbury ('03) that in its origin the columella is independent of the ear capsule. It arises as a cord of cells extending between the squamosum and the fenestra vestibuli, but at all times in these early stages is clearly independent of both ear capsule and fenestral membrane. While all stages are necessary in tracing the development history of the fenestral structures, larvae from 35 to 70 mm. long are most important in determining the morphology of the plate itself.

The definitive plate in *Necturus* has a double origin, a suggestion made by Kingsbury and Reed ('09) as one of two possible interpretations of its nature.

Previous work renders it unnecessary to describe any given stage in detail. Only a brief sketch, therefore, will be given of the developmental changes taking place during the larval period. In larvae 23 mm. long the nature and relations of the columella are shown in figure 1. It consists in this stage of a well-defined chondrified rod extending from the level of the cephalic lips a third of the way across the fenestra. It is entirely free from all other skeletal structures, a relation which exists from its first appearance up to this stage. No stilus is present, the suspensorial connection being effected by the usual cord of cells. The structure and relations of the columella at this stage are very suggestive of similar stages in *Spelerpes* and as much in contrast

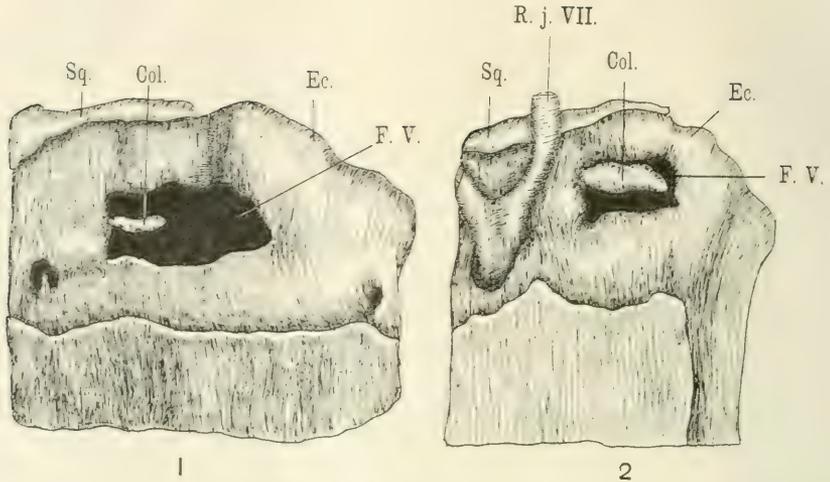


Fig. 1 Drawing from a wax model of the ear capsule of a larval *Necturus* 23 mm. long. *Col.*, columella resting against the fenestral membrane; it is rod-like, without stilus, and is free from the skeletal parts of the ear capsule; *Ec.*, ear capsule; *F.V.*, fenestra vestibuli; *Sq.*, squamosum.

Fig. 2 Drawing from a wax model of the ear capsule of a larval *Necturus* 25 mm. long. *Col.*, columella which has increased greatly in length and vertical diameter but is still without a stilus; *Ec.*, ear capsule; *F.V.*, foramen vestibuli; *R.j.VII*, ramus jugularis of the nervus facialis; *Sq.*, squamosal.

to the conditions which obtain in *Cryptobranchus* and *Amblystoma*, where the fenestral end of the columellar cord even before chondrification begins, spreads out over the membrane to form the plate portion of this element.

An examination of a specimen 25 mm. long (fig. 2) shows that the columella has increased greatly in size as regards both diameter and length. Its increase in size is relatively greater than that of the fenestra. It is still free from the ear capsule and without a stilus. The growth of the columella combines the features of *Amblystoma* (Kingsbury and Reed '08) and *Spelerpes* (Reed '14) a condition which is not found in other urodeles, so far as they have been studied, unless the growth of this structure in *Amphiuma* is to be so interpreted. Increase in the size of the stilus is at first apparently uniform, so that the cylindrical shape is maintained. But beginning with the 25 mm. stage, growth is most active along the dorsal side and particularly

in the cephalic end. This method of growth causes the former rod to become more plate-like and while it lies wholly outside the fenestral membrane it rests against it. The nature of the whole plate is best shown in a series of sections of a specimen 40 mm. long. A section near the cephalic end (fig. 3) shows the columella filling the fenestra at that level. The shape of the plate indicates its rapid dorsal and slight ventral growth, and further, this stage (and others younger) shows clearly from the position of the columella *upon* the fenestral membrane and the method of growth of its peripheral cells that no otic tissue has taken part in its formation. Such is not the condition further caudad. In following this series in that direction one notes that the thickened central part gradually narrows with a corresponding increase in the width of the thin portion both above and below (fig. 4). Finally, behind the middle of the fenestra, the thicker portion comes to a point and disappears entirely. Thus the thick portion forms a triangular area, with the apex pointing caudad and the base filling the cephalic part of the fenestra. In models as well as in sections the thick and thin regions are clearly marked (fig. 5). These two areas possess a deeper significance than that of mere topography. The thickened area represents columella resulting from the growth of the original extraotic rod, mentioned above and illustrated in figures 1 and 2. The thin portion of the plate arises as chondroblasts within the fenestral membrane independent of the columellar element. Figure 6 is from a section a little caudad of the middle of the plate. Here the thick columellar portion is in strong contrast with the thinner part of the plate found above and below. Both figures 4 and 6 show the mode of development of the thin part of the fenestral element. In the fenestral membrane near the edge of the previously formed plate, chondroblasts arise and through the subsequently secreted matrix come in contact with the edge of the plate itself. Thus new layers of tissue, the cells of which are derived from the fenestral membrane, are gradually added to the margin of the plate. This process, illustrated in figures 4 and 6, is quite different from that which obtains in the cephalic part of the plate, where it spreads out

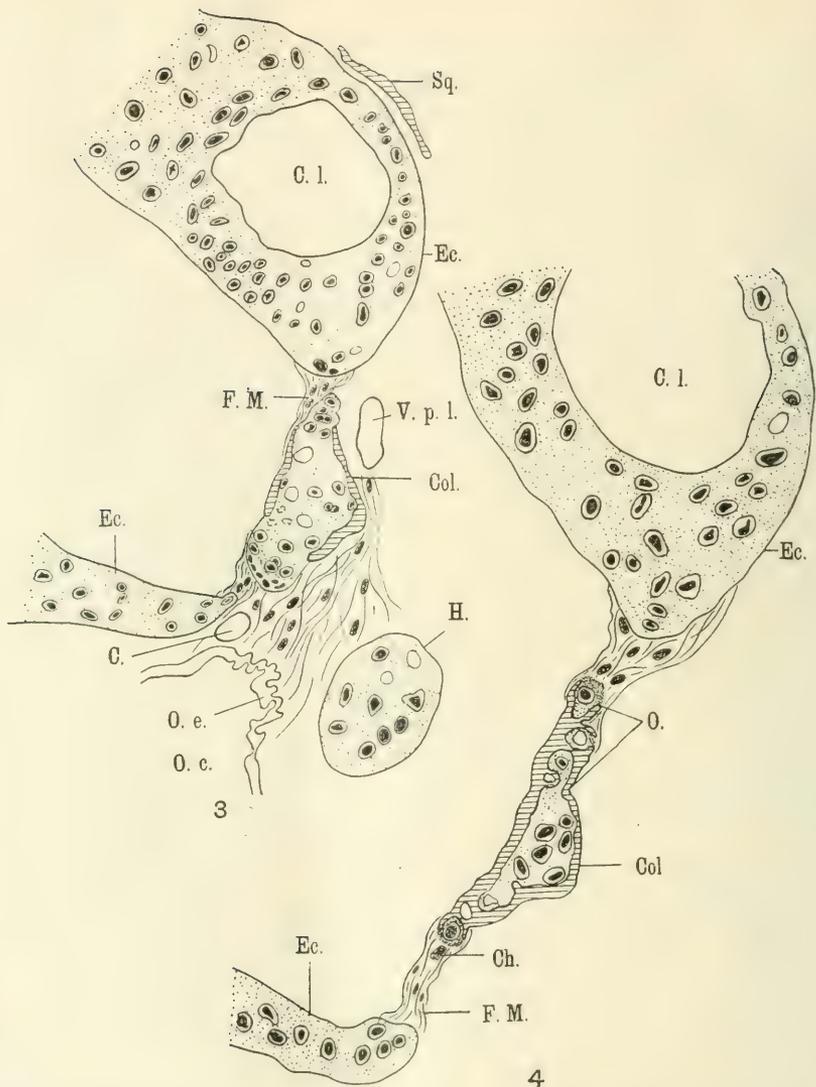


Fig. 3 Transsection of the ear capsule of a larval *Necturus* 40 mm. long, through the cephalic part of the fenestra. *C.*, arteria carotis interna; *C.l.*, canalis lateralis; *Col.*, columella spreading out over the fenestral membrane due to the proliferation of cells from its dorsal and ventral borders; *Ec.*, ear capsule; *F.M.*, fenestral membrane applied to the ental side of the columella only; *H.*, hyoid; *O.c.*, oral cavity; *O.e.*, oral epithelium; *Sq.*, squamosum; *V.p.l.*, vena petrosolateralis.

Fig. 4 Transsection of the ear capsule of a larval *Necturus* 40 mm. long, at a level of the middle of the fenestra. *Ch.*, chondroblasts forming in the fenestral membrane independent of columellar tissue; *Col.*, columella; *C.l.*, canalis lateralis; *Ec.*, ear capsule; *F.M.*, fenestral membrane; *O.*, otic portion of fenestral plate (operculum) formed by chondroblasts in the fenestral membrane, which are later surrounded by bony tissue and thereby joined to the definitive plate.

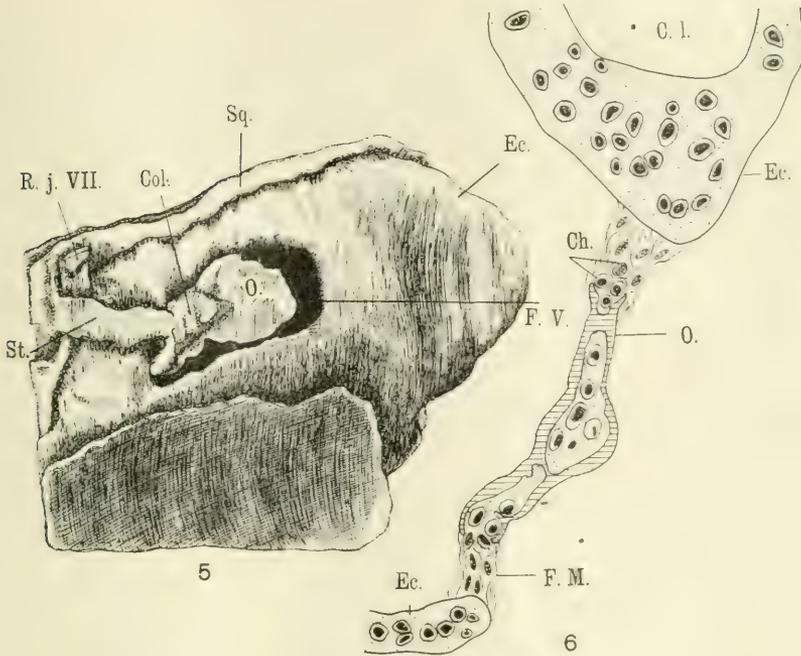


Fig. 5 Drawing from a wax model of the ear capsule of a larval *Necturus* 48 mm. long. *Col.*, columella portion of fenestral plate derived from tissue outside the ear capsule; *Ec.*, ear capsule; *F.V.*, foramen vestibuli; *O.*, otic portion of fenestral plate derived from chondroblasts in the fenestral membrane and comparable to the operculum of *Amblystoma* in origin and position; *R.j.VII.*, ramus jugularis of the *N. facialis*; *Sq.*, squamosal; *St.*, stylus columellae.

Fig. 6 Transsection of the ear capsule of a larval *Necturus* 40 mm. long, just caudad of the middle of the fenestra. *Ch.*, chondroblasts in the fenestral membrane; *C.l.*, canalis lateralis; *Ec.*, ear capsule; *F.M.*, fenestral membrane; *O.*, otic portion of the fenestral plate. At this level the columellar portion has almost disappeared; compare with figure 5.

over the fenestral membrane through the growth of its own tissue, as is shown in figure 3.

Transsections during this stage of development show that the fenestral plate of *Necturus* possesses numerous areas of cartilage encircled by bone, so that it exhibits a decidedly ringed appearance. This is due to the mode of ossification in connection with the formation of cartilage about the periphery of the plate. As in *Amblystoma*, two plates of bone are formed,

one upon the inner surface of the columellar portion and the other upon the outer. When growth of the cartilage ceases these plates meet above and below, thus forming a complete shell to this element. New chondroblasts in the membrane are consequently prevented from fusing with the previously formed cartilage and in the end become joined only through the extension of bony tissue about them in groups, or individually, as may be seen by a glance at the figures, where various stages in the extension of bone are shown. In many cases every stage in the formation and growth of cartilage and its inclusion by bony tissue may be seen in the same section. It is this method of independent origin of cells in the membrane and their fusion through ossification into a connected whole that accounts for the peculiar relations of the two elements shown in figures 4 and 6.

It appears evident from a study of the material available that the fenestral plate in *Necturus* is of double origin. The anterior end and a portion of the center being formed from the columella proton represents that structure in *Amblystoma*. The caudal half being formed by the chondrification of the fenestral membrane belongs to the ear capsule and must be likened, therefore, to the operculum of *Amblystoma*, although the *M. opercularis* never appears. The plate, as a whole, in its morphological nature is like that of *Spelerpes*, the difference being found in degree only.

*Otic connections of the fenestral plate.* There appears in the literature (Wilder '03) some comment concerning a connection between the fenestral plate in *Necturus* and the otic capsule. Since this element is free from the ear capsule in young larvae and since there is no continuity between the two structures in the adult it appeared advisable to examine the various series carefully with this point in mind, for in the light of such connections in other forms, an early fusion and a later separation of the parts did not seem probable. As stated above, the position of the columellar proton is along the fenestra, outside the membrane. Chondrification begins in larvae about 22 mm. long and at this stage the columella is clearly distinct from the ear capsule. Very soon, however, it comes to lie close to the

fenestral membrane, in which a decided impression is made. In somewhat older larvae (25 to 26 mm.) the cephalic and dorsal growth causes the columella to press closely against the lips of the fenestra, bringing about a relation which is maintained throughout life. In most instances the perichondrium, where the two structures meet, can be made out and in no place does there appear what might be considered a true fusion, but rather a firm articulation. An examination of the articulation in the adult strengthens the view that the lips of the ear capsule do not fuse with the fenestral plate and contribute nothing to its formation. The close relations may result from physical causes alone, since the plate of the adult exactly fills the fenestra, or may be considered as reminiscent of such fusions as occur in *Amblystoma* and others.

*The stilus.* It is a well established fact that the stilus in *Necturus* and *Proteus* is found below the jugularis branch of the facial nerve, a relation which is not the usual one among urodeles. It is believed, however, that this relation does not affect its homology with the stilus of other forms, a view which seems to be supported by the relations of columella and nerve which obtain in certain of the plethodontids. One observes that in the *Amblystomidae* the ramus jugularis VII comes forth freely underneath the stilus columellae and follows a course caudad across the ventral portion of the fenestra and its plate. In the *Plethodontidae*—as *Gyrinophilus*, for example—it emerges close against the ventral border of the stilus, and turning somewhat abruptly, takes a dorsal course across the fenestral plate. Whatever may have been the cause of the difference in the relative position of nerve and skeletal parts in these groups, it offers a possible explanation of the existing relations in *Necturus*. The nerve not only has a more dorsal position, but is well defined before the mesenchyme becomes concentrated into the forerunner of the columella. The latter naturally develops underneath instead of above it. The apparent mingling of the R. jugularis VII and the cells of the columellar proton in some plethodontid embryos tends to strengthen such a belief.

*Summary.* The fenestral plate in *Necturus*, while of the single type, is double in its origin. The columellar portion is extra-otic, having no early developmental connection with the ear capsule. At about the beginning of larval life it spreads out over the fenestral membrane and completely fills the cephalic portion of the fenestra, from which position it gradually narrows, coming to a point and disappearing near the center of the oval window. The remaining portion (by far the larger) of the fenestra is filled by tissue originating from chondroblasts in the fenestral membrane and therefore strictly otic. The columellar portion including the stilus is the homolog of the columella of *Amblystoma*. The otic part of the plate represents the operculum. The larval characteristics of the plate are shown *not* in its morphology but in the absence of the *M. opercularis*. The sound-transmitting apparatus of this species is a true morphologic intermediate between that of *Amblystoma* and the *Plethodontidae*.

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ON THE COMPARATIVE OSTEOLOGY OF THE  
LIMPKIN (*ARAMUS VOCIFERUS*) AND ITS  
PLACE IN THE SYSTEM

R. W. SHUFELDT

SIXTEEN FIGURES

In the world's avifauna there are two birds contained in the genus *Aramus*. They have the appearance of large rails, and, very much like these, they inhabit marshes and extensive swamps and bogs. One species—the *Aramus scolopaceus* of Gmelin—ranges through Brazil, Guiana, and Venezuela, while our United States species—*A. vociferus*—occurs in Florida, the Greater Antilles, Central America, northward to South Carolina, and, very rarely, westward into Texas.

A number of years ago I prepared an illustrated account of *Aramus vociferus*, comparing its skeleton with those of rails, cranes, and allied birds; but as no good opportunity offered at the time for its publication, the manuscript and figures were set aside with others I was at work upon then. Later on this material was taken up again, and I published a brief, illustrated synopsis of it, which, while useful in some ways, was quite inadequate for working avian osteologists.<sup>1</sup> In that contribution, however, there was presented the schemes of classification of the super-suborders Gruiformes and Ralliformes of a number of the most eminent avian taxonomists, of this and the last generation, as Merrem, Huxley, Garrod, Selater, Newton, Reichenow, Fürbringer, Sharpe, Gadow, and others.

The crane-rail group at that time I considered to form the suborder Paludicolae, an opinion at variance with the one I now hold.<sup>2</sup>

<sup>1</sup> Shufeldt, R. W. on the osteology of certain cranes, rails, and their allies, with remarks upon their affinities. *Jour. Anat. and Phys.*, Lond. October, 1894, vol. 29; N.S., vol. 9, pt. 1, art. 5, pp. 21-34; text figures.

<sup>2</sup> Shufeldt, R. W. An arrangement of the families and the higher groups of birds. *Amer. Nat.*, vol. 37, nos. 455-456, November-December, 1904, pp. 833-857, figs. 1-6.

There are three little tables given in the above cited article in the *Journal of Anatomy*, arranged in parallel columns. These tables present, in a comparative way, the salient osteological characters of *Rallus longirostris*, *Aramus vociferus*, and *Grus americanus*, and are very useful. Moreover, the article is illustrated by figures of the skull of the limpkin, a rail, and a crane, which, while they show the characters pretty well, are by no means as good as similar figures made by me of more recent dates. It is not my intention here to cite any of the numerous articles which have been published on the osteology of either the rails or the cranes and their allies, a number of which are from my own pen, contributed many years ago.

On the other hand, it is the specific object of this contribution to give a detailed account of the skeleton of *Aramus vociferus*, which is a chapter in avian osteology hitherto unpublished, and one which will prove to be highly useful to students of the subject in the future, particularly to paleontologists who may require such information at any time.

For many years the fact has been more or less generally recognized that *Aramus* is related to the cranes (*Grus*) on the one hand, and to the rails (*Rallidae*) on the other. This, however, is a question to be more thoroughly touched upon in the concluding remarks at the close of the present article. Further, it is fair to presume that many species of birds of past ages and eras have become extinct, which, were they in existence now, would not only fill in the above-mentioned gaps, but would render it at once clear exactly what the aforesaid relationships were among all these now most puzzling gruine and ralline genera and species of birds.

In so far as I am aware, none of the fossil cranes or rails discovered up to date have thrown much light upon this part of the subject, though there is no telling what future discoveries along such lines may have in store for us.

Unfortunately there are, at this writing, but few skeletons of the *Gruidae* at my disposal for examination and comparison; while among the *Rallidae* there are a larger number of species and genera of birds, both existing and extinct, which stand in

need of osteological description and comparison with *Aramus*, especially of the genera *Rallus*, *Limnopardialis*, *Gymnocrex*, *Aramides*, *Aramidopsis*, and other ralline groups, or birds supposed to be more or less nearly related to the typical forms we designate as rails.

Upon comparing the skeleton of such a species as *Grus mexicana* among the cranes or *Gruidae* with that of the limpkin, it would seem that the gap between these two genera and their affines upon either side is at least partially bridged over—that is, in so far as two representatives species can demonstrate it. Take the *skull* of *Aramus* for example. With equal truth one might say that it belonged to some bird that was either a rail-like crane or a crane-like rail; with such equality are the characters represented in it, that is, ralline and gruine ones. Especially is this true when we come to compare this skull with that part of the skeleton of *Rallus longirostris crepitans* on the one hand, and *Grus mexicana* on the other.

One of the most evident characters distinguishing it from *Rallus* is its having a pretty well marked supraoccipital prominence, with an occipital foramen upon either side of it; this is a gruine character. The vacuity in the interorbital septum is smaller than it is in either *Grus* or *Rallus*. *Aramus* has a short pterygoid, much dilated behind, and not seen in either the crane or the rail, where the pterygoids, although short, do not show this dilation. Its palatines and its laterally compressed, sharp-pointed vomer, with its inferior edge cultrate, are exactly what we find in *Grus*, this latter bone being more spreading in the clapper rail.

Its maxillo-palatines, being plate-like, are decidedly more gruine than they are ralline, as is also the broader interorbital frontal region above. The merest paring is taken off the edge of the superior orbital margins, to indicate the presence of the nasal glands, while those depressions are better marked in *Grus*, and still better in *Rallus*. In type the quadrates and lacrymals agree in all three of these genera. The broad orbital process of the first-mentioned bones are squarely truncated, exactly as we find them in *Porzana*. *Aramus* has its temporal fossae on the



Fig. 1 Right lateral view of the skull of *Aramus vociferus*; natural size. Photograph by the author of specimen No. 19667, Collection U. S. National Museum; ♂ adult

lateral aspects of the skull concaved precisely as they are in *Grus* and *Rallus*, and all its supero-parietal region is beautifully rounded, as we likewise see it in the genera mentioned. Passing to the foramen magnum of *Aramus*, we find it to be quite circular in outline, while in *Grus* and *Rallus* it is cordate. Again, it differs from either in having the anterior wall of its brain-case thoroughly completed in bone, a feature, by the way, not often met with in birds. In its fronto-premaxillary region above *Aramus* exhibits the most perfect type of schizorhinalism, the sutures among the several bones there being very distinct, and remaining persistent during the life of the individual. The descending limbs of the nasals are antero-posteriorly broader than they are in either *Rallus* or *Grus*, while the nasal processes of the premaxillary—which extend from those bones and the palatine forwards—are more or less rounded and rod-like, not showing the supero-longitudinal grooving seen in the latter genus.

*Pars plana* is small and very distinct on either side, and lacks that elegant scroll-like process seen above and in front of it in *Grus*, in which it connects upon either side the lateral ethmoidal wing with the nether side of the fronto-nasal roof above. In all the birds thus far considered, the basitemporal region of the skull is, with respect to its characters, in very close agreement, and in them, too, the osseous aural entrance is very open, lacking the bony protecting walls so generously supplied by the squamosal and its neighboring bones in not a few other birds.

The long, acutely V-shaped *mandible* of *Aramus*, with its symphysis extending back for at least one-fourth its length, more nearly approaches the bone as seen in *Rallus* than the mandible in *Grus*. Its articular ends are abruptly truncated behind, and a good-sized ramal vacuity exists at the usual site upon either side. Borders or edges of either ramus above and below are rounded, as is also the under side of the mandibular symphysis, the upper part of the latter being longitudinally grooved. The hyoidean apparatus, the sclerotical plates of the eyeballs, and the ossiculae auditus require no special description.

In all the North American cranes and rails, including *Aramus*, the thyro-hyal rods seem to be the only part of the skeleton of the tongue that ossify, and the ring of bony platelets in an eye are all small for the size of any particular species to which they may belong.

*Of the remainder of the axial skeleton:* Between the skull and pelvis, *Aramus* has 23 vertebrae. With the possible exception of the *atlas* they are all thoroughly pneumatic, as in *Grus*. The postero-external angles of the neural arch of the first cervical are produced as processes, extending backwards, while the superior periphery of its cup is roundly notched out. In the *axis vertebra* we find a very low, tuberous neural spine, and a well-developed hypapophysial one. But what is unusual is that it has a good pair of parapophysial processes directed backwards. Its neural canal is small for the size of the bird. This is also the case in the third vertebra, after which this canal gradually enlarges, to become small again as it passes through the dorsal series, where it is of markedly small caliber, as it is in the pelvis. In the third vertebra the neural spine is very inconspicuous, likewise in the fourth, to be entirely absent in the fifth to the eleventh inclusive; whereupon, in the twelfth, it gradually begins to make its appearance once more, until it assumes the well developed quadrate plate seen in the dorsal vertebrae. In the third vertebra we see interzygapophysial bars connecting the pre- and post-zygapophyses, while in the fourth these are long and reduced to the most hairlike dimensions. The lateral vertebral canals also begin in the third vertebrae, and persist as such to the 16th cervical inclusive. Commencing, as I have said, in the *axis*, the narial parapophysial spines are present down the chain to include the *tenth*, they being very long and slender from the fifth. In the *eleventh* they suddenly disappear altogether, which is an interesting fact. From the fifth to the thirteenth cervical a hypapophysial open channel is developed for the passage of the carotid arteries; a small spine takes its place in the fourteenth vertebra. But this never becomes very large thereafter, and disappears entirely as we pass to the last four dorsal vertebrae. This hypapophysial

spine exhibits a tendency to trifurcate in the seventeenth segment of the column, but at the best it is but feebly accomplished. The 18th, 19th and 20th (dorsal) vertebrae are completely fused together, while the 21st, 22d and 23d are freely articulated. In these last three, long osseous metapophysial spines or ossified tendons of the spinal muscles ornament the neurapophyses and transverse processes, extending, as they do, both forwards and backwards. A slender pair of free ribs are suspended from the 17th vertebra; on the coössified 18th these connect with the sternum, as they do in the succeeding five dorsals. There is also a pair of pelvic ribs that meet the sternum by means of rather long hemapophyses. All these ribs are highly pneumatic, and the true thoracic ones have free unciform appendages upon their posterior borders. The second and third pairs of ribs are short and slightly stoutish, but they soon become, in the succeeding pairs, long, slender and narrow.

The *pelvis* of *Aramus*, in some important particulars, more closely resembles that bone in *Grus* than that in the *Rallidae*, though it is not lacking in the characters seen in the pelvis of the latter. Regarded upon superior view, we note that the faintly emarginated anterior ends of the ilia are squarely cut across, while the mesial margins of these bones rise up to fuse with the superior border of the sacrae crista, thus completely sealing in the 'neural canals' behind.

The surface of the preacetabular portion of either bone faces almost directly outwards. There are present no intervertebral foramina of the sacrum, such as we find in rails; and the postacetabular part of the pelvis, with the included sacrum, is bent downwards, so as to make a considerable angle with the fore part of the bone. Posteriorly, the ischio-iliac extremities extend far back of the last sacral vertebra, the posterior margin of the latter lying in the curve formed by the mesial edges of the ilia.

Viewed laterally, it will be seen that the external iliac borders of the postacetabular region extend over the outer aspects of the ischia, but, proportionally, not to the extent they do in some of the *Rallidae*, though rather more than in *Grus*. The planes in which the ischia are found are nearly parallel to each other,

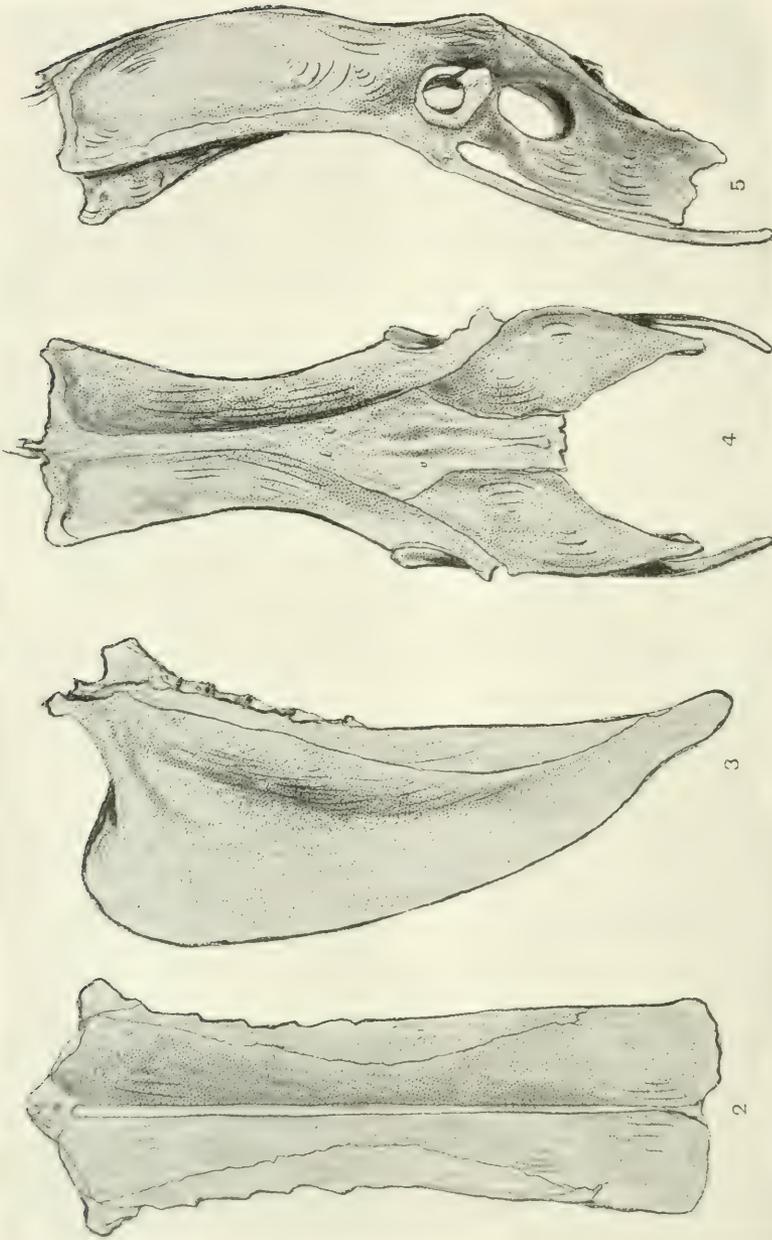


Fig. 2 Ventral view of the sternum of *Aramus vociferus*; adult; No. 11795, Collection U. S. National Museum.

Fig. 3 Same bone shown in figure 2, seen on left lateral view.

Fig. 4 Pelvis of *Aramus vociferus*; dorsal view; same specimen as before.

Fig. 5 Same bone as shown in figure 4, viewed on left lateral aspect. All specimens drawn natural size by the author.

and the ischiadic foramen is relatively small. The flat, rather broadish pubic style is separated by an open line coming in contact with the lower edge of the ischium above, and its distal extremity is produced well beyond the latter bone, to run out as it slightly curves towards the fellow of the opposite side. The posterior ilio-ischiadic margin exhibits hardly the semblance of a 'notch,' the edge of the former being in a line nearly perpendicular to the plane of the postacetabular region, or to the superior edge of the pubic style that extends beyond it. Seen upon ventral aspect, we find that the *five* leading sacral vertebrae throw out their lateral processes, to fuse with the ventral surface of the ilium upon either side; this agrees with *Crex*. In *Grus*, *seven* of the leading sacrals behave in this manner. In *Aramus* the next four vertebrae have their elevated diapophyses completely concealed upon this view by the much swollen sacral body. Then follow five more vertebrae, which have stout transverse processes thrown out as braces against the inner margins of the ilia. The first pair of these are the longest, and they rapidly graduate down to the ultimate sacral. In the case of the first pair, too, the outer ends of their transverse processes meet, upon either side, the superior periphery of a cotyloid ring, and posterior to this point they fuse with the outer ends of the diapophyses of the next vertebra behind. But these two only—the two true sacrals—are thus linked together. The renal fossae are deep and circumscribed, especially by the folding forwards of the postero-ventral parts of the ilia, as they do both in *Grus* and all typical *Rallidae*.

There appear to be *six* free *caudal vertebrae*, but they are small, and have all their outstanding processes considerably aborted. A sub-quadrilateral *pygostyle* of better proportions finishes off the distal extremity of the spinal column in this remarkable bird.

Passing to its *shoulder-girdle*, we find coracoids, scapulae, and os furcula all highly endowed with pneumaticity. The first-mentioned are rather short bones; antero-posteriorly compressed, and lacking an epicoracoidal process, but developing an elegant, forward-curling scapular wing from the mesial side

of the shaft), which presents, well up, that foraminal perforation seen in the coracoids of several other groups of birds. A generous articular facet occupies the mesial two-thirds of the lower border of the expanded sternal part of either one of these coracoids, and this facet is grooved for its entire extent in the transverse direction. This groove admits of perfect articulation with the rounded transverse eminence occupying nearly the entire length of either coracoidal facet of the sternum. Disagreeing with both *Grus*, and the *Rallidae*, the coracoids of *Aramus* slightly decussate in their sternal beds.

*Os furcula* is a broad U, without a hypocleidium, and with its transversely flattened rami, of no mean width, extending, without narrowing in the least, to include the symphysis, the anterior and posterior plane surfaces of which latter have gradually come to face the other way. The free, upper clavicular ends are bluntly truncated, and support each an articular face, for a coracoid and scapula of the corresponding side.

A *scapula* is a rather short bone, elegantly curved outwards, drawn gradually to a point behind, and its outer blade decidedly flattened in the vertical direction. Its head is large, and is occupied, at their usual sites, by extensive facets for the os furcula, the coracoid, and the demi-facet of the glenoid cavity. At the under or ventral side of this end of the bone, just within the coracoidal facet, we always find a pneumatic foramen, which is very large in *Grus*.

The *sternum* of *Aramus* is very long and correspondingly narrow. Its carina is inclined to be shallow, and it extends the entire length of the sternal body, which latter is distinctly quadrilateral in outline, without any xiphoidal processes behind, but showing there a slight median emargination. The carinal angle is handsomely rounded off; the anterior carinal border above it is concave and somewhat thickened; while the manubrial process is quite rudimentary. Either costal process is very much turned outwards, and is, comparatively, of no great height. The costal borders are transversely narrow; the haemaphysial facets upon them rather far apart, and their intervals occupied by deep little concavities. On the ventral aspect of the

sternal body, on either side of the keel, a strong, rough, muscular line is seen, extending nearly its entire length. It is uniformly concaved outwards, the surface of the bone being roughened within it and smooth without. The convexity of this 'pectoral line' is well separated by an interval from the base of the keel.

On the thoracic aspect of this sternum we find it deeply concaved in front, gradually shallowing as we pass posteriorly. A median line of small pneumatic foramina are seen, and others are found, upon this view of the bone, in various localities. Between the costal borders, where the thoracic concavity is deepest, the two sides of the sternal body are *flat*, and face each other at an open angle. In front, the anterior wall, which bears the coracoidal grooves upon its outer side, is deep and nearly vertically disposed.

*Of the appendicular skeleton:* Aside from the great difference in size, the *humerus* of *Aramus* is the very counterpart of that bone as we find it in *Grus mexicana*—even to the most trivial characters. In the former it has a length of 11 cm., just double that in the latter, and they differ from the humerus of the Rallidae in their being more completely pneumatic. The pneumatic foramen is a single hole, and the ulnar and radial crests are strongly developed; so is the thick humeral head, which is separated from the ulnar crest by a deep valley. The smooth shaft shows the double sigmoid curve and is subcylindrical on section. At the distal end we find the articular trochleae and of the usual form. There is a very rudimentary epicondylar process.

Having compared in detail the remaining bones of the pectoral limb of *Aramus* with the corresponding ones in *Grus mexicana*, I find them likewise, as in the case of the humerus, to agree, character for character, throughout, except in the matter of size. As a rule, the various bones have in *Grus* rather more than double the lengths of their counterparts in the antibrachium and pinion of *Aramus*. They are not pneumatic but inclined to be stoutish in proportions. An *ulna* is concavely bowed along its anconal border, while the palmar one shows a row of papillae for the quill-butts of the secondary wing feathers. The proximal end is, in proportion, larger than ordinary, as compared with the

distal extremity. This is so in order to support the articular cavities for the large trochleae of the humerus. An olecranon process is pretty well developed also. The radius is slightly bowed along its inferior border, and its carpal end is larger than its head. On the whole it exhibits the usual ornithic characters. This also applies to the two small bones of the wrist, and the various ones that make up the skeleton of the pinion. In the *carpo-metacarpus* the medius metacarpal is bowed and a trifle longer than the stouter and straight one of index. There is constantly present in both *Grus* and *Aramus* a minute hole on the anconal aspect of the head, between the tubercle that occurs there and the trochlear surface, which, inasmuch as it is not a pneumatic one, must be for the entrance of a nutrient vessel. The central portion of the expanded part of the proximal phalanx of index digit is very thin, and occasionally shows a single pin-hole perforation in the limpkin, but not in the crane, as a rule. Nothing peculiar is exhibited on the part of the terminal finger-joints, and pollex digit bears a claw.

None of the bones of the *pelvic limb* in either *Aramus* or *Grus* are pneumatic. In the majority of their essential characters, the corresponding ones in the two genera agree, except, of course, in the matter of size, thus being in *Grus* considerably longer and larger in every way. As to lengths, however, they differ in proportions, as will be seen from table 1, given in millimeters.

TABLE 1

	FEMUR	TIBIOTARSUS	TARSO-METATARSUS
<i>Grus</i> .....	129	314	185
<i>Aramus</i> .....	84	181	136
Differences.....	45	133	49

In other words, twice the length of the femur in *Aramus* would be 168 mm. against 129 mm.—the length of the femur in *Grus mexicana*—showing the bone to be considerably more than twice the length in the former, as compared with the length of that in the latter. Whereas, in the case of the tibiotalarsus,

twice its length in *Aramus* would be 362 mm. against 314 mm. of the bone in *Grus*, showing it to be nearly double the length—as 39 mm. is to 48 mm., the differences as thus compared. The differences in the tarso-metatarsi are nearer what they are in the femora, as may be seen from the above table.

In *Aramus* the *femur* has a shaft that for its middle third is nearly cylindrical, the bone as a whole being slightly bowed in the anterior direction. Its great trochanter is very broad transversely, and its crest rises above the articular summit, being continued round on to the anterior aspect of the upper third of the shaft. A deep pit exists for the ligamentum teres on the semiglobular caput femoris. At the distal end the condylar portion is greatly developed; the rotular channel in front is very marked, as is its continuation below as the intercondyloid fossa. The inner projection of the external condyle is sharp and prominent, and the pits in the neighborhood for muscular and ligamentous attachment very distinct. The lowermost points of the condyles are nearly in the same horizontal plane, and the popliteal depression is not especially concaved. On the surface of the shaft we find the usual muscular lines well marked.

If *Aramus* and *Grus* possess *patellae*, they have been lost from all the material at present at my command, and at this writing I cannot speak with certainty upon that point.

The shaft of the *tibio-tarsus* is very straight, being flattened in front and rounded behind. Its pro- and ectocnemial crests are well developed, but they do not extend down on the shaft, the latter process being hooked with its plane at right angles to the former, which stands out about perpendicular to the surface of the anterior aspect of the shaft. The rotular crest is only very moderately developed. At the distal end of this bone we meet with the usual characters found there in all ordinary birds. For the confinement of some of the anterior tendons, we find the little osseous bridge spanning a deep groove, with the tubercles above it, one on either side, for the attachment of the ends of a ligament that fulfils a similar purpose. Of ordinary size, the condyles are separated by a well marked intercondyloid groove, it being especially so in front. The internal condyle

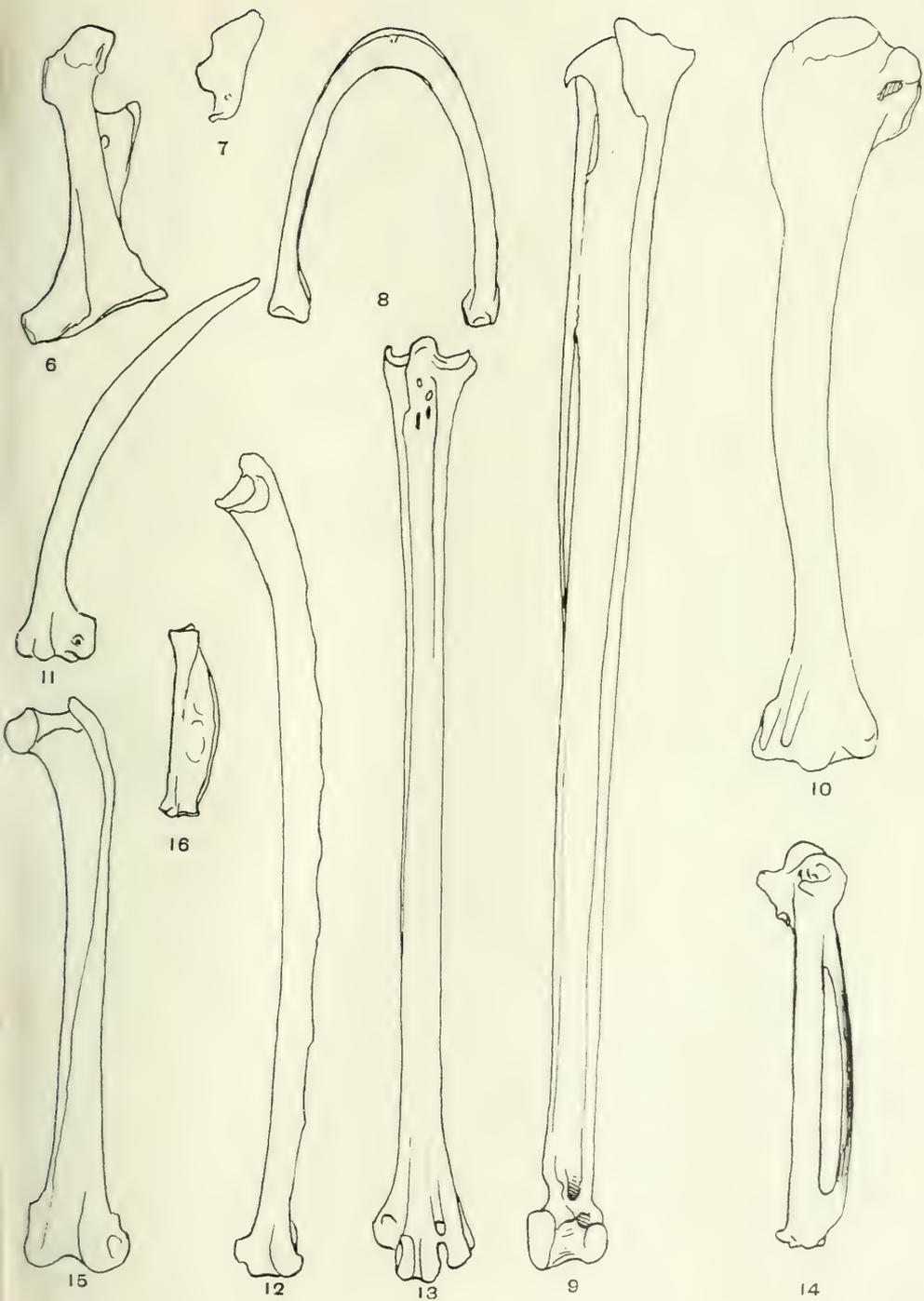
is sharp behind, where it projects; is shallow in the vertical direction, and long in the antero-posterior—this being less the case in the external one. Both have the usual reniform outline.

Aramus has an incomplete *fibula*, its lower extremity fusing indistinguishably with the outer side of the tibio-tarsal shaft at about its middle. The articular fibular ridge is high up on the latter, and has a length of about 2.5 cm.; fusion does not take place at this point. The head of this bone is rather large, transversely flattened, and produced backwards. On the outer side of the upper part of its shaft is a small, deep, spiral groove for the tendon of the *biceps flexor cruris* muscle.

*Tarso-metatarsus* has its shaft quite as straight as that of the tibia. Its sides are flat, but before and behind it is longitudinally grooved for the passage of tendons. In either case, this grooving extends the whole length of the bone, but is deepest on the anterior aspect for its upper third. On the summit of the bone the articular concavities are deep, and at the fore part between them the interarticular rounded prominence is conspicuous. The hypotarsus, though fairly well developed, is not especially so, and it is hardly at all extended down upon the back of the shaft. It has one main groove which is deep and nearly closed over posteriorly. Within, this groove is partially subdivided into two by a more subordinate median longitudinal crest. Upon either side of the hypotarsus we find a small perforating foramen. The anterior exits of these are close together just above the tubercle for the tendon of the tibialis anticus muscle in front. At the distal end of the shaft the trochlear processes are rather large and distinctly separated from each other. The lateral ones curve towards the median

Fig. 6-16 Various bones of the limpkin (*Aramus vociferus*); natural size. Drawn by the author in outline from specimen No. 11795, collection U. S. National Museum.

- Fig. 6 Anterior aspect of right coracoid. Fig. 7 Coccyx, left surface.  
 Fig. 8 Front view of os fureula. Fig. 9 Anterior aspect, right tibio-tarsus and fibula.  
 Fig. 10 Anconal aspect, left humerus.  
 Fig. 11 Right scapula, upper surface. Fig. 12 Left ulna.  
 Fig. 13 Left tarso-metatarsus, from in front. Fig. 14 Left carpo-metacarpus.  
 Fig. 15 Left femur, anterior surface. Fig. 16 Proximal phalanx, index digit.



plane behind, particularly the inner one of the two, which is at the same time the highest on the shaft. The next in this latter respect is the external one, the lowest on the shaft being the mid-trochlear process. Between this and the outer one is found the usual perforating foramen for the anterior tibial artery.

The small free *first metatarsal* is but slightly twisted upon itself (about half a turn), and presents nothing beyond what we usually see in that bone in birds; it has a length of 9 mm.

Aramus has the normal type of foot for birds, that is, 2, 3, 4, 5 joints to 1 to 4 toes respectively. As a rule the phalanges are long and slender, agreeing in this and other respects better with this part of the skeleton in *Rallus* than with *Grus mexicana*. Ungual joints are well developed and somewhat curved. All the sides of these latter are longitudinally marked by very distinct groovelets.

In both *Aramus* and *Grus* there is a great disposition for most of the tendons of the muscles of the pelvic limb below the thigh to ossify. In many cases they form stout, osseous rods, of very considerable length; others are beautifully dilated for half their lengths, like a small, partly opened fan, composed of the most delicate radii, being formed by that part of the tendon which is spread out in or on the muscle. In a disarticulated skeleton of *Grus mexicana* before me, there are between 60 and 70 of these ossified tendons, and about half that number in a similarly prepared skeleton from a specimen of *Aramus*.

From this description it will be seen that this rail-like bird belongs in a different family from the *Rallidae*—that is, in the family *Aramidæ*, as pointed out by me in my classification of Aves, published in *The American Naturalist* in 1904 (vol. 38, nos. 455–456, Nov.–Dec., p. 852), which stands thus:

Supersuborder	XIII	RALLIFORMES
Suborder	XX	Fulicariæ
Superfamily	I	Heliornithoidea
Family	I	Heliornithidæ
Superfamily	II	Ralloidea
Family	I	Rallidæ
	II	Aramidæ

# THE PARAPHYSIS AND PINEAL REGION OF THE GARTER SNAKE

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FORTY-ONE FIGURES

The pineal region of the vertebrates has been studied by a large number of investigators whose interest has been directed especially to the parietal organ and epiphysis, the significance and functions of which are still so problematical. On account of the relatively high state of development of the parietal organ in *Sphenodon* and many lizards, the reptiles have received special attention and the papers of Warren ('11) on the development of the pineal region in the lizard and turtle, Dendy ('99) on *Sphenodon*, and Voeltzkow ('03) on the crocodilian furnish rather complete pictures of the development of the region in each one of these groups. The snakes have received very little attention from this point of view and are only very imperfectly known. It was for the purpose of filling up certain serious gaps in our knowledge regarding this group that the present study was undertaken.

The literature dealing with the pineal region in snakes is very restricted. The earliest reference to this region is Hoffmann's ('85). In describing briefly the pineal region in *Tropidonotus natrix*, he called attention to a thickening of the roof of the brain at the boundary between the telencephalon and diencephalon. He offered no suggestion as to its significance but there can be little doubt that it was the anlage of the paraphysis which he saw. Hanitsch ('88) in a paper which I have not been able to see for myself, describes according to Leydig ('97) an organ which he supposed to be a well defined parietal eye in an embryo *Vipera berus*. He described the eye as provided with a lens in

which were pigment masses. Leydig thinks that Hanitsch was in error regarding this structure, and in his own investigations on *Coronella* he found nothing comparable to a parietal eye. In the stage described by Leydig, the paraphysis is characterized by the presence of many budlike evaginations and the epiphysis has become solid though it still retains its connection with the roof of the diencephalon by means of a hollow stalk penetrating the posterior commissure. Studnicka ('93) studied the epiphysis and paraphysis of an advanced embryo and a full-grown specimen of *Tropidonotus* and found the epiphysis to consist of a massive, ellipsoid body connected with the roof of the diencephalon by a thin stalk. The epiphysis itself was of a glandular structure. It was divided into lobes by connective tissue septa and exhibited a small cavity at the junction of the stalk and the body proper. In the adult form the same condition was found except that the cavity was wanting. Sorensen ('94) figured a sagittal section of the diencephalon of an embryo black snake in which the epiphysis was connected by an attenuated stalk with the roof of the diencephalon and was inclined caudad. He also figured a cross-section of the epiphysis of the garter snake which was a globular body. The failure of these investigators to find a parietal organ is probably because of the advanced age of the embryos studied and the temporary occurrence of that organ. The most complete account of the development of the pineal region in the snakes is that of Ssobolew ('97) on *Tropidonotus* and *Vipera*. In the former the epiphysis is described as a double evagination of the cerebral vesicle at the point of division between the di- and mesencephalon. The lumen of the epiphysis is still in wide open communication with the brain when the paraphysis first appears, and at its distal end exhibits "eine kleine Vertiefung mit der Bildung von zwei rundlichen Körnern, -dem vorderen und hinteren." The former he interprets as the anlage of the parietal organ, the latter as that of the epiphysis. At this stage the wall separating the two does not quite reach the level of the wall of the diencephalic roof. Ssobolew's figures fail to show the parietal organ so that a clear picture of the relationship of this organ to the epiphysis is still

lacking. In view of my own findings in *Thamnophis* and the irregularities which appear in the epiphysis, there may still be some question as to the exactness of his interpretation. In *Vipera* he described, without a figure, the anlage of the parietal organ as an anterior evagination from the epiphysis resting directly on the anterior epiphysial wall and accordingly elevated through the growth of the latter while not itself growing materially. All trace of the parietal organ disappeared later in both snakes. So far as I have been able to find, these are the only papers devoted to the development of the epiphysis and parietal organ in the snakes, although several have described the condition in the adult animal, for instance, Rabl-Rückhardt's ('94).

For a full account of the literature on this subject reference should be made to Studnicka ('05) and Gaupp ('98).

The observations upon which this study is based were made upon a series of some 20 embryos of *Thamnophis radix*, the ordinary garter snake, ranging in length from about 10 to 100 mm. The lengths of the younger specimens could be obtained only approximately on account of their being coiled in a tight spiral. These embryos were projected and drawn by means of a camera at a magnification that was exactly determined in each case and was about 10 diameters; the length was then scaled off on the drawing by means of dividers. In the stages in which the cerebral flexures were marked, the most prominent point of the mesencephalon was regarded as the most anterior point. The older embryos were sufficiently flexible to permit a straightening of the coils and direct measuring. These embryos I have grouped into five stages for convenience of description. Stage I is characterized by its  $2\frac{1}{2}$  spirals and length of 11 mm. The choroidal fissure of the eye is invisible from the exterior; the mandibular, second, and third visceral arches are evident. The otocyst from the lateral aspect has a simple triangular form. In Stage II the olfactory pits have become constricted to form small nostrils. The body length is 18 mm. and there are  $3\frac{1}{2}$  coils. Stage III has an approximate length of 30 mm. In Stage IV the length is about 42 mm. and the number of coils is  $4\frac{1}{2}$  or 5. The phalli are well developed and everted at this time in the

males. Stage V has a length of 80 to 100 mm. and is characterized by the disappearance of the mesencephalic flexure of the brain.

The roof of the forebrain included in the present study comprises the following parts enumerated from posterior to anterior:

(a) The posterior commissure, occupying the roof of the synencephalon (or pars intercalaris of Burekhardt),

(b) The epiphysis or pineal body arising as an evagination immediately anterior to the posterior commissure,

(c) The superior commissure or commissura habenularis which connects the two ganglia habenulae, and which is situated immediately cephalad to the stalk of the epiphysis,

(d) The parietal organ or pineal eye situated in front of the epiphysis but separated later from it in the garter snake by the superior commissure,

(e) The postvelar arch (or dorsal sac of Goronowitsch or Zirbelpolster of Burekhardt),

(f) The choroid plexuses of the third and the lateral ventricles, arising from the roof of the anterior portion of the postvelar arch and sides of the telencephalon medium respectively,

(g) The velum transversum projecting into the encephalic cavity and separating the paraphysis from the postvelar arch,

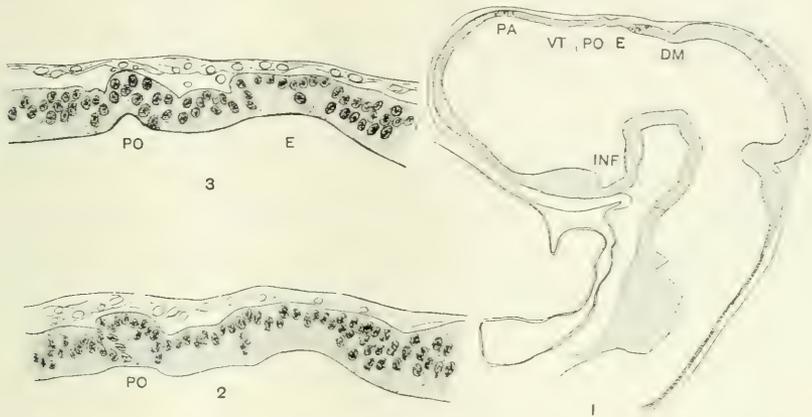
(h) The paraphysis, a median diverticulum from the roof of the telencephalon medium.

### *Stage I*

At this stage the roof of the diencephalon exhibits two minute, inconspicuous evaginations separated from each other by a short interval (fig. 1). The anterior one of the pair, I believe, represents the anlage of the parietal organ, the posterior one, the epiphysis. The posterior one of the two is broader at its base than the anterior one, but both are of the same height and are inclined slightly so that the front wall of each makes somewhat less than a right angle with the roof of the diencephalon and the posterior wall rather more than a right angle. The anterior evagination is much compressed so that the lumen is very small,

## ABBREVIATIONS

<i>ACC</i> , process from the roof of the diencephalon marking the attachment of the accessory parietal organ.	<i>INF</i> , infundibulum
<i>ACP</i> , accessory paraphysis	<i>LCP</i> , lateral choroid plexus
<i>BV</i> , blood vessel	<i>P</i> , paraphysis
<i>CH</i> , cerebral hemisphere	<i>PA</i> , paraphysal arch
<i>DCP</i> , diencephalic choroid plexus	<i>PC</i> , posterior commissure
<i>DI</i> , diocoel	<i>PO</i> , parietal organ
<i>DM</i> , di-mesencephalic groove	<i>SC</i> , superior commissure
<i>E</i> , epiphysis	<i>TM</i> , telencephalon medium
<i>FM</i> , foramen of Monro	<i>TP</i> , tuberculum posterius
	<i>TT</i> , torus transversus
	<i>VT</i> , velum transversum



Figs. 1-3 Sagittal sections of an embryo (C2) having a length of 11 mm. Stage I. Figure 1 is a combination of two adjacent sections through the brain, showing the relation of the epiphysis, parietal organ, velum, paraphysal arch, postvelar arch, and di-mesencephalic groove.  $\times 20$ . Figures 2 and 3 are detailed drawings of the sections of the epiphysis and parietal organ from which figure 1 was made.  $\times 230$ .

the posterior one opens by a wide mouth into the roof of the diencephalon. The distal ends of the two evaginations come in contact with the overlying external epithelium of the head. The velum transversum is present as a low ridge on the dorsal wall which continues laterally and ventrally and becomes more prominent at the sides than on the middle line and extends downward to the postoptic prominence. Externally ridgethis is manifest as a shallow groove.

The paraphysis is not visible at this stage. The paraphysal arch is simply a low, broad dome extending cephalad from the velum and passing over without interruption into the lamina terminalis as Tandler and Kantor have shown in their Stage II of *Gecko*. The position of the recessus neuroporicus is indicated by a space anterior to the paraphysal arch in which the encephalic wall is only about one-half as thick as it is immediately dorsal and ventral to it and where the basement membrane of the epithelium has disappeared and the space between it and the external epithelium of the embryo is filled up with a mass of polyhedral cells among which are many blood cells. The postvelar arch is very low. The diencephalon is separated sharply from the mesencephalon by a deep groove externally and a slight ridge internally.

The histological structure of the roof of the diencephalon exhibits several noteworthy features. In the postvelar arch there are two or three layers of nuclei situated toward the outer side of the brain. The preparations did not allow the outlines of the cells to be seen so that it is still a question how many layers of cells are represented by these nuclei. In the evaginations of the epiphysis and parietal organ the wall is only about two-thirds as thick as the rest of the postvelar arch and apparently is only one layer of cells in thickness. In the interval between the two evaginations the wall is thin, as in the evaginations themselves. The thickness of the roof behind these evaginations becomes gradually and uniformly thicker toward the di-mesencephalic groove; but the roof of the mesencephalon becomes suddenly nearly twice as thick as the thickest part of the diencephalic roof. In the evaginations the nuclei lie rather toward the outer ends of the cells. The front wall of both evaginations appear somewhat thinner than the posterior one, as Tandler and Kantor have observed in *Gecko*.

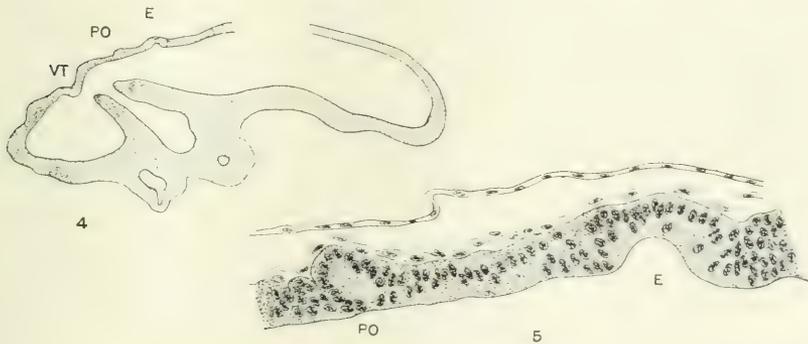
The line of demarcation between the anterior parencephalic and the posterior synencephalic portions of the diencephalon is marked by the epiphysis which arises from the extreme posterior end of the former region. The anlage of the posterior commissure is characterized at this stage by a zone of cells in the roof

of the synencephalon in which the cytoplasm is less dense than elsewhere. At this stage no traces of the other commissures of this region are visible.

The lateral and ventral prolongation of the velum transversum exhibits on its posterior aspect a very pronounced thickening having the form of a ridge that extends posteriorly and ventrally to the infundibulum. This ridge is very evidently anterior to the di-mesencephalic ridge which marks the division between the diencephalon and mesencephalon.

### *Stage II*

This stage exhibits several slight advances over the preceding one in the region of the diencephalon. The epiphysis opens widely into the diocoel and is bowl-shaped, the cavity having a depth about equal to its antero-posterior diameter (fig. 5). The parietal organ has the form of a solid outgrowth from the roof of the diencephalon. Its cells are elongated and placed perpendicular to the surface so that it has the appearance of having been originally an evagination, as in the preceding stage, which has suffered a compression obliterating the lumen. In this



Figs. 4-5 Sagittal sections through the brain of an embryo (A2) Stage II, having a length of 18 mm., showing the parietal organ apparently constricted from the roof of the diencephalon so that its lumen is obliterated. The epiphysis is further removed from the parietal organ than in the embryo previously described.  $\times 25$ . Figure 5 shows the epiphysis and parietal organ of the same embryo.  $\times 230$ .

embryo the axis of the epiphysis is perpendicular to the roof of the diencephalon but that of the parietal organ is inclined forward as before.

The histological structure of the epiphysis is very different from that of the surrounding portions of the encephalic wall. The cells composing it are arranged in a single layer with the nuclei toward the outer ends of the cells. The wall of the parietal organ is not as thick as that of the epiphysis, but like the latter organ, its walls are of a single layer of cells with their nuclei toward their bases. The cytoplasm of these cells differs slightly from that of the epiphyseal cells in being more dense.

The velum transversum is visible as a slight ridge in the cavity of the brain and as a corresponding groove on the exterior. The paraphysis is just visible as a slight outpocketing of the apex of the paraphysal arch. Immediately dorsal to it is a large blood vessel. The cells composing it have a less dense cytoplasm than those forming the brain generally. The posterior commissure is quite clearly differentiated and the very slight constriction between the parencephalon and synencephalon is clear.

In another embryo of essentially the same size as the one just described, the paraphysis is apparently more highly developed. It has the form of a fingerlike evagination which is directed posteriorly. Its length is somewhat less than that of the epiphysis. Its posterior wall exhibits several wrinkles, as Ssobolew described in his *Vipera* embryo number 4. In this embryo I cannot be sure of the presence of a parietal organ. The plane of the sections (parallel to the roof of the synencephalon) was not favorable for the display of such an outgrowth, but a careful study of the series revealed nothing that could be interpreted as a parietal organ. The epiphysis has become somewhat larger than in the previous embryo described in this stage and slants distinctly backwards.

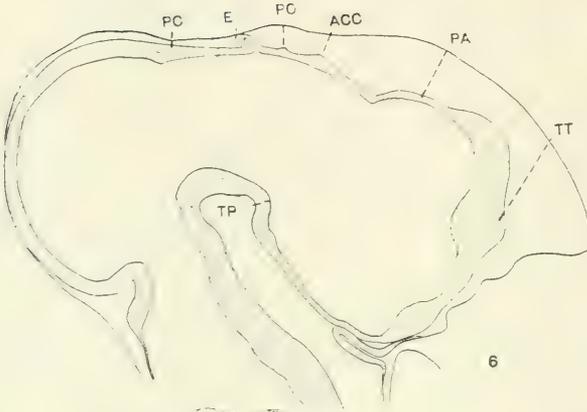
The telencephalon exhibits the two hemispheres and the telencephalon medium which is posterior and dorsal to the foramina of Monro. The paraphysis projects from the roof of this as a fingerlike evagination or, as in another embryo, as two very small evaginations. The telencephalon medium is greatly

compressed laterally and its sides are parallel so that the velum transversum is very narrow. It projects very slightly into the ventricle.

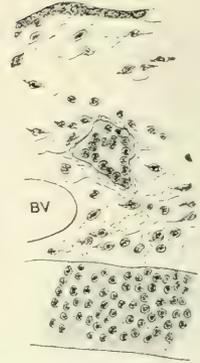
The posterior commissure in this stage seems very distinctly to lie in the second diencephalic segment of the brain and not in the mesencephalon as it has been usually described. It is situated a short distance caudad to the epiphysis. *Thamnophis* agrees in this regard completely with *Lacerta* and *Chrysemys* as described by Warren. In its earliest manifestation in the reptiles, the posterior commissure is wholly diencephalic. As will be shown later, however, it apparently encroaches upon the mesencephalon.

### *Stage III*

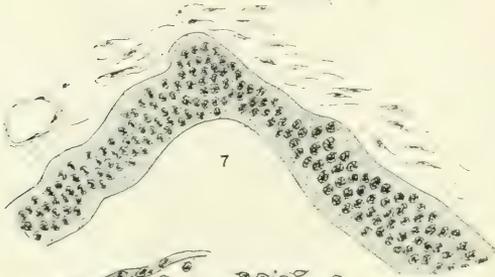
The changes that have taken place in the pineal region at this stage are slight, but nevertheless perfectly evident. The epiphysis has increased in length so that it is now about twice as long as wide, and its axis inclines dorsally and posteriorly. Its cells are arranged in several layers and the posterior wall continues to be thicker than the anterior one and is slightly wrinkled. In one section the posterior wall bears a small budlike projection (fig. 11) the proximal half of the slender lumen is larger than the distal half although at the apex itself it is again slightly larger so that in sagittal section it looks almost as if it were divided into a cephalic and caudal branch. The apex of the epiphysis lies in contact with the external epithelium of the head and is surrounded by numerous large blood sinuses in the mesenchyme. In another embryo having a length of 33 mm. the epiphysis communicates with the ventricle of the diencephalon by a constricted neck (figs. 12 and 17). The parietal organ shows an appreciable advance in development over that of the earliest stage observed where it consists of a budlike outgrowth in wide open communication with the diencephalon on the middle line a short distance in front of the epiphysis. In this stage, as in the one immediately preceding, it has become solid, apparently by a pinching together in an antero-posterior direction. It has increased somewhat in



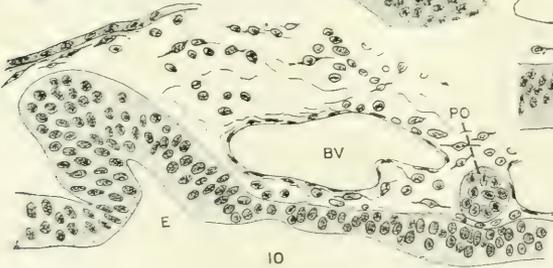
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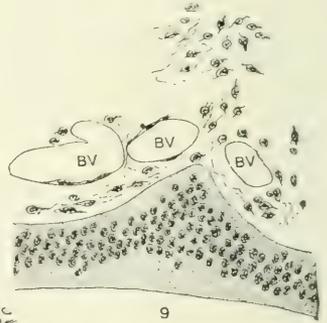
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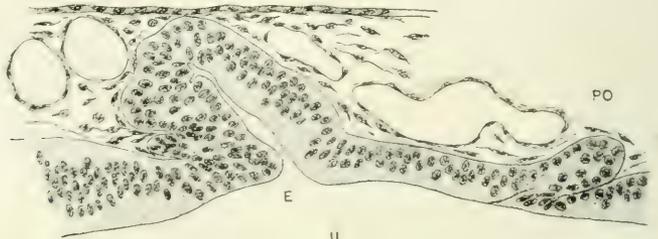
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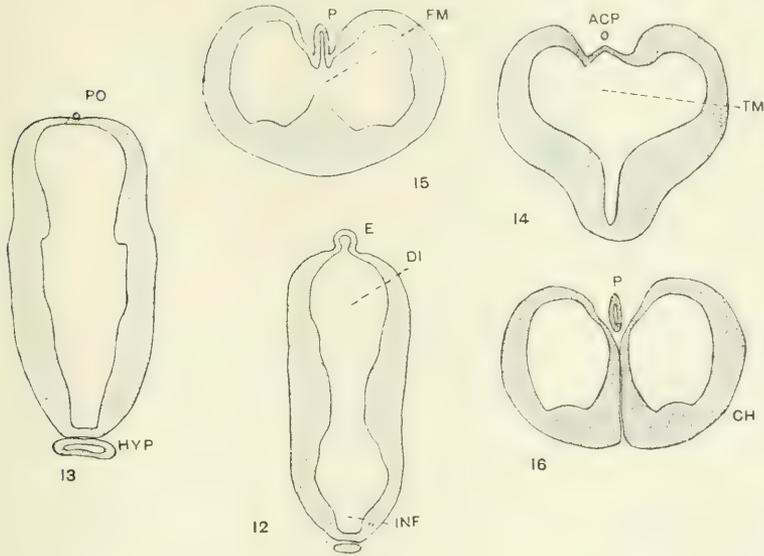
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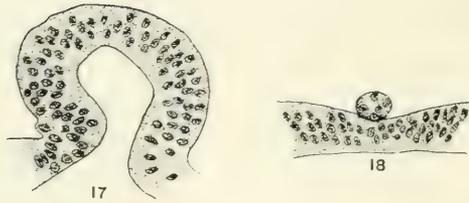
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Figs 6-11 Sagittal sections of an embryo (J) having a length of 29 mm. Stage III. Figure 6 is a section through the entire head to show the general topography of the roof of the diencephalon. The section does not pass exactly through the paraphysis.  $\times 20$ . Figure 7 is a detailed drawing to show the histology of the paraphysis which in this specimen was very slightly developed.  $\times 230$ . Figure 8 shows the accessory parietal organ situated to the left of the mesial plane.  $\times 230$ . Figure 9, the roof of the diencephalon on the mesial line at the level of the accessory parietal organ.  $\times 230$ . Figures 10-11, adjacent sections through the epiphysis and parietal organ. The epiphysis is directed posteriorly; the parietal organ is suffering a lateral constriction from the roof of the diencephalon but is still in continuity with it; the epiphyseal wall has become much thicker than in Stage II.  $\times 230$ .

Figs. 12-16 Transverse sections through an embryo (G) having a length of 33 mm. Stage III.  $\times 25$ . Figure 12 is a section passing through the epiphysis and showing it slightly constricted off from the roof of the diencephalon. Figure 13 is a section  $105 \mu$  in front of the preceding and passing through the parietal organ. Figure 14 passes through the posterior portion of the accessory paraphysis derived from the roof of the telencephalon medium. Figure 15 shows the paraphysis cut through its attachment to the telencephalon. Figure 16 passes through the middle portion of the paraphysis.

length and inclines cephalad so that its anterior face is for the most part in contact with the roof of the diencephalon in front of it. The portion of the roof of the diencephalon between the two evaginations continues to be made up of a single layer of

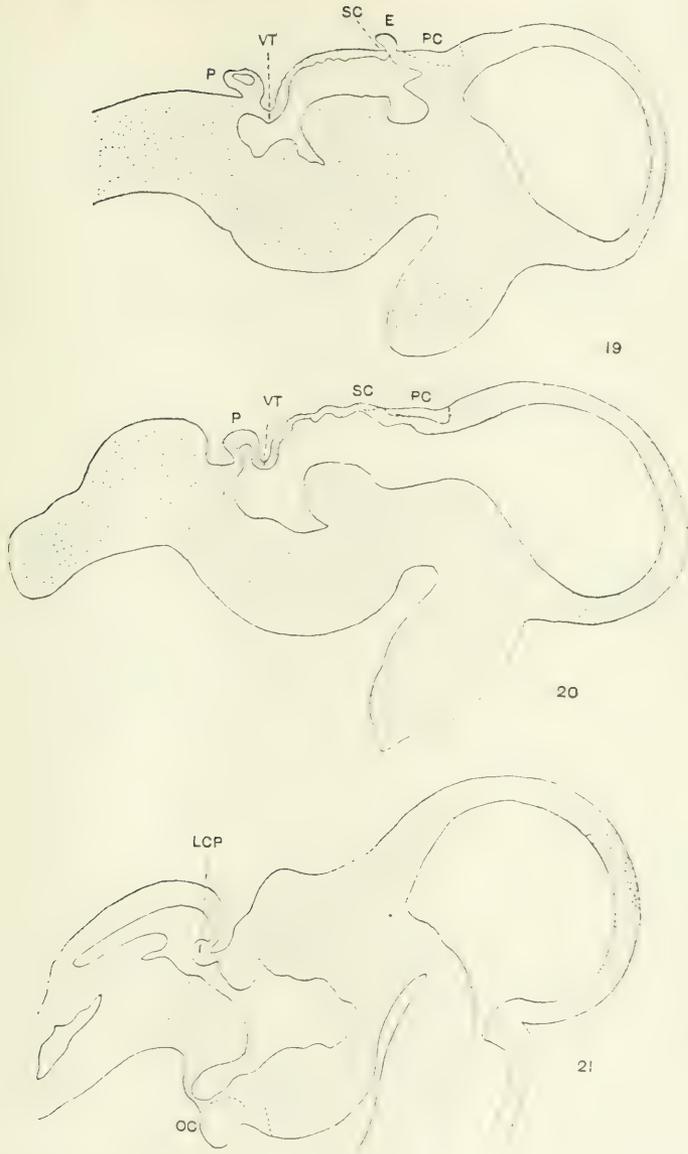


Figs 17-18 Portions of figures 12 and 13 respectively, to show the histological structure of the epiphysis and parietal organ.  $\times 230$ .

cells. The parietal organ is made up of a single layer of cells in which the nuclei lie toward the basement membrane. In one embryo of this stage I could find no trace of a parietal organ.

At some distance in front of the parietal organ—about midway between the epiphysis and the velum transversum—the roof of the diencephalon exhibits a curious structure (fig. 9) whose significance may still be somewhat doubtful. Partly by an increase in the number of cell layers and partly by the increased length of the innermost layer whose clear cytoplasmic portion is relatively taller than elsewhere, the roof thickens to form a conical projection of small size. Separated by a short distance from the thickening mentioned there is a tiny nodule of cells lying in the mesenchyme similar in staining qualities to the roof of the brain (fig. 8). It lies 0.06 mm. to the left of the apex of the thickening but at such a level that its ventral limb is on a level with it. There is very distinctly a complete separation between the two structures but at the same time their form and position leave little room for doubt as to their earlier continuity. This evidently is an unusual structure for in my whole series of embryos it appeared only once. For the present it may perhaps be looked upon as an accessory parietal organ.

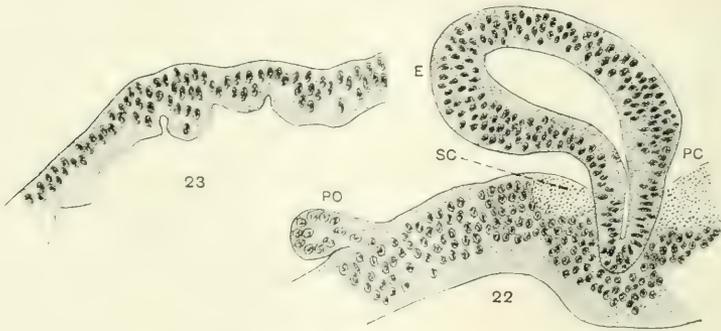
The velum transversum is in the form of a broad fold of the roof and sides of the brain projecting into the ventricles. The anterior and posterior limbs of the fold make an obtuse angle with each other. The anterior limb bears two very slight budlike evaginations which may become involved in the anlage of the paraphysis. In *Lacerta muralis* embryos 23. and 4.5 mm. long.



Figs. 19-21 Sagittal sections through the brain of an embryo (D2) 35 mm. in length. Stage III.  $\times 20$ . Figure 19 shows the relation of the paraphysis and velum transversum and the earliest trace of the diencephalic choroid plexus. Figure 20 passes  $45 \mu$  to the right of figure 19, to show the connection of the paraphysis and telencephalon medium. Figure 21 shows especially the prominence formed by the ganglion habenulae and the development of the choroid plexus of the lateral ventricle.

Warren has shown three similar evaginations which he regards as the anlage of the paraphysis.

The paraphysis in an embryo having a length of 33 mm. consists of a triangular pocket which is very much compressed from side to side and which opens into the ventricle of the telencephalon medium by an opening greatly extended anteriorly and posteriorly. In another embryo of slightly greater length, 33 mm., the paraphysis extended as a fingerlike pouch somewhat compressed laterally and extending forward between the two cerebral hemispheres (fig. 16). The roof of the telencephalon medium



Figs 22-23 Sections of the same embryo as figures 19 to 21.  $\times 230$ . Figure 22 shows the parietal organ in continuity with the roof of the diencephalon in front of the superior commissure. Figure 23 shows the anlage of the diencephalic choroid plexus.

slants laterally from the middle line at a low angle which increases to a right angle immediately behind the origin of the paraphysis.

In one of the specimens of this stage there is a small outgrowth behind the paraphysis, but in front of the velum transversum which resembles closely the organ described in another embryo of this stage as an accessory parietal organ (fig. 8). It has the form of a solid bud only  $30 \mu$  in diameter which is slightly constricted from the roof by a groove on its posterior side. Figure 14 shows a section immediately posterior to its connection with the roof of the brain. It may become involved in the paraphysis later, but the paraphysis is so well advanced in this stage

that it hardly seems likely. On the other hand it can scarcely be regarded as an accessory parietal organ since it originates from a segment of the brain anterior to the diencephalon.

The posterior commissure at this stage is clearly outlined. It does not lie immediately behind the epiphysis as at first, but is separated by a short portion of the roof, in this respect resembling *Lacerta* and *Chrysemys* as described by Warren. Later this portion of the roof of the synencephalon between the commissure and epiphysis becomes invaded by transverse fibers. In one embryo of this stage the transverse fibers seem to extend very slightly behind the groove separating diencephalon and mesencephalon.

#### *Stage IV*

At this stage the development of the pineal region exhibits considerable variation. Although the various specimens appeared externally to be of the same age, the relative development of the parts of this region varied greatly.

The posterior commissure has extended both caudad and cephalad, so that it reaches the stalk of the epiphysis in front and passes beyond the di-mesencephalic groove behind and occupies the entire roof of the synencephalon. Its fibers are very clearly differentiated by this time.

The epiphysis has still a simple form, being cylindrical or club-shaped, showing a differentiation into body and stalk. In two cases there was a wide open connection between the lumen of the epiphysis and the ventricle of the diencephalon; but in two other instances the pinching off seemed to be complete. The wall of the stalk is thinner than that of the body and in several instances the anterior wall is slightly thinner than the posterior one. Generally the body and stalk make an obtuse angle with each other, but in one case the stalk itself is bent. In one embryo in which the cavity of the epiphysis was completely severed from that of the diencephalon, a large blood vessel presses obliquely into the anterior wall (fig. 25).

The superior commissure can be distinguished as a small band of transverse fibers immediately cephalad of the stalk of the epi-

physis in the three embryos of this stage in which the epiphysis is still in communication with the diocoel, but in the other specimens which are more advanced in regard to the epiphysis, no trace of a superior commissure could be seen (fig. 28).

As might be expected, the parietal organ exhibits the greatest diversity of development. In one instance it was simply a solid budlike evagination as has been noted already in several of the earlier stages (fig. 22). In a second instance it was in the form of a solid pyriform mass of cells with the smaller end reaching almost to the roof of the diencephalon but not coming in contact with it (fig. 24). The axis of the organ in this embryo is inclined upward and forward at an angle of about forty-five degrees with the roof. The stem is a single column of cuboidal cells, the body itself exhibits a single layer of spherical nuclei around the margin with only a few situated internally. It has no trace of a lumen. It is notable in this embryo that the roof of the diencephalon is made up of only a single layer of tall columnar cells in the region from which the parietal organ apparently has separated whereas in the other specimens of this stage there are distinctly several layers. In a third embryo, the parietal organ has separated completely from the roof of the diencephalon and is in the form of a solid ovoid mass of cells with its long axis extending forward and upward. There was no trace of a lumen or stalk in this case (fig. 26). The roof of the diencephalon, however, was characterized by a slight irregularity in which the nuclei were rather crowded and squeezed toward the outer side of the roof,

Figs. 24-28 Sagittal sections of the roof of the diencephalon of three different embryos of 42 mm. length (Embryos B, R, and S). Stage IV. Figure 24 shows the parietal organ as a pyriform outgrowth separated from the roof of the diencephalon.  $\times 230$ . Figure 25 is from the same embryo as the preceding and shows the lumen of the epiphysis closed off from the diocoel and a large blood vessel entering the anterior wall of the epiphysis.  $\times 230$ . Figure 26 shows the parietal organ completely separated from the roof of the diencephalon in the form of a solid ovoid mass of cells and the epiphysis in open communication with the diocoel.  $\times 230$ . Figure 27 shows the general relation of the parts of the brain of the same embryo as the preceding.  $\times 20$ . Figure 28 shows the lumen of the epiphysis completely cut off from the diocoel and limited to the body of that organ, and the parietal organ as a hollow sphere of cells completely separated from the brain.  $\times 230$ .



indicating probably a very recent separation. Several blood vessels were in close relation to the organ. Still a fourth condition of the parietal organ was met with in another embryo in which it was in the form of a hollow spherical vesicle (fig. 28). It was in this instance made up of a single layer of cells and was completely removed from the roof of the diencephalon. The postvelar arch is large and domelike in this stage and is characterized especially by the numerous small wrinkles especially in its anterior portion (figs. 19 and 20). The anterior and posterior limbs make approximately a right angle with each other but the posterior limb is much longer than the anterior one. The velum transversum is well differentiated, with its two faces making an angle of about thirty degrees with each other. The epithelium of the front wall in the middle line is conspicuously thicker than that of the posterior wall.

The paraphysis has the form of an irregular conical or oval evagination in open connection with the cavity of the telencephalon medium. Usually it is slightly constricted at the base. It is considerably more voluminous than the epiphysis and its walls are more irregular. At this stage the blood vessels surrounding the paraphysis are very conspicuous.

The choroid plexuses exhibit at this stage marked advance in development over that of previous stages. The diencephalic plexus apparently appears later than the telencephalic, for at this time it is evident only as several bud-like thickenings of the postvelar arch projecting into the ventricle of the brain, and separated from each other by fairly deep clefts (fig. 23). The telencephalic plexus consists of an outgrowth extending anteriorly into the lateral ventricle from the wall immediately dorsal and lateral to the foramen of Monro (fig. 21). It is rounded at its free anterior margin and contains a large blood vessel. The histological differentiation of the telencephalic plexus is marked. The cells which are arranged in a single layer are more slender and taller than in the paraphysis and the cytoplasm is denser, and their nuclei are more elongated.

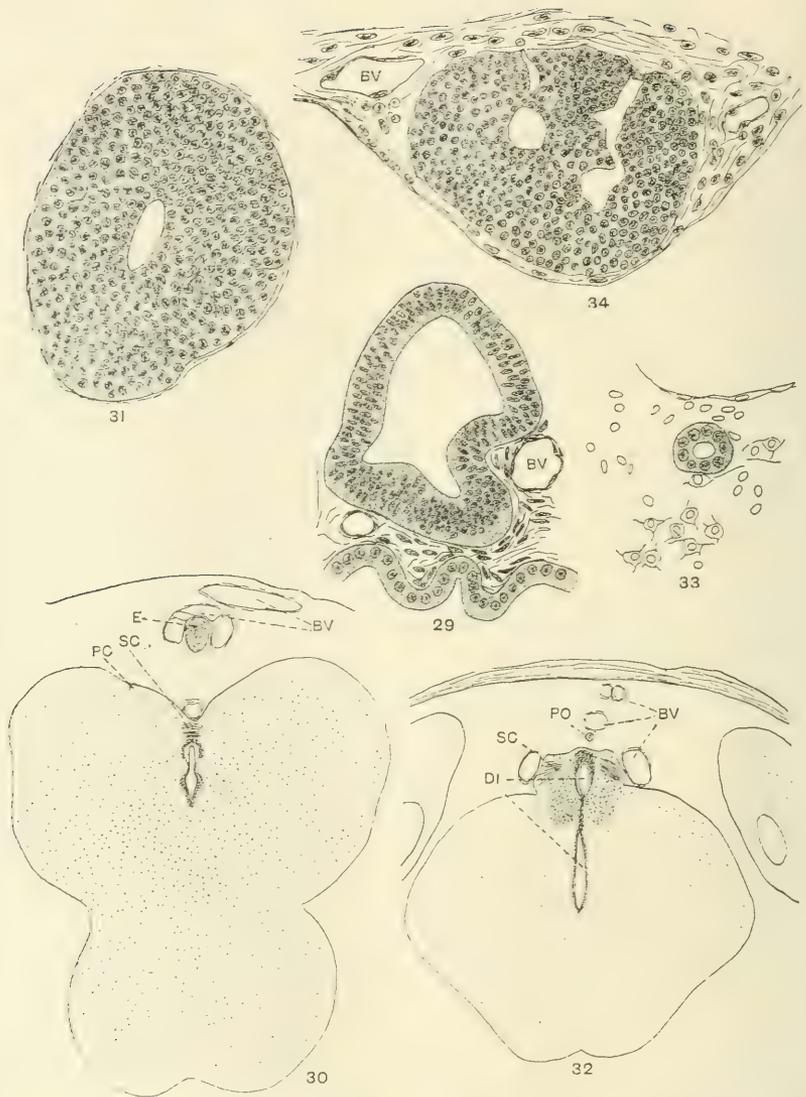
*Stage V*

The paraphysis at this stage has become much longer and has a horizontal position, its blind end pointing caudad (fig. 39), the choroid plexus of the lateral and the third ventricles are much folded and the epiphysis has become greatly elongated and its stalk has become solid.

The paraphysis extends from the roof of the median telencephalic ventricle at first dorsally and then turns at a right angle so that its distal half is horizontal. Its lumen is of somewhat irregular form. Proximally it is T-shaped, distally it becomes flattened dorso-ventrally. It lies in contact with the postvelar arch causing a distinct depression along the median line. The opening of the paraphysis into the ventricle of the telencephalon medium is exactly at the level of the opening of the foramina of Monro so that the ventricle widens quite suddenly immediately ventral to the opening of the paraphysis.

The velum transversum is difficult to delimit accurately. Its cephalic wall passes directly into the paraphysis and its caudal wall into the choroid plexus of the diencephalon. It is rather narrow from side to side, and at its sides it is very short. Medially it exhibits a longitudinal groove with parallel sides so that in cross-section it appears like a bilobed tongue depending from the dorsal wall of the brain. In general the velum has a vertical position. Its caudal surface bears several large oval or irregular prolongations which represent the choroid plexus of the diencephalon. These processes are not to be distinguished from those which hang down from the postvelar arch. They are in fact continuous with them.

The choroid plexuses of the telencephalon and diencephalon at this stage are quite complex in form. The latter has already been mentioned as made up of a number of irregular masses or folds from the caudal wall of the velum and roof of the diencephalon. It extends caudad as far as the level of the parietal organ and almost completely fills up the dorsal portion of the third ventricle which is nearly circular in cross-section. A study of the transverse series of sections of this region showed so

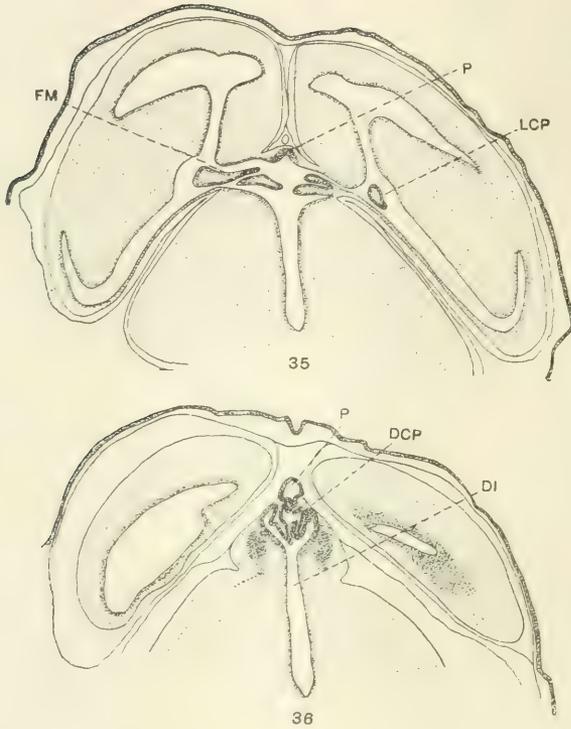


Figs. 29-34 Transverse sections through the brain of two embryos (D and C) having a length of 80 and 90 mm. respectively. Stage V. Figure 29 represents a section through the paraphysis of embryo D.  $\times 230$ . Figure 30 passes through the epiphysis and the superior and posterior commissures of embryo C.  $\times 20$ . Figure 31 represents a portion of the previous figure to show the histological structure of the epiphysis.  $\times 230$ . Figure 32 is a section through the same embryo as the preceding but further anterior, passing through the parietal organ, ganglia habenulae, and superior commissure.  $\times 20$ . Figure 33 shows the histological structure of the parietal organ of the same embryo.  $\times 230$ . Figure 34 shows the histological structure of the epiphysis of embryo C, showing several clefts within.  $\times 230$ .

great irregularity on the two sides that there can be no definite arrangement of parts. The telencephalic plexuses are likewise complicated. That of the lateral ventricle has the form of a plate of nearly horizontal position with its distal, lateral margin much thickened and turned up dorsally so that it has a concave upper surface. This plate extends anteriorly from the posterior side of the foramen of Monro so that the latter is partially obliterated by it. Warren describes the choroid plexus lateralis as springing from the paraphysal arch immediately in front of and lateral to the mouth of the paraphysis, invaginating the dorso-mesial wall of the hemispheres. The plexus extends medially from its connection with the margin of the foramen of Monro so that it projects freely into the ventricle of the telencephalon medium and unites with the lateral wall of this ventricle by a slender stalk, ventral and lateral to the origin of the paraphysis (fig. 35). This median prolongation of the lateral plexus represents probably the telencephalic choroid plexus of Warren or the choroid plexus inferioris. It is wanting, according to Warren, in *Lacerta* but in *Chrysemys* there are described two paired masses growing back from the origin of the lateral plexus into the diencephalon. In *Thamnophis* they are confined to the unpaired ventricle of the telencephalon and do not extend posterior to the velum. The telencephalic plexus of the Amphibia, in which group it is most highly developed, arises from the paraphysal arch in front of the paraphysis. Warren thinks that these paired masses in *Chrysemys* may be the homolog of the amphibian telencephalic plexus.

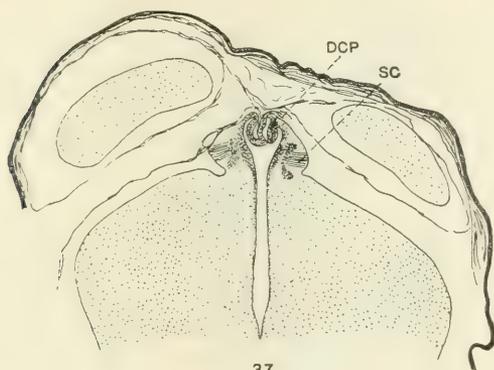
The parietal organ is present in an embryo having a length of 90 mm. as a hollow, ovoid body entirely separated from the roof of the third ventricle (figs. 32-33). Its long axis extends antero-posteriorly. It is situated about  $50\mu$  dorsal to the diencephalon, slightly nearer the stalk of the epiphysis than the distal end of the paraphysis (fig. 39). It is composed of a single layer of cuboidal cells with large spherical nuclei. A large blood vessel lies immediately dorsal to it.

The stalk of the epiphysis is very slender and has attained a considerable length. It passes into the roof of the diencephalon

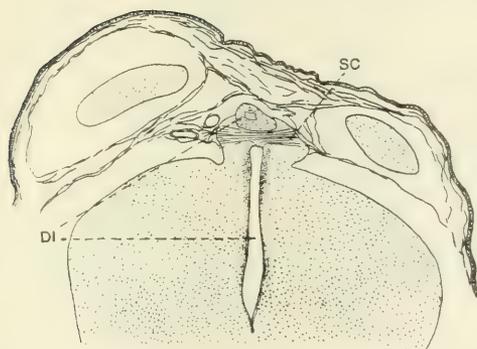


Figs. 35-38 Transverse sections through the dorsal portion of an embryo (D), having a length of 80 mm., arranged from anterior to posterior, showing the paraphysis, lateral, median, and diencephalic choroid plexuses, ganglia habenulae, superior commissure, and a pocket of the roof of the diencephalon cut off by the superior commissure.  $\times 20$ .

between the superior and posterior commissures and is directed almost exactly dorso-ventrally. The body of the epiphysis is large and pear-shaped. In one specimen it exhibited a small lumen in the anterior and ventral portion near its connection with the stalk, as has already been described in the adult serpent by Studnicka ('93). Its histological structure has changed markedly for now it is made up of an irregular mass of cells packed closely together with occasional connective tissue fibers. In another specimen there were several irregular clefts instead of a single lumen (fig. 34).



37



38

The posterior commissure has increased much in size and occupies the entire roof of the third ventricle from the very attenuated stalk of the epiphysis posteriorly to the anterior portion of the mesencephalon. The superior commissure is strongly developed, forming a distinct projection from the roof of the diencephalon (fig. 32). The fibers of the superior commissure are much longer than those of the posterior and curve forward toward their ends, presenting thereby a concavity in front.

The diencephalon is very much compressed laterally. Anteriorly in the immediate region of the velum its sides are parallel, but further caudad it becomes widened in its dorsal portion around the choroid plexus so that it becomes nearly circular in cross section. Immediately ventral to the widened portion of

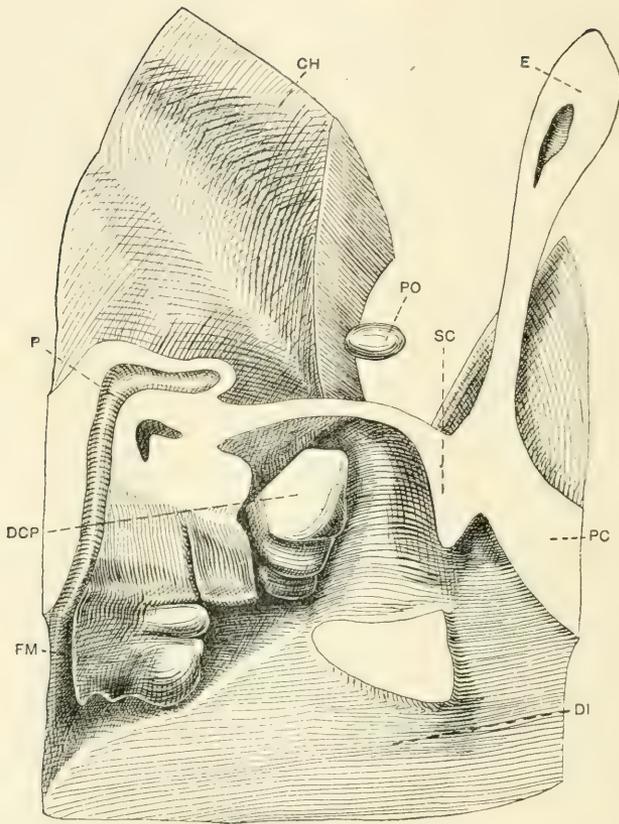


Fig. 39 Reconstruction of the pineal region of an embryo (C) having a length of 90 mm. Stage V. The right half of the model is viewed from the middle line.  $\times 36$ .

the ventricle it becomes greatly narrowed by the development of the optic thalami until the space between the two walls is obliterated. The ependyma at the line of fusion becomes flat and indistinguishable except as a layer of cells which appear like fibers in cross-section. Dorsal to this line of fusion and extending caudad to it, in the region caudad to the superior commissure, the ependymal cells become very different from elsewhere, increasing greatly in height. These taller cells pass over directly into the cuboidal cells of the stalk of the epiphysis (fig. 40).

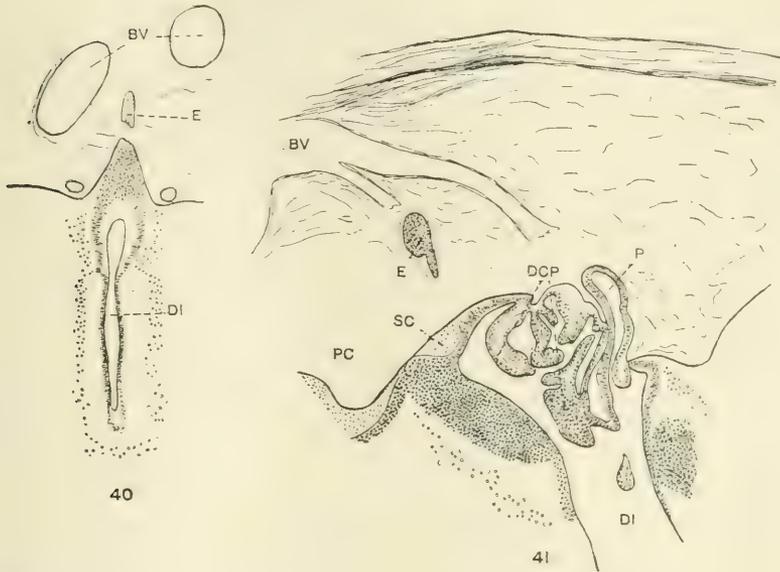


Fig. 40 Represents a transverse section through the stalk of the epiphysis of an embryo having a length of 90 mm. The section was accidentally broken across the stalk.  $\times 50$ .

Fig. 41 Represents a sagittal section through the pineal region of an embryo having a length of 100 mm., showing the large blood vessel dorsal to the epiphysis.  $\times 50$ .

Each ganglion habenulae forms a dorsal projection on the diencephalon in the region of the posterior end of the paraphysis. These two ganglia are separated dorsally by the much folded roof of the diencephalon making up the choroid plexus of the third ventricle. In one specimen the superior commissure apparently cuts off a portion of the roof of the diencephalon between the two ganglia so that there is a short pocket formed extending posteriorly above the commissure (fig. 38).

#### DISCUSSION

The present study on the development of the parietal region in the garter snake has thrown light upon several matters which have been the subject of much debate.

First of all, there can be little doubt left regarding the presence of a parietal organ in certain stages of the ophidians. Either

the organ described in this paper is the parietal organ or it is an organ entirely different from anything else described in the vertebrates. In favor of the interpretation here placed upon it, there may be mentioned; first, the method of origin as an evagination from the roof of the diencephalon anterior to the epiphysis and posterior to the postvelar arch; second, its form when at its maximum development; namely, a hollow spheroid consisting of a single layer of cells. Against this interpretation may be mentioned; first, the appearance of the parietal organ later than the epiphysis; second, its wide separation from the epiphysis with the superior commissure separating the two; and third, its failure to migrate dorsally to lie in close proximity to the dorsal side of the head. The first and third objections are not significant since in those groups in which there is no doubt whatever regarding the identity of the parietal organ there is much difference in the relative time at which the parietal organ and epiphysis appear, although the parietal organ in the lizards always seems to be in advance of the epiphysis. The failure of the parietal organ to pass far dorsally may be explained by its distance in front of the epiphysis which does not lie ventral to it and which otherwise by its growth might push the parietal organ dorsally as it does for example in *Lacerta*. Besides this, the extreme development of the paraphysis caudad in such forms as *Lacerta* may also tend to displace the parietal organ toward the dorsal surface of the head. This last factor is wanting in *Thamnophis* because of the shorter paraphysis which does not insinuate itself beneath the parietal organ. The wide separation of the epiphysis and parietal organ constitutes a valid objection to the notion here put forth, for a similar relation has not been noted in any other type. There are two facts, however, which seem to make this objection less significant. The parietal organ is known in various forms to originate in slightly different places, as will be shown later; and it has also been noted in this study that the segment of the roof of the diencephalon lying between the epiphysis and parietal organ in the earlier stages of the garter snake exhibits a different histological structure from the rest and resembles more nearly that of the epiphysis itself. It

seems reasonable, on the whole, to regard the present organ as a parietal organ rather than one *sui generis*.

Another matter upon which evidence has been produced by the present study is the independence of the parietal organ and epiphysis.

The earlier view regarding the relationship between the two was that the parietal organ was pinched off from the distal end of the epiphysis (Strahl '84). Thus it would appear that the two organs were in the closest possible relation. A few years later Baldwin Spencer ('86), as a result of an extensive study of the parietal organ of many lizards, confirmed Strahl's conclusion, for in all the species studied the parietal organ and epiphysis seemed to be connected by means of the 'parietal stalk.' Beraneck ('87) studying the development of the parietal organ and epiphysis in *Lacerta agilis* was the first investigator to regard the two as independent, arising from two separate anlagen, one of which lay close in front of the other. Francotte ('96) recognized that the parietal organ might arise in two different ways. According to the first type of origin the anlagen of the parietal organ and epiphysis appear as independent buds from the roof of the diencephalon. The anterior one, the parietal organ, appears first and is larger than the posterior one, which develops into the epiphysis. In the beginning the parietal organ is larger than the epiphysis. According to the second type of origin, the posterior bud does not arise from the roof of the diencephalon but from the postero-dorsal border of the anterior one. Francotte regarded this latter type to be derived from the former which he thought was the more primitive. This apparent origin from the same outgrowth Francotte explained by assuming that the parietal organ elongates more rapidly than the epiphysis at first and drags the latter along with it so that the lip between the two at an early stage really represents the segment of the roof of the diencephalon which lay between them in the first place. This is also confirmed in a measure by Klinekowström ('93) on *Iguana*. In this lizard the roof of the diencephalon at one time exhibits a single evagination directed anteriorly, which is secondarily separated into two parts by a

ringlike furrow. The parietal organ is in this way cut off from the epiphysis. The subsequent growth of the epiphysis is not, however, in the direction at right angles to the furrow just mentioned, but dorsally so that the scar marking the earlier connection of the parietal organ and epiphysis comes to lie on the anterior wall of the epiphysis. This means, as can readily be seen, that the material from which the greater part of the definitive epiphysis is derived, lay originally posterior to that of the parietal organ just as is the case where there are two separate anlagen for these organs. The fundamental difference between the condition in Iguana and Francotte's first type of origin of these organs lies in the greatly retarded appearance of the epiphysis in Iguana, until after the parietal organ's anlage has grown to considerable size.

Further evidence of the independence of these two structures is afforded by a study of the innervation of the epiphysis and parietal organ in certain vertebrates. The parietal organ may be supplied by the parietal nerve whose fibers enter the brain by way of the superior commissure, in front of the epiphysis, and from there may be traced to the right ganglion habenulae (Strahl and Martin '88); the epiphysis may be supplied by a nerve, the pineal nerve, which enters the posterior surface of the epiphysis and arises from the roof of the brain from the posterior commissure (Klinckowström '93, on Iguana).

The development of the parietal organ and epiphysis in the garter snake leaves no room for doubt as to the complete independence of these organs in this form; since there is a considerable interval between them on the roof of the diencephalon from their earliest appearance, in which space the superior commissure later appears.

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## THE GASTRIC VASA BREVIA

H. M. HELM

*From the Anatomical Laboratory of the University of Wisconsin*

THIRTY-SEVEN FIGURES

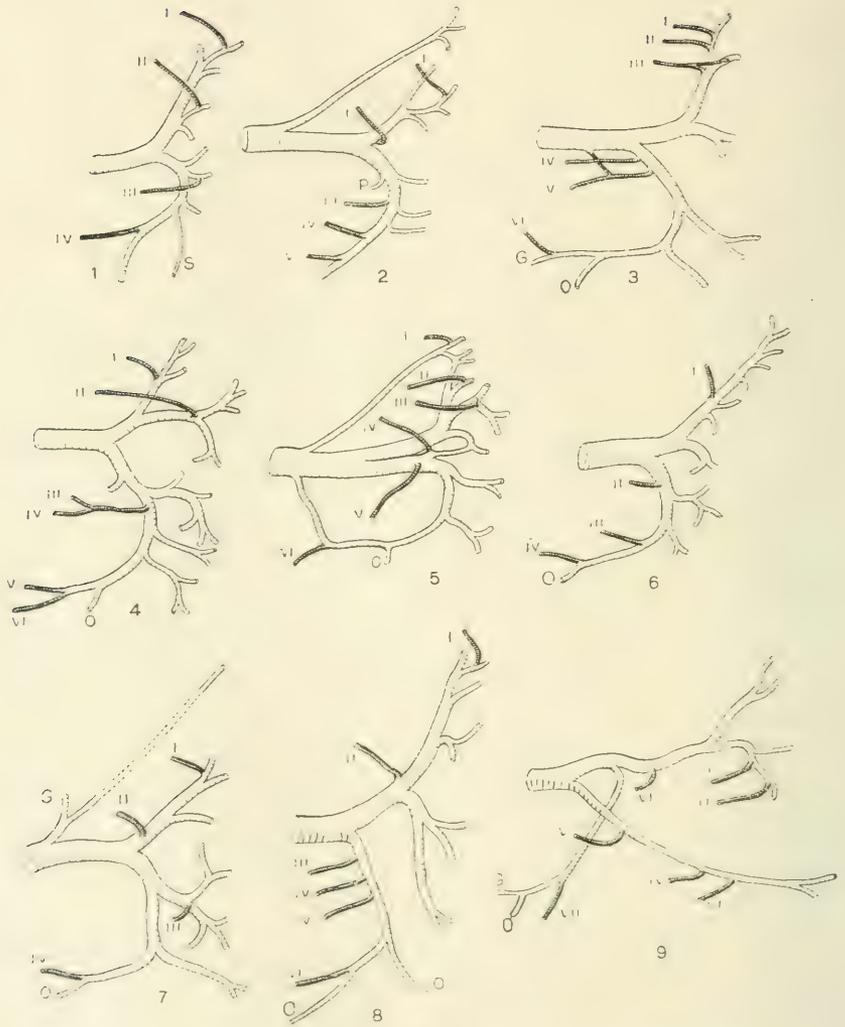
The gastric vasa brevia are those branches of the splenic artery and veins and their terminal divisions which pass by way of the gastro-splenic omentum to the fundus of the stomach.

In this consideration of the gastric vasa brevia the aim has been: first, to determine the most usual arrangement of these vessels in the adult, as regards number, size, origin, and distribution; and, second, to ascertain the time and manner of their development in the embryo. Conclusions regarding the adult arrangement are based on a series of twenty-five drawings of the spleens and splenic vessels of as many dissecting-room subjects. The first eight drawings were made by Dr. Bunting, and it was at his suggestion that the study was carried farther.

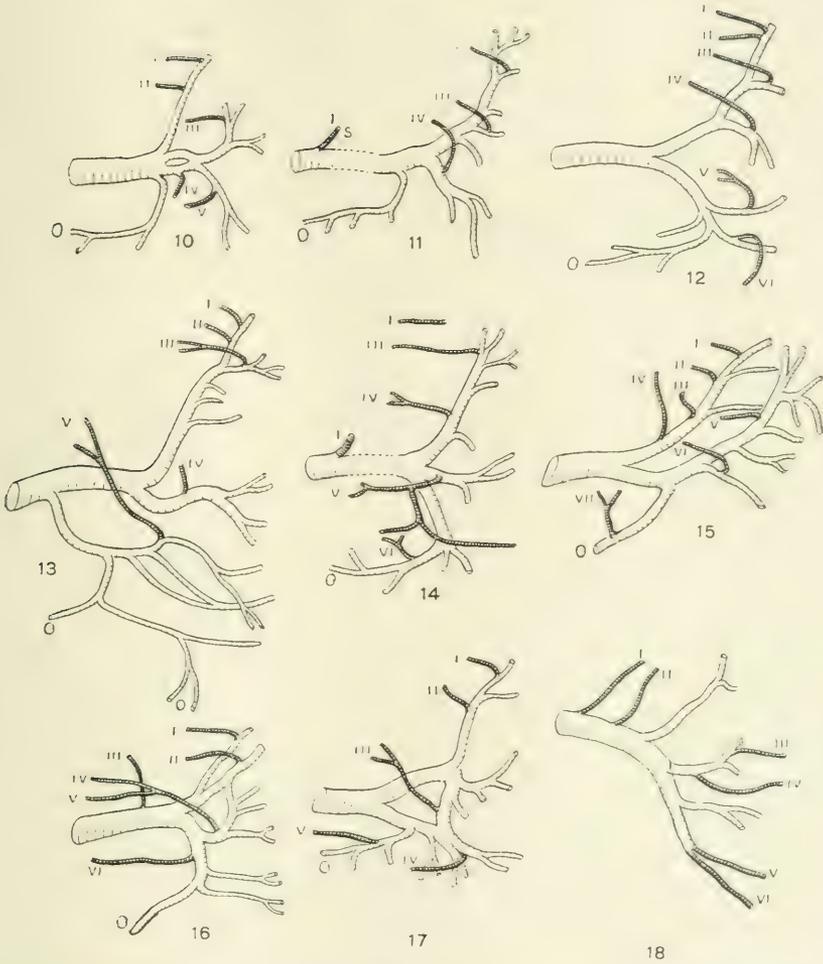
*Number.* A glance at the outstretched arteries (figs. 1-25), shows that there were three in two cases, four in six, five in six, six in eight, and seven in three. Thus there are usually more than three and seldom as many as seven; the most usual number is six, but four and five are hardly less frequent. The average number in the series was a little over five.

*Size.* The vasa brevia are always small. They were measured in fifteen of the subjects examined and in no case were they more than 4 mm. in diameter. Not infrequently they were mere threads, less than 0.5 mm. in diameter; the most common size was about 2 mm.

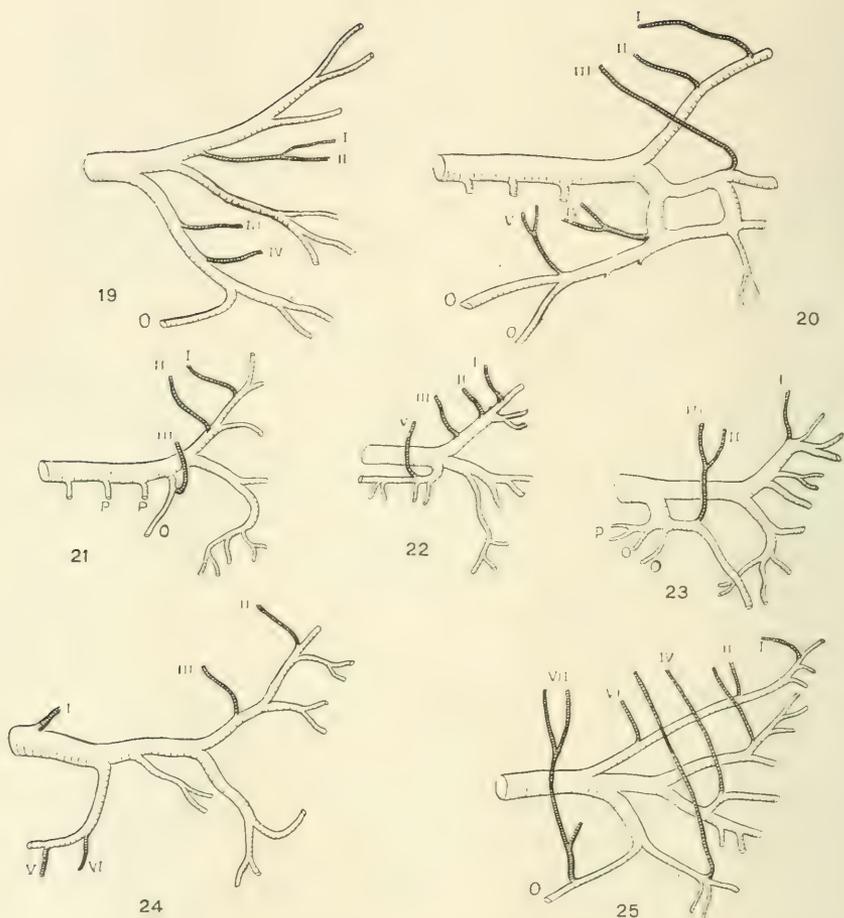
*Origin.* The splenic artery ordinarily divides into two main divisions, a superior for the supply of the upper half of the spleen, and an inferior for the supply of the lower half of the spleen, a part of the great omentum and a part of the greater curvature



Figs. 1-25 Diagrammatic sketches of twenty-five specimens showing the origin of the vasa brevia arteries of the stomach from the splenic artery and its branches. The vasa brevia are shown dark. The lighter branches not otherwise labeled are splenic branches. O, gastro-epiploic; P, pancreatic branch S (fig. 1), branch to accessory spleen.



of the stomach. The left gastro-epiploic may be a branch of the inferior division, as was true in thirteen cases, or it may be a branch of the main splenic trunk, occurring before the division into superior and inferior divisions. In any case the gastro-epiploic trunk usually gives off vasa brevia (e.g., this was true in thirteen of the seventeen gastro-epiploic vessels examined). It was true in every one of the eight instances in which the gastro-epiploic arose from the splenic artery proper.



There may be anastomosis between the superior and inferior divisions, as in figures 16 and 17, and there may be accessory superior branches from the splenic trunk, as in figures 2 and 5, or accessory inferior branches as in figure 9.

As we have seen, the vasa may arise from (1) the splenic artery itself; (2) the accessory splenic branches springing from the main splenic trunks midway between the coeliac axis and the spleen, and (3) the superior and inferior divisions (the latter including the gastro-epiploic) and their secondary branches.

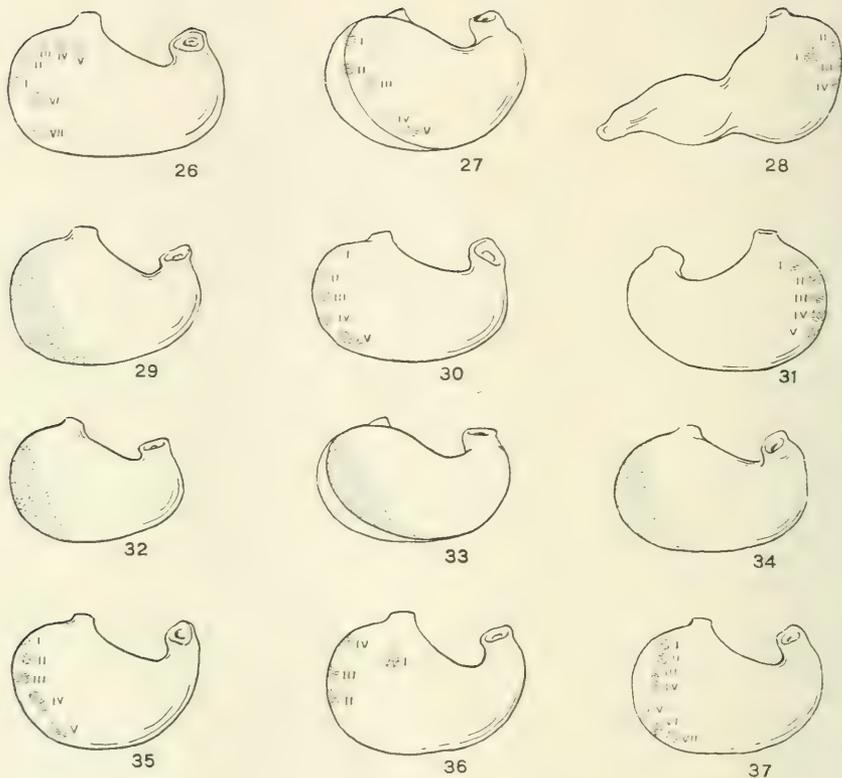
Vasa brevia arising from the splenic artery itself are the exception; figures 9 and 16 show them. That shown in figure 9 was about 1 mm. in diameter and it passed to the dorsum of the stomach, low down on the fundus (VI, fig. 26). That shown in figure 16 was larger (3 or 4 mm.) as were all the vasa brevia in this specimen. It passed to the dorsum of the stomach toward the cardiac orifice. Both these vessels arose close to the spleen. More frequently the splenic artery gives off a gastric branch near its origin. This branch may be a typical *vas breve*, but as often it is a coronary or accessory coronary branch destined for the supply of the lesser curvature. Such a branch was present in four of the twenty-five specimens, as shown in figures 11, 14, 18 and 24. In figure 11 the vessel was 1.5 mm. in diameter and about 5 cm. long. It arose 3 cm. from the origin of the splenic artery and 9 cm. from the hilus of the spleen, and passed to the dorsum of the fundus about 3.5 cm. below and 2 cm. to the left of the cardiac orifice. It did not anastomose with any other vessel; it was a true *vas breve*.

The vessel shown in figure 18 was similar in size, origin, and distribution; it was also a true *vas breve*. This specimen also showed a *vas breve* originating nearer to the spleen and similar to those described as occurring in figures 9 and 16.

In case of those splenic arteries which give off accessory splenic branches before reaching the spleen, the accessory branches almost always give rise to vasa brevia. Such branches were present in five cases, and all but one (fig. 2) gave off vasa brevia. But since these branches occur in but twenty per cent of the cases, we come to the rather self-evident conclusion, that typically, the vasa brevia arise from the superior and inferior splenic divisions and their branches.

We have said the usual number of vasa brevia is five or six. Table 1 shows the number of branches arising from the superior and inferior divisions.

The vasa brevia arising from the superior division tend to be smaller than those arising from the inferior. This was true in ten of the fifteen cases in which comparative measurements were made. In another case the smallest vessel arose from the supe-



Figs. 26-37 Diagrammatic sketches of twelve stomachs, showing the distribution of the vasa brevia of twelve of the specimens illustrated in figures 9 to 25. The distribution of the vasa brevia of the specimen shown in figure 9 is illustrated in figure 26; that of figure 10 in figure 27; figure 11 in figure 28; figure 12, in figure 29; figure 13, in figure 30; figure 14, in figure 31; figure 15, in figure 32; figure 17, in figure 33; figure 18, in figure 34; figure 20, in figure 35; figure 24, in figure 36; figure 25 in figure 37. In figure 36 the area of distribution of branch V was not determined accurately and is not shown. This is also true of branch VI in figure 31. In figures 29, 32, 33 and 34 the general area of distribution is shown instead of the approximate area of each branch. In figures 27 and 33 the stomach is turned so as to show the line of omental attachment. Figures 28 and 31 show the ventral, the other figures the dorsal surface of the stomach. In figures 28 and 31 the area of distribution of branch I is really on the dorsal surface so that the stomach is represented as transparent over this area. The other branches are distributed near the line of omental attachment.

TABLE 1

Figs.....	1	2	3	4	5	6	7	8	9	10	11	12	13
Sup.....	2	2	3	2	3	1	2	2	3	3	2	4	3
Inf.....	2	3	3	4	2	3	2	4	4	2	1	2	2

Figs.....	14	15	16	17	18	19	20	21	22	23	24	25
Sup.....	3	4	2	2	2	2	3	2	3	1	2	4
Inf.....	2	3	2	3	2	2	2	1	1	2	2	3

rior division, but the other superior branches were as large as the inferior. In the other four instances all the vessels were of about the same size. In many cases the superior branches, notably the first two, are mere threads, less than 0.5 mm. in diameter, whereas the inferior branches are apt to be 1.5 to 3 mm. in diameter.

A glance at the sketches shows that the point of origin of most of the vasa brevia is very uncertain so soon as one attempts to localize it to a secondary branch of the superior or inferior division. This is because the secondary splenic branches themselves are so variable. However, the first vas breve is relatively constant. It is small, as we have said, and usually arises from the highest splenic branch of the superior division—the terminal branch, virtually—close to where it sinks into the spleen. This was the case in sixteen of the twenty-five spleens examined; in another it arose slightly lower, from the superior division itself; in another it arose from the second instead of the first splenic branch; and in two others it arose from a superior accessory splenic branch. Its distribution is likewise relatively constant. Typically, it passes to the highest point on the fundus. It is not always the highest branch, however; thus in figure 15, vessel IV had the highest position on the stomach.

The second vas breve usually arises from the superior division or from one of its uppermost branches close to the first, runs parallel with the first, and has the next lower position on the fundus; like the first it is usually small. The third also usually

risers from the superior division in case there are five or six vessels in all. The fourth arises close to the bifurcation of the splenic artery, sometimes from one main division, sometimes from the other. The fifth and sixth arise from the inferior division, frequently from the gastro-epiploic trunk. The vessels tend to run parallel and to reach the stomach in the order of their origin.

*Distribution.* A consideration of the distribution of the vasa brevia gives a somewhat more satisfactory result. Since the vessels run in the gastro-splenic omentum, they reach the greater curvature of the stomach in the region of the fundus. Some small twigs may pass to the fundus just ventral to the line of omental attachment, but in every case virtually the whole area of vasa brevia supply was dorsal to the line of omental attachment: i.e., on the dorsal or original right side of the fundus. The uppermost vasa brevia tended to remain practically in the line of omental attachment; the lower branches, on the other hand, usually passed well onto the dorsum. In no case did a vessel pass much distance onto the ventral surface of the fundus.

In no case did a vas breve form anastomotic loops with other vessels. Thus they differ from all other gastric vessels, i.e., the coronary and gastro-epiploic branches; they are end arteries.

A glance at the sketches, figures 26 to 37, will indicate the location and extent of the area of distribution of the vasa brevia. In figure 31 the vessels are confined to the region of omental attachment, as many twigs passing anteriorly as posteriorly. No anterior twigs pass far, however, while the first vessel is distinctly dorsal in position. In figures 27 and 28, likewise, the vessels remain close to the line of omental attachment, though the tendency is toward dorsal distribution. In all other cases the area is distinctly dorsal to the line of attachment of the omentum. Furthermore, it is to the left of a line dropped from the esophagus to the greater curvature. That is to say, the vasa brevia are confined to the fundus. Figure 26 illustrates the fact previously mentioned that vessels arising from the inferior division do not necessarily take the lower positions on the stomach.

*Development.* The vasa brevia develop very early in the embryonic life as primary branches of the splenic artery. They

later become tributary to the splenic arteries as these are differentiated and in adult life are always small. Their number and origin is variable, but their distribution is constant: they pass practically wholly to the dorsum of the fundus. They are end arteries and in no case anastomose with other vessels. In a pair of duplicate twin fetuses there were in one body five vessels, in the other six and the origins of the vessels differed in the two specimens.

To summarize: In the adult there are usually five or six vasa brevia, but there may be fewer or more. The vessels usually arise from the superior and inferior divisions of the splenic artery, but they may arise from the main trunk of the splenic artery or from accessory splenic branches; the branches of the superior division tend to be smaller, more numerous and to take a higher position on the fundus than the inferior branches, but the reverse may be true. The vasa brevia are never very large—at least under normal conditions; they are terminal or end arteries; they pass to the dorsum of the fundus of the stomach.

MEMOIRS  
OF  
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY  
No. 5

THE DEVELOPMENT OF THE ALBINO RAT, *MUS  
NORVEGICUS ALBINUS*

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of Embryology, the Wistar Institute of Anatomy and Biology*

I. FROM THE PRONUCLEAR STAGE TO THE STAGE OF MESODERM  
ANLAGE; END OF THE FIRST TO THE END OF THE NINTH DAY

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# ON THE INFLUENCE OF EXERCISE ON THE GROWTH OF ORGANS IN THE ALBINO RAT

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For the past three years experiments have been carried on to determine the effect of long-continued exercise on the growth of organs in the albino rat. The main object of the present investigation was twofold: (1) to repeat the observations of Donaldson ('11) who found a slight increase (2.6 per cent) in the brain weight in albino rats which had been subjected to exercise for the period of six months, and (2) to extend the observations to other organs besides the central nervous system. The present paper includes the data obtained by the previous study, just mentioned, as well as the results of my own investigations.

## TECHNIQUE

The opportunity for exercise was given by placing the test rats in a form of cage which was used by Slonaker ('08) in his studies on the daily activity of the albino rat. The revolving cage consists of a large cylindrical drum 58.5 inches in circumference, made of  $\frac{3}{16}$ -inch wire mesh, which revolves on a stationary axle. On the axle was fastened the nest box and the food and water pans. The number of revolutions of the cage is registered by a cyclometer.

Albino rats one month old were placed in these cages for a period of three to six months, which is equivalent to seven to fourteen years of human life. Each cage contained a single rat. The rats used for the control were litter brothers and sisters of those in the revolving cages and were placed in the ordinary laboratory cages (one foot high, one foot wide and six feet long). The total number of rats examined was 36 controls

and 42 test animals. For the material of the 1914 series the present writer is under great obligation to Miss Caroline Holt, a graduate student at The Wistar Institute, who permitted the use of her exercised rats with their controls, and I wish to thank her for her courtesy in this matter.

#### METHOD OF COMPUTING THE AMOUNT OF ALTERATION

In determining the deviations of the organs in the exercised rats from those in the controls, the following method was used: As a first step, the weights of the various organs corresponding to the observed body length of the rats were computed by means of formulas (for various formulas see Hatai '13 and '14). This computation was made for both the controls and the exercised rats. The differences between the observed and computed values of both control and exercised are now transformed into percentages by taking the computed values as 100 per cent. Thus we obtain two sets of percentage values, one expressing the difference between the computed and observed values in the control rats, and the other expressing the difference between computed and observed values in the exercised rats. If exercise has not altered the organs of the animals at all, then these two sets of percentages should be alike within the limits of the normal fluctuations. If, on the other hand, exercise has altered the organs, these two sets of percentages should differ more or less according to the nature of the response to exercise. If in the case of any organ we now take the difference between the percentage value obtained for the controls and that for the exercised rats, this difference represents the amount by which the exercised rats, as compared with controls, depart from the value obtained by the formula. By the use of this procedure, successive series are thus referred to the formula values in each instance and since the deviations are measured by this means always from the same standard, they may be directly compared with one another.

In the case of the thymus gland, the age of the rats was taken as the basis for the computation, since the weight of the thymus is much more highly correlated with the age than with either the

weight or the length of the body (Hatai '14). The example taken from the 1911 male series (the second series in table 3), may serve to illustrate the method of comparison described above (table 1). Expressing in words the results as given in the last line of this table, these show that the exercised rats are 13.4 per cent heavier than the controls and have a tail 1.62 per cent longer.

AMOUNT OF EXERCISE TAKEN

The rats in the revolving cages often take a large amount of voluntary exercise. To illustrate this I have chosen an example from the 1912 series and given the average distance run during every 24 hours for the entire period of 93 days.

As will be seen from table 2, the distance run is almost incredible in some instances, the greatest average run for 93 days being 10 miles per 24 hours; a record made by one female. Since the rat is most active during the night (Slonaker '12) this daily run

TABLE 1

	BODY LENGTH (MM.)	TAIL LENGTH (MM.)	BODY WEIGHT (GMS.)
Controls: observed values	212	176	238.3
Controls: values calculated from formulas (body length taken as basis of computa- tion)		180	238.1
Percentage deviation of ob- served from calculated val- ues.....A		-2.22%	0.08%
Exercised: observed values	195	164	202.0
Exercised: values calculated from formulas (body length taken as basis of computation)		165	178.1
Percentage deviation of ob- served from calculated val- ues.....B		-0.60%	13.48%
Amounts by which the percent- ages for the exercised animals differ from those for the controls; i.e., B - A		1.62%	13.40%

TABLE 2

*Showing the distance run by the exercised rats in a period of 93 days*

EXERCISED RAT, NO.	NO. OF MILES PER 24 HOURS	SEX	EXERCISED RAT, NO.	NO. OF MILES PER 24 HOURS	SEX
8 B <sub>10</sub>	0.4	M.	7 A <sub>5</sub>	7.6	M.
7 A <sub>17</sub>	0.5	M.	7 A <sub>4</sub>	7.6	M.
8 B <sub>9</sub>	0.6	M.	7 B <sub>17</sub> <sup>2</sup>	6.2	F.
7 B <sub>16</sub> <sup>2</sup>	0.6	M.	7 B <sub>20</sub>	7.4	F.
7 B <sub>19</sub>	4.8	M.	6 B <sub>92</sub> <sup>2</sup>	7.7	F.
7 B <sub>48</sub>	6.5	M.	6 B <sub>83</sub> <sup>2</sup>	8.3	F.
6 B <sub>82</sub> <sup>2</sup>	6.7	M.	8 B <sub>11</sub>	10.2	F.

was accomplished for the most part within a twelve-hour period. With the exception of the first four sluggish male rats, the average run was about  $6\frac{1}{2}$  miles for males and 8 miles for females. Slonaker found that female rats were more active than the males and he further noticed considerable individual variation in the activities. The present results agree nicely with the observations of Slonaker. It is interesting to note also that the maximum average run by Slonaker's rats was 11 miles (average for one month) while that of mine was 10 miles per day for 93 days, thus showing a close agreement even in this respect. We do not know how active the wild Norway rats may be, but a considerable curtailment of the normal activity in the albino rats under domestication seems highly probable.

The effects of long-continued exercise on the body of the Albino as a whole, and on the organs, was the object of the present investigation.

#### INFLUENCE OF EXERCISE FOR 90 TO 180 DAYS ON THE EXTERNAL MEASUREMENTS AND ON THE ORGAN WEIGHTS

##### *1. Alterations of the external measurements*

The external measurements of the exercised albino rats contrasted with those of the controls are given in table 3.

To prevent misunderstanding or misinterpretation, a word of explanation touching the tables is in order. Using the series for 1911 males, the second one in table 3 (the same which has been used as an illustration for procedure on page 649), attention

is called to the following points: The entries for the control are the *observed* values—the entries for the exercised are the *observed* values. The percentages deviations of both controls and exer-

TABLE 3

*Showing the external measurements in both control and exercised rats*

	LENGTH (mm.) OF		WEIGHT OF BODY: GRAMS	DURATION OF EXERCISE: DAYS	AGE: DAYS	NO. OF RATS USED
	Body	Tail				
<i>Stock albino rat (Donaldson, '11) M. and F.</i>						
Control.....	199	165.00	199.60	180	242	31
Exercised.....	195	163.00	192.80		242	24
Per cent: Exerc.-cont.		0.42	2.90			
<i>Stock albino rat, 1911 series M.</i>						
Control.....	212	176.00	238.30	180	215	21
Exercised.....	195	164.00	202.00		195	11
Per cent: Exerc.-cont.		1.62	13.40			
<i>Inbred albino rat, 1912 series M.</i>						
Control.....	212	184.00	217.30	180	212	9
Exercised.....	214	181.00	245.30		213	10
Per cent: Exerc.-cont.		-2.77	8.42			
<i>Inbred albino rat, 1912 series F.</i>						
Control.....	191	174.00	155.40	180	215	6
Exercised.....	198	173.00	179.80		213	7
Per cent: Exerc.-cont.		-4.77	2.52			
<i>Stock albino rat, 1914 series M.</i>						
Control.....	176(F)	167.00	145.60	90	135	3
Exercised.....	198	178.00	220.90		135	4
Per cent: Exerc.-cont.		-2.56	11.35			
<i>Stock albino rat, 1914 series F.</i>						
Control.....	176	167.00	145.60	90	135	3
Exercised.....	182	171.00	159.40		135	4
Per cent: Exerc.-cont.		-1.60	-1.87			
Percentage by which exercised rats differ from controls. Same weight given to each series.....		-2.02	6.76			

cised from the formula values have been determined but *are not entered* in the table. The difference between these two percentage deviations is alone given in the table after ‘%: exerc.-contr.’ To test the correctness of these last figures it is always necessary to carry out the operations which have been described (p. 648). This same explanation applies to the other series in this table and also to the other tables 3, 4 and 5.

*Body length.* The average absolute length of the body is practically identical in both the exercised and control rats. The difference amounts to 0.5 per cent in favor of the controls. The data given by Donaldson ('11) for a single series show also a small difference of 2 per cent in favor of the control. We may conclude therefore that exercise does not alter the growth of body in length to any noticeable extent.

*Tail length.* The tail length with respect to body length tends to be slightly shorter in the exercised than in the non-exercised rats. The average difference is 2.02 per cent in favor of the control. The data given by Donaldson show practical identity in his series. However, the difference is noted in four out of my five series and I conclude therefore that exercise tends to retard the growth of the tail in length.

*Body weight.* A slight increase of 6.76 per cent is given in the average body weight for the exercised rats when compared with that of the non-exercised. This difference appears in four series out of five. Data given by Donaldson shows also an increase of 2.90 per cent in favor of the exercised. This relative gain in body weight of the exercised rats may be due in part to the absence of lung disease in the exercised rats. As will be seen later, most of the control rats were suffering from a lung infection, while most of the exercised rats were free from this. It is therefore possible that the increased body weights shown in table 3 are in some measure due to the slightly emaciated condition of the control rats, thus raising the relative value in favor of the exercised rats. I am therefore inclined to believe that in the case of the albino rat the body weight is not much affected by the form of exercise here given—though it may be slightly increased.

Considering these three external characters together, we may say that long-continued exercise does not modify significantly any of the characters here mentioned with the possible exception of the tail length, which shows a slight tendency to a deficit with respect to the body weight.

2. Alterations of the viscera

TABLE 4

Showing the weights of viscera of the exercised rats compared with those of the controls. Arrangement of data explained on p. 650.

	BODY LENGTH (mm.)	HEART	WEIGHTS (GMS.) OF				ALIM. TRACT	NO. OF RATS USED
			Kidneys	Liver	Spleen	Lungs		
<i>Stock albino rat, 1911 series, M.</i>								
Control.....	212	0.886	1.768	10.21	0.611	1.796		21
Exercised.....	195	0.904	1.867	10.35	0.589	1.588		11
Per cent: Exerc.-cont.		26.980	32.170	23.15	25.980	17.250		
<i>Inbred albino rat, 1912 series M.</i>								
Control.....	212	0.784	1.627	8.50	0.445	2.325	7.86	9
Exercised.....	214	0.874	2.032	11.36	0.415	1.228	9.15	10
Per cent: Exerc.-cont.		7.380	17.300	22.46	-6.790	-82.990	9.49	
<i>Inbred albino rat, 1912 series F.</i>								
Control.....	191	0.587	1.246	6.48	0.395	2.205	6.77	6
Exercised.....	198	0.787	1.530	8.85	0.363	0.999	8.12	7
Per cent: Exerc.-cont.		17.430	8.310	17.24	-14.900	-89.970	7.52	
<i>Stock albino rat, 1914 series M.</i>								
Control.....	(F)176	0.595	1.054	6.19	0.651	1.000	8.27	3
Exercised.....	198	1.044	1.790	9.06	0.444	1.128	8.82	4
Per cent: Exerc.-cont.		37.680	25.280	13.12	-61.870	-15.160	-14.80	
<i>Stock albino rat, 1914 series F.</i>								
Control.....	176	0.595	1.054	6.19	0.651	1.000	8.27	3
Exercised.....	182	0.822	1.317	7.74	0.457	1.105	8.09	4
Per cent: Exerc.-cont.		27.130	11.930	12.72	-63.210	1.390	-9.76	
Average percentage by which exercised albino rats differ from controls. Same weight given to each series.....		23.320	19.000	17.74	-24.160	-33.090	-1.89	

From table 4 we note the following modifications.

*Heart.* In all the series the weight of the heart is considerably heavier in the exercised rats than in the controls. The average difference is 23.32 per cent in favor of the exercised rat.

*Kidneys.* The kidneys of the exercised rats are also heavier than those of the controls in all the series. We note the average difference of 19 per cent in favor of the exercised.

*Liver.* The weight of the liver is also heavier in the exercised rat in all the series. The difference is 17.74 per cent in favor of the exercised rat.

*Spleen.* On the other hand, the weight of the spleen is greater in the control than in the exercised. The difference amounts to as much as 24.16 per cent in favor of the controls. This difference does not appear to be due to a greater variability of the spleen, since it occurs in four series out of five, and furthermore, the same phenomena occurs in another series, which will be considered later (table 7). The reason why exercise retards the normal growth of the spleen with respect to body length is not clear.

*Lungs.* We notice the average difference of minus 33.90 per cent in the exercised lungs compared with the controls. In the case of the lungs, however, those heavier than normal for the body weight are associated with a lung infection. As a matter of fact, most of the control rats were infected, while the exercised rats were free from infection. It has already been noted that the infection of the lungs in the control rats was probably responsible in part for the relatively small body weight of the controls. It is highly interesting to see that exercise prevents, or at least delays, an onset of a very prevalent pulmonary infection in the albino rat.

*Alimentary tract.* The alimentary tract as here designated includes not only the digestive tract proper, such as the stomach, intestine, etc., but also all attached structures, as the pancreas, omentum, as well as fat deposited in them. As is shown in table 4, the alimentary tract is not evidently modified. We note an average difference of 1.89 per cent in favor of the controls. This small difference, associated as it is with the bal-

anced distribution of plus and minus variations, justifies our conclusion that exercise has not affected this part of the visceral system.

### 3. Alterations of ductless glands

*Testes.* The testes of the exercised rats show an average increase of 12.33 per cent when contrasted with those of the controls. This increase occurs in both the male series, thus showing the significance of the reaction.

*Ovaries.* The ovaries in the exercised rats show an increase of 84.33 per cent when contrasted with those of the controls. The difference between the exercised and controls in these organs is evident even at a glance. It is interesting to note that the sex glands of the Norway rats are normally heavier than those of the Albinos. The increase here shown as the result of exercise may possibly indicate a return to the wild form, not in size alone but also in fertility.

*Hypophysis.* The hypophysis responds to exercise differently according to sex. We note an increase of 10.25 per cent in the case of the male and a deficit of 22.23 per cent in the case of the female. The approach of the weights in the two sexes and the larger loss in the female bring about relations which I have observed in the wild Norway (Hatai '14 b).

*Suprarenal glands.* As in the case of the hypophysis, the suprarenal glands show also dissimilar reaction to exercise according to the sex. Thus we note practically no alteration in the male (0.84 per cent), while there is an increase of 47.76 per cent in the female—again relations such as the wild Norway shows (Hatai '14 b).

*Thyroid.* We note a difference of nearly 13.44 per cent in favor of the control rats.

*Thymus gland.* The thymus of the exercised rat shows a slight relative increase of 4.80 per cent when contrasted with that of the control. It occurs, however, in only two cases out of four, and furthermore the greater variability in the alteration suggests that the difference here noted may not be significant at all. We must await the results of future experiments to make any positive statement.

TABLE 5

Showing the weights of the ductless glands in the exercised rats compared with those in the controls. Arrangement of data explained on page 650.

	BODY LENGTH (mm.)	SEX GLANDS	WEIGHTS (GMS.) OF				NO. OF RATS USED
			Hypophysis	Suprarenals	Thyroid	Thymus	
<i>Stock albino rats, 1911 series M.</i>							
Control.....	212	2.2280					21
Exercised....	195	2.3380					11
Per cent: Exerc.-cont.		19.7800					
<i>Inbred albino rat, 1912 series M.</i>							
Control.....	212	2.3430	0.0085	0.0328	0.0344	0.0984	9
Exercised....	214	2.5030	0.0096	0.0339	0.0354	0.1085	10
Per cent: Exerc.-cont.		4.8800	10.2500	0.8400	0.2700	7.1800	
<i>Inbred albino rat, 1912 series F.</i>							
Control.....	191	0.0368	0.0113	0.0425	0.0273	0.1051	6
Exercised....	198	0.0602	0.0127	0.0622	0.0282	0.1698	7
Per cent: Exerc.-cont.		46.4200	-2.0200	27.3200	-5.4600	41.7000	
<i>Stock albino rat, 1914 series M.</i>							
Control.....	176 (F.)				0.0282	0.1931	3
Exercised....	198				0.0290	0.1355	4
Per cent: Exerc.-cont.					-25.8100	-25.4900	
<i>Stock albino rat, 1914 series F.</i>							
Control.....	176	0.0557	0.0076	0.0417	0.0282	0.1931	3
Exercised....	182	0.1145	0.0058	0.0656	0.0247	0.1836	4
Per cent: Exerc.-cont.		122.2500	-42.4300	47.7600	-22.7400	-4.2000	
Average percentage by which exercised albino rats differ from control. Same weight given to each series		12.33(M.) 84.33(F.)	10.25(M.) -22.23(F.)	0.84(M.) 47.76(F.)	-13.4400	4.8000	

From the above it is clear that most of the ductless glands are subject to a considerable alteration as the result of exercise. Beyond pointing out that the exercised rats show, in the case of the testes, ovaries, hypophysis and suprarenals, relations similar to those found in the wild Norway, I am unable to give an interpretation of the changes observed. The data are therefore presented without further comment except to repeat that the alterations here noted are constant and are not the result of a great inherent variability of these organs.

#### *4. Alterations of central nervous system and of eyeballs*

*Brain weight.* On the average the brain weight of the exercised rat is 4.02 per cent heavier than that of the controls. This relatively greater weight of the exercised rat is true not only on the average, but also for all the series given in table 6. A variation of 4 per cent is not usually regarded as a large figure, nevertheless it is certainly significant for this particular organ. Indeed, this gain of 4 per cent is the largest plus alteration so far obtained from our experiments. It seems safe to conclude from its constancy, as well as from relative uniformity of the value, that exercise increases the brain weight with respect to the body length. It should be noted also that the present results agree with the finding of Donaldson ('11) in this respect. It is not clear, however, whether this gain was due to a uniform enlargement of an entire mass, or to the increase of special divisions or structural components of the encephalon. A detailed analysis may settle this question in the future.

*Spinal cord weight.* The weight of the spinal cord evidently is not significantly altered. This is shown not only by its slight average modification of 0.88 per cent but also by the fact that the variation is not uniform in all the series. Donaldson found a difference of 0.65 per cent in favor of the control rat. All we can say about this part of the central nervous system is that exercise produces no significant alterations.

*Amount of water in the brain and spinal cord.* As shown in table 6, the percentage of water in the brain and spinal cord is

TABLE 6

Showing the weights of brain and spinal cord and of eyeballs of the exercised rats compared with those of the controls. Arrangement of data explained on p. 650.

	BODY LENGTH (mm.)	WEIGHTS (GMS.) OF		PERCENTAGE OF WATER		WEIGHT OF EYEBALLS (GMS.)	NO. OF RATS USED
		Brain	Sp. cord	Brain	Sp. cord		
<i>Stock albino rat (Donaldson '11) M. and F.</i>							
Control.....	199	1.920	0.597	78.41	71.45		31
Exercised.....	195	1.951	0.580	78.12	71.37		24
Per cent: Exerc.-cont.....		2.570	-0.650	-0.29	-0.08		
<i>Stock albino rat, 1911 series M.</i>							
Control.....	212	1.872	0.614	78.09	70.39		4 21
Exercised.....	195	1.866	0.564	78.08	71.05		5 11
Per cent: Exerc.-cont.....		3.670	3.000	-0.01	0.66		
<i>Inbred albino rat, 1912 series M.</i>							
Control.....	212	1.784	0.610	78.57	71.40	0.294	9 6
Exercised.....	214	1.868	0.603	78.40	71.20	0.299	10 7
Per cent: Exerc.-cont.....		3.940	-2.420	-0.17	-0.20	0	
<i>Inbred albino rat, 1912 series F.</i>							
Control.....	191	1.715	0.547	78.24	70.89	0.288	6
Exercised.....	198	1.826	0.558	78.57	71.65	0.279	7
Per cent: Exerc.-cont.....		4.430	-3.220	0.33	0.76	-9.920	
<i>Stock albino rat, 1914 series M.</i>							
Control.....	176(F)	1.648				0.288	3
Exercised.....	198	1.836				0.288	4
Per cent: Exerc.-cont.....		5.990				-16.950	
<i>Stock albino rat, 1914 series F.</i>							
Control.....	176	1.648				0.288	3
Exercised.....	182	1.701				0.276	4
Per cent: Exerc.-cont.....		2.060				-10.910	
Percentage by which exercised albino rats differ from controls.							
Same weight given to each series.							
		4.020	-0.88	-0.02	0.41	-9.45	

not modified. This result agrees with the findings of Donaldson ('11).

*Eyeballs.* As a sample of the sense organs, the eyeballs were examined. As will be seen from table 6, the average weight of the eyeballs of the exercised rats is 9.45 per cent less than that of the non-exercised rat. The meaning of the smaller eyeballs in the exercised rats is not at all clear. I may mention, however, that from the data so far accumulated, the wild Norway rat has somewhat smaller eyeballs than the Albinos of the same body length.

THE EFFECT OF EXERCISE TAKEN FOR A PERIOD OF 30 DAYS

We have found that exercise taken for a period of 90 days produces changes in the organs to the same extent as exercise given for the period of 180 days (tables 3 to 6). It was thought interesting to determine the minimum period necessary to produce all the typical alterations. For this purpose the rats one month old were kept in the revolving cages for 30 days. The number of rats used was 6 controls and 6 experimented.

Without discussing the individual characters separately, we may make the following general statement.

(1) In no case are the alterations as large as those shown by the rats which had been kept in the revolving cages for the period of 90 or 180 days.

TABLE 7

*Showing percentage values by which the several characters of the rats exercised for 30 days differ from those of the non-exercised. Males=3 controls and 4 exercised. Females=3 controls and 2 exercised. In this table only the final percentage values (i.e., for "% exerc.-contr.") are given.*

	PER CENT		PER CENT		PER CENT
Tail length.....	0.4	Heart	8.0	Thyroid	2.0
Body weight.....	2.4	Kidneys	8.2	Thymus	-1.1
Brain.....	0.2	Liver	-9.5	Hypophysis { M.	25.7
Eyeballs.....	4.2	Spleen	-52.9	{ F.	10.6
Alimentary tract....	-7.7	Testes	9.2	Suprarenals { M.	8.0
Lungs.....	4.7	Ovaries	51.4	{ F.	30.1

(2) The amount of time necessary to produce the typical alterations varies according to different organs.

(3) While the heart and kidneys show a typical change, the liver shows a contrary modification. This may be due to a rapid utilization of reserve materials following a rapid growth, as well as the greater activity of the animal at this younger period.

(4) The early response of the sex glands to exercise may be the result of two combined factors; a greater supply of nutrition following the rapid circulation, and a strong tendency for growth of sex glands at this period of 50 to 60 days of age.

(5) Changes shown by other organs, particularly by the hypophysis, suprarenals and spleen, are very large. It is to be noted, however, that the weights of the hypophysis in the two sexes, despite the fact that both show a gain, tend to come together as in the 90 and 180 day series, while in the case of the suprarenals, the increase in the female is much the greater, a result again agreeing with the earlier series.

We may conclude from this short experiment that the effect of exercise is clearly shown in the rats which have been kept in the revolving cages for one month only, though the amount of modification varies considerably according to different organs.

TABLE 8

*Showing the relation between heart weight and amount of exercise taken*

DESIGNATION OF RAT	BODY LENGTH (mm.)	HEART WEIGHT (GMS.)	NO. OF MILES PER 24 HOURS	SEX
8 B <sub>10</sub>	222	0.725	0.4	M.
7 A <sub>47</sub>	212	0.771	0.5	M.
8 B <sub>9</sub>	215	0.799	0.6	M.
7 B <sup>2</sup> <sub>16</sub>	218	0.853	0.6	M.
7 B <sub>19</sub>	201	0.720	4.8	M.
7 B <sub>48</sub>	220	0.888	6.5	M.
6 B <sup>2</sup> <sub>82</sub>	217	1.149	6.7	M.
7 A <sub>5</sub>	205	0.907	7.6	M.
7 A <sub>4</sub>	212	0.915	7.6	M.
7 B <sup>2</sup> <sub>17</sub>	198	0.745	6.2	F.
7 B <sub>20</sub>	191	0.724	7.4	F.
6 B <sup>2</sup> <sub>92</sub>	205	0.838	7.7	F.
6 B <sup>2</sup> <sub>83</sub>	201	0.891	8.3	F.
8 B <sub>11</sub>	197	0.762	10.2	F.

WEIGHT OF HEART IN RELATION TO THE AMOUNT OF EXERCISE TAKEN

The variability in the activities of rats in the revolving cage suggested that there might exist a definite relation between the heart weight and the amount of exercise taken. To test this point table 8 was prepared. The data were taken from the 1912 series.

Among normal rats kept in the ordinary cages the correlation between heart weight and body length is very high (Hatai '13). Table 8 shows, however, that the correlation between the heart weight and body length in the exercised rats is almost zero, while, on the other hand, the correlation between the heart weight and amount of exercise taken is very high. To illustrate these points, I have divided the male records into three groups and the females into two groups according to the following plan:

- Male* Group 1 Average for the first four sluggish rats
- Group 2 Average for the rats which ran 4.8 to 6.7 miles
- Group 3 Average for the rats which ran 7.6 miles
- Females* Group 1 Average for the rats which ran 6.2 to 7.7 miles
- Group 2 Average for the rats which ran 8.3 to 10.2 miles

These average values are tabulated below.

		Body length (mm.)	Heart weight (gms.)	Mean distance run (miles)
<i>Males</i> .....	Group 1	217	0.787	0.5
	Group 2	213	0.919	6.0
	Group 3	209	0.911	7.6
<i>Females</i> .....	Group 1	198	0.769	7.1
	Group 2	199	0.827	9.3

From the above we notice that despite a greater body length in Group 1 (males) the corresponding heart weight is less in accordance with the least distance run. On the other hand, Group 3 (males) which has the least body length (209 mm.) gives almost as large a heart weight as Group 2, whose body length is 213 mm. In coincidence with this non-correlation:

between the body length and heart weight, we notice a harmonious relation between the heart weight and amount of exercise taken.

In the female series we notice that despite the practical identity in their body length, Group 2, which had run the greater distance, surpasses in heart weight Group 1, which had run the lesser distance. These facts indicate clearly that the heart increased in weight in relation to the amount of exercise taken, as was anticipated.

#### GENERAL REMARKS

It is clear from the foregoing that long-continued exercise (equivalent to a period of 7 to 14 years in man) in the albino rat produces many striking alterations in the organs. The modifications here given are found to be true not only for all the series, but in most cases even for the contrasted pairs of rats, and thus the results are not dependent on the variability of these organs. I am confident that the alterations are the result of the long-continued exercise. It may not be out of place to mention here that a careful analysis of data has been made to see whether or not the infected lungs are in any way responsible for the changes observed in the organs. The results were negative.

The medical literature abounds with writings on the subject of 'exercise.' We find a universal recognition of hypertrophy of the heart following severe and long-continued exercise. I am not aware, however, that there are any similar statements concerning the modifications of other organs. Although from the results of physiological investigations on metabolism during or after severe physical exercise in man and in mammals, the occurrence of modifications in organs (such as the kidneys, liver, lungs, etc., besides the heart) are quite conceivable, yet we still lack the anatomical data for man.

From a purely biological standpoint, long-continued exercise has a special interest in the case of the domesticated albino rat. Former investigation has established the fact that the albino rat is a strain of the Norway rat (Hatai '07) and further, that these

two forms of the rat possess central nervous systems of a dissimilar weight, the Norway having an absolutely heavier brain and spinal cord for a given body weight (Donaldson and Hatai '11). Again, recently, I have shown that the relative weights of some of the ductless glands differ also in the Norway and albino rats (Hatai '14 b).

In rabbits, Darwin ('83) noted several physical differences, particularly in the cranial capacity between the wild and domesticated forms. The wild rabbits possessed a noticeably greater cranial capacity than the domesticated variety. More recently Lapique and Girard ('07) have accumulated extensive data on the brain weights in wild and domesticated races. These two authors conclude also that the wild forms surpass the domesticated in their relative brain weights. Domestication, however, involves numerous interrelated factors which can be only slowly isolated by systematic study, and this experiment was devised to test the value of exercise, which seems to be one of the important factors forming the complex of domestication. The present investigation shows that at least in such organs as the brain, eyeballs, sex glands, hypophysis and suprarenals, the exercised Albinos show an approach to the Norway rat.

In conclusion, I may repeat that from the anatomical side the question of exercise is usually taken rather lightly in the case of man, nevertheless when we consider its striking effect on some of the organs of the rat, a further careful investigation of the subject, not only in the rat but in man also, seems certainly worth while, both from the general biological standpoint and for its bearing on hygiene.

#### CONCLUSIONS

1. The following determinations were made on exercised as compared with non-exercised rats: (1) External measurements; body and tail length and body weight. (2) Visceral organs; heart, kidneys, liver, lungs, spleen and alimentary tract. (3) Ductless glands; testes, ovaries, hypophysis, suprarenals, thyroid

and thymus. (4) Nervous system and sense organs; brain and spinal cord, and eyeballs.

2. The albino rats allowed to exercise in the revolving cages for 90 or 180 days show modifications in most of the organs. Among them, the following may be mentioned:

(a) The heart, kidneys and liver show an average excess of about 20 per cent, while the spleen shows a similar amount of deficiency.

(b) The brain weight shows an average excess of 4 per cent, while no change is noticed in the case of the spinal cord. (This result agrees with the observation of Donaldson '11).

(c) The ovaries give an excess of 84 per cent, while the testes give an excess of 12 per cent.

(d) The hypophysis, as well as the suprarenals, respond differently to exercise according to sex. Furthermore, these two organs show, as the result of exercise, an approach to the relations characteristic for the Norway rat.

(e) The exercised rats were either entirely free from lung infection or but slightly affected. The control rats, on the other hand, had badly infected lungs and in some series several of them were lost, presumably from the lung disease. Analysis of the data shows that the lung infection is not responsible for the changes observed in the organs.

3. Exercise for the period of 30 days showed in most organs modifications similar to those observed in rats exercised for 90 to 180 days.

4. In the exercised rats the heart weight and amount of exercise taken are highly correlated.

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No. 6

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THE RAT

COMPILED AND EDITED BY  
HENRY H. DONALDSON

REFERENCE TABLES AND DATA FOR THE ALBINO RAT (*MUS  
NORVEGICUS ALBINUS*) AND THE NORWAY RAT  
(*MUS NORVEGICUS*)

To be published in October

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PREFACE

For a number of studies on the growth of the mammalian nervous system made by my colleagues and myself we have used the albino rat. In the course of the work we frequently felt the need of referring to other physical characters of the rat to which the nervous system might be related. This led us to collect such data as were already in the literature and also led us to make further investigations. The facts gathered in this way have proved useful to us and are here presented in the hopes that they will be useful to others also.

CONTENTS

Preface. Introduction. Classification. Early records and migrations of the common rats.

Part 1. Albino rat—*Mus norvegicus albinus*. Chapter 1—Biology. Chapter 2—Heredity. Chapter 3—Anatomy. Chapter 4—Physiology. Chapter 5—Growth in total body weight according to age. Chapter 6—Growth of parts or systems of the body in weight. Chapter 7—Growth of parts and organs in relation to body length and weight in relation to age. Chapter 8—Growth in terms of water and solids. Chapter 9—Growth of chemical constituents. Chapter 10—Pathology.

Part 2. *Mus norvegicus*. Chapter 11—Life history. Chapter 12—Growth in weight of parts and systems of the body. Chapter 13—Length of tail and weights of body, brain and spinal cord in relation to body length. Chapter 14—Growth in terms of water and solids. Chapter 15—References to the literature. Index

# THE GROWTH OF THE FETUS OF THE ALBINO RAT FROM THE THIRTEENTH TO THE TWENTY- SECOND DAY OF GESTATION

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TWO FIGURES

For the prenatal growth in weight of the human body Jackson ('09) has presented data gathered by himself and compared these with such data as had already been published. Similarly, Lowrey ('11) has studied the prenatal growth of the pig. In both cases the authors have found it necessary to depend in part on preserved material. While preservation may not alter materially the relative weights of parts of the body, it undoubtedly does alter the total body weight and the records for that character must be interpreted, therefore, with this fact in mind.

The following study on the growth of the fetus of the albino rat forms another series of observations on the prenatal growth of the mammal and has the special virtue of being made throughout on fresh specimens.

The fetuses were all from second litters, the female having been allowed to breed once and to raise her litter under observation, so that we might be assured of her normal behavior as a breeding animal. The data have been gathered from the colony at The Wistar Institute between 1907 and 1913.

## METHODS

The female was mated for the second time, under observation, and after the lapse of the desired interval, was killed with ether. The fetuses were removed, cleared of membranes, and then each placed in a previously weighed stoppered vial and the weight determined to a tenth of a milligram. The operation was al-

ways conducted within a protecting chamber to prevent the loss of moisture by evaporation. The fetus of 13 days was found to be the youngest which would stand manipulation without damage and the observations begin with that age. The litters of 38 females have been thus studied. The total number of fetuses removed was 336, giving an average of 8.8 per litter, with a range of from 3 to 16 fetuses per litter. For 330 of these the exact weights have been obtained.

The observations furnish records for the weights of the fetus from the 13th to the 22d day inclusive—the latter being about the time of birth—under usual conditions, and within these limits they give the weights of the fetus at approximately twenty-four-hour intervals. The observed weights for this series are entered in table 1.

When the data of table 1 are combined and the means taken, we obtain the mean fetal weights given in table 2. The values given in table 2 are the means of the averages for the several litters of like age. Thus the average value for each litter was given the same weight irrespective of the number of fetuses in the litter. When the data of table 2 are plotted they furnish the graph in chart 1. The form of this graph illustrates the rate of growth as given in table 2 and this agrees with the general observation that in the growing fetus the rate tends to diminish with advancing age.

It has been pointed out by Donaldson ('06) that we may assume the span of life in the albino rat to be three years—between birth and natural death—and that this span in the rat is equivalent to ninety years in man. On this assumption the rat grows thirty times as rapidly as man. If we apply this ratio to the gestation period it follows that one-thirtieth of the human gestation period is about 9 days, but the rat has a gestation period of some 22 days. The explanation of this discrepancy between the rate of prenatal and that of postnatal growth is still wanting but the recent observations of Huber ('15) on the early stages of development in the Albino show that the first phases go very slowly. Taking 22 days for the gestation period of the Albino and 271 days (Mall, in Keibel and Mall '10) for

TABLE I

Giving the observed weights of the fetuses at different ages from the 13th to the 22d day of gestation. Where the horn of the uterus from which the fetus came and its relative position in the horn were not noted, the fetus weights are given in ascending values under the heading 'Horn and position not noted.' When these facts were noted the weights of the fetuses are given under the respective horns and in serial order, no. 1 being the fetus nearest to the ovary. In a few cases the horn was noted but the order of the fetuses not determined. All the weighings were made to the tenth of a milligram, but in the table only three digits are entered. The diet of the mother, which appears to influence the number of fetuses, is also given

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
39....	13		Scrap	0.036 1 0.038 2 0.044 3 0.029 4 0.031 5	0.045 2 0.038 3 0.029 4 0.031 5	
21....	13	2	Bread and milk	0.— 1 0.— 2 0.041 3	0.010 1 0.055 2 0.028 3 0.068 4 0.037 5 0.042 6 0.047 7	
22....	13	2	Bread and milk	0.032 0.034 0.034 0.036 0.038 0.041 0.—	0.033 0.043 0.050	
23....	13	2	Bread and milk	0.046 1 0.046 2 0.036 3 0.049 4 0.041 5 0.046 6	0.058 1 0.049 2 0.046 3 0.038 4	

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED	
	Days	Hours		Left	Right		
42....	14		Scrap	0.092	1	0.081	1
				0.088	2	0.108	2
				0.093	3	0.107	3
				0.107	4	0.085	4
				0.092	5	0.101	5
						0.091	6
						0.097	7
						0.104	8
						0.059	9
17....	14	2	Bread and milk	0.122	1		
				0.145	2		
				0.098	3		
				0.127	4		
				0.116	5		
				0.136	6		
				0.101	7		
20....	14	2	Bread and milk	0.117	1	0.085	1
				0.—	2	0.099	2
				0.135	3	0.131	3
24....	14	2	Bread and milk	0.103	1	0.108	
				0.144	2	0.101	
				0.115	3	0.091	
				0.120	4	0.096	
				0.100	5	0.102	
				0.104			
14....	14	2	Bread and milk				0.080
							0.109
							0.118
							0.119
							0.121
							0.124
				0.126			
43....	15		Scrap	0.104	1	0.119	1
				0.109	2	0.098	2
				0.114	3	0.111	3
				0.094	4	0.088	4
				0.132	5	0.109	5
				0.118	6	0.097	6

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
38....	15		Scrap	0.223 1 0.202 2 0.205 3 0.217 4 0.206 5	0.228 1 0.217 2 0.244 3	
16....	15		Bread and milk	0.148 0.167 0.182 0.183 0.197 0.226	0.158 0.170 0.176 0.189 0.190	
7....	15		Bread and milk			0.119 0.143 0.176 0.186 0.193 0.196
41....	16		Scrap	0.319 1 0.306 2 0.336 3 0.329 4 0.315 5 0.256 6	0.342 1 0.360 2 0.336 3 0.327 4 0.322 5	
15....	16		Bread and milk	0.348 1 0.310 2 0.322 3 0.347 4 0.300 5	0.258 1 0.306 2 0.306 3 0.353 4 0.288 5	
12....	16		Bread and milk			0.257 0.291 0.336
25....	16		Bread and milk			0.320 0.326 0.328 0.332 0.351 0.352 0.356 0.373 0.390

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
5.....	16		Bread and milk			0.220 0.233 0.237 0.252 0.263 0.269 0.274 0.276 0.298 0.304 0.311
18....	17		Bread and milk	0.536 1 0.474 2 0.617 3 0.543 4	0.419 1 0.529 2 0.608 3	
40....	17		Scrap			0.482 0.491 0.493 0.508 0.530 0.531 0.543 0.625
6.....	17		Bread and milk			0.518 0.536 0.580 0.595 0.649 0.650
13....	18		Bread and milk			0.898 0.934 0.955 0.101 0.105 0.105 0.106 0.108

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
30....	18		Scrap			0.825 0.938 0.941 0.944 0.958 0.961 0.962 0.973 0.978 0.980 0.983 0.986 1.000 1.010 1.020 1.090
4.....	18		Bread and milk			0.930 0.950 1.030 1.029 1.090 1.130 1.170 1.230 1.250
36....	18		Scrap			0.819 0.859 0.866 0.939 0.943 0.954 0.961 0.967 1.010 1.150
37....	19		Scrap	1.480 1 1.550 2 0.530 3 1.450 4	1.310 1 1.690 2 1.540 3 1.340 4	

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
19....	19		Bread and milk	1.930 1	1.560 1 2.020 2 1.740 3 1.910 4 1.630 5 1.860 6 1.900 7	
2.....	19		Bread and milk	1.020 1.440 1.440 1.550		
31....	19		Scrap			1.510 1.590 1.670 1.670 1.690 1.700 1.710 1.730 1.730 1.740
35....	20		Scrap	2.280 1 2.310 2 2.700 3 2.520 4 2.390 5 2.510 6	2.780 1 2.480 2 2.520 3 2.690 4	
33....	20		Scrap			2.130 2.280 2.470 2.480 2.500 2.550 2.600
26....	20		Scrap			2.770 2.830 2.870 2.900

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
26....	20		Scrap			2.940 3.000 3.060 3.200
34....	21		Scrap	3.750 3.950 4.000 4.020 4.160 4.420	3.980 4.050 4.280	
35....	21		Scrap			3.920 3.990 4.030 4.110 4.150 4.220
32....	21		Scrap			3.580 3.720 3.790 3.810 3.930 4.080 4.090 4.260 4.370
28....	21		Scrap			3.030 3.400 3.470 3.470 3.550 3.580 3.590 3.720 3.760 3.950
27....	21		Scrap			4.000

TABLE 1 (Continued)

SERIAL NO.	AGE OF LITTER		DIET	HORN OF UTERUS AND POSITION IN HORN		HORN AND POSITION NOT NOTED
	Days	Hours		Left	Right	
27....	21		Scrap			4.020 4.120 4.150 4.220 4.330 4.380 4.460
44....	22		Scrap	4.460 1 4.710 2	3.910 1 4.710 2 4.950 3 4.700 4 4.540 5 4.820 6 4.750 7 4.710 8	

TABLE 2

*Derived from the data in table 1, and showing the mean weights of the fetuses at ten ages during gestation*

AGE IN DAYS	NUMBER OF FETUSES	AVERAGE WEIGHT OF FETUS IN GRAMS	RATE OF INCREASE IN WEIGHT
			<i>per cent</i>
13.....	34	0.040	
14.....	44	0.112	179
15.....	37	0.168	50
16.....	44	0.310	83
17.....	21	0.548	77
18.....	43	1.000	83
19.....	30	1.580	58
20.....	25	2.630	65
21.....	42	3.980	51
22.....	10	4.630	16

Fetus of albino rat

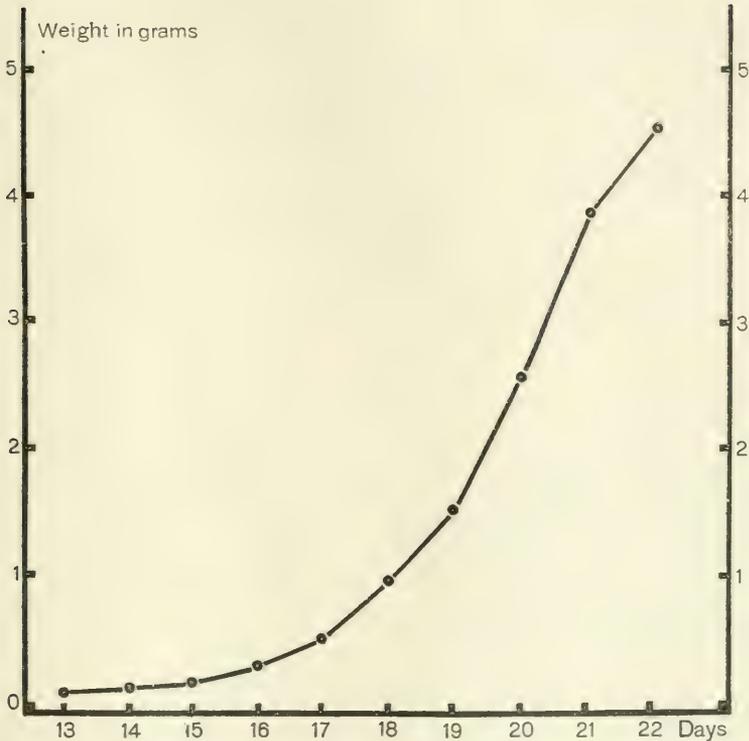


Chart 1 Showing the mean weights in grams of the fetuses of the albino rat at 24-hour intervals, from the 13th to the 22nd day of gestation, inclusive, based on the values given in table 2. The weights here given would be slightly increased if the mothers had all been fed on 'bread and milk,' and slightly diminished had the mothers all been fed on 'scrap.'

that of man we find the actual time ratio to be 1 to 13. Applying this ratio to the human records, the 13th day of gestation in the rat would correspond to the 169th day in man. If the weights in the two species—man and the rat—correspond during the gestation period, then at birth, 4.6 grams for the rat would represent 3250 grams for man. At the 13th day the rat fetus weighs 0.04 grams, so by proportion we would obtain a weight for the human fetus of 283 grams at 169 days. Speaking broadly, this seems to be too small a fetal weight for man (Jackson '09).

TABLE 3

*Crown-rump length of fetus in millimeters; scrap diet only*

SERIAL NO.	AGE IN DAYS	NUMBER IN LITTER	AVERAGE WEIGHT OF FETUS IN GRAMS	AVERAGE CROWN-RUMP LENGTH IN MM.	RANGE OF LENGTH IN MM.
42.....	14	8	0.093	9.5	9 -10
43.....	15	12	0.107	9.4	9 -10
38.....	15	8	0.218	12.1	12 -12.5
41.....	16	11	0.322	13.0	12.5-13
40.....	17	7	0.525	16.3	16 -17
36.....	18	9	0.947	19.1	18 -21
37.....	19	8	1.490	22.7	20.5-24
35.....	20	10	2.510	27.7	24 -32
34.....	21	9	4.070	36.7	35 -39
44.....	22	10	4.630	39.2	36 -41

It might be interpreted, however, as evidence for a still greater slowness of growth in the rat during the earlier period of gestation, but any attempt to follow the matter further must await better data on the weight of the human fetus at different ages.

The data contained in table 1 are sufficient to justify some further discussion of the characters and relations of the fetus during the period covered by the observations.

It is often desirable to have the data for fetal weight correlated with fetal length. Table 3 gives in a number of cases the crown-rump measurements *of the fresh fetus* after the membranes had been cleared away. The litters from scrap-fed mothers only have been used for this purpose.

A word of explanation touching the diets is here in place. In the course of these observations the general diet of the colony was changed. The earlier litters were from females fed on a diet in which bread and milk were the chief features—this is designated 'bread and milk,' while the later records were from females fed on a 'scrap' diet—i.e., table scraps from which materials known to be injurious to the rats had been excluded—this is designated as 'scrap.'

Two differences which are apparently related to the diet, appear, as can be seen from table 4, in which the data are arranged according to the diet of the mother.

In seven out of eight comparisons the litter number for the scrap diet is greater, while the average weight of the fetus is less for the scrap diet litters. Also in seven out of eight comparisons; the exceptional records are in parentheses. The lower average weights of the fetuses from the scrap-fed rats are about what we should expect to follow from the increase in the number in the litter (King '15) but the appearance of the larger number per litter in the scrap-fed series was an unexpected result. The distribution of the litter size (number of individuals) is a fairly symmetrical one and is shown in chart 2.

The mean value for the litter size is 8.8. Our laboratory records show a mean litter size of about 7.0 for the general

TABLE 4

*Effect of diet on the number of fetuses in the litter and on the mean fetus weight*

AGE, DAYS	DIET	NO. OF LITTERS	LITTER DESIGNATION	AVERAGE NO. IN LITTER	AVERAGE WT. OF FETUS, GRAMS
13.....	Bread and milk	3	(21) (22) (23)	9	0.041
	Scrap	1	(39)	(7)	0.037
14.....	Bread and milk	4	(20) (17) (14) (24)	8	0.117
	Scrap	1	(42)	14	0.093
15.....	Bread and milk	2	(7) (16)	8.5	0.174
	Scrap	2	(38) (43)	9.5	0.162
16.....	Bread and milk	4	(12) (25) (15) (5)	8	0.305
	Scrap	1	(41)	11	0.322
17.....	Bread and milk	2	(6) (18)	6.5	0.560
	Scrap	1	(40)	8	0.525
18.....	Bread and milk	2	(13) (4)	8.5	1.05
	Scrap	2	(36) (30)	13	0.95
19.....	Bread and milk	2	(2) (19)	6	1.59
	Scrap	2	(37) (31)	9	1.58
20.....	Bread and milk	1	(26)	8	2.95
	Scrap	2	(33) (35)	8.5	2.47
General average: Bread and milk.....				7.8	0.848
Scrap.....				10.0	0.781

population of the colony (King and Stotsenburg '15). In the present case, however, it is to be remembered first, that we are dealing only with second litters, which tend to be large (King and Stotsenburg '15) and second, that there may be some tendency also for the fetuses to be more numerous than are the young actually born.

Litter size—albino rat

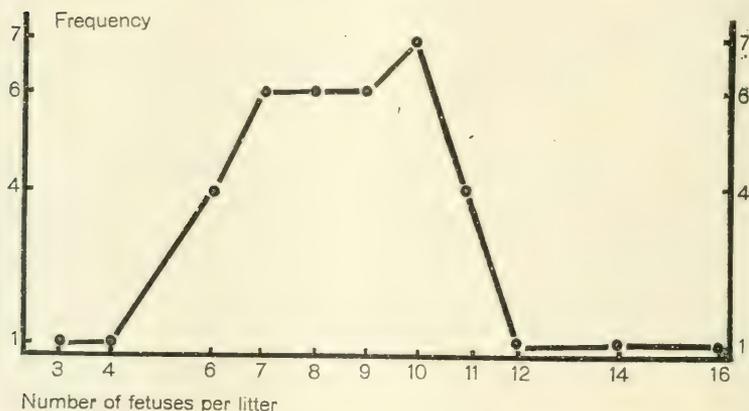


Chart 2 Showing the frequency, as indicated on the ordinate, of the litters containing from 3 to 16 fetuses, as indicated on the abscissa. The mean value is 8.8 fetuses per litter.

#### DISTRIBUTION OF THE FETUSES BETWEEN THE TWO HORNS OF THE UTERUS

This distribution was noted in the case of 20 litters and the details are given in table 5. *In toto* there were 90 fetuses in the right horn, 94 in the left. In two cases the right horn was sterile, and in four cases there was the same number of fetuses in each horn. If the comparison is made between the average weights of the fetuses in the two horns it is seen that in nine out of the fourteen possible comparisons the average weight of the fetus is greater in the horn containing the smaller number.

## THE WEIGHT OF THE FETUS ACCORDING TO POSITION IN HORN

An examination of the fetal weights according to the position of the fetus in the horn has not revealed any correlation. At the same time, inspection of table 1 shows that marked variations in the weights of the fetuses in the same litter and even within the same horn may occur.

For the growth of the Albino from the beginning to the end of gestation, we already have the observations of Huber ('15) giving weight data for the first 3 days and 17 hours, so that there still remains to be filled the interval of about 10 days between the end of Huber's weight records, and the 13th day, which marks the beginning of the records here presented.

TABLE 5

*Showing the number of fetuses in each horn of the uterus and their average weight*

SERIAL NO.	AGE OF LITTER		NO. IN LITTER	LEFT HORN		RIGHT HORN	
	Days	Hours		No.	Weight	No.	Weight
21.....	13	2	10	3	0.041	7	0.041
22.....	13	2	10	7	0.043	3	0.042
23.....	13	2	10	6	0.044	4	0.048
39.....	13		8	3	0.039	5	0.036
17.....	14	2	7	7	0.121	0	
20.....	14	2	6	3	0.126	3	0.105
24.....	14	2	11	5	0.116	6	0.100
42.....	14		14	5	0.094	9	0.092
16.....	15		11	6	0.184	5	0.176
38.....	15		8	5	0.210	3	0.229
43.....	15		12	6	0.112	6	0.104
15.....	16		10	5	0.325	5	0.302
41.....	16		11	6	0.310	5	0.337
18.....	17		7	4	0.542	3	0.519
2.....	19		4	4	1.36	0	—
19.....	19		8	1	1.93	7	1.80
37.....	19		8	4	1.50	4	1.47
35.....	20		10	6	2.45	4	2.62
34.....	21		9	6	4.05	3	4.08
44.....	22		10	2	4.58	8	4.63
Total				94		90	

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OBSERVATIONS ON THE DIFFERENTIATION OF  
THE GRANULES IN THE EOSINOPHILIC  
LEUCOCYTES OF THE BONE-MARROW  
OF THE ADULT RABBIT

PRELIMINARY NOTE

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In a recent paper<sup>1</sup> the writer has described the mast myelocytes and mast leucocytes in the bone-marrow of the rabbit. No evidence could be found in support of the theory that the mast cell of the rabbit represents a young or 'unripe' eosinophil or special cell,<sup>2</sup> or for the view expressed by Pröscher that the mast granules are products of a mucoid degeneration of the spongioplasm of a lymphocyte. The preparations show that mast leucocytes are true granular cells, equivalent in all respects to the other granular cells, with both the myelocyte and fully differentiated forms represented in the marrow.

The supposed relationship of mast leucocytes to eosinophil and special leucocytes necessitated a detailed study of their development also. The same material which was used for the study of the mast leucocytes was found to be excellent for the investigation of the other granulocytes, and of these the eosinophil leucocytes were of special interest on account of the many theories regarding the origin of their granules.

The exact origin of the eosinophil leucocytes of mammals has been the subject of considerable investigation, and up to the present day there is no unanimity of opinion among investigators as to the source and nature of the granules of these cells. The literature relative to the subject is enormous; it shows that

<sup>1</sup> *Anat. Rec.*, vol. 9, no. 3, 1915.

<sup>2</sup> As claimed by Pappenheim's students: Benacchio, Kardos, and Szécsi.

the most divergent theories and explanations are held with reference to the histogenesis of these cells.

Various investigators of the eosinophil problem have come to recognize that the supply of eosinophilic leucocytes in the adult animal is not necessarily limited to mitosis of pre-existing eosinophilic myelocytes, but that a heteroplastic means of regeneration of these cells must also be taken into consideration. The investigations of Tettenhamar ('93), Sacharoff ('95), Brown ('98), Weidenreich ('01), Howard and Perkins ('02), Ascoli ('04), Maximow ('09), Pappenheim ('09), Badertscher ('13), Downey ('13, '14), and Barbano ('14), have shown the importance of the heteroplastic form of development. Downey confined his studies to the differentiation of the eosinophilic leucocytes of the bone-marrow of the guinea-pig and found that heteroplastic development of these cells from non-granular cells is by no means an exceptional process.

Haematologists who believe in a heteroplastic form of differentiation of eosinophils, however, do not agree on the derivation of the granules, and consequently a number of views have been advanced which have sought to account for their source and nature. The older view, that eosinophils are formed from polymorphonuclear neutrophils by a direct transformation of their granules, has within recent years lost support. Brown ('98) has described such a direct transformation of neutrophil granules into eosinophil granules in human muscle infected with trichinae. This theory has been advanced a number of times by different investigators, but it appears that the conclusion for such a direct transformation of one type of granule into another type is not based on sufficient evidence. The mere fact that Brown found a marked increase in the number of eosinophils, with a corresponding decrease in the number of polymorphonuclear neutrophils, does not necessarily indicate that a direct transformation process was going on.

The eosinophils are so widely distributed throughout the tissues, and in certain pathological conditions become so numerous, that it seems quite reasonable to believe that they multiply in these situations by homoplastic means also, since the cor-

responding myelocytes are often present (Gulland and Goodall, Herzog).

At the present day the literature relative to the origin of the eosinophil granules during heteroplasmic differentiation of eosinophilic leucocytes may be said, in the main, to be rather sharply centered about the belief that the granules are of an exogenous origin. Weidenreich is the chief exponent of this theory; he believes that the granules of all eosinophils are hemoglobin-containing products of degenerated erythrocytes,<sup>3</sup> i.e., he believes that the granules are not the products of protoplasmic activities of the cells which contain them.<sup>4</sup> Weidenreich states explicitly that this is the only source of the eosinophil granules, and furthermore, that there are no observations on record which prove a gradual differentiation of eosinophil granules in the protoplasm of non-granular cells.<sup>5</sup>

The theory that the eosinophil granules are of an exogenous origin, and that they are not related to hemoglobin, has been given additional support by the recent observations of Badertscher ('13), who believes that the granules of eosinophil leucocytes seen in the neighborhood of degenerating muscle fibres and erythrocytes in *Salamandra atra* during metamorphosis are products of the degenerating fibers and red cells, and that they are, therefore, related to hemoglobin or its dissociation products.

Weidenreich's hemoglobin theory has met with many staunch supporters. Downey ('13, '14), however, has recently taken exception to the theory in so far as the eosinophils of the bone-marrow are concerned. He states that his preparations showed nothing which would indicate hemoglobin was concerned in the elaboration of these granules; he believes that the eosinophil

<sup>3</sup> *Anat. Rec.*, 1910, vol. 4, p. 327, "Die eosinophilen Granula der Säugetiere sind als exogene Plasmaeinlagerung zu bezeichnen und zwar als hämoglobinhaltige Teile, grösstenteils von Erythrocyten herrührend, die durch hämolytische Vorgänge zerstört, oder in toto phagocytiert wurden."

<sup>4</sup> *Anat. Anz.*, 1901-1902, vol. 20, p. 197, "Die eosinophilen Leucocyten sind also nichts anderes als sog. Lymphocyten, welche die durch den Zerfall roter Blutkörperchen entstehenden feinen Trümmer in ihren Plasmaleib aufnehmen, wobei ihr Kern in die polymorphe Form übergeht."

<sup>5</sup> *Die Leucocyten und Verwandte Zellformen*, p. 250.

granules are real intracellular formations (endogenous differentiations), which are the products of specific activities of the protoplasm. Downey's view is, therefore, in strict opposition to that of Weidenreich and others, who believe in an exogenous origin for all eosinophil granules.

Barbano ('14) has also recently favored the view that the granules are endogenous formations; he believes that they are secretory granules which may be extruded from the cells. His conclusions are based entirely on a study of local eosinophilia in various pathologic conditions.

Under normal conditions the eosinophil leucocytes have been reported by various investigators as being widely distributed throughout the tissues, appearing in great numbers in the gastrointestinal tract, in the walls of the trachea, in the connective tissue surrounding the bronchi, in lymph glands, the thymus, and hemolymph glands. Under certain conditions the number of these cells may be materially increased, so that great numbers of them may appear throughout the section. It now seems certain that many of them are the products of local development, while others have emigrated from the vessels.

The local development of eosinophils, however, is still denied by many authors. Barbano believes that, in local eosinophilia, he can exclude the emigration of myelocytes from the vessels, and that the cells which are found in these local accumulations are, therefore, new differentiations from non-granular cells. The latter are typical small and large lymphocytes. That they may differentiate into granulocytes is shown by the fact that Barbano finds many mononuclear eosinophils whose nuclei are identical with those of the lymphocytes. That lymphocytes, especially small lymphocytes, are concerned in the production of acidophil granules was shown also by Downey and Weidenreich,<sup>6</sup> Howard and Perkins,<sup>7</sup> and others.

<sup>6</sup> Downey, H., and Weidenreich, Fr. 1912, Über die Bildung der Lymphozyten in Lymphdrüsen und Milz. *Arch. f. mikr. Anat.*, Bd. 80.

<sup>7</sup> Howard, W. T., and Perkins, R. G. 1902, Observations on the origin and occurrence of cells with eosinophile granulations in normal and pathological tissue. *The Johns Hopkins Hospital Reports*, vol. 10.

The theory that the eosinophil granules are derived from phagocytosed material (Weidenreich, Badertseher, Brown, and a great many others), is based largely upon the presence of free eosin-staining granules among erythrocytes and muscle tissue which are undergoing degeneration. These free granules are believed to be ingested by lymphocytes which are then converted into eosinophils, many of which are distinctly mononuclear.<sup>8</sup>

Benacchio ('09), in considering the bone-marrow of the rabbit, was primarily interested in the origin of the mast leucocytes of this animal; consequently, he did not make detailed investigations as to the histogenesis of eosinophils and special cells. He concluded, however, that the myelocytes with basophilic granules which he found in great numbers in his preparations were not real mast myelocytes, but simply 'unripe' stages of young eosinophil and special cells.<sup>9</sup> Pappenheim, Kardos, and Szécsi also regard all the basophilic granulocytes in the marrow of the rabbit as immature eosinophils and special cells. Many haematologists, in fact, believe that the early myelocyte stages of eosinophil and special cells have a granulation, which has a predominant basophilic element when first differentiated. These granules, however, do not retain their basophilic element, as they should if they were real mast granules, but they undergo a gradual transformation or 'ripening process,' during which they change their staining reactions and are finally transformed into eosinophil granules. That such transformation actually takes place has been reported by Ehrlich ('78, '79), Schwarze ('80), Hirschfeld ('98), Benacchio ('09), Kardos ('09), Pappenheim and Szécsi ('09), Maximow ('10, '13), and Downey ('13, '14).

The early basophilic granules of eosinophils and special cells, however, are in no way related nor similar to the basophilic

<sup>8</sup> Sternberg claims (Ueber die Entstehung der eosinophilen Zellen. Beitr. z. path. Anat. und allg. Pathol., Bd. 57) that he can distinguish real eosinophil granules from erythrocyte fragments.

<sup>9</sup> Mast myelocytes or fully differentiated mast leucocytes could not be found when Benacchio's methods were used. For the detection of these cells in the marrow of the rabbit, methods of technique must be used in which water is absolutely avoided. After lucidol-acetone fixation, however, the granules are more resistant to water and are able to withstand its action while being stained in watery staining combinations.

granules of mast leucocytes. The mast granules are endowed with certain specific and diagnostic characters at their first appearance within the cell body, and they are readily distinguished from the granules of eosinophils and special myelocytes. This is contrary to the statements of Weidenreich, who believes that if all of the granules of the eosinophil and special leucocytes were basophilic when first formed, it would be impossible to distinguish their myelocytes from the basophilic myelocytes of mast leucocytes. I have found, however, that mast granules can always be distinguished from the granules of other basophilic myelocytes, provided that the proper methods of fixation have been applied to the marrow.

Mention has already been made of the fact that the methods of technique employed by Benacchio failed completely to demonstrate mast leucocytes, although his preparations did show great numbers of eosinophil and special cells. These results would indicate that the chemical composition of mast granules in the rabbit, at least, is quite different from that of the ordinary basophilic granules of young eosinophils and special cells. Mast granules are more soluble in water than are the basophilic granules of either eosinophil or special myelocytes. As far as resistance to water is concerned, it also appears that the granules of the mast leucocytes of the circulating blood are quite different from the granules of the mast myelocytes and the more fully differentiated mast cells of the marrow. The granules of the mast leucocytes of the blood are less soluble in water than are the granules of the mast myelocytes and the corresponding leucocytes of the marrow. This difference in the constitution of the granules is shown by the fact that the most ordinary methods will preserve the granules of the blood mast cells, while the strict elimination of water is necessary for the preservation of the granules of both the mast myelocytes and the fully differentiated mast leucocytes found in the bone-marrow. In the older mast leucocytes, or those found in the blood stream, there may be some chemical change within the cell body—initiated by the blood plasma—as soon as the leucocytes are thrown out into the circulation, which in turn acts upon the mast granules

changing their composition to a greater or less extent and thus rendering them more resistant to the action of water. The mononuclear mast leucocytes are never found in the blood of the normal adult rabbit, but are confined under ordinary conditions to the marrow. In these cells the basophilic granules are very sensitive to the action of water. As the number of granules increase the nucleus gradually becomes polymorphous, while in the fully differentiated mast leucocyte of the circulating blood the nucleus is very polymorphous and the granules are comparatively resistant to the action of water.

The presence of basophilic granules in eosinophil myelocytes is no longer doubted nor questioned by haematologists, their occurrence having been reported by a number of investigators, including Arnold, Hirschfeld, Hesse, Benacchio, Kardos, and others. Maximow and Pappenheim have called particular attention to the very decided basophila of young eosinophil and special granules in the eosinophils and special cells of the rabbit.

An early 'primitive' granulation which is also basophilic has been reported by several investigators. Pappenheim regards it as an early or 'prodromal' granulation that is not related to the final eosinophil or special granulation which appears later as a new differentiation. According to Pappenheim the 'prodromal' granulation is derived from the nucleus of the cell and is basophilic; it disappears when the specific granulation appears later. He believes that the eosinophil granules are a new development and that they too are basophilic when first differentiated. Weidenreich admits the presence of basophilic granules in some of the eosinophils, but claims that they are either fragments of the nucleus or endogenous differentiations which are in no way related to the eosinophil granules. Maximow described a primitive azurophil granulation in eosinophil myelocytes, and he also claims that it is not related to the specific granulation which is developed later. Hertz and Pappenheim have also described an azurophil granulation in the leukoblasts and myelocytes of myelogenous leukemia.

As early as 1895, Arnold, in studying the morphological features of the cells of the marrow of the rabbit, observed that many granulocytes contained basophilic granules. He also noticed that many myelocytes of the same type contained both basophilic and acidophilic granules within the same cell body. Arnold thought it probable that the basophilic granules were transformed into acidophil granules, since so many of the former showed considerable variation in their staining reactions even within the same cell body. Arnold, in fact, seems to have made the correct interpretation of his preparations; however, he gave no detailed descriptions of the gradual changes in the morphology of the granules during the transformation process; neither did he work out in a detailed manner the gradual changes in the staining reactions of the early basophilic granules.

Other investigators of the bone-marrow have also noted changes in the staining reactions of the basophilic granules of eosinophilic myelocytes and have, in fact, reported a transformation of the basophilic into the acidophilic type, but they have not made a particular study of the transformation process itself and of the other phenomena which are seen to accompany it. The life-history of the eosinophil granule has not been worked out with sufficient detail to warrant the statement that all basophilic granules in eosinophilic myelocytes represent unripe eosinophil granules. No attempt has been made to give minute descriptions of the gradual changes in staining reactions which the basophilic granules pass through in becoming transformed into acidophil granules.

Maximow, in his earlier investigations of the bone-marrow, was interested primarily in the rôle which lymphocytes played in the elaboration of granules in their protoplasm, and in the regeneration of the granular leucocytes, consequently, he also failed to make a detailed study of the histogenesis of the eosinophil series. In 1913, however, his figures show that the very youngest granules of eosinophil myelocytes are basophilic when first differentiated, and that they are gradually transformed into acidophil granules, although Maximow does not emphasize this point in particular. He used cover-glass preparations (Helly

fixation followed by staining in alcoholic thionin) and found that the granules of the eosinophils showed considerable variation in their staining reactions depending on the stage of differentiation that they had attained. The older or more fully differentiated granules were stained green in the alcoholic thionin, while the myelocytes contained granules which were stained blue, in addition to other granules which were of a green color.

Downey ('13, '14) has made a special study of the life-history of the eosinophil granules based on the variations in staining reactions and form of these granules. He also finds that the eosinophil granules are basophilic when first differentiated. Gradually, however, these early basophilic granules change their staining reactions, taking on the eosin of the stain when subjected to the action of the indulin-aurantia-eosin staining combination, instead of staining in the indulin, as Downey found to be the case when the granules were first differentiated. Furthermore, he found that in the later stages of differentiation the granules lost their avidity for the eosin of the staining mixture and stained with the aurantia of the same staining combination. In regard to the staining reactions, Downey states that

Some of the granules change their staining reactions while they are still small and basophilic, while others remain basophilic until they have reached a size even greater than that of the fully differentiated granule before such change takes place. That these larger granules do not disappear, and that they are transformed directly into the eosinophil granules is shown by the fact that many of the largest ones are stained in the acid component of the staining mixture, while others are of a mixed tone.

These gradual progressive changes in the staining reactions of basophilic granules in eosinophilic myelocytes together with changes in their shape and size have led Downey to conclude that as far as the bone-marrow is concerned, eosinophil granules are true endogenous formations resulting from special activities of the protoplasm, and that they are not related to hemoglobin or its dissociation products, as maintained by Weidenreich and others.

Barbano, who also regards the eosinophil granules as real endogenous differentiations, has also noted differences in the

-staining reactions of eosinophil granules, although he does not give detailed descriptions of these changes. In using the hemalum and eosin staining combination, he found that there were great individual differences in the avidity with which the granules stained in the eosin. In an epithelioma of the uterus, also in other similar cases, Barbano found that many of the granules in cells of the lymphocyte type stained only slightly in the eosin, while others scarcely stained at all with this dye. The latter appeared clear and refractive and were colored by only the slightest tinge of eosin. Barbano, however, does not interpret these differences in staining reaction as indicating a gradual 'ripening' process of the eosinophil granules. He believes that lymphocytes under certain conditions differentiate granules which are typical eosinophil granules from the very beginning, although in some instances the early formed granules may not exhibit a remarkable affinity for the acid component of the staining mixture.

Downey, however, has shown that the first granules of the eosinophil myelocytes are not the typical granules of the fully differentiated cells. He believes that the fully differentiated granule is the end product of a series of gradual, complex changes in chemical constitution, as well as in form and size, of the small 'unripe' granule which first appeared in the protoplasm of the myelocyte.

In view of the varied opinions concerning the origin and nature of the eosinophil granules, and since the majority of those authors who believe in the hemoglobin nature of the granules have based their studies on local eosinophilia, it is of importance that new studies of the bone-marrow be undertaken, with their results in mind, in order to determine whether the eosinophils of the marrow develop under conditions which might indicate that their granules are also related to hemoglobin products. Fortunately I have had the great pleasure of carrying on an investigation of this kind under the direction of Professor Downey, to whom I am greatly indebted. As far as the marrow of the rabbit is concerned, my observations on the life-history of eosinophil granules do not differ essentially from those of Professor

Downey. In strict corroboration with his findings, my preparations showed nothing which would indicate that hemoglobin was a contributing factor in the formation of these granules.

Many of the myelocytes, in smears prepared according to Pappenheim's method,<sup>10</sup> contain basophilic granules only. These cells might easily be taken for mast myelocytes if it were not for the fact that other similar cells contain oxyphilic granules also. The oxyphilic granules may be very numerous or there may be only a few of them in any one cell, and, in general, the presence of a greater number of oxyphilic granules in a cell seems to be conditioned on a corresponding diminution in the number of basophilic granules. This, together with the fact that many of the granules are intermediate in staining reaction, shows that there is a gradual change in the staining reaction of the granules from basophilic to oxyphilic. The intermediate stages in this gradual process of 'ripening' are so numerous that there is no question but what all of the basophilic granules seen in preparations prepared according to this method are eventually transformed into granules whose chemical constitution becomes such that they finally stain only in the acid component of the staining combination. Such granules are surely not mast granules, for the latter have never been known to change their staining reactions in this way. They remain basophilic throughout their existence.

No other type of basophilic granules, besides those which eventually become oxyphilic, could be found in the preparations prepared by the above mentioned method. We must, therefore, give Benacchio credit for a correct interpretation of the nature of these granules when he stated that the cells which contain them are young 'unripe' eosinophil and special leucocytes. Benacchio, however, did not go into the details of the gradual differentiation and transformation of these granules, and such detailed study would have been impossible with his methods, because with them the vast majority of the cells are distorted, their granules are swollen, and the outlines of the nucleus are usually indistinct.

<sup>10</sup> *Folia Haem.*, Archiv, Bd. 13.

The results obtained by Kardos ('09), in working with sections of bone-marrow of the rabbit fixed in 100 per cent alcohol and in Helly's mixture, are difficult to understand. He found neither mast cells, nor cells of any kind which contained basophilic granules. Contrary to the findings of Kardos, I find that in sections of bone-marrow (material fixed in 100 per cent alcohol and stained in alcoholic thionin) myelocytes with basophilic granules are very numerous, and furthermore, that in these same preparations it is also possible to demonstrate mast myelocytes and fully differentiated mast leucocytes. Sections stained in May-Giemsa not only show many granulocytes which contain basophilic granules, but also other cells in which both basophilic and acidophilic granules are intermixed, and still others in which all of the granules are of the acidophil type. The preparations also show the various other types of granulocytes which are characteristic of the marrow of the rabbit. The basophilic granules of the eosinophil and special myelocytes are also seen in sections of material fixed in Helly's fluid. True mast granules, however, are not preserved by this method, and with their granules dissolved it is difficult to identify the mast cells.

From the above it is seen that it is not a difficult matter to demonstrate basophilic granules in the marrow of the rabbit even with the most ordinary methods. These granules, however, are not the mast granules. If the latter are also desired it is necessary to avoid the use of fixing fluids which contain water. For material fixed in bulk absolute alcohol proved most satisfactory, and for smears the lucidol-acetone method of Szécsi.

Of the various methods tried for working out the life-history of the eosinophil granule, none gave sharper and more decisive results than did Benacchio's method of staining bone-marrow smears in a mixture of indulin-aurantia-eosin. These smears were fixed in Helly's fixative for fifteen minutes and then washed in running water from three to four hours. The preparations were dehydrated and finally stained in the indulin-aurantia-eosin mixture, in a thermostat at a temperature of 38°C. This gave excellent results for the study of both the eosinophil and special myelocytes. The chief advantage of this method is that it

practically eliminates the special myelocytes from our consideration, at least in the later myelocyte stages, since the granules of the special cells at no time in their evolution show any great affinity for the eosin of the staining mixture. The vast majority of the special cells have dark-grayish-black granules, but in the youngest myelocytes these granules also have a slight affinity for the eosin of the mixture, a condition which often makes it difficult to distinguish the earliest eosinophil myelocytes from those of the special cells. The special granules, however, very soon develop their strong affinity for the indulin to the complete exclusion of the eosin, while many of the granules of the eosinophil myelocytes become strongly oxyphilic, causing them to stain intensely with the eosin.

In the earlier stages of the eosinophil myelocytes in which there are only a few acidophil granules, there are a great many small basophilic granules, with a few medium-sized and large basophilic granules scattered among them. In the later myelocyte stages, however, most of the granules are large and many of them are acidophilic. However, the later stages, including those in which most of the granules are acidophilic, contain a few small basophilic granules as well as a few larger ones. It is very probable that these smaller granules are the youngest ones: all of them probably increase in size before developing an affinity for the eosin of the stain. The presence of the small indulinophilic granules in the later myelocyte stages in which the eosinophilic granules are very numerous could not be accounted for by Hirschfeld. Downey, however, believes that these smaller granules represent recent differentiations, since they are small and still basophilic.

In addition to the changes in the staining reactions of young eosinophil granules which at first are basophilic, there is further evidence in favor of the view that these young basophilic granules are the precursors of eosinophil granules. The life-history of the eosinophil granule, in the rabbit at least, is not completed with the change in staining reaction, since the granules in the fully differentiated eosinophil leucocytes of the blood of this animal are typically spindle shaped, while the early granules

are spherical. Downey has already shown (as far as the eosinophil leucocytes of the guinea-pig are concerned, and the histogenesis of these cells seems to be very similar in the bone-marrow of the rabbit) that in addition to the changes in the staining reactions there are further changes in the morphological features of these granules which prove conclusively that the basophilic granules are in reality the younger eosinophil granules.

The granules of both the eosinophil and special leucocytes are very small when they are first formed, and both types of granules are stained dark with the indulin, with possibly a slight tinting with the eosin of the mixture. This uniformity in size and staining reaction frequently make it impossible to distinguish the earliest special myelocytes from those of the eosinophil leucocytes. Maximow encountered the same difficulty in rabbit embryos, but claims that he could always distinguish the two types of cells in the marrow of post-natal animals. In the latter he finds that the first eosinophil granules are from the very beginning brighter and coarser than are those of the special cells; at first the youngest granules possess a clear basophilic quota and stain a bluish tinge, but are not metachromatic as are the granules in the special cells. In spite of these slight differences there are times when it is almost impossible to classify basophilic myelocytes with any degree of certainty. Maximow admits that in alcoholic thionin preparations it is very difficult to distinguish between eosinophil and special myelocytes. He states, however, that the granules of the eosinophils can often be distinguished from the granules of the special cells by the fact that some of the eosinophil granules enlarge very rapidly "und dass man infolgedessen schon in den noch ganz granulaarmen Zellen typische, grobe, glänzende azidophile Granula neben nur sehr spärlichen feineren erblicken kann."

Downey also states that when the granules are few in number and of small dimensions there may be considerable difficulty in distinguishing between eosinophil and special myelocytes. He also found that the granules of eosinophil myelocytes enlarged shortly after they were differentiated and that some of them changed their staining reactions, while others remained basophilic

for a longer period of time. The basophilic granules in young special myelocytes, on the other hand, remained basophilic for a longer period of time which was followed by a rapid change in staining reaction involving all of the granules.

The same difficulty in determining the exact position of the very earliest myelocytes, i.e., those with a few granules only, was encountered in the present investigation.

In the indulin-aurantia-eosin preparations there is no difficulty in finding basophilic myelocytes which contain a few decidedly oxyphilic granules. Myelocytes containing the latter can be diagnosed as eosinophil myelocytes, since the granules of the special cells are never stained intensely with the eosin of the mixture. It must be admitted, however, that there were always a few cells which it was difficult to classify. With more intensive study it might be possible to properly place these cells also. However, it is not the object of the writer to describe the morphological features of the very earliest myelocyte stages of eosinophil and special myelocytes. The indulin-aurantia-eosin preparations show beyond all doubt that, in so far as the adult animal is concerned, the bone-marrow contains two distinct types of myelocytes, the precursors of eosinophils and special cells respectively. The inability to diagnose the specific type of basophilic myelocyte in every case—before some of the granules stain in the eosin—does not invalidate the conclusions in regard to the life-history of the eosinophil granules.

The results of the study outlined above show that these granules are differentiated gradually from the basophilic protoplasm of non-granular cells, and that they pass through a gradual progressive development which is expressed in changes in staining reaction, as well as in shape and size. When first formed they are indulinophilic (with Ehrlich's triglycerine mixture) or basophilic with heterogeneous mixtures containing a basic dye. At first there are only a few granules in the cell, but their number is gradually increased, the youngest granules always being basophilic (or indulinophilic), while the older ones have become distinctly acidophilic. The number of granules which are intermediate in staining reaction, i.e., which have an affinity for

both the acid and the basic component of the heterogeneous mixtures (or for indulin and eosin of the triglycerine mixture), shows that there is a gradual transformation from one type of granule to the other, and not a replacement of one kind by another. There is no evidence for Weidenreich's view that the basophilic granules, which he admits are present in the myelocytes of eosinophil leucocytes, are endogenous formations which are not related to the eosinophil granules which are supposed to be developed later from products of hemoglobin dissociation.

The changes in the character of eosinophil granules are similar to those which are seen during the development of the special granules. For the latter these changes are universally conceded to indicate a process of gradual differentiation, progressive development and 'ripening.' It is difficult to see why the same conclusion should not apply to the eosinophil granules, especially when there is nothing to indicate that in the normal bone-marrow fragmenting erythrocytes or other hemoglobin products are in any way concerned in their development.

The development of eosinophil leucocytes in the tissues is a different question. For them there is much evidence to show that their granules are closely related to hemoglobin products. Their granules apparently do not pass through the same series of changes during their development as was outlined above for the eosinophil granules of the bone-marrow, at least no such changes have ever been described. This may be due to the fact that local eosinophilia has never been investigated from this same standpoint. Barbano's recent study of the subject indicates merely that the granules of the tissue eosinophils are quite variable in their staining capacity with eosin. These variations, however, do not seem to be bound with any particular stage in the development of these leucocytes, and there was nothing to show that those granules which had the least affinity for the eosin were the youngest ones. Barbano, however, has peculiar ideas about eosinophil leucocytes and his results do not seem very trustworthy.

Gütig believes that there are two types of eosinophils, those of the tissues and those of the marrow and blood. The granules of the hematogenous eosinophils are true endogenous differenti-

ations, while those of the tissues are of an exogenous origin. Downey agrees with this conclusion, provided that the observations of Weidenreich and others on the development of eosinophil granules in local eosinophilia are correct. With these questions in mind a renewed study of local eosinophilia would be of great importance.

#### SUMMARY

The bone-marrow of the normal adult rabbit shows no evidence for the support of the theory that the eosinophil granules are exogenous formations which are derived from hemoglobin or its dissociation products (Weidenreich and others).

Application of the proper methods of technique, however, show that these granules are real manifestations of protoplasmic activities, and that they are gradually differentiated in the cytoplasm of mononuclear cells (Downey).

The indulin-aurantia-eosin preparations show that the youngest granules of the myelocytes are indulinophilic. They do not remain indulinophilic for any length of time, but pass through a series of progressive evolutionary processes during which they change their shape and staining reactions. Finally they are transformed into typical eosinophilic granules. In the fully differentiated eosinophil leucocyte of the marrow all the basophilic (with Giemsa, etc.) granules have been converted into acidophilic granules and no new basophilic (indulinophilic with triglycerin) granules are formed.

These gradual changes in the staining reactions and morphology of basophilic granules in the myelocytes of eosinophil leucocytes must be interpreted as indicative of progressive evolution on the part of the eosinophil granules.

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MEMOIRS  
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THE RAT

COMPILED AND EDITED BY  
HENRY H. DONALDSON

REFERENCE TABLES AND DATA FOR THE ALBINO RAT (MUS  
NORVEGICUS ALBINUS) AND THE NORWAY RAT  
(MUS NORVEGICUS)

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PREFACE

For a number of studies on the growth of the mammalian nervous system made by my colleagues and myself we have used the albino rat. In the course of the work we frequently felt the need of referring to other physical characters of the rat to which the nervous system might be related. This led us to collect such data as were already in the literature and also led us to make further investigations. The facts gathered in this way have proved useful to us and are here presented in the hopes that they will be useful to others also.

CONTENTS

Preface. Introduction. Classification. Early records and migrations of the common rats.

Part 1. Albino rat—*Mus norvegicus albinus*. Chapter 1—Biology. Chapter 2—Heredity. Chapter 3—Anatomy. Chapter 4—Physiology. Chapter 5—Growth in total body weight according to age. Chapter 6—Growth of parts or systems of the body in weight. Chapter 7—Growth of parts and organs in relation to body length and weight according to age. Chapter 8—Growth in terms of water and solids. Chapter 9—Growth of chemical constituents. Chapter 10—Pathology.

Part 2. The Norway Rat—*Mus norvegicus*. Chapter 11—Life history. Chapter 12—Growth in weight of parts and systems of the body. Chapter 13—Length of tail and weights of body, brain and spinal cord in relation to body length. Chapter 14—Growth in terms of water and solids. Chapter 15—References to the literature. Index.

## AN ABNORMAL FROG'S HEART WITH PERSISTING DORSAL MESOCARDIUM

JAMES CRAWFORD WATT

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SIX FIGURES

The frog forming the subject of description in this paper belongs to the species *Rana pipiens*, bred in tanks in the Medical Building of the University of Toronto. After pithing, the sternum was cut away to expose the heart, by a student working in the Pharmacological Laboratory and the abnormal heart was so striking in appearance that the frog was sent to Prof. J. Playfair McMurrich who kindly turned it over to me for investigation.

After observing the beating of the heart, the frog was placed in strong formalin solution, and the heart was injected with warm wax. The wax on hardening enabled the necessary dissection to be easily performed, and was easily removed from the heart later, to admit of examination of the cavities. The heart and great vessels were also dissected in a couple of normal frogs for purposes of comparison with this abnormal specimen.

After opening the pericardium the heart is seen (fig. 1) as a long tubular organ extending forward under the floor of the mouth, with its anterior portion displaced to the left of the median line by the hyoid bone and mass of the tongue.

The sinus venosus is situated in its usual location and receives the postcaval and the two precaval veins. It extends forward to open into the right atrium which lies directly cephalad of it. The atria are only incompletely divided from each other on the surface, and they are marked out from the sinus venosus at one end, and the ventricle at the other, by constrictions in the wall. They are of large capacity and appear as long as the whole heart

of a normal frog of equal body size, and form one-half the total length of this heart. They have an oblique position, being nearer the median line posteriorly, as they are forced out to the left side under the floor of the mouth as they proceed forward. The groove on the ventral surface, marking off the left atrium from the right, starts just cephalad of the sinus venosus on the left side, and runs obliquely forward toward the origin of the conus arteriosus, but is lost before reaching this point, so that there is no visible division externally of the atria in the part nearest the ventricle.

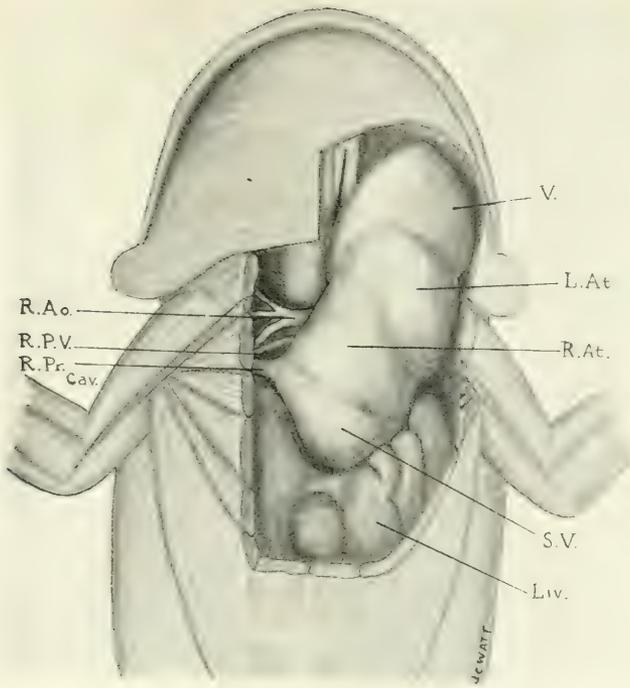
The ventricle continues forward in the line of the atria and is of normal size. Its apex is situated almost in contact with the inner side of the mandible. No conus arteriosus and no aorta can be seen with the heart undisturbed, but by lifting the atria and ventricle slightly, and pulling them out to the left, the conus arteriosus is seen leaving the ventricle on the right side, and running medially and dorsally (fig. 2). Immediately back of the hyoid bone it reaches the left side of the esophagus and bifurcates, sending the left ventral aorta directly dorsally alongside the esophagus, where it divides, and the right one horizontally across the esophagus, after which it divides, giving off the

#### ABBREVIATIONS

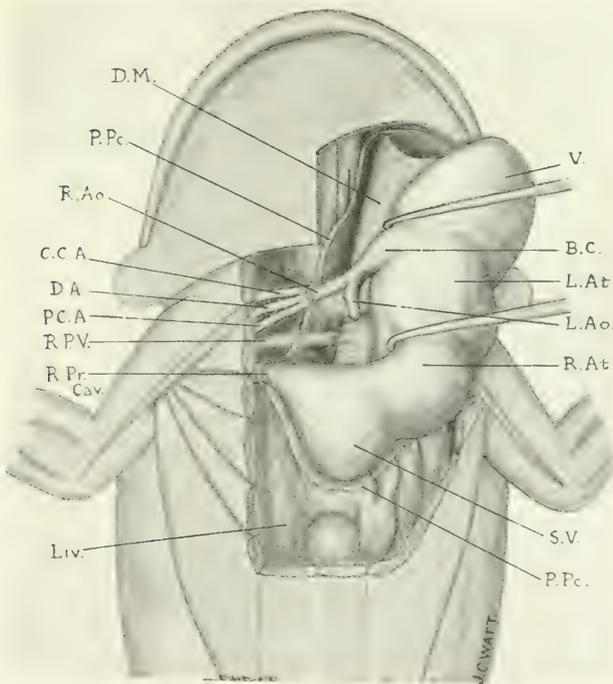
<i>Ao.</i> , aortae	<i>P.C.A.</i> , pulmocutaneous artery
<i>At.</i> , atrium	<i>Post.Cav.</i> , postcaval vein
<i>B.C.</i> , bulbus cordis or conus arteriosus	<i>P.Pc.</i> , parietal pericardium
<i>C.C.A.</i> , common carotid artery	<i>R.</i> , right half of pericardial cavity
<i>D.A.</i> , dorsal aorta	<i>R.Ao.</i> , right aorta
<i>D.M.</i> , dorsal mesocardium	<i>R.At.</i> , right atrium
<i>L.</i> , left half of pericardial cavity	<i>R.Pr.Cav.</i> , right precaval vein
<i>L.Ao.</i> , left aorta	<i>R.P.V.</i> , right pulmonary vein
<i>L.At.</i> , left atrium	<i>S.V.</i> , sinus venosus
<i>Liv.</i> , liver	<i>V.</i> , ventricle
<i>L.Pr.Cav.</i> , left precaval vein	

Fig. 1 Dissection of frog to show abnormal heart. Sternum and ventral muscles have been cut away, also the parietal pericardium; the heart lies undisturbed.

Fig. 2 Same as figure 1 except that heart has been drawn over to left by hooks, to show persistent dorsal mesocardium attached to its dorsal surface. Cut edge of parietal pericardium is also shown.



1



2

common carotid trunk, the rest turning dorsally to form the pulmo-cutaneous artery and dorsal aorta. No abnormalities exist beyond this point, all the arterial trunks appearing normal.

When the heart is lifted a structure which is probably the mechanical cause of the abnormality comes into view. This is a double fold of pericardium (fig. 2) reflected from the wall of the sac to the dorsal surface of the heart. It extends all the way from the sinus venosus to the apex of the ventricle, and at this latter point exhibits a free edge. Running off at right angles to this fold as it lies over the ventricle, is a process of the fold passing to the right over the conus arteriosus and ventral aortae, right out to where these latter structures leave the pericardial cavity, so that this smaller fold has no free border.

I interpret this membrane as a completely persistent dorsal mesocardium. Its presence may be the cause of the abnormal shape of the heart, for the membrane normally disappears as flexion begins in the heart tube, and its continued existence would I think, be an obstruction to this flexion, especially to the bend which would project the heart toward the ventral surface (see arrow, fig. 3). It would thus hold the heart in the extended tubular condition. There has been a certain amount of flexion, however, in this heart, shown by the conus arteriosus coming off the ventricle dorsally and running caudally and medially. The free anterior border of the dorsal mesocardium is thus accounted for, as that part of the membrane lying in the long axis of the heart represents the mesocardium only up to the point of flexion which forms the apex of the ventricle. The mesocardium (fig. 6) originally anterior to this point has been carried back with the conus arteriosus, over which it is still found, and now appears as a process running off from the right side of the rest of the membrane.

The presence of this flexion seems to be an argument against regarding the mesocardium as a force resisting the folding up of the heart tube, but on analysis of the nature of the bend exhibited, this objection is found to be more apparent than real. The bend which has occurred, it will be remembered, is one which brings

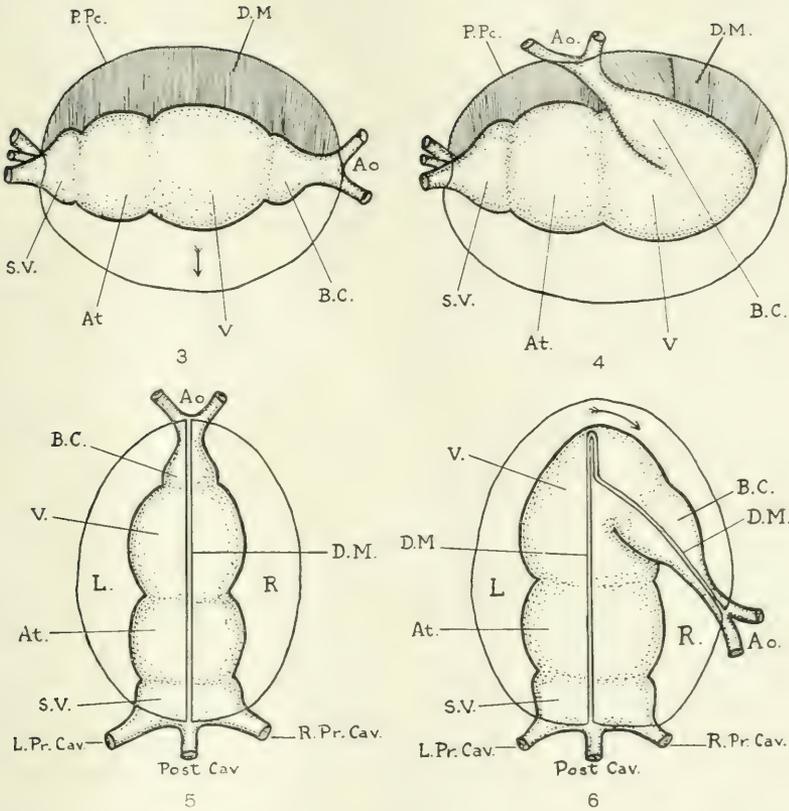


Fig. 3 Diagram of normal unflexed heart tube seen from the right, lying in pericardial cavity, showing dorsal mesocardium. Movement of heart tube in direction of arrow would be resisted. (See under figures 1 and 2 for list of abbreviations).

Fig. 4 Diagram of abnormal frog's heart seen from right, lying in pericardial cavity and showing persistent dorsal mesocardium.

Fig. 5 Diagram of normal unflexed heart seen from above to show line of attachment of dorsal mesocardium.

Fig. 6 Diagram of abnormal frog's heart seen from above, to show line of attachment of persistent dorsal mesocardium. Arrow indicates direction in which flexion has occurred.

the part of the tube displaced, slightly above and to one side (figs. 4 and 6) of the part still retaining its original position. This means that no stretching of the suspending membrane of the tube—the mesocardium—is necessary, but only a folding of this layer on itself to one side. There is no increase in the distance between any part of the heart and the roof of the pericardium. There would of necessity be a great increase in this distance over a part of the heart if the fold had occurred which projects the ventricle (see arrow, fig. 3) into a position ventral to the atria, and this would of course bring great strain and stress to bear on the mesocardium, in order to stretch the part attached to the regions of the heart involved, sufficiently to permit of the flexion. As the mesocardium forms a continuous double-layered membrane suspending the whole heart tube, its strength would be much greater than any adventitious bands or adhesions. Its thickness, also is considerable, relatively to the size of the heart in early stages of development, and so I think it is probable that it was possessed of sufficient strength to resist stretching, and so to prevent the normal atrioventricular bend. The fact that the conus bend occurred was because it could take place without stretching the attached membrane, merely folding it on its side.

The left pulmonary vein comes directly into the left atrium through the dorsal mesocardium. The right vein runs across the roof of the pericardial cavity (figs. 1 and 2) in which it forms a transverse fold lying caudal and parallel to the right ventral aorta, and in this fold it runs to the mesocardium which it enters to reach the atrium.

The flexion of the anterior portion of the heart can be accounted for by the recession of the branchial region during development, carrying back the aortic arches, and necessitating a bending back of the arterial end of the forwardly directed tubular heart. This bend is in the normal direction for that always found between the ventricle and conus arteriosus, but appears on the dorsal surface instead of the ventral because of the absence of the atrioventricular bend, which would have projected all the heart cephalad of it to the ventral surface.

From the foregoing description it will be seen that there is nothing in the condition of this heart that cannot be explained on embryological grounds. The primary cause of the deformity appears to be the failure of the dorsal mesocardium to disappear after the formation of the simple tubular heart. Development in the heart tube has proceeded in a normal way throughout, and the final form of the heart has been influenced almost solely by the mechanical action of the dorsal mesocardium, accompanied by the recession of the branchial arches.

Of course there is another explanation for the condition found here. It is that there was a primary failure of the atrioventricular region to undergo any flexion, and that the presence of the dorsal mesocardium is a second and distinct anomaly, not related in any way to the failure of flexion to occur. Both of these abnormalities are exceedingly rare. As far as I have been able to ascertain neither of these conditions has been previously described, and from the previous discussion I do not see why they cannot be regarded as cause and effect.

I have tried to find accounts of any similar conditions recorded in the literature on the heart and pericardium. Anomalies of the pericardium mentioned are fringes, apertures communicating with the pleural cavity, also complete absence of the pericardium. I have seen no description of a persistent mesocardium or any membranous septum or band which could be interpreted as such. Hundreds of cases of cardiac abnormalities are listed, but none of them were due to incomplete flexion of the heart tube. I have not read all the papers entitled simply 'Cardiac anomaly' as there are scores of them, and experience showed that what were investigated were common occurrences, such as failures of septa, and the like; thus I may possibly have missed a case similar to the present.

There is no doubt, however, that both the conditions here present are extremely rare. Both the disappearance of the mesocardium and the flexures of the heart tube are quite early occurrences in embryonic life. Usually, of course, the earlier a process occurs, the more fundamental it is, the more likely it is not to present abnormalities, being more firmly impressed on

the organism, and the graver are the consequences of departures from the normal. If the supposition is correct that the dorsal mesocardium would offer mechanical opposition to flexion in the heart, then its persistence is fraught with much graver consequences than at first thought would be supposed, and will lead to most extreme cardiac deformity. The deformity in the frog was not incompatible with full activity and functional efficiency, but it is conceivable that in higher animals it might be incompatible with existence, at least beyond fetal life. An homologous deformity in man would postulate a heart tube partly at least located in the neck, subject to compression and to stretching from the movements of this part of the body, and lacking all protection from the outside, such as is afforded by the ribs.

If the dorsal mesocardium should persist, and yet permit of flexion going on normally in the heart, it should be found in the adult as a membrane forming a complete cephalocaudal partition across the sinus transversus of the pericardial cavity, and I have seen no mention whatever of any membrane in this region. Text-books of embryology and of pathology pay scant attention to the mesocardium, unite in describing its very early and complete disappearance, and recount no case of its persistence in whole or in part. It is evidently worthy of consideration in view of the fact that it can be retained and may have far-reaching effects on the heart.

In conclusion I wish very heartily to thank Professor Henderson and Professor McMurrich, through whose kindness this interesting specimen has come into my possession: and for friendly criticism modifying the opinions expressed in this paper I am also indebted to Professor McMurrich.

April 21, 1915

# A MECHANICAL DEVICE TO SIMPLIFY DRAWING WITH THE MICROSCOPE

RAPHAEL ISAACS

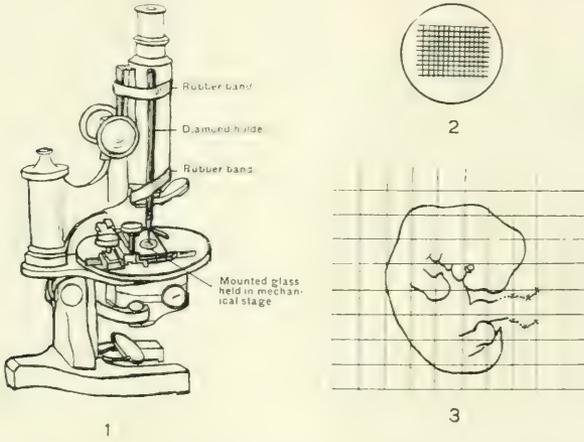
*From the Anatomical Laboratory, University of Cincinnati*

## THREE FIGURES

It is often desirable in making rapid drawings of objects under the microscope, to have some inexpensive mechanical aid, especially when the outline is complex. For some purposes the camera lucida is either inconvenient or undesirable, being too expensive for use in large classes, besides giving a limited field. A simple accessory to aid in making fairly accurate outlines of any desired magnification, from note-book to chart size regardless of the size of the field, should thus find a place in the laboratory equipment, especially if at the same time it can be furnished at a low cost.

The device here described makes use of the fact that the mapping out of the outlines of an object is easier if some fixed points of reference on the specimen can be established and related to corresponding points on the drawing paper. These requirements will be fulfilled if a piece of glass, ruled in squares (fig. 2) is placed in the eyepiece of the microscope so that the squares appear superimposed on the object. The enlarged outlines of the object can then be made on a similarly ruled sheet of drawing paper, by noting the position at which any point of the object appears in the squares in the microscope and placing a mark on the corresponding square and position on the paper. The squares on the paper are lightly ruled in pencil and therefore easily erased. The magnification of the drawings can be regulated at will by making squares of appropriate size on the paper. To rule the squares accurately on a small piece of glass, a mounted 'writing diamond' (a small diamond mounted at the end of a piece of wood or metal) is necessary, although no doubt a carborundum pencil or other substitute may be used. The diamond, in its holder, is fastened at the side of the body tube of a microscope with two wide rubber bands (fig. 1) so that although firmly held, the diamond will have some freedom when pressure is used. The microscope is used merely as a convenient way of holding the diamond so that it can be raised or lowered without changing its position, or throwing it out of adjustment. The lines are ruled by moving the glass beneath the point of the diamond with a mechanical stage. This makes it possible to secure lines at right angles to each other and squares correct to the tenth of a millimeter. The following procedure will be found advisable:

A smooth, preferably thick piece of glass is selected and fastened to a slide with a drop of thick xylol-balsam which has been evaporated and melted on the slide. On pressing the glass down so as to insure a flat upper surface, the balsam soon cools and sets firmly. If the upper surface slants or is uneven, the ruled lines will not be of uniform thickness. A thick round cover-glass or a square cut from a slide can be used for this purpose. The slide, carrying the glass to be ruled is now fitted tightly into the mechanical stage so that there is very little free movement. By using the vernier to mark off the distances, lines



Figs. 1-3 Method of using the mechanical stage for ruling an eyepiece scale to be used in making drawings with the microscope.

Fig. 1 Microscope, with diamond holder in position.

Fig. 2 Ruled glass.

Fig. 3 Method of mapping out in drawing.

can be ruled one millimeter apart (a convenient distance for most purposes) accurate to the tenth of a millimeter. To rule the lines, the diamond is lowered with the coarse adjustment and brought into contact with the slide using very light pressure with the fine adjustment (fig. 1). The reading of the fine adjustment, when the conditions are ideal, will help to give the same pressure for every line. The pressure is tested by starting the line outside the field to be ruled. The elasticity of the rubber bands will give the diamond enough freedom to overcome any slight unevenness of the glass. The slide is moved using the mechanical stage with a steady, sweeping motion. The diamond is then lifted with the fine adjustment, and the slide brought back to the starting position, the slide being moved up and the new position adjusted until the reading on the vernier shows it to be correct. The horizontal lines should be made by moving the slide towards the side

having the fixed support, thus giving it the backing of the solid shoulder of the mechanical stage. For the same reason, vertical scratches should be made by pushing the slide forward. To make the lines at right angles to the ones first drawn, the pressure of the diamond must be as light as possible, as too great pressure in crossing scratches is not considered good for the diamond. It is unwise to go over a line once drawn, as this will often start cracking of the surface. Care should be used in removing the glass, as it is liable to break. The balsam is warmed and when soft the glass is slid off. It should then be mounted with the ruled surface exposed, on another piece of glass of a size to fit neatly into the eyepiece, using melted balsam. The ruled lines may be made more distinct by rubbing them lightly with a lead pencil, so that some of the black graphite is deposited in the grooves. In case it is desirable to cut the ruled glass into smaller pieces, special lines slightly deeper should be ruled along the places to be broken, as it is unsafe to try to break the glass along the light lines already ruled.

The whole operation of ruling takes but a short time, and a good slide can be finished in less than five minutes. With a little practice, especially if the diamond is good, the lines will be fairly even. In a properly adjusted eyepiece, the lines will be in focus, when the ruled glass is placed on the diaphragm inside, the ruled surface being placed facing downward. The glass can be used equally well in a compound microscope or in a binocular. In the latter, where a single picture of a solid object is desired, it is well to put the ruled glass on the side of the eye usually used with the ordinary microscope. Of course the two tubes will give different pictures.

To use the glass in drawing, squares of any desired size are lightly ruled on the drawing paper. It is usually simpler if the squares on the paper appear the same size as those in the microscope. Students can get good results if they use the corner of a slide as a right angle and measure the distance on the lines with a ruler. The approximate position of the object is noted on the squares in the microscope and the corresponding position found and mapped off on the squares on the paper (fig. 3). For low-power work, the lines appear better if the light is cut down. Further details of the drawing can be filled in later and the squares erased. As these squares are only a help in drawing, and as the process may tend to become mechanical, the instructor should be somewhat reserved in placing it freely in the hands of elementary students, where the efforts to draw a microscopic object are often an aid in analysing the specimen. Under high power, however, the squares are a decided help in analysing a complex field and the real educational value of the specimen comes in the synthesis of the parts in the finished drawing.

MEMOIRS  
OF  
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY  
No. 5

THE DEVELOPMENT OF THE ALBINO RAT, *MUS  
NORVEGICUS ALBINUS*

G. CARL HUBER

*From the Department of Anatomy, University of Michigan, and the Department  
of Embryology, the Wistar Institute of Anatomy and Biology*

I. FROM THE PRONUCLEAR STAGE TO THE STAGE OF MESODERM  
ANLAGE; END OF THE FIRST TO THE END OF THE NINTH DAY

THIRTY-TWO FIGURES

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## A SIMPLE FORM OF DRAWING APPARATUS<sup>1</sup>

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*From the Harvard Medical School, Boston*

### ONE FIGURE

This apparatus was designed for use by students in the Harvard Embryological Laboratory, where it has served with success during the courses just closed. It has effected a saving in time spent on outlines, and a gain in accuracy. By the addition of an inexpensive lens to our ordinary set of microscope objectives, it has also been utilized in neurology. The apparatus has the advantages of simplicity, cheapness, and freedom from the care and dirt of carbon arc lamps. No special room or electrical connections are needed, since it is attached to an ordinary socket in the open workroom, the back of the box being towards the windows. It must be understood, however, that it is not designed to be used in place of an Edinger, or similar apparatus for higher powers.

The apparatus (fig. 1) consists of a box, blackened on the inside, measuring 32 inches in height by 18 inches in width and depth. One side is left open, and in the center of the upper end is a hole for the reception of the main condenser system. This system consists of two plano-convex lenses, 3 inches in diameter, mounted in a cell around the upper rim of which is a flange. The cell hangs by the flange in the aperture mentioned above. The focal length of the combination is 2 inches. Above the condenser is held a Mazda projection bulb of 100 watts, mounted in an Edison keyless socket at the end of a horizontal tube, through which the conducting cord passes. This tube is held by means of a right angled sliding body on a perpendicular rod, which is mounted on top of the box; this gives an adjustment of the light source in all directions. It is found better to turn light off and on at wall fixture, and to avoid disturbing lamp when once adjusted in optical axis. A cheap tin shield around the lamp prevents light from escaping into the room.

Inside of the box are two cleats, one on either side, to support a wooden rack or frame upon which rests the stage and microscope. These cleats (b) are so placed that the upper surface of the microscope stage is 5 inches from the lower surface of the condenser. The stage is 6 inches square, with a center opening of  $2\frac{1}{4}$  inches, which may be

<sup>1</sup> This apparatus was designed during the fall of 1914 while working under a grant from the Carnegie Institution of Washington, D. C.

reduced by a washer to  $\frac{3}{4}$  inch. Beneath the stage is fixed, in inverted position, the arm and body tube of a microscope. The one pictured is from a discarded instrument of the type found lying idle in most laboratories. It has a coarse adjustment by means of the slip tube and sleeve, and a fine adjustment by micrometer screw. When once the optical axis is found, the rack is prevented from being displaced backward by means of small screws inserted behind the ends of the rack and into the upper surfaces of the cleats. This merely acts as a check to

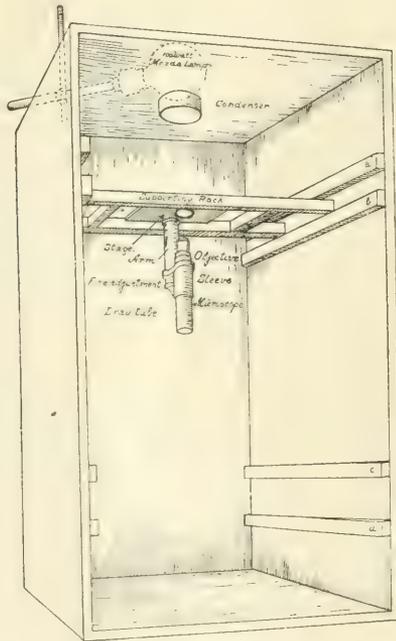


Fig. 1

backward movement, and permits removal of rack from the box in front. When working with large slides, as of brain stem, the larger stage opening is used, and in order to cover the slide, the rack and stage are moved to the upper cleats (a) about 2 inches closer to the condenser.

The lenses used are microscope objectives of 48, 32, 25, 4, 16 and 6 mm. focus, without eyepieces, and a special achromatic lens of  $4\frac{1}{2}$ -inch focus, obtained locally from Pinkham and Smith, for neurological work. The mounting of this lens is threaded to fit the eyepiece end of the body tube. A 3- or 4-inch diaphragm of cardboard may be held in place by objectives to cut out rays from around object and tube. No

curtains have been used, since the body of the worker blocks the light from without fairly well.

An accessory condenser is mounted above the stage. This is a single lens of 2-inch focus which is adjustable up and down a pillar by means of a sleeve. This, however, is not needed in the ordinary routine and may be omitted. The image is received upon paper placed either upon the lower end of the box, or upon a blackened compo-board shelf placed on either of the lower cleats, depending upon the magnification desired.

The cost of each apparatus, based upon figures for the lot of eight, in use in this laboratory, is as follows:

Boxes, painting, racks, etc. ....	\$3.00
Main condensers, in flanged cell.....	2.50
Accessory condenser in ring.....	1.00
Brass stage and washer, with pillars for accessory condenser, etc..	4.50
100-watt Mazda projection bulb.....	1.50
Lamphouse, tubing, socket, cord and plug.....	2.85
Special 4½-inch lens in threaded mount.....	2.50
Total.....	\$17.85

After the above article was in the hands of the printer, we had an opportunity to try the 250-Watt and 400-Watt gas-filled bulbs as a source of light. These lamps are highly satisfactory, the higher power light being almost equal to a 4-ampere arc in brilliancy, and of course much superior in the matter of convenience.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

**THE DEVELOPMENT OF THE HUMAN BODY**, A manual of human embryology, J. Playfair McMurrich, A.M., Ph.D., LL.D., Professor of Anatomy in the University of Toronto; formerly Professor of Anatomy in the University of Michigan. Fifth edition, revised and enlarged, with two hundred and eighty-seven illustrations, several of which are printed in colors. Philadelphia, P. Blakiston's Son & Co., 1012 Walnut Street, 1915.

From preface to the fifth edition: The increasing interest in human and mammalian embryology which has characterized the last few years has resulted in many additions to our knowledge of these branches of science, and has necessitated not a few corrections of ideas formerly held. In this fifth edition of this book the attempt has been made to incorporate the results of all important recent contributions upon the topics discussed, and, at the same time, to avoid any considerable increase in the bulk of the volume. Several chapters have, therefore, been largely recast, and the subject matter has been thoroughly revised throughout, so that it is hoped that the book forms an accurate statement of our present knowledge of the development of the human body.

# THE USE OF GUIDE PLANES AND PLASTER OF PARIS FOR RECONSTRUCTIONS FROM SERIAL SECTIONS: SOME POINTS ON RECONSTRUCTION

WARREN H. LEWIS

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FIVE FIGURES

Owing to the generosity of the Carnegie Institution of Washington, funds were made available for reconstructing the head of a 21 mm. human embryo, No. 460, Mall collection. During the process of this reconstruction, some rather helpful methods were developed, which others who are engaged in similar work may find useful, and with this in view I have been urged to publish the methods employed.

In regard to the preservation and staining of the embryos, as well as the cutting and mounting, it is assumed that these operations have been carried out in such a manner that the series is practically perfect, and that no unavoidable shrinkage has occurred during the dehydration and embedding; and that in cutting the orientation is such as to give as nearly perfect horizontal, sagittal or frontal sections as is possible. Great care must be taken in mounting the sections on the slides in order to avoid distortions. Reconstruction is undoubtedly greatly facilitated by the use of guide lines in the sections, such as are produced by the ordinary camphor black method. Unfortunately, such guide marks were not present on any of the series of sections used and it was necessary to depend for the form on photographs or camera drawing of the whole embryo, made before cutting. These should be as nearly as possible from lateral, frontal or horizontal views.

## PHOTOGRAPHS OF SECTIONS

I have been able to abandon the laborious and time-consuming method of drawing or tracing each section projected in the usual manner onto paper, and have substituted the more expensive but far better method of photographing on large plates, and using the line bromide or azo G hard (matte) prints. While this method is more expensive as regards the immediate outlay of money, it is much cheaper in the end than the old method of tracing, when account is made of the time involved. The photographs are far superior to any drawing that can possibly be made and greatly facilitate the work, both on account of

the greater accuracy and the greater wealth of detail. The various structures to be reconstructed may first be colored on the photograph, thus enhancing the clearness and sharpness of the picture. The ordinary Sussner creta polycolor pencils were used. The photographs of the sections were made in a dark-room with the ordinary Zeiss projection apparatus and for ordinary diameters, 40 or 50, the Zeiss 5 cm. planar lens was used. This is an ideal lens for such work since there is no measurable distortion of the image. In the place of an ordinary arc lamp, we substituted a 250-watt mazda stereopticon bulb, a round bulb with filaments grouped together in a small ball at the center. To avoid unequal illumination from the spiral filaments a ground glass plate was interposed directly in front of the light. The advantages of the mazda light over the arc are (1) it remains constant, and (2) it can easily be turned on or off for time exposures.

The arrangement of the Zeiss optical bench is shown by the diagram, figure 1. I am aware that this may not be the most perfect arrangement, still we were able to obtain excellent results. The most important point consists in focusing each section by moving the slide carrier back and forth, the lens remaining fixed, instead of the usual method of moving the lens back and forth. When the magnification is once adjusted to the required diameter, the lens (7) is securely fixed in position and the plate-holder (14) likewise. Thereafter, the object or slide is brought into focus by means of a fine adjustment connected with the focusing-rod (10). Magnification is thus not altered from section to section or from slide to slide, since the sections are thus brought in the focus of the lens. This insures equal magnification of every section, the most important condition for accurate reconstruction. This method of focusing was introduced into the laboratory by Doctor Essick.

The dark-room by this method becomes the camera, in which a perpendicular board (14) with slots for the plates takes the place of the plate-holder. This board is pivoted in the center and can be freely turned at any angle in a perpendicular plane and clamped there by means of a thumb-screw at the back.

The plate-holder-board is attached to a movable stand, which can be moved to or from the lens in a straight line only, and can be securely clamped in position when in the proper place. At 50 diameters' magnification with the 50 mm. planar lens the plate-holder should be about 6 ft. and 8 in. from the lens. A series of holes can be so placed on the plate-board, as at a, b, c, figure 1, into which the clamp bolt can be changed and the board given a new center for different sized plates. An old plate having the same thickness as those about to be exposed was used for focusing, after having had a fine piece of white paper tightly pasted over it. An undeveloped plate is still better.

Standard orthonon and Stanley commercial plates were used. The latter are apparently just as good and much cheaper than the former. An exposure of about 5 seconds gave the best results; diaphragm of the planar lens at 4. Flat negatives with very little contrast give the best

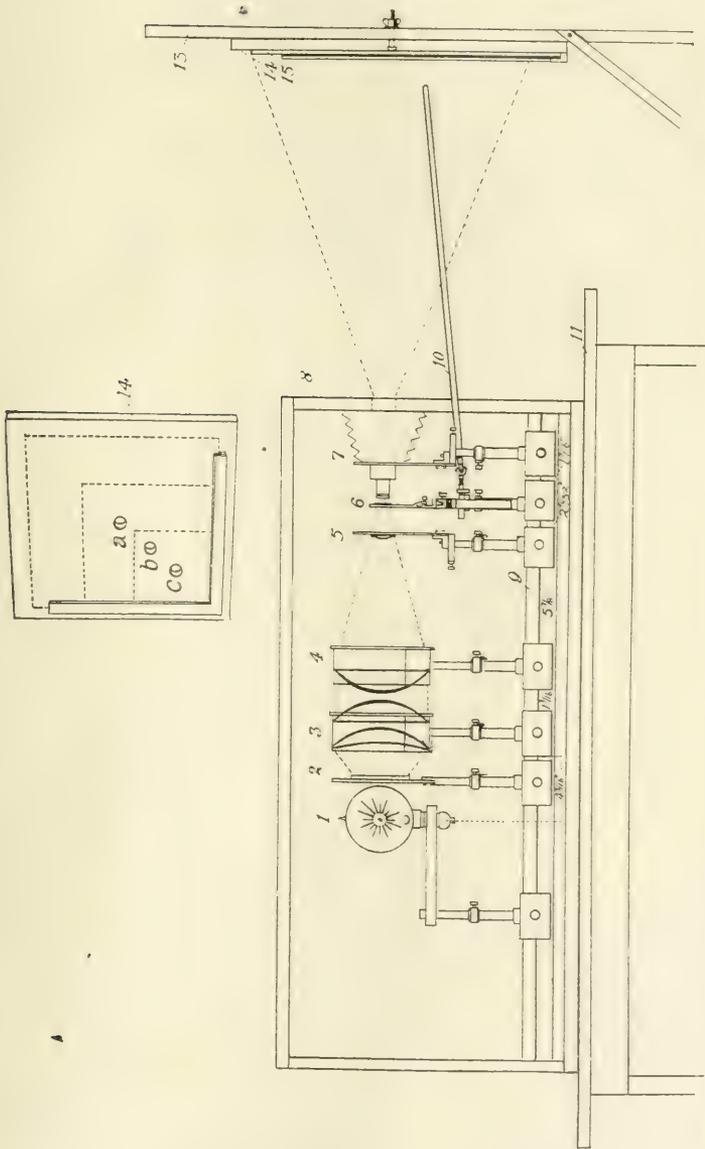


Fig. 1. Arrangement of Zeiss optical bench for photographing: 1, 150-watt mazda stereopticon bulb; 2, ground-glass plate; 3, 5-in. concave, convex condensing lens; 4, 5-in. plano-convex lens; 5, 5-in. condensing lens; 6, mechanical slide carrier; 7, 50 mm. Zeiss planar lens; 8, light-proof hood over entire bench with curtain on operating side; 9, optical bar, distances between the feet of the various lens, etc., shown in inches and fractions; 10, movable stands; 11, plate holder board; 12, plate.

prints, while 'contrasty' plates, which appear very beautiful, give very poor prints since the shadows and high lights are in too strong contrast. When the shadows and high lights are in strong contrast in the sections—as was the case in the series used, since the central nervous system was deeply stained with alum cochineal and the delicate connective tissue but faintly stained—it is not easy to get negatives which will give prints showing detail in both regions. With full development, a strong light and short exposure give flatter, softer negatives than a dim light and long exposure. The diaphragm of the lens should be as wide open as is consistent with a sharp image, to increase light and reduce time of exposure. It is important to use a developer that will help to give softness to the negative and the following formula is recommended. There is a decrease in the amount of sodium carbonate usually employed for the purpose of increasing the softness of the negative:

*Formulae for pyro developer*

Solution No. 1	Solution No. 2	Solution No. 3.
Water.....470 cc.	Water.....470 cc.	Water.....470 cc.
Oxalic acid...700 mg.	Sod. sulph..... 60 gms.	Sod. carb..... 60 gms.
Pyrogal. acid. 30 gms.		

For tank development use 30 cc. each of solutions 1, 2, 3, to 1000 cc. water; develop 20 minutes at 70° F. For tray use 60 cc. each of solutions 1, 2, 3, to 1000 cc. water; develop 5 minutes at 70° F.

Add 1 drop of a 10% solution of Potassium Bromide to every 30 cc.

*Fixing bath formula*

Hypo.....	480 gms.
Water.....	1920 cc.

*With acid hardener*

Water.....150 cc.	Acetic acid (28%).....90 cc.
Sulphite soda.....30 gms.	Powdered alum.....30 gms.

The azo G hard (matte) paper should be given short exposure with bright light and developed with formula recommended for azo portrait prints. If the amount of elon is doubled and the hydrochinon decreased one-half, a still softer print with more detail is obtained. For contrast plates a still softer developer may be used for the prints with the following formula:

Water.....300 cc.	Hydrochinon..... 4 gms.
Elon..... 4 gms.	Carbonate of soda.....22 gms.
Sulphite of soda.....30 gms.	Potassium bromide..... 1 gm.

## MODEL OF THE EXTERNAL FORM

With the photographs or drawings of the sections complete, our next step is to make a model of the external form of the embryo or of a large enough part of it to insure that we have as accurate a reproduction as possible. For this the ordinary Börn wax plate method is used. Wax plates of the proper thickness are cut out for the external form and piled either by the orientier guide lines or according to the photographs of the external form. It is extremely important that this external form shall be as perfect as possible, for on it all subsequent reconstructions are based, as will be seen later.

The wax plates should be piled without fusing so that they can be easily unpiled later. In making this external form of a whole embryo, it is usually best to begin piling with the larger plates from the middle of the trunk towards the top of the head, and another pile from the same region towards the tail, as when horizontal sections are used. In fact, two or three piles may be used, provided the guiding curves coincide. Or one may pile the head with the trunk, then lift off the head and turn the trunk piece upside down and pile the tail end on it. In this case the guide curves will overlap.

It is a great help to use a guide curve from a negative outline in cardboard of the external form of the embryo, made by magnifying the photograph of the external form to the proper diameter, as shown in figure 2. In horizontal series this curve should be made from a direct sagittal view of the embryo, and will of course be in the same plane as the median sagittal plane of the model. It is also important to establish the relation of the plane of the sections to this enlarged outline in order to give the proper angle to the guide curve.

The sections of different embryos, for example, may be cut at somewhat different angles to the *frontal plane* of the embryo, as in figure 3. So if one were piling the head end of the embryo from the middle of the trunk, it would be necessary to determine the plane of the sections in relation to the whole embryo, whether in the direction of *a* or *b* or *c*, etc., in order that the base-line of the cardboard guide curve may coincide with the proper plane *a* or *b* or *c*, etc., when it rests on the base-board. It may also happen that the sections in a horizontal series are cut obliquely to the median sagittal plane, as is often the case. In piling the plates for the external form from such a series the median sagittal plane of the plates must be made to start at a corresponding angle to the base-board, leaning either in the right or left, as the case may be. In this way the plates are piled up with their flat surfaces parallel to a horizontal base-board.

In a similar manner, the proper angle should be used in piling plates from sections cut more or less obliquely to the other planes of the embryo. It is important that the piling be done on a board with a true surface. Overhanging parts which are likely to sag should be supported from the base-board.

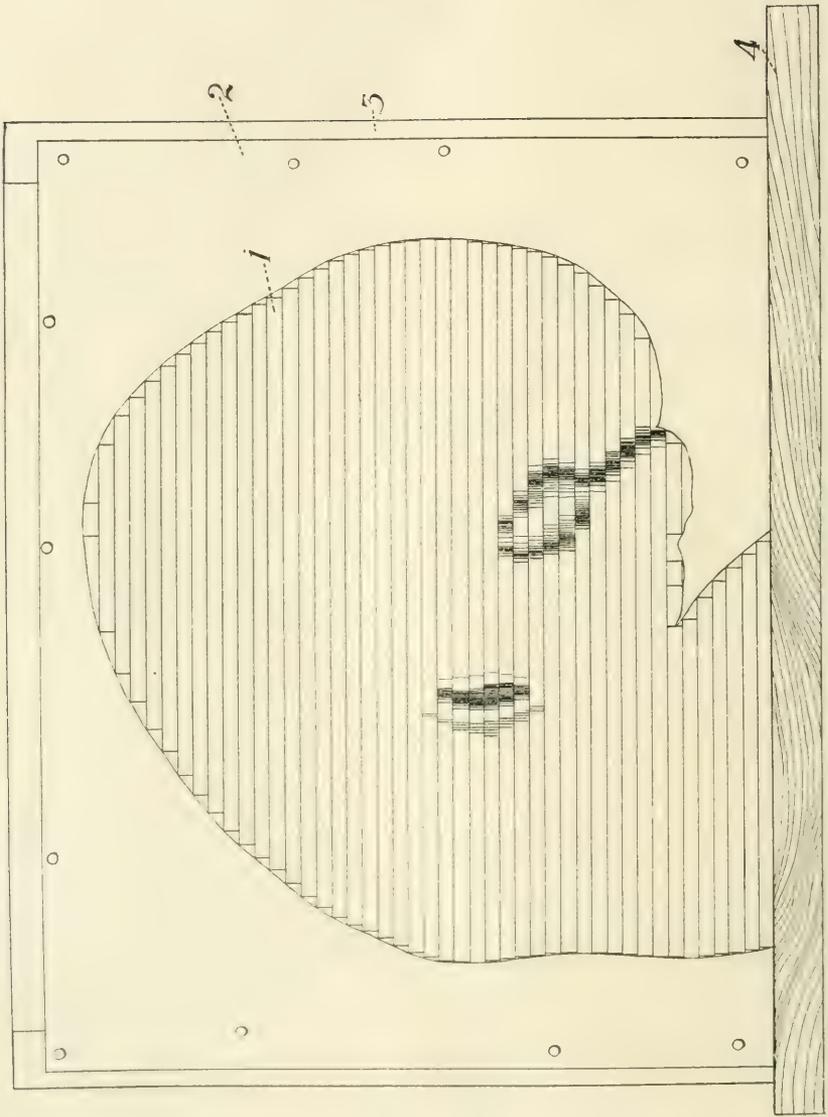


Fig. 2 Method of piling with cardboard outline as guide; 1, external form piled in wax plates; 2, cardboard outline guide; 3, posts for attaching same; 4, baseboard.

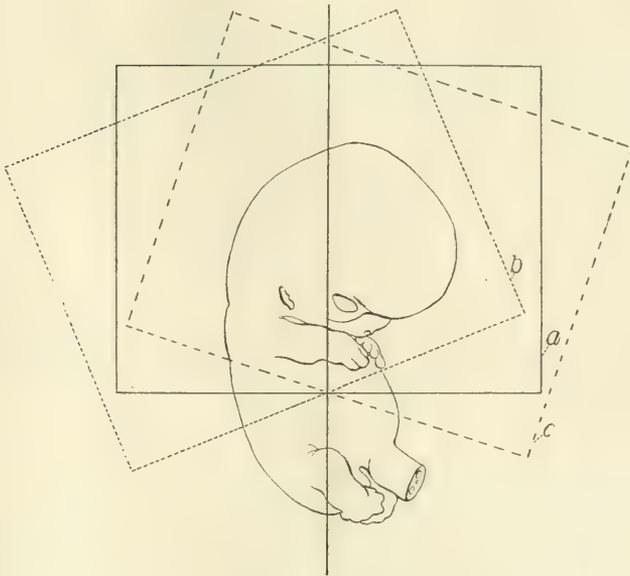


Fig. 3 Outline of embryo to show different arrangements of cardboard guide *a, b, c*, for cross-sections, cut at different angles to the frontal plane of the embryo.

#### ESTABLISHING GUIDE PLANES

Since in most series there are no guide marks, it was necessary in some manner to establish guide lines on our photographs that could be used for all subsequent reconstructions. I first thought of drilling two perpendicular holes through the entire series of plates as they stood together in the piled-up form, and then by placing each plate on its own photograph the position of the hole could be traced onto the photograph and we would thus have two orientier marks on each photograph (or drawing) that could be utilized in piling plates for future models.

A somewhat different and better method was finally adopted, which has proved very successful. After the piling of the external form was complete and satisfactory and the cardboard outline removed from the head end (for example, from a series of horizontal sections) two perpendicular posts were erected from the base-board in such a manner that a line drawn between them passed along the median plane of each plate or parallel to it (fig. 4). With a straight-edge rule a line was then drawn with a needle across the top wax plate, the straight edge resting against the edges of the two perpendicular posts, and a second line was drawn at right angles to this at a given distance from one of the perpendicular posts. The top plate was then carefully lifted off and similar lines drawn on the next and each succeeding plate in turn.

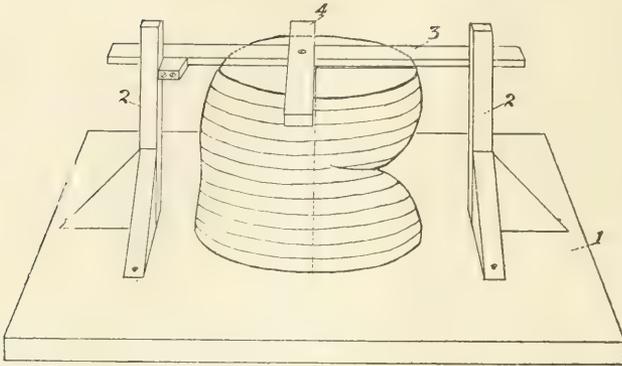


Fig. 4 Method of making guide lines: 1, baseboard; 2, perpendicular posts; 3, straight edge; 4, piece at right angles to it.

Care must be taken, of course, not to disturb the position of the plates until the lines are drawn. Thus there are established on each wax plate two lines, at right angles to each other, which coincide with two planes through the model or embryo that are perpendicular to the plane of the sections. One of the *principal planes* either coincides with the median plane or is parallel to it, while the other is at right angles to this and at a given distance from one of the perpendicular posts. With these two *principal planes* established, it is possible to repile the external form or any other part of the embryo with mathematical precision, since these two planes are likewise perpendicular to the plane of the sections.

After the guide lines have been drawn on each wax plate, they must next be transferred to the photographs or drawings by super-imposing each wax plate on its photograph or drawing and marking at the ends of the guide lines. The wax plate is then lifted off and the points on the photograph connected by lines similar to those on the plates. When the two *principal guide lines* are established we have found it convenient to establish secondary guide lines by drawing other lines, 5 cms. apart, parallel to those over the entire surface of the photographs.

We have introduced lately a still better method: namely the printing of lines in red ink over the photographs from a lithographic stone. These lines form squares of 1 cm. with slightly heavier lines every 5 cms. The lines are printed to correspond with the two principal guide planes. Such lines greatly facilitate not only the plastic reconstruction work but are of especial value for graphic reconstructions. These lines will of course coincide with various planes through the embryo. The advantage in having such a number of planes will become apparent when one wishes to reconstruct small structures that are limited to a particular part of the embryo. The whole section or any part of it will thus be included in rectangles or squares of various sizes depending upon the extent of the part.

## WAX MOLD FOR PLASTER OF PARIS CAST

With the development of these guide planes we have abandoned the usual Born wax plate method of making models, and use instead wax plate negatives into which plaster of Paris is poured. The method is as follows: Structures to be modeled are outlined on wax plates in the usual manner, and at the same time, while the plate is still in proper position under the photograph, points are pricked through into the

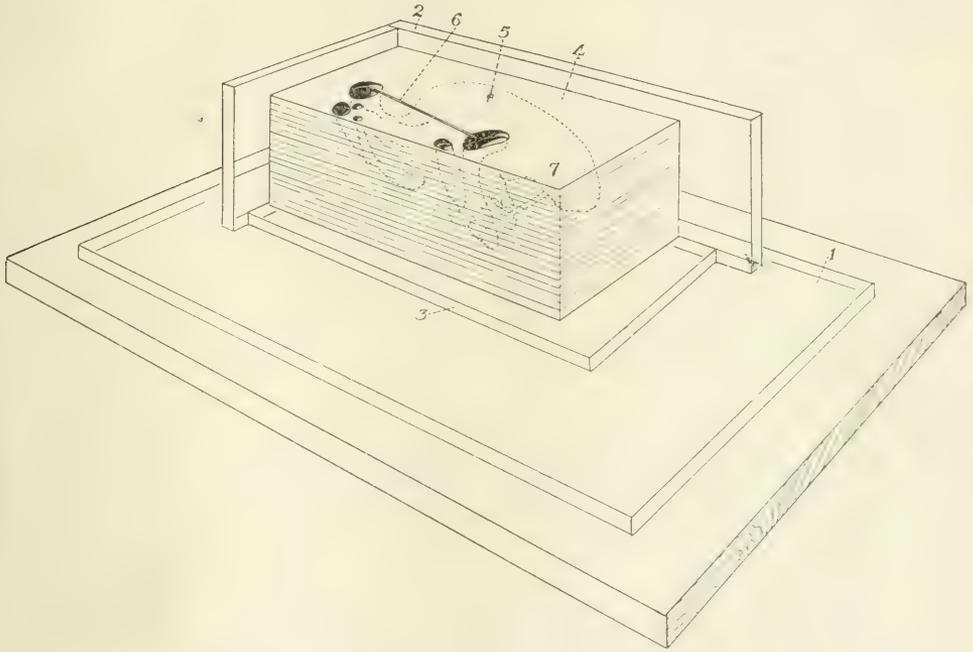


Fig. 5 Method of piling wax mold: 1, baseboard; 2, perpendicular right angle corner; 3, glass plate; 4, wax mold; 5, vent; 6, galvanized iron wire bridge; 7, gate for plaster between parts of mold.

wax with a fine needle at the corners of the rectangle in which the structure or structures outlined are included. The outline is transferred from the photograph onto the wax plate by the use of carbon paper; tracing on the photograph with a smooth glass point. Each plate is then carefully trimmed to the rectangular shape corresponding to that outlined by the four needle points. The outlined structures are then cut out, leaving holes in the plates. The plates are then piled into a perpendicular rectangular corner (fig. 5). Bridges of galvanized iron wire (ordinary iron wire will rust and discolor the cast) can be placed

in position as the piling progresses to hold the various parts of the cast together. Wire, string, or cloth may be inserted into the holes of the finer structures to give strength. Gates and vents to carry plaster from one part of the model to the other and to allow air to escape were cut through suitable places as the piling progressed. It is better not to smooth the inside of the mold. Owing to the fact that the plates were trimmed into rectangles, having the four sides in the same perpendicular planes, the structures which are represented by the holes in the plates must necessarily come into the proper relation with each other. If some of these structures come to the edge of the plate at any place, they would necessarily be cut off by one of these planes. After the piling is completed the outside edges of the plates are fused together to prevent the plaster from leaking out. A wax plate is also fused over the side of the block if any holes come to the edge. In piling the wax plates, it is necessary to measure the height of the block of plates after the addition of each new plate in order to be sure that the plates are piled properly as regards height. It is usually necessary to scrape off a slight burr which comes along the edge of the cut.

We have often found it advisable to build up models in rather small blocks, usually about 50 mm. in thickness, and where the models are large these blocks can be limited in other directions as well and the casts later fused together or merely fitted together as a dissectable model. Such combinations can be varied to suit special conditions.

#### THE PLASTER OF PARIS CAST

After the piling is completed and the edges of the plates are fused, the bottom of the pile which rests on the glass plate is made fast. Plaster of Paris is then poured into mold until it rises above the top and before it has completely hardened the excess on top is usually scraped off level with the top plate.

We used a grade of plaster known as potter's plaster. The mass consists of about equal weights of water and plaster. The latter is sifted into the water until it just begins to show dry on the top. It is then stirred a little and poured into the mold. After setting for an hour or so, the wax is melted off in boiling water. The cast is taken out and washed in very hot water and dried in the air. Plaster of Paris is wonderful material to work with and requires but little experience to handle it with considerable facility. The plaster cast of course shows the lines of the plates just as wax models do unless considerable polishing is done. It is easier to smooth off a plaster cast than a wax model. The plaster is easily trimmed with a knife or sandpaper and the angles remaining between the edge of the plates can easily be filled in with fresh plaster painted on with a brush to any desired thickness. Corrections and additions can also be made on such plaster models by cutting off and building up with fresh plaster, using wire, string, cloth, etc., if necessary. The different structures are easily tinted with water colors (suspensions in water or ordinary commercial house paint pig-

ments; such as, ultramarine blue, yellow ochre, burnt sienna, chrome yellow, English vermilion, etc.). A better method if one wishes to polish the model, is to mix up fresh plaster by using water colored with the pigment and to do the final smoothing with this mixture. If models are made in sections there is no very great difficulty in putting these together in the proper relation to each other. Finally the entire model can be toughened by soaking in hot paraffin until all the air is driven out by the paraffin which penetrates through the plaster. It is best to have the paraffin bath somewhere between 95 and 100°C. when the model is lifted out, in order that the surface will not be heavily coated with paraffin.

#### WAX PLATES

The wax plates were made according to the following formula:

Bees' wax.....	6 parts
Paraffin.....	4 parts
White lump rosin.....	2 parts

The ordinary 56°C. Standard Oil paraffin was used; lump rosin is much better than powdered rosin. 2000 grams poured on very hot water—surface 3 by 4 feet—gives plate 2 mm. in thickness. The hotter the wax and water the better. The air bubbles which form in the wax are driven off before the plates cool by playing a Bunsen flame over the surface.

The points which I wish to emphasize are: first, the use of photographs; second, the use of the series of guide lines which coincide with planes that are at right angles to each other and perpendicular to the plane of the sections; and third, the use of plaster of Paris. Although the preliminary steps are somewhat more complicated than those usually employed, they are nevertheless essential and in the end save both time and expense. The advantages of a plaster model are obvious to those who have worked with wax and realize the dangers of distortion and the difficulties involved in strengthening the wax and in modeling fine structures.

# MEMOIRS

The publication of this series of Anatomical Monographs has been undertaken with the purpose of presenting the results of original investigation in anatomy which are too extensive for incorporation in the already over-crowded current periodicals.

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COMPARATIVE OSTEOLOGY OF CERTAIN RAILS AND  
CRANES, AND THE SYSTEMATIC POSITIONS OF  
THE SUPERSUBORDERS GRUIFORMES AND RALLI-  
FORMES

R. W. SHUFELDT

NINE FIGURES

Owing to the existence of such peculiar birds as the Kagu (*Rhinochetus*); the finfeet (*Podica*); the *Ortygometra*; the sun bittern (*Eurypyga*); the trumpeter (*Psophia*); the Madagascan genus *Mesites*, and the *Seriema* (*Dicholophus*), the exact limits of the rail and crane groups of birds still existing in nature have long been a mooted question with avian taxonomers; and far more light from anatomical sources is required before the true relationships of many of the groups and species just enumerated can be definitely determined.

Such classifications of the Class Aves as have appeared within comparatively recent times are much at variance with respect to opinions upon the groups to be discussed in the present article.

In order to demonstrate this, it is but necessary to present in brief several of the classifications of birds, which have been offered by writers of authority on the subject during the last century. For example, in the year 1813 the Academy of Sciences of Berlin, in its *Abhandlungen* (pp. 237-250), published a scheme of bird classification proposed by Blasius Merrem, entitled "*Tentamen Systematis Naturalis Avium.*" That gifted writer distinguished his fourth group of birds as '*Aves palustres,*' and thus divided it:

4. *Aves palustres*:

A *Rusticolae*: (a) *Phalarides*—*Rallus*, *Fulica*, *Parra*; (b) *Limosugae*—*Numenius*, *Scolopax*, *Tringa*, *Charadrius*, *Recurvirostra*

B *Grallae*: (a) *Erodii*—*Ardeae ungue intermedis serrato*, *Cancroma*; (b) *Pelargi*—*Ciconia*, *Alycteria*, *Tantali quidam*, *Scopus*, *Platalea*; (c) *Gerani*—*Ardeae cristatae*, *Grues*, *Psophia*

C *Otis*

For the time it was given, this classification is by no means lacking in merit, and there are taxonomers of the present day who may, with profit, still contemplate it.

Fourteen years later l'Herminier, in his "Recherches sur l'appareil sternal des oiseaux," published in the 'Actes' of the Linnean Society of Paris (T. 6, pp. 3-93), showed upon osteological grounds that the Rallidae and the true cranes (*Grus*) were affined; that neither family was especially related to the herons (*Ardeidae*), and so should be separated from them.

Passing to the classifications of many of the other early writers—always more or less conflicting in their views—we come to the famous scheme of Professor Huxley, so frequently quoted in previous papers of mine (*P. Z. S.*, 1867). In his Order III (*CARINATAE*, Merrem), Group 2 (*Geranomorphae*), Huxley arrays two families thus:

- Family 1: *Gruidae*  
Intermediate forms: *Psophia*, *Rhinochetus*
- Family 2: *Rallidae*  
Intermediate forms: *Otis*, *Cariama*

He remarks thereon that he considered the cranes and the rails as constituting the typical forms of the group. The herons he places in a distinct suborder, the *Desmognathae*, in a group *Pelargomorphae*, containing the *Ardeidae*, the *Ciconiidae*, and the *Tantalidae*.

In the curious and unique classification of Garrod (*P. Z. S.*, 1874, p. 116), we find his subclass *Homalagonatae* divided into numerous orders, the first of which is the *Galliformes*. This latter he divides into 'cohorts,' *x*, *B*, *y*, etc.,—Cohort B being the *Gallinaeae*, and it is thus divided:

- Family 1: *Palamedeidae*  
2: *Gallinae*  
3: *Rallidae*  
4: *Otidae*  
Subfamily 1: *Otidinae*  
2: *Phoenicopterinae*
- Family 5: *Musophagidae*  
6: *Cuculidae*  
Subfamily 1: *Centropodinae*  
2: *Cuculinae*

It is not surprising, in a classification of this nature, to find the herons in another Order, placed between the Cathartidae and Steganopodes, while the Gruidae are consigned to still a different order, from the rails, for example, and placed between the plovers and gulls (among the Limicolae), the only other cohort in the same Order being the Columbæ.

Order XVI of Doctor Selater's scheme is the Fulicariæ, which is created to contain (a) the Rallidæ, and (b) the Heliornithidæ. In the XVIIth he places the (a) Aramidæ; (b) Eurypygidæ; (c) Gruidæ; (d) Psophiidæ; (e) Cariamidæ, and (f) Otidæ. The Parridæ he places in Order XVIII—the Limicolæ.

Professor Newton, ever cautious and far-seeing in everything he did in ornithology—although the present writer does not always coincide with him in all his views, as in the present instance—places in the Herodiones the Ardeæ, the Ciconiæ, and the Plataleæ. Fulicariæ and Grues—the latter made to include the Gruidæ, Psophiidæ, Aramidæ, Eurypyga, and Phinochetus—make up the Grallæ.

Doctor Reichenow, who in 1882 gave us "Die Vögel der zoologischen Gärten," divides the Class Aves into 'Series.' Series III is the Grallatores, split up into orders, suborders, families, etc., and I extract the following from it:

- Suborder B: Arvicolæ
  - Family 19: Otididæ
  - 20: Gruidæ
- Suborder C: Calamocolæ
  - Family 21: Rallidæ
    - Subfamily A: Rallinæ
    - B: Gallinulinæ
    - C: Parrinæ
  - Family 22: Eurypygidæ
- Suborder D: Deserticoeæ
  - Family 23: Thinocoridæ
  - 24: Turnicidæ
  - 25: Pteroclidæ
- Order VII: GRESSORES
  - Family 26: Ibirdæ
  - 27: Ciconidæ
  - 28: Phoenicopteridæ
  - 29: Scopidæ
  - 30: Baloenicipidæ
  - 31: Ardeidæ

In 1884 appeared the second edition of Doctor Coues's 'Key' to North American birds, and in it we meet with the following scheme:

<i>Order</i>	<i>Suborders</i>	<i>Families</i>	<i>Subfamilies</i>
ALECTORIDES	{ Gruiformes { Ralliformes	{ Gruidae { Aramidae { Parridae Rallidae	{ Rallinae { Gallinulinae { Fulicinae

This is one of the most natural arrangements I have met with, and in most particulars closely agrees with what I will probably have to suggest in the sequel, excepting the position of the Parridae.

Dr. Leonhard Stejneger in 1885 proposed a classification in that now wellknown work "The Standard Natural History." In it he places the Jacanidae in his superfamily Scolopacoideae of the order Grallae, and the Gruidae, Aramidae, and Rallidae in another superfamily, the Gruioideae of the same order. The storks and herons are removed to another order, the Herodii.

In the first edition of the 'Check-List' of the American Ornithologists Union, the following scheme is given:

<i>Order</i>	<i>Suborders</i>	<i>Families</i>	<i>Subfamilies</i>	<i>Genera</i>
	Grues	Gruidae Aramidae		Grus (3 sp.) Aramus (1 sp.) Rallus (6 sp.) Porzana (5 sp.) Crex (1 sp.)
PALUDICOLAE	Ralli	Rallidae	Gallinulinae Fulicinae	Ionornis (1 sp.) Gallinula (1 sp.) Fulica (2 sp.?)

Here the Jacanidae are placed as the last family of the Limicolae.

Fürbringer places the Parridae in a gens *Parrae* of a suborder Charadriiformes, which belongs to his order Charadriornithes. Between this order and the order Alektorornithes we find inserted two intermediate suborders, thus:

<i>Suborder</i>	<i>Genus</i>	<i>Family</i>
Gruiformes	Eurypygae	Eurypygidae Rhinochetidae Aptornithidae
	Grues	Gruidae Psophiidae Cariamidae
	Fulicariae	Heliornithidae Rallidae
	Hemipodii	Mesitidae Hemipodiidae

The late Doctor Sharpe, in his exceedingly useful "Review of recent attempts to classify birds," also places the *Parrae* in an order *Charadriiformes* (Order XVIII), while the *Grues* and *Arami* are in an order *Gruiformes*, along with the *Rhinochetides*, *Mesitides*, *Eurypygae*, *Psophiae*, and *Dicholophi* (Order XIX).

Dr. Hans Gadow, who has accomplished so much in the morphology of birds, has given us at least two schemes of classification for the class. One of these appears in Brown's 'Thierreich' (*Aves*), and the earlier one in the *P. Z. S.* ('92, p. 229), a paper "On the classification of birds." In this latter he places the *Parridae* among the *Limicolae*, and divides his *Gruiformes* into the *Eurypygae*, *Ralli*, *Grues*, *Dicholophi*, and *Otides*.

There are a number of others we might quote, but enough has been presented for my purpose: to show that a great variance of opinion still exists among the best authorities on the subject, but that the tendency seems to be to keep the *Gruidae*, the *Rallidae*, and the *Aramidae* more or less closely together, and well removed from the herons and storks and ibises, while the *Parridae* are placed among the *limicoline* forms, more or less near the plovers.

We will next proceed to examine the osteology of several forms representing such genera as *Grus*, *Rallus*, *Crex*, *Ionornis*, *Gallinula*, and *Fulica*. As long ago as July, 1888, I printed an account of the osteology of the sora rail; and as that contribution is now probably well known to comparative anatomists, I will not reproduce any part of it here beyond making references to

it in the course of the comparisons to be made with *Crex*, *Rallus*, etc., further on in the present paper.<sup>1</sup>

My ability to compare the skeleton of our sora rail (*Porzana carolina*) with the corn crake of Europe (*Crex crex*) is entirely due to the kindness of Dr. F. E. Beddard, F.R.S., Prosector of the Zoological Society of London, who, with great generosity, presented me with a fine skeleton of the latter ralline form. Such a comparison need not detain us long, for the osteological characters that distinguish *Crex* and *Porzana* are but a kind of distinguishing two genera osteologically, while, as a matter of fact, in many of their skeletal characters, these two short-billed rails are very much alike. Indeed, to such an extent is this the case, and so gradually do the skeletal characters of *Crex* shade through those of *Porzana* to typical *Rallus*, that the distinction between 'land-' and 'water-rails' possesses, in such premises, no foundation in fact, the opinion of some authorities to the contrary.

Essentially, the corresponding characters of the skull and associated parts of the same are identical in *Crex* and *Porzana*, the former simply being of greater size, as the corn crake is about one-third larger than the Carolina rail. The same observation applies to all the elements of the shoulder-girdle. The scapulae in *Porzana* are, however, relatively somewhat longer and more curved. With regard to the ribs and spinal column, they are the same in these two genera, while a few differences are to be found in the *pelvis*. These are of interest. The most important one is the rising up of the mesial borders of the ilia anteriorly, to meet the superior edge of the sacral crista in *Crex* and not in the Carolina rail.

Passing to the sternum, we find but two good distinctive characters worthy of mention. Upon its dorsal aspect in *Crex* there is a median osseous bar extending from its anterior border abruptly downwards, and but slightly backwards, to fuse at its narrower end with the sternal body; this is absent in *Porzana*.

<sup>1</sup> R. W. Shufeldt. Osteology of *Porzana carolina*, Jour. Comp. Med. and Surg., New York, July, 1888, vol. 9, no. 3, art. 17, pp. 231-248; numerous cuts in text.

In *Crex*, too, the body of the sternum is both actually and relatively larger than it is in the Carolina rail, while its lateral xiphoidal processes are drawn more closely towards the free external margins of the sternal body behind. This last character is to be well noted. Aside from the matter of size, the characters seen in the skeleton of the *pectoral limb* are essentially the same in the two genera, while a few distinctive ones are found in the *pelvic limb*. For example, the cnemial crests of the tibio-tarsus are more conspicuously developed in *Porzana* than in *Crex*, while the several joints of pes in *Porzana* are comparatively slenderer and actually longer than they are in the corn crake.

In comparing the lengths of the corresponding long bones of the *upper extremity* in these two genera, the differences are very well marked. In the case of the corresponding bones in the *lower extremity*, the difference, with respect to lengths, is but slightly in favor of *Crex* in any particular case. The femora form an exception to this last statement. It can be shown thus:

	<i>Humerus</i>	<i>Femur</i>	<i>Tibio-tarsus</i>	<i>Metatarsus</i>
<i>Crex</i> .....	45 mm.	49 mm.	65 mm.	41 mm.
<i>Porzana</i> .....	36 mm.	37 mm.	60 mm.	38 mm.
Differences in length.....	9 mm.	12 mm.	5 mm.	3 mm.

The *patellae* are absent both in *Porzana* and in *Crex*.

*Fulica*, *Ionornis*, and *Gallinula* are all genera that have characters in their skulls and associated skeletal parts which essentially agree with the corresponding ones in *Porzana*. So slight are the differences that, were we to be guided by these parts of the osseous system alone, it would be impossible to find even characters for generic distinctions to separate the forms in question. They will each and all constitute examples to show that the skull in birds is not always an all-sufficient guide to correct taxonomy, much less a never-failing index of remote or near affinities in this class of vertebrates. The skull in *Fulica* might be attached to a skeleton of a *Porzana*, of a size corresponding to that of the former; and there is not an ornithotomist, living or dead, who would for an instant believe it was the slightest shade out of the way. It would simply be regarded as having come

from a very large-sized Porzana, and assigned to that genus as a matter of course.

Coots (Fulici) and Gallinules (Gallinula) have, too, the same character in their vertebral column, ribs, shoulder-girdle, and sternum that we found in Crex and Porzana. In the sternum of Fulica, the lateral ziphoidal processes are long, and slightly inclined to flare outwards more than they do in the Carolina rail; and a manubrial process is also better developed, although it is still very small.

Coming to the *pelvis*, we find Fulica has the preacetabular parts of the ilia as we find them in Porzana; but the pelvis is actually as well as relatively longer and narrower in the first-named genus. The lateral postacetabular projections are not so well marked, while the pubic styles are quite different from what we have described for those parts in Porzana. They each become broader as they proceed backwards, until they are met by a conspicuous out-turned process, given off by either ischium at its postero-inferior angle. At this point, a pubic style turns abruptly downwards, and is continued for some little distance to its truncate free posterior extremity. This downbent portion is broader and flatter than the style is at its commencement beneath the cotyloid cavity. On the dorsal postacetabular surface of the bone, the parial and scattered inter-diapophysial foramina are usually entirely absent in Fulica, and always so in the skeletons of adult Gallinules. Otherwise the pelvis in Gallinula closely coincides with that part of the skeleton in Porzana, with the exception of the ilia meeting the sacral crista; in that particular it is more like the pelvis of Crex.

The *appendicular skeleton* in the coots and Gallinules is distinctly ralline in character. They lack patellae in the pelvic limbs, the several bones in Gallinula being more like the corresponding ones in Crex than in Porzana; the reverse of this is the case for Fulica. An example of this is well seen in the development of the *cnemial crests* of the *tibio-tarsus*, they being much suppressed in Gallinula, as they are in the corn crake, while in a coot they are conspicuously developed, as we find them in the Carolina rail.

From these short thick-billed ralline birds, the passage to the long and comparatively slender-billed representatives of the genus *Rallus* is easily made. To illustrate this latter genus I have before me skeletons of both *Rallus longirostris crepitans* and *R. l. obsoletus*.<sup>2</sup> A glance at either of them is sufficient to satisfy us that they are closely related to the forms we have already passed in review above.

In *Rallus l. crepitans* all the characters of the skull and associated skeletal parts are typically ralline, agreeing with what we found in *Porzana*. The chief difference to be noted is an elongation of the bones of the frontal portion of the skull, but more particularly the bones of the face and the mandible. This latter gives the bird its long beak and the elongate external narial apertures. Another point to be noticed is the well-marked, though narrow supra-orbital glandular depressions. These are confined to the entire superior margin of either orbit, and are very faint in *Porzana* but slightly better marked in *Fulica*.

As in *Fulica*, *Rallus* has much better defined temporal fossae; they are entirely restricted to the lateral aspects of the skull. In this species of *Rallus*, the maxillo-palatines may be in *contact* with each other in the median line; they are always very close to each other there in all true rails. The number and characters of the vertebrae between the skull and the pelvis, as well as the number and general arrangement of the ribs, agrees with the Carolina rail and with *Crex*.

The *shoulder-girdle*, the *sternum*, and the *pelvis* of this species of rail also agree exactly with what we find in *Crex*, and this is an interesting fact. The limb bones are more like those in the corn crake also than those of either *Porzana* or the coots and *Gallinules*. In other words, *Crex* stands between *Rallus* and *Porzana*, rather than between *Porzana* and the *Gallinulinae*, to which latter place it has been incorrectly assigned by some authorities.

<sup>2</sup> For the first-named species I am indebted to Mr. Philip Laurent, of Philadelphia, and for the last-named to Dr. T. S. Palmer, of the U. S. Dept. of Agriculture, who collected the material for me at Berkeley, California, for use in the present connection, and my thanks are here tendered to him and to Mr. Laurent for their timely assistance.

With respect to the osteology of *Aramus vociferus*, I have already written a complete and fully illustrated account of the skeleton of that puzzling species (The Anatomical Record, vol. 9, no. 8).

OSTEOLOGY OF *GRUS AMERICANUS* AND OTHER CRANES  
OF THE GENUS *GRUS*

Of the genus *Grus* I have before me, at this time, a complete, disarticulated skeleton of *G. americanus*, a complete skeleton of *G. mexicana*, and two extra skulls of *G. canadensis*. For this

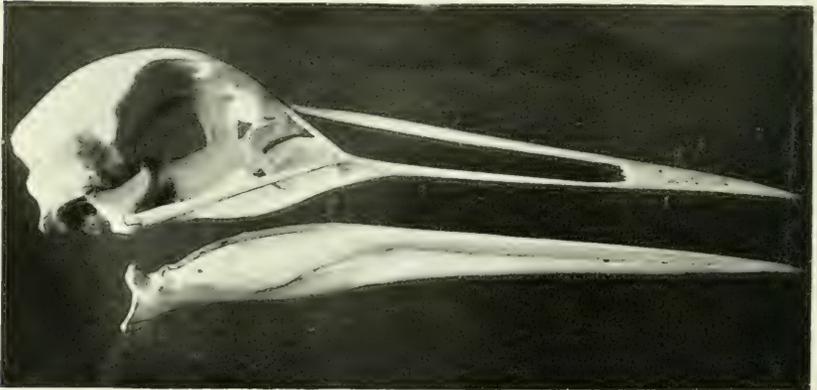


Fig. 1 Left lateral view of the skull and mandible of the little brown crane (*Grus mexicana*); photographed by the author, natural size; reduced in reproduction to three-fifths; Specimen No. 820, Coll. U. S. Nat. Museum.

material I am indebted to the U. S. National Museum, of Washington, D. C., and from its examination I am enabled to demonstrate the following facts:

*The skull.* Except in point of size, the skull of *Grus canadensis* presents the same characters as those found in *G. americanus*, the latter being about one-third larger, and lacks the sculpturing along the superior orbital margins for the nasal glands.

There is but little else to be said here about the skull of this crane, as I incidentally described it when writing out the above-mentioned account of the skull in *Aramus*. Special attention is invited, however, to the additional perforation above the central



Fig. 2 Left lateral view of the sternum and os furcula of *Grus mexicana*. Fig. 3 Left coracoid, anterior aspect. Fig. 4 Left carpo-metacarpus, anconal aspect. Fig. 5 Left tibio-tarus, distal portion on anterior aspect. Figures 3-5 are all from the same skeleton that furnished the sternum in figure 2 (No. 820, Coll. U. S. Nat. Mus.). Drawn by the author, natural size, and here reduced to two-fifths.

one in the interorbital septum, and to the large, leaf-like maxillo-palatines; these are entire, though very thin. Another point is the peculiar manner in which the descending part of the *lacrymal* stands out at right angles to the rest of that bone, not approaching the zygoma as it does in so many other birds where it is found, and as it does to a great degree in *Aramus*. This is likewise the case in *Grus mexicana* (fig. 1).

In the *mandible* of this crane I find the ramal vacuities nearly filled in by the splenial elements, and its symphysis is wider than it is in the limpkin, which gives rise to a wider longitudinal groove upon its upper side. Hardly any evidence of a coracoid process exists in the lower jaw of either of these birds, the merest tubercle being present at the site where it is commonly found on the superior ramal border. In both *Aramus* and *Grus*, this part of the skull is only partially pneumatic, and the pneumatic foramina at the supero-mesial aspects of the inturned processes of the articular ends are always single and small.

*Of the remainder of the axial skeleton.* When Garrod gave us his account of the anatomy of the limpkin, in a paper in the Proceedings of the Zoological Society of London ('76, pp. 275-

277), which he entitled "On the anatomy of *Aramus scolopaceus*," he remarked of the bird: "The sternum is completely Gruine, as are the other parts of its skeleton" (p. 275), by which he meant, I presume, in a general way; for if he meant anything else by the word 'completely,' what he said will by no means strictly apply to *Grus americanus*, as may be seen from what follows.

Now I have shown in my former paper that *Aramus* has 23 vertebrae between skull and pelvis; *Grus americanus* has one more than this—24. They are all highly pneumatic, and each one is about double the size of its corresponding representative in the spinal column of *Aramus*, so far as the latter can be satisfactorily ascertained or determined.

The first *eleven* cervical vertebrae in *G. americanus* have exactly the same characters as the first eleven in the column of *Aramus*; but from that point on, as we pass from vertebra to vertebra, we come to appreciate the fact that a change is slowly taking place. The 12th cervical is relatively shorter in *Aramus*, and its neural spine, or rather eminence, is longitudinally divided; not so in *Grus*. These differences still obtain in the 13th, while in the 14th the neural spine in *Aramus* is represented by a remarkable saddle-shaped enlargement, and the hypapophysial canal is replaced by a spine. This canal in the 14th vertebra of the crane is still continued, and the neural spine is a low, median eminence near the center of the centrum. Again, these differences are carried on to the 15th vertebra, wherein the peculiarly enlarged neural eminence of this one in *Aramus* lends to the bone a most extraordinary appearance. Strange to relate, the 16th cervical in these two birds have the same identical characters. To some extent, this also applies to the 17th in each; but the 17th in *Aramus* bears a pair of free cervical ribs of no mean length, while the pleurapophyses in this vertebra in *Grus* are short and fused with the bone. In *Grus* the 18th and 19th vertebrae are still free, and the 18th supports the first pair of free ribs; they do not connect with the sternum as they do in the case of the 19th. In *Aramus* the 18th vertebra is fused with two others, and, by means of haemapophyses, its ribs do con-

neet with the sternum. From this point on, to include the pelvis, the sternum, and other parts of the axial skeleton, the skeleton in *Grus* differs so much from what we find in *Aramus*, that a more exact description is required for the former. In *Grus americanus*, the 20th, 21st and 22d vertebrae of the spinal column are coössified so as to form a single bone. In it the neural canal is markedly small in caliber, while a large *pneumatic canal*, of fully three times its size, occurs above it. It is almost entirely unobstructed by any slender osseous trabeculae. Nothing whatever of this nature occurs in *Aramus*. This canal is open at both ends, and the entire bone otherwise is more or less riddled with pneumatic openings. Very extensive ossification of the tendons attached to it upon its neural aspect has taken place, and they lead forwards and backwards as lengthy interlacements.

The 23d and 24th vertebrae in the column of *Grus americanus* are again free, and are remarkable bones in many particulars. The lofty neural spines are quadrilateral in form, and great, lengthy, osseous rodlets, with fringed ends, project directly forwards and backwards from their superior borders. Similar coössified tendons, directed in the same manner, are found on the supero-external aspects of the diapophyses. The neural canal is particularly small, when we come to take the size of the bird into consideration. The *pneumatic canal* above it, which is completed by the neural arches, is, like the neural canal, a cylindrical passage, but has certainly five or six times the caliber, and is filled with a very open, spongy, osseous tissue. Of especial interest are the forms assumed by the post- and prezygapophyses. Antero-posteriorly, they are exceedingly narrow, while at the same time they are very long. Both in front and behind the mesial ends of the pair meet, and make an angle with each other of about ninety degrees. Lying in the middle line, the apex of this angle is found in the periphery of the entrance to the neural canal, at its highest dorsal point. The aperture of the angle embraces the immediate entrance to the 'pneumatic canal' described above—the whole being in a plane perpendicular to the longitudinal axis of the centrum. The mesial ends of the prezygapophyses of the 23d vertebra do not quite meet with each

other; the articular surfaces are upon their upper aspects. This is their character also in the next succeeding vertebra; but in both of these the mesial ends of the postzygapophyses do meet, and the articular surfaces of them have the appearance of being continuous.

The *general form* of the *pelvis* in *Grus americanus* is the same as we see in the pelvis of *Aramus*, but there are to be found a few differences in details. In front, the antero-mesial margins of the ilia are so rounded off that they fail to meet the superior border of the fore part of the sacral crista to more than the extent of the neural spine of the first vertebra composing it. In the post-acetabular region small perforating foramina are seen among the diapophyses of the vertebrae. Some of these have a parial arrangement, and some are scattered. Upon lateral aspect of the bone we are to note how, upon either side, the ilium is produced as a conspicuous ledge overhanging the antitrochanter and the ischiadic foramen. A much smaller ledge of this kind is found just before the ilium terminates posteriorly; the two are separated by a considerable interval. This formation is the very reverse of what we find in the *Rallidae*. Turning to the under side of the pelvis of this crane, we find that it is *seven* of the leading vertebrae that throw out their lateral processes, to fuse by their other ends with the ventral surface of the ilium upon either side, instead of only *five* as in *Aramus*. Then follow *three* more, which have their apophyses thrown directly upwards against the pelvic roof, being thus concealed entirely upon direct ventral view. The 35th vertebra of the spinal column in this specimen, on its left side only, sends out a weak parapophysis, to reach over to the pelvic wall just above the cotyloid ring. But in the last *six* (*five* in *Aramus*) 'sacrals,' or true sacral vertebrae plus 'urosacrals,' these lateral apophysial braces are big and strong, and have their outer ends, and the adjacent ventral surfaces of the pelvic basin, all indistinguishably fused into one mass at their several points of meeting. Other characters of the renal fossae in *Grus* agree, in the main, with what we find in *Aramus*.

Both the pygostyle and the last two or three caudal vertebrae,

in this museum specimen of *Grus*, have been lost (or cut off by the taxidermist), and I have but the leading four vertebrae of the skeleton of the tail before me. Judging from these, however, one is enabled to say that they are more highly pneumatic than they are in *Aramus*, but that there is the same stunting of the outstanding processes and spines as in that genus. And all these vertebrae are very small compared with those in the pre-pelvic part of the vertebral chain.

It has already been said above that the 18th vertebrae supports a pair of free ribs. They are pneumatic, nearly 3 cm. long, and without unciform processes and haemapophyses. Both these latter characters pertain to the ribs of the 19th vertebrae, and the unciform appendages may anchylose to the borders of the ribs, though this is not the case with those in the middle of the dorsal series. Vertebrae 20 to 23 also have fully developed, highly pneumatic ribs, all connecting with the sternum by costal ones.

Then there are three pairs of pelvic ribs; but the haemapophyses of the ultimate pair fail to have their costal ribs quite reach the sternum. Nothing peculiar marks any of these free pleura-pophyses of the dorsal region; if we select one from the middle of the series, we find it considerably curved so as to conform to the outline of the thorax. It is constricted opposite the facet for the large, flake-like epipleural process; while from this point, both in the dorsal and ventral direction, it very gradually widens again. But at the best, these are narrow, though at their lower ends they become sufficiently broad to support a pretty good-sized facet for the costal rib.

The last pelvic pair of all are very long and slender, and at their vertebral ends they are more or less rudimentarily developed—the tubercles being entirely aborted.

*Grus americana*, as well as *Grus mexicana* (fig. 2), has its os furecula completely fused with the *sternum* at the carinal angle, the point of fusion being broad and firm.

The 'body' of the sternum is long and narrow as in *Aramus*; its xiphoidal margin is inclined to be a little jagged, and presents no definite pattern of notching. The thoracic aspect of the

sternal body is more generally and roundly concaved than it is in *Aramus*, and the pneumatic foramina far more open. Small air-holes of this kind absolutely riddle the bone upon this surface in front. They are found upon either side of a prominent median mound which exists in this locality, it being the supero-external evidence of the coiling of the windpipe within the carina, to which reference will be made presently. This crane has *seven* haemaphysial facettes upon either costal border, to the *six* found there in *Aramus*. Its costal processes are very low, curl outward, and each is squarely truncated. The anterior border of the of the body of the bone is thickened, and upon either side of the median mound spoken of above, it overhangs the fossae it contributes to form in those localities; and it is within these fossae or recesses that the very numerous pneumatic perforations are seen, to which reference has just been made.

The 'coracoidal grooves' are very extensive, and are the same in general character as they occur in the limpkin, except that in *Grus* they *do not decussate*.

Below, the carina extends the entire length of the sternal body; transversely it is very thick, but this part is entirely hollowed out for two very different purposes. These are: the admission of air to the posterior moiety, while anteriorly a chamber is created in which the trachea is once or twice looped and coiled.

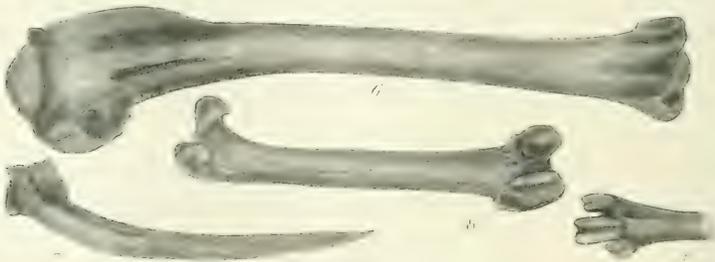


Fig. 6 Anconal aspect of the right humerus of *Grus mexicana*. Fig. 7 Right scapula, dorsal surface. Fig. 8 Left femur, posterior aspect. Fig. 9 Distal extremity of the right tarso-metatarsus, anterior view. These bones are all from the same skeleton that furnished those seen in figures 2-5 (antea; No. 820, Coll. U. S. Nat. Mus.). Drawn by the author, natural size, and here reduced to two-fifths.

The latter room, however, is not sufficient for the purpose just mentioned, so the cavity has ossified by extension far out in front, appearing as a median, rounded, transversely-compressed, closed protuberance between the costal grooves and the point below where the trachea enters the carina. The tracheal loop which is lodged in this part is the continuation of the same that passes round through the median mound on the thoracic aspect of the body of the sternum, to which I have already made reference above.

The sternum I have just described is, as stated above, from a specimen of *Grus americanus*, but the arrangement of the tracheal coils within the carina exist very much as they are seen in the figure showing them for *Grus mexicana* (fig. 2). Age and sex may have something to do with this; and a great many more sterna of these birds, at all ages, must be examined before the complete and full account of this very interesting feature can be written out. No such material is at hand at this writing.

*Os furcula*, as I have already stated, is, in *Grus americanus*, fused below with the angle of the carina of the sternum. It is quite a differently characterized bone as compared with the *os furcula* of *Aramus*. In the first place, it is more on the V-order of pattern than on the U; its rami are far more cylindrical; indeed, in the limpkin the rami are very decidedly flattened, and its free clavicular ends are very differently fashioned from those parts as found in the latter bird. Here, in *Grus*, they are pointed at their extremities. The articular facets situated *below* these points are comparatively very small; while below them, again, about a centimeter down either ramus, we meet with a moderate, more or less abrupt swell in the bone, which gradually dies away about half way down towards the symphysis. These enlargements occur upon the antero-external border of the clavicular ramus of either side—a border the more easily defined here by the very distinct muscular ridge-like lines that pass longitudinally down over the ramal surface. This clavicular fourchette of *Grus* differs quite as much from that bone as seen in *Aramus*, as the one in the latter differs from the bone in *Rallus*.

Apart from the great differences in size, the *coracoids* and

scapulae in *Grus americanus* simply repeat, in all their essential characters, the corresponding elements of the shoulder-girdle as they are found in *Aramus*. When articulated *in situ*, however, the end of the clavicle on either side does not come in contact with the scapula.

A *scapula* in *Grus americanus* has just twice the length of that bone in the limpkin, and it is not as much curved; while the pneumatic foramen on the under side of its head is very large and deep. There is a large pneumatic foramen, too, on the posterior aspect of the expanded sternal portion of either *coracoid* in *G. americanus*, and these bones, in this species of crane, are relatively much shorter and stouter than are the coracoids of *Aramus*; otherwise they have the same form and character.

So far as the axial skeleton is concerned, then, in the genera here compared, we can hardly say with Garrod that the "sternum in *Aramus* is completely Gruine, as are the other parts of its skeleton," for the differential characters are too marked, too important, and too numerous, when we come to regard them carefully in such representatives as *Aramus vociferus* and *Grus americanus*.

*The appendicular skeleton.* Little requires to be said here upon this subject, as the various bones were practically described during the comparisons made, on a former page, with those of the pectoral and pelvic limbs of *Aramus*. All the essential characters of the skeleton of the arm as seen in *A. vociferus* are repeated in the corresponding bones of that limb in *Grus*.

In the lower extremity we find, in the *femur* of *Grus*, a very deep 'rotular channel' between the condyles in front, and posteriorly there is constantly seen above the fibular groove of the external condyle a small, concave excrescence, to which is attached in life the femoral head of the *flexor perforans digitorum profundus* muscle.<sup>3</sup> Only a slight roughness occurs upon the femur at the same site in *Aramus*.

*Tibio-tarsus* and *fibula* have identically the same morphological characters in the two genera. In the *tarso-metatarsus*, the groove seen in the hypotarsus of that bone in *Aramus* is com-

<sup>3</sup> Myology of the raven, p. 167, fig. 46, a. Macmillan and Co., London, 1890.

pletely sealed over in *Grus*, converting it into a cylindrical perforation passing vertically through the center of the projection. In addition, the back of the hypotarsus is shallowly grooved in two or three places for the passage of tendons. At the posterior aspect of the shaft the outer edge of the tendinal groove is, for its middle third, elevated as a conspicuous crest in this crane, and it serves well to keep the ossified tendons in the channel where they belong. For the rest, these bones in the two genera under consideration have similar characters.

Joints of the phalanges of *pes* are relatively much stouter and shorter in *Grus* than they are in *Aramus*; in other words, the skeleton of the foot in *Aramus*, in so far as the toes are concerned, is markedly rilline in type, notwithstanding the fact that all the other bones of its pelvic limbs, with the exceptions mentioned, are so typically gruine.

In my former brief abstract of the osteology of the birds which have been compared in the present paper, I published a 'synoptical table,' in which the skeletal characters of *Rallus longirostris*, *Aramus vociferus*, and *Grus americana* were critically compared in parallel columns. As stated above, this paper appeared a great many years ago in the *Edinburgh Journal of Anatomy*; and as that publication is to be found in the majority of the larger scientific libraries in America, and is therefore accessible to students of avian osteology in this country, the aforesaid table has been omitted in the present contribution.

#### CONCLUSIONS

In so far as the rails, cranes, and their allies, are concerned, as they are represented in North America and other regions where they occur, they are allied or related to each other as I have, in the main, provisionally pointed out in another contribution,<sup>4</sup> thus:

<sup>4</sup> R. W. Shufeldt. An arrangement of the families and higher groups of birds. *Amer. Nat.*, vol. 38, nos. 455-456, Nov.-Dec., 1904, pp. 833-857. The part of the classification referred to is to be found on pages 851-852. As this classification of birds first appeared (that is, in the paper here cited), the family *Aramidæ* was arrayed among the *Ralliformes*, while here it is placed among the *Grues*, where it really belongs.

Supersuborder	XII	GRUIFORMES	Supersuborder	XIII	RALLIFORMES
Suborder	XIX	Grues	Suborder	XX	Fulicariae
Superfamily	I	Gruioidae	Superfamily	I	Heliornithoidea
Family	I	Gruidae	Family	I	Heliornithidae
	II	Aramidae	Superfamily	II	Rallioidae
	III	Psophiidae	Family	I	Rallidae
Superfamily	IV	Cariamoidea			
Family	I	Cariamidae			
Superfamily	III	Eurypygoidea			
Family	I	Eurypygidae			
	II	Rhinochetidae			
	III	Mesitidae			
	IV	Aptornithidae			

These groups are arrayed between the supersuborders Stereornithiformes and the Apterygiformes, where they naturally belong.

My examination of the skeleton of the courlans demonstrates the fact that, in that part of their anatomy at least, those birds have the gruine characters predominating. But these gruine characters are not always typical, and the departures seen are frequently of a class that distinguish families among birds rather than genera. This settles the position of the courlans in the system as a family—the Aramidae of the crane-group (Grues).

In the A. O. U. "Check-List of North American Birds," published in 1895 (second edition), it will be noted that the Aramidae was placed as a family among the Ralli or rail-group; and that after my paper appeared in the *American Naturalist* ('04) it was removed, as a family, to the crane-group (Grues), where up to this writing, it has been very properly retained. There are not a few similar errors yet to be rectified in that surely not-up-to-date volume. However, its last edition has the Rallidae properly and naturally arranged—a fact upon which we may congratulate ourselves, perhaps all the more for the reason that the Grues have also been relegated to their true position in the system in that work ('10).

As elsewhere pointed out, Fürbringer is of the opinion that the Apteryges are far more closely related to the Ralliformes than has heretofore been realized; and if this proves to be true, another linking line for the cranes and rails leading to the generalized struthious types is in evidence, with all the gallinaceous birds more or less related.

# THE GROWTH AND VARIABILITY IN THE BODY WEIGHT OF THE ALBINO RAT

HELEN DEAN KING

*From the Wistar Institute of Anatomy and Biology*

FIVE FIGURES

For several years investigations have been in progress in the animal colony of The Wistar Institute for the purpose of ascertaining the environmental and nutritive conditions most favorable for the development of the rat. The very flourishing state of the colony at the present time seems to indicate that these investigations have solved the problem of the care and breeding of this animal, and that in future it will be possible to supply a 'standardized' type of rat for laboratory use.

Growth records for the body weight of the albino rat have already been given by Donaldson ('06) and by Jackson ('13), but it seems worth while to publish additional data obtained from a study of a series of rats bred in The Wistar Institute colony in order to show the rapid and continuous growth of this animal in response to a particularly favorable set of environmental conditions. It is hoped that these records may also serve as standards with which the body weights of various strains of rats, raised under similar conditions, may be compared.

The thirteen litters used in this study were taken from the general colony of albino rats kept as 'stock' supply. In choosing the litters care was taken to select only those in which the individuals were of good size at birth and appeared strong and vigorous. The animals used, therefore, represent the best stock in the colony. A random selection of any stock litters available for the purpose in mind was not feasible, as experience has shown that rats that are small and weak at birth do not, as a rule, grow at a normal rate and that they usually die at an early

age: records from litters of this kind would have seriously affected the results.

Since the removal of young rats from the nest at or soon after birth often results in their being destroyed by the mother when returned, the first weighings were made when the litters were thirteen days old. As at this age differences in the weights of the members of the litter are usually very slight, individuals of the same sex were weighed together and the average weight of the group recorded. The rats were weighed in a similar way when they were thirty days old, but thereafter the individuals of the litters were weighed separately at intervals of one month. Records were taken over a period of sixteen months, by which time so many of the rats had died that the investigation was brought to an end.

Members of the same litter were kept together and allowed to breed. This, of course, introduced the possibility of an error in the records for the body weights of the adult females. In the rat pregnancy can usually be detected by the twelfth or thirteenth day, at which time, as Stotsenburg's ('15) records show, the average weight of each fetus is only about 0.04 grams. At this stage the increase in the weight of the female as a result of pregnancy is comparatively slight, and as females known to be pregnant were never weighed, the records have probably not been affected to any great extent by this factor. Only a very few of the litters cast were reared, and the weights of nursing mothers were not recorded if they were below those of the last weighing before pregnancy was noted.

In any series of weighings of live animals there is always an unavoidable error due to the presence of a greater or less quantity of undigested matter in the alimentary tract. To minimize this source of error in these records the rats were always weighed in the morning before they had been fed.

An illness of any kind has a marked effect on the body weight of the albino rat, and cases are not uncommon in which animals have lost 100 grams in weight in the course of two or three weeks. Except in very old rats, a steady loss in weight for a period longer than one month is an almost certain indication of a chronic disease

that will eventually kill the animal. The body weights of animals obviously ill were not used in making up the final records, and as far as known the body weights given in the accompanying tables are those of animals in good physical condition.

All the rats were reared under similar environmental conditions. The food given them was a 'scrap' diet consisting of carefully sorted table refuse which was fed once each day, while corn on the cob was always available as an extra ration. Each cage had an abundant supply of water, which was renewed daily to prevent contamination.

#### GROWTH IN BODY WEIGHT OF THE ALBINO RAT

In order to obtain a series of records that would represent the normal increase in the body weight with age it was considered advisable to take a sample of the stock at two different periods rather than to rear a large number of litters at one time. This plan has extended the work of collecting records over the greater part of three years, but it has amply justified itself since it has shown that, when environmental conditions are uniform, there is comparatively little variation in the rate and in the extent of growth of rats born in different years. Records for the growth in body weight obtained from one set of stock albino rats can therefore be used as 'standards' for the colony, as long as the animals are reared under similar external conditions.

The first series of rats used in this investigation comprised seven litters born between December 26, 1912 and March 10, 1913. These litters contained a total of 46 individuals, 23 males and 23 females. The average weight of the individuals of each sex, together with the extreme body weights for each age at which records were taken are given in table 1. Individual data for this and also for the second series of rats studied are filed at The Wistar Institute.

In a series of investigations recorded in a previous paper (King '15), it was found that the average body weight of stock albino males at birth is 4.6 grams, and that the average birth weight of the females is 4.5 grams. According to these records the male

rat is heavier than the female at birth, and, as shown in table 1, the male also exceeds the female in body weight at all ages at which data were collected, the difference in the weight of the two sexes becoming more marked as the animals grow older.

The growth of the albino rat is very rapid during the first 120 days of postnatal life, as Donaldson has already shown. After this age growth in body weight is relatively slow, as is

TABLE 1

*Data on seven litters of stock albino rats, showing the increase in the weight of the body with age (Series 1)*

AGE IN DAYS	MALES				FEMALES			
	Body weight in grams			No. individuals	Body weight in grams			No. individuals
	Average	Lowest	Highest		Average	Lowest	Highest	
13.....	18.2	16	21	23	16.0	14	20	23
30.....	49.7	43	60	23	47.4	40	57	23
60.....	131.0	87	170	23	113.2	77	153	23
90.....	188.5	125	238	23	148.9	99	178	16
120.....	230.3	146	284	23	173.1	125	197	17
151.....	252.1	196	307	23	181.0	152	215	18
182.....	268.0	195	343	23	197.5	147	245	21
212.....	276.0	221	366	22	192.3	136	245	18
243.....	281.7	194	355	21	200.2	141	256	19
273.....	270.6	202	344	20	203.0	158	248	16
304.....	281.4	198	366	18	201.1	165	230	18
334.....	291.1	238	368	16	212.4	178	239	17
365.....	301.7	248	370	12	214.2	176	247	15
395.....	310.0	254	381	9	213.7	178	247	15
425.....	316.3	255	368	8	207.9	169	238	12
455.....	316.0	249	349	6	215.2	196	242	7
485.....	313.5	255	374	3	219.5	210	241	4

indicated by the data in table 1. Increase in body weight does not entirely cease when the rats have reached maturity, and it usually continues as long as the animals are in a healthy condition. The later weight increase, however, is not true growth, but chiefly the accumulation of adipose tissue, as is the case in many other forms.

The second series of rats used in this study consisted of six litters, born during the first week of October, 1913. These lit-

ters contained a total of 54 individuals, equally divided as to sex. Growth data for this series of rats are given in table 2.

The growth records obtained for this series of rats are so nearly like those for the rats of the first series that the rate and extent of body growth in the individuals of the two series can best be compared through the growth graphs constructed from the average body weight of the animals as given in table 1 and in table 2.

TABLE 2

*Data on six litters of stock albino rats, showing the increase in the weight of the body with age (Series 2)*

AGE IN DAYS	MALES				FEMALES			
	Body weight in grams			No. individuals	Body weight in grams			No. individuals
	Average	Lowest	Highest		Average	Lowest	Highest	
13.....	16.4	13	18	27	15.3	13	18	27
30.....	47.4	41	54	27	44.2	39	50	27
60.....	116.0	64	149	27	101.8	66	124	27
90.....	181.6	103	226	27	147.7	95	177	23
120.....	217.1	151	274	27	173.7	132	212	25
151.....	238.6	169	294	27	189.8	147	225	27
182.....	250.1	172	303	27	195.1	149	232	21
212.....	261.3	176	324	26	201.4	167	238	24
243.....	277.8	206	334	23	216.5	171	256	24
273.....	290.7	218	349	21	216.6	170	254	22
304.....	310.8	220	379	18	235.2	177	262	20
334.....	309.8	240	385	17	231.8	170	276	18
365.....	310.6	245	377	16	231.6	177	276	16
395.....	317.4	246	376	15	226.9	175	284	16
425.....	310.0	243	397	15	221.0	178	271	18
455.....	329.2	289	414	9	223.4	198	264	11
485.....	330.3	276	437	9	241.4	197	324	9

Chart 1 shows the growth graph for the 23 males belonging to the first series and also the growth graph for the 27 males of the second series of animals studied. In this, as in other charts where the graphs would properly run very close together at the beginning, the distance between the graphs has been slightly exaggerated in order to keep the lines distinct.

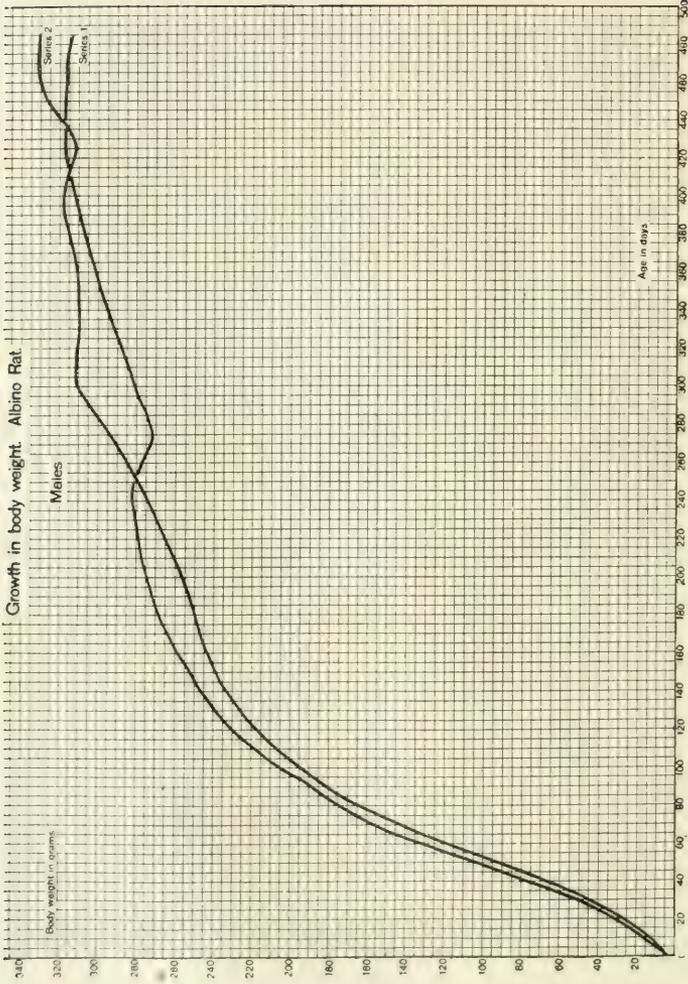


Chart 1 (graphs showing the growth in body weight of two series of stock albino males (data in tables 1 and 2)).

In this chart the growth graph for the males belonging to the second series runs somewhat below that for the males of the first series until the rats have reached 240 days of age. The two graphs cross at this point and the graph for the second series then runs above the other until the end, except for a slight dip at the 425 day period. The pronounced drop in the graph for the first series that occurs at 280 days is not to be considered as normal. It is due to the fact that, when the majority of the rats in the series were about eight months old, the animals were removed to new quarters and the change, which unfortunately took place during a spell of cold, damp weather, so affected the animals that many of them, particularly the males, did not show a normal gain in body weight for about two months.

Graphs for the females of the two series, constructed from the average body weights as given in table 1 and in table 2 are shown in chart 2.

Growth graphs for the females belonging to the two series bear about the same relation to each other as do those for the males. The graph for the second series runs slightly beneath that for the first series in the beginning, it meets the other graph at the 120 day period, and subsequently runs higher for the remainder of its course. There is no drop in the graph for the females of the first series comparable to that found in the graph for the males at the 280 day period. The change of quarters which so adversely affected the growth of the males seemed to have had so little effect on the females that the growth graph has not been lowered at any point.

In both chart 1 and in chart 2 the growth graphs for the two series of rats born nearly a year apart run very close together throughout their entire length. This indicates that, when environmental conditions are uniform, the growth of stock albino rats takes a very definite course and tends to produce animals having a like weight at any given age.

To summarize the results the growth data for all the individuals in the two series are combined in table 3. From the average body weights given in this table the graphs in chart 3 have been constructed.

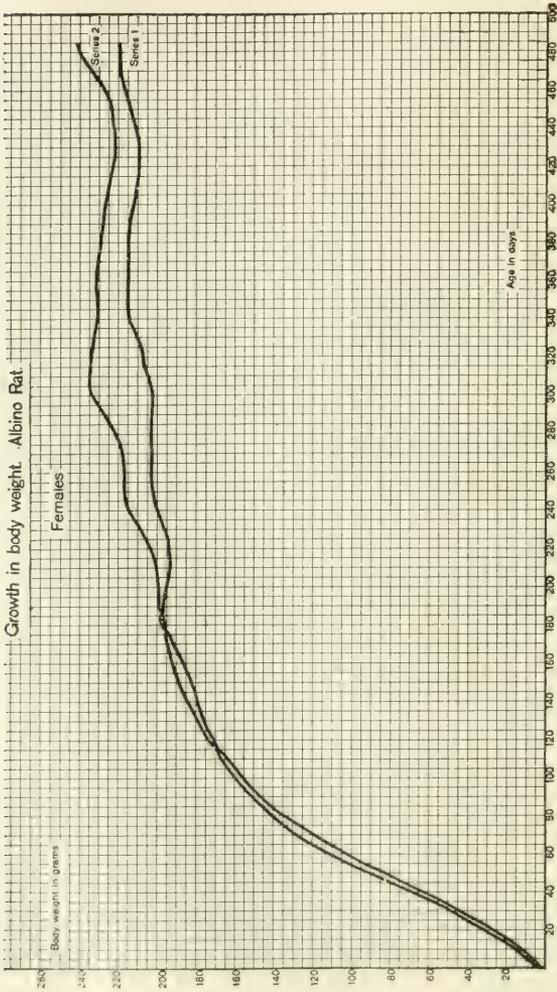


Chart 2 Graphs showing the growth in body weight of two series of stock albino females (data in tables 1 and 2).

A comparison of the graphs shown in this chart brings out very clearly the great difference between the growth of the male and that of the female rat. The graphs run very close together until the 60-day period. At this point they begin to diverge rapidly, the graph for the males soon appearing far above that for the females. At 200 days of age the average weight of the males is

TABLE 3

*Data on thirteen litters of stock albino rats, showing the increase in the weight of the body with age (summary of the data in table 1 and in table 2)*

AGE IN DAYS	MALES				FEMALES			
	Body weight in grams			No. indi- viduals	Body weight in grams			No. indi- viduals
	Average	Lowest	Highest		Average	Lowest	Highest	
13.....	17.2	13	21	50	15.7	13	20	67
30.....	48.5	41	60	50	45.7	39	57	50
60.....	122.9	64	170	50	107.1	66	153	50
90.....	184.8	103	238	50	148.0	95	178	39
120.....	223.2	146	284	50	173.4	125	212	42
151.....	244.8	169	307	50	186.3	147	225	45
182.....	258.4	172	343	50	196.5	147	245	42
212.....	268.0	176	366	48	197.3	136	245	42
243.....	279.7	194	355	44	209.6	141	256	43
273.....	280.9	202	349	41	210.8	158	254	38
304.....	296.1	198	379	36	219.1	165	262	38
334.....	300.8	238	385	33	222.4	170	276	35
365.....	306.1	245	377	28	223.1	176	276	31
395.....	314.1	246	381	24	220.5	175	284	31
425.....	312.2	243	397	23	215.8	169	271	30
455.....	323.9	249	414	15	220.2	196	264	18
485.....	326.0	255	437	12	234.7	197	324	13

about 50 grams more than that of the females (table 3), and this difference, as the growth graphs in chart 3 show, tends to become still greater as the animals grow older.

The growth of the albino rat in body weight has been studied by Donaldson, by Ferry ('13), and by Jackson. Growth graphs constructed from data obtained by the first two of these investigators and graphs from my own series of animals are given in chart 4 and in chart 5.

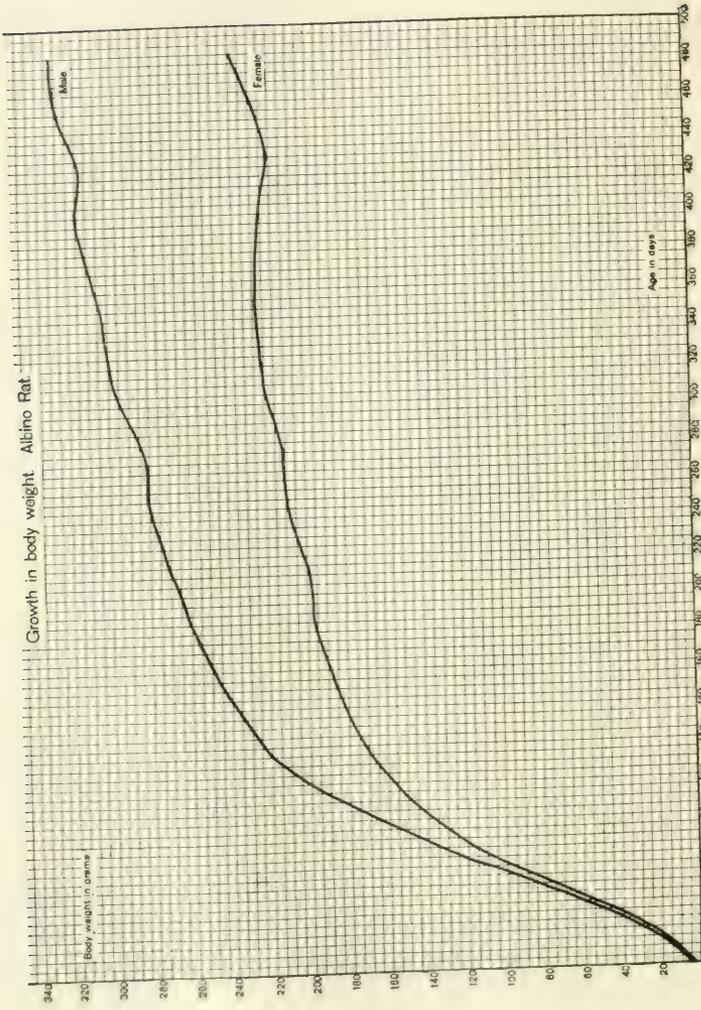


Chart 3 Graphs showing the growth in body weight of male and female stock albino rats (data in table 3).

The first data recorded for the growth of the albino rat in body weight were obtained by Donaldson from a series of animals reared in the laboratory of the University of Chicago. Bread and milk formed the staple food of the rats used in this investigation: this diet being varied occasionally by the addition of vegetables, meat, corn and sunflower seeds.



Chart 4 Graphs showing the growth in body weight of three series of male albino rats reared under different environmental conditions. A, graph constructed from data obtained by Donaldson; B, graph constructed from data furnished by Ferry; C, graph constructed from data recorded by King.

Donaldson's growth graph for the male albino rat (A, chart 4) is based on data for 19 animals weighed at varying intervals for one year. This graph has practically the same form as that for the male rats reared in The Wistar Institute colony (C, chart 4), but it runs considerably lower throughout its entire length. The space between these graphs represents, for the adult rats,

a difference of about 20 grams in the average body weight of the two series of animals.

Graph B in chart 4 was constructed from growth data for about 50 male albino rats bred in the laboratory of Yale University. These data were kindly furnished by Miss Ferry, who used them in her study of "The rate of growth of the albino rat." As regards the food given the rats Miss Ferry states in a letter: "The diet of the rats consisted of Austin's dog biscuit, sunflower seeds with fresh vegetables (chiefly carrots or corn and string beans) two or three times a week, and a small amount of cooked meat twice a week. A little salt was always kept in the cages."

The growth graph for the albino males based on Ferry's data closely follows Donaldson's graph, running a little above it in the beginning but falling below after the 60-day period. The drop in graph B at the end is due to the fact that Ferry included in her records the body weights of some adult animals that were probably ill.

The body weight of the albino rat at any given age depends to a considerable extent on the character of the food, as has been shown experimentally by Hatai ('07) and by Osborne and Mendel ('11). The fact that the male rats from which the data for Graph C were obtained grew more rapidly and attained a greater absolute weight than the rats reared by Donaldson and by Ferry can doubtless be ascribed in great part to the difference in the food that the animals received. The 'scrap' food given the rats in The Wistar Institute colony contains a relatively larger amount of meat and of fresh vegetables than the diet supplied to the rats used in the other two series of investigations noted. Considerable quantities of these substances are evidently needed by the rat to supply the materials needed to stimulate vigorous and continued growth.

Growth graphs for the body weights of the female albino rats used in these three series of investigations are given in chart 5.

The growth graphs for the three series of female rats bear very different relations to each other from those shown by the graphs for the males of the series (chart 4). The graph constructed from the data furnished by Miss Ferry (B, chart 5) is consider-

ably lower than either of the other two graphs. This indicates either that exceptionally small rats were used for her investigations, or that the animals were not in good physical condition and so did not gain in weight at a normal rate. Whether these females were allowed to breed is not stated.

As investigations made by Watson ('05) had shown that female rats that are allowed to breed are heavier than unmated females of the same age, the growth graph for female albino rats as given by Donaldson, and reproduced as graph A in chart 5,

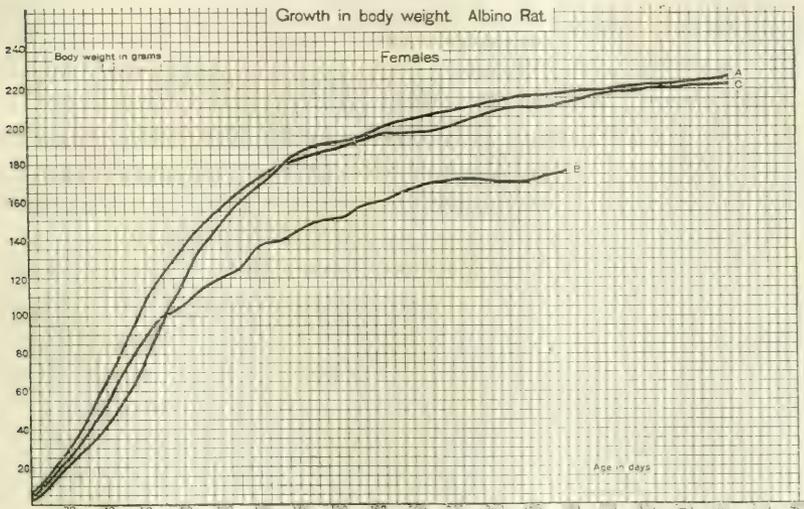


Chart 5 Graphs showing the growth in body weight of three series of female albino rats reared under different environmental conditions; lettering as in chart 4.

was constructed from the actual weight data of unmated females up to the 90-day period, and beyond this point the data used were the body weights of unmated females corrected to accord with the weights of breeding females as calculated by Watson's formula.

The female rats belonging to Donaldson's series grew very slowly at first, as is shown by the position of graph A in chart 5. At 120 days of age the average body weight of the females used in my investigations was 173 grams. This is practically the es-

timated weight for breeding females of this age as calculated by Donaldson. In chart 5, graph A meets graph C at this point and subsequently runs parallel with but slightly above it. It is, of course, impossible to obtain, at stated intervals, the true body weights of albino females that are allowed to breed. Pregnancy produces a temporary increase in weight that may amount to 50 grams or more, while the nursing of a large litter usually causes the female to lose considerable weight. To weigh females only after they have recovered from nursing a litter makes the intervals between the weighings too long to give weight records of much value. Positive errors in the weighings of females during early pregnancy undoubtedly occur in my records, but, as previously stated, such errors would be very slight, and they have probably been balanced by negative errors due to the weighing of nursing females or of females that were ill. Graph C is probably, therefore, fairly representative of the average body growth of albino females that are allowed to breed. It is of interest to note that the graph constructed from actual weight records for breeding females runs close to the theoretical graph.

In his paper on the postnatal growth of the rat, Jackson gives some data for the body weight of male and of female albino rats of various ages, but these data are not extensive enough to make it possible to construct from them growth graphs similar to those in chart 4 and in chart 5. From the records given it would appear that the rats used by Jackson were relatively small, since the males had an average weight of 213.0 grams when they were one year old, while the average weight of the females at this age was 163.7 grams: corresponding figures for the rats in my series give 306.1 grams as the average weight of the males, and 223.1 grams as the average weight of the females at one year of age.

An interesting comparison between the rate of growth of the male and of the female albino rat during the early stages of development has been made by Donaldson. His records show that as early as the seventh day after birth the female rat grows more actively than the male and is, as a rule, a relatively heavier animal up to about 55 days of age. At this point the male

shows a very rapid gain in its rate of growth and he is much heavier than the female at all subsequent ages.

Jackson found that "the excess of average weight was invariably in favor of the male at birth, and also in the majority of cases at all succeeding ages." In general, however, his data seem to confirm Donaldson's conclusion that the growth of the female is more vigorous than that of the male during the first two months of postnatal life.

In the thirteen litters of rats used in my investigations there was only one litter in which the average weight of the females at thirteen days of age exceeded that of the males. In this case the average weight of the females was 18 grams while that of the males was 17 grams. In one litter the average weight of the females at 30 days of age was exactly the same as that of the males, but in all other litters the males averaged from one to eight grams heavier than the females. At 60 days of age a few of the females weighed slightly more than the smallest males in the same litter, but in no case did any female have a weight equal to that of the largest male. In this series of animals the male tends to exceed the female in body weight in early as well as in late stages of development, but up to about 60 days of age the difference is very slight. Growth graphs for the two sexes, shown in chart 3, run very close together at first and begin to diverge only after the animals have reached 60 days of age. Female rats attain their maximum weight sooner than do the males, thus indicating that their rate of growth exceeds that of the males, although their absolute body weights may be less than those of the males at any given age. Evidence that the female rat tends to develop somewhat more rapidly than the male is also shown by the fact that, as a usual thing, the female rats in a litter open their eyes several hours sooner than do the males.

#### VARIABILITY IN THE BODY WEIGHT OF THE ALBINO RAT

Even with environmental and nutritive conditions as nearly uniform as possible, rats of the same sex belonging to the same litter show marked differences in body weight that must be attributed to factors inherent in the 'germplasm' from which the individuals were derived. By studying the magnitude of the

variation in these body weights we can obtain some idea as to the range in the action of these unknown intrinsic factors of growth even if we get no hint as to the nature of the factors themselves.

The relative extent of variability in body weight, as well as in other characters, is at the present time best determined through the coefficients of variation obtained for the body weights at each age at which weighings are taken. Since the frequency curves for both male and female rats when plotted from the data given in table 3 are fairly symmetrical, it was possible to obtain the coefficients of variation for the body weights according to Pearson's formula as given by Davenport ('14).

The index of variation ( $\sigma$ ) was first obtained by the following method:

$$\sigma = \sqrt{\frac{\text{sum of } [(deviation \text{ of class from mean})^2 \times \text{frequency of class}]}{\text{number of varieties}}}$$

The coefficient of variation ( $C$ ) was then found by the following formula in which the number of the varieties is indicated by the letter  $N$ .

$$C = \frac{\sigma}{N} \times 100 \text{ per cent.}$$

The coefficients of variation for this series of body weights are relatively small, being in many cases less than 10 per cent. It was possible, therefore, to calculate the probable error in these coefficients ( $EC$ ) by the formula:

$$EC = 0.6745 \frac{C}{\sqrt{2N}}$$

Table 4 gives the coefficients of variation with their probable errors for the body weights of the male and of the female rats used in the two series of investigations described above. For the 13- and for the 30-day periods grouped data were used in making the calculations, as only the average body weight of the individuals of each sex was recorded in the weighings of the various litters at these ages: for all other ages the individual data were used.

The range of variability in the body weights of the male rats is practically the same for the two series of litters weighed, as the difference between corresponding coefficients of variation is not more than three in any instance (table 4). The coefficients given in table 4 for the body weights at 13 and also at 30 days are doubtless lower than would be the case had the coefficients been calculated from individual and not from average body weights. The high coefficients for the males at 60 and at 90

TABLE 4

## ERRATA

766<sup>a</sup>

THE ANATOMICAL RECORD, volume 9, number 10, October, 1915, in the middle of page 766, substitute for the lines and formulas there printed these corrected formulas and lines.

$$\sigma = \sqrt{\frac{\text{sum of [(deviation of class from mean)}^2 \times \text{frequency of class}]}{\text{number of variates}}}$$

The coefficient of variation (C) was then found by the following formula in which the mean of the variates is indicated by the letter A.

$$C = \frac{\sigma}{A} \times 100 \text{ per cent.}$$

days indicate that the maximum variability for this sex comes at this period. For the adult males the range of variability in body weight is practically constant for all the ages studied up to one year. From this point it tends to diminish slightly, as Minot ('91) found to be the case in guinea-pigs.

The high coefficients of variation for the body weights of very old males, as shown in table 4, can have little significance although they occur in both series, owing to the small number of individuals

weighed at this time and to the fact that the probable errors in the coefficients are relatively large.

Corresponding coefficients for the body weights of the female rats in the two series do not accord as well as do those for the males. There is, in fact, no agreement whatever between the coefficients for the first three ages at which the animals were weighed. In the first series the highest coefficient (16.1) is that for the females at 60 days of age; in the second series the highest coefficient (14.9) is that for the 13-day period. Coefficients for the body weights of the females of the first series at 90 and at 120 days of age are practically the same as the corresponding coefficients for the second series, and the coefficients for later stages show no significant differences. The range of variability in the body weights of the females that lived to 485 days of age is curiously unlike in the two series. Females of the first series that lived to this age varied little in their body weights, as the coefficient of variation is only 5.6; while the females of the second series exhibited a very wide range of variability in their body weight, as is indicated by a coefficient of 14.1. The relation of body size to longevity in the rat is a point that will be considered in detail when a larger series of records is available for analysis.

The coefficients of variation for body weights at different ages, calculated from the data for all of the individuals in the two series, are given in the last two columns of table 4. From these coefficients it is possible to compare the range of variability in the body weight of 50 albino males with that of 50 females. At 13 and at 30 days of age the females seem to be quite as variable in body weight as the males, as the coefficients for body weight of the sexes at these ages are much the same. Males and females show their maximum range of variability in body weight at 60 days of age, but the coefficient of variation for the body weight of the males is 17.0 against 15.7 for that of the females. The males are more variable in body weight than the females from this time on, as at every subsequent age the coefficients for the male weights are higher than those for the female weights, although when the probable errors in these co-

efficients are taken into consideration the difference in favor of the males is very slight in many cases.

Other studies on the rat also seem to show a greater variability in the males than in the females. Hatai ('08) found that in skull measurements the males show a greater tendency to variability than do the females. Jackson's data show that males are more variable than females in body weight except at 20 days of age, and he concludes that "variability in body weight is lowest at birth (the coefficient being about 12) and is not much higher at seven days (16). It appears highest at three weeks (28), and at later periods varies from 19 to 21. The average coefficient, taking all ages together, is 19." In my investigations I find that the maximum coefficient of variation for the body weight of the rat comes at 60 days in both sexes, and that the average coefficient is much smaller than that calculated by Jackson, being 13.6 for the males and 12.1 for the females. The fact that Jackson's calculations were based on data obtained from rats that represented "for the most part a random sample of the general population at each age," while mine were based on the weighings of the same series of individuals throughout the entire period of observation, undoubtedly accounts for the differences in our results.

It has been held by many investigators, among whom are Darwin ('71) and Brooks ('83), that throughout the organic world the male tends to be more variable than the female. The known facts regarding variability in man have been collected by Ellis ('11) who sums up his discussion of the subject with the following statement: "In man, as in males generally, there is an organic variational tendency to diverge from the average; in woman, as in females generally, an organic tendency \* \* \* to stability and conservatism involving a diminished individualism and variability." Pearson ('97) attempts to refute this theory, which he states is based chiefly on the fact that pathological variations seem to occur more frequently in man than in woman. After a critical examination of a large mass of growth statistics for various races Pearson concludes that, although men are more variable than women at certain ages and in certain characters,

yet there is not a pronounced difference between the sexes as regards variability, the weight of evidence indicating slightly greater female than male variability. Data regarding variability in the rat as collected by Hatai, by Jackson and by myself do not support Pearson's contention, since these data show that, in the characters tested, there is decidedly greater variability in the male than in the female rat.

Boas ('97) and Porter ('05), among others, have shown that in man variability in body weight is correlated with rapidity of growth. A similar correlation in the rat is not shown by Jackson's data, although it seems to be indicated by my results judging from the relative size of the coefficients for body weight at various ages as given in the last two columns of table 4. Since in this table the coefficients given for the 13- and for the 30-day periods were calculated from the average body weights of groups of rats and not from individual data they cannot justly be used to give evidence on this point. At 60 days of age, when the rats are still growing very rapidly as is indicated by the growth graphs in chart 3, the coefficients of variation are higher for both males and females than at any subsequent period. The rate of growth is beginning to slacken at 90 days of age, and the coefficients indicate a corresponding lessening in the range of variability of the body weights. At 120 days of postnatal life the period of rapid growth is at an end, and it is significant that at this point the coefficients for both sexes drop to the level that is maintained, with no important change, up to one year of age, when growth has practically stopped. Correlation between the rate of growth and variability in body weight exists in the rat in a late period of adolescence according to these records, but further investigations will be necessary in order to determine whether this correlation also exists at birth and during the early stages of development.

Two of the litters of rats used for this study contained an unusually large number of individuals of the same sex: one litter was composed of nine males and three females; the other had seven females and two males. For the purpose of comparing the variability within the litter with that of the general popula-

tion as determined from the data for all of the litters weighed, growth records for these two litters are given in table 5 and in table 6. Data for the males, together with their coefficients of variation, are presented in table 5.

On comparing the data in table 5 with the corresponding data in table 3, it is found that the average body weight of these nine males greatly exceeds that for the males of the general pop-

TABLE 5

*Showing the increase in body weight with age and the coefficients of variation for nine males belonging to the same litter of stock albino rats*

AGE IN DAYS	BODY WEIGHT IN GRAMS			COEFFICIENTS OF VARIATION	NO. INDIVIDUALS
	Average	Lowest	Highest		
13.....	15.0				9
30.....	50.0				9
60.....	115.1	98	135	9.6±1.52	9
90.....	177.7	162	206	7.9±1.25	9
120.....	220.5	199	242	6.5±1.03	9
151.....	246.6	231	269	5.1±0.80	9
182.....	262.2	231	287	7.1±1.12	9
212.....	273.0	234	298	6.8±1.14	8
243.....	296.0	241	329	10.1±1.69	8
273.....	311.5	252	349	9.5±1.59	8
304.....	338.1	269	379	10.0±1.68	8
334.....	341.5	278	385	8.6±1.44	8
365.....	334.0	270	377	10.0±1.68	8
395.....	336.3	273	376	9.8±1.65	8
425.....	331.2	267	397	13.3±2.23	8
455.....	345.6	289	414	13.7±2.66	6
485.....	341.4	297	437	15.0±3.19	5

ulation after the animals reach the age of 120 days. This result may be due, possibly, to the fact that the litter to which the males belonged happened to be by far the best of the 13 litters weighed, judging from the size and vigor of the individuals and from their longevity.

Growth data for the seven females belonging to the same litter with the coefficients of variation for their body weights at various ages, are given in table 6.

After the age of 60 days the average weight of these seven females exceeded that of the females representing the general population, as may be seen by comparing the corresponding data in table 6 and in table 3. The largest females in the series studied were not contained in this litter, but were members of the litter containing the nine males whose growth data are given in table 5. Whether there is any correlation between the number of individuals of the same sex in a litter and the size of the indi-

TABLE 6

*Showing the increase in body weight with age and the coefficients of variation for seven females belonging to the same litter of stock albino rats*

AGE IN DAYS	BODY WEIGHT IN GRAMS			COEFFICIENT OF VARIATION	NO. INDIVIDUALS
	Average	Lowest	Highest		
13.....	12.5				7
30.....	41.0				7
60.....	105.5	99	121	6.4±1.15	7
90.....	164.0	143	177	9.2±1.78	6
120.....	176.5	160	195	6.5±1.16	7
151.....	204.2	163	220	8.9±1.59	7
182.....	205.4	190	223	6.6±1.40	5
212.....	215.6	197	228	4.9±1.04	6
243.....	225.0	204	256	7.7±1.38	7
273.....	231.4	193	254	8.5±1.53	7
304.....	242.8	212	265	7.4±1.32	7
334.....	240.0	215	259	6.3±1.22	6
365.....	239.0	220	255	5.3±1.12	5
395.....	221.6	198	243	6.5±1.68	5
425.....	224.4	203	250	5.4±1.15	7
455.....	220.1	198	239	6.0±1.16	6
485.....	234.8	226	250	4.3±0.91	5

viduals, as the records from these two litters seem to indicate, can only be determined from the data of a much larger series of litters.

The coefficients of variation for the males of one litter, as given in table 5, are considerably lower than those for the males of the general population (table 4) up to the time that the animals were 425 days old: the small number of individuals weighed at an advanced age make it impossible to draw any conclusions from

the data given. Coefficients of variation for the body weight of the seven females from the one litter are likewise considerably below those for the females of the general population (table 4). From these facts it follows that variability within the litter unit is less than that of the general population, as Jackson has already shown.

The variability in the body weights of the male members of a litter is greater than that in the female members of a litter, as is shown by comparing the coefficients of variation given in table 5 with those in table 6. The difference, as might be expected, is about the same as that between the males and females of the general population.

From an examination of the coefficients of variation for the body weights of the individuals belonging to several litters, Jackson concludes that "in general the variation in body weight within a given litter of albino rats is probably less than half that of the general population of the same age under similar environment." Taking all ages together I find that the average coefficient of variation for the body weights of the nine males from the one litter is 9.5, while that for the males of the entire series is 13.6 (table 4); for the seven females belonging to the same litter the average coefficient of variation is 6.7, that for the females of the general population being 12.1. In this instance the range of variability within the litter is about 70 per cent that of the general population in the case of the males, while for the females it is about 55 per cent. These figures seem to indicate that the relation between fraternal variability and racial variability for the body weight of the rat is much the same as that for human stature which, according to Galton ('94), is approximately 62 per cent. Definite conclusions regarding this point cannot be drawn from a consideration of the records from such a small number of litters, but must be deferred until a larger series of data is available for analysis.

## SUMMARY

1. The present paper gives the data for the increase in body weight with age for two series of stock albino rats reared under similar environmental conditions. Altogether thirteen litters containing 100 individuals, 50 males and 50 females, were used in this study.

2. Growth graphs for each sex, constructed from the average body weights at various ages, are practically the same for the two series of rats used (charts 1 and 2). From this fact it follows that, when environmental conditions are uniform, the growth of albino rats within a given colony tends to follow the same course and to produce individuals having a like weight at any stated age.

3. As a rule, the male rat is heavier than the female at birth and also at all subsequent ages at which records were taken. During the first 60 days of postnatal life the body weight of the female tends to approach that of the male, but after this age the male grows more rapidly than the female and soon greatly exceeds her in body weight. At 200 days of age the male rat weighs, on the average, about 70 grams more than the female of the same age (chart 3).

4. The female rat tends to increase in body weight at a much more rapid rate than does the male during the early stages of development, and she reaches her maximum weight much earlier than does the male.

5. The environmental and nutritive conditions under which rats are reared have a marked influence on their body weights, as is indicated by the relation of the growth graphs constructed from data obtained from three different series of rats reared under different conditions (charts 4 and 5).

6. Variability in the body weight of the albino rat, as measured by the coefficients of variation, is greatest when the animals are about 60 days of age. It decreases slightly at 90 days, and

after 120 days remains practically constant until the animals are about one year old.

7. Very young female rats seem to show as great a range of variability in body weight as do the males, but the males are more variable than the females at all later stages of growth.

8. The average coefficient of variation for the body weights of the 50 male rats used in this study is 13.6; that for the females is 12.1.

9. In the rat there is apparently a direct correlation between the rapidity of growth and the variability in body weight after the animals have reached 60 days of age. The records collected are not in a form to give evidence regarding the correlation that exists at earlier stages of growth.

10. Fraternal variability in the rat is less than racial variability. For the male rat the fraternal variability is about 70 per cent that of the general population; for the female it is about 55 per cent.

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# TAILLESSNESS IN THE RAT

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*From the Wistar Institute of Anatomy and Biology*

THREE FIGURES

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## INTRODUCTION

There seems to be a general opinion that when a rat appears without a tail it means the loss of the tail by accident early in the animal's life, and it is usually suggested that it was bitten off by another rat at the time of birth or soon after. The objects of this paper are to describe skeletal conditions in the region posterior to the thoracic vertebrae of several tailless rats, and to correct the existing impression that a tailless rat occurs through the accidental loss after birth of a once existing tail. As to the word tailless; by tailless we mean here "with no caudal vertebrae." There are cases, of course, where, from disease or from some accident after birth, the tail has become simply a stub, but in these cases some caudal vertebrae remain.

## HISTORICAL SURVEY

Little data seems to have been recorded concerning the tailless condition of the higher animals. Short tails have been noted among cats, fowls, and dogs, while the number of caudal vertebrae has been found to vary in some other animals, but only

among dogs have cases been recorded where the caudal vertebrae of a mammal were completely absent.

Hind ('89), Anthony ('99), Kennel ('01), and Davenport ('05) all give accounts of mating Manx cats or short-tailed cats with the long-tailed variety and having the short tail appear in many of the offspring. The ordinary cat, according to Flower ('85), has twenty-two caudal vertebrae, and according to Jayne ('98), eighteen to twenty-six. As to the number of caudal vertebrae of the short tailed cats, Flower ('85) gives three for the Manx cat, Anthony ('99) describes six, and Kennel ('01) speaks of six 'post-sacral' vertebrae in a so-called tailless cat.

Concerning fowls, Godron ('65) in a footnote says that complete lack of coccyx has been observed in a large number of fowls and that the character is very readily transmitted. He does not give any details of vertebral conditions in these cases, but, from descriptions of other 'rumpless' fowls, we conclude that the coccyx here probably was not completely lacking. In the ordinary fowl Davenport ('06) gives the number of free, caudal vertebrae as five, followed by a fused portion, the uropygial bone. Davenport ('06) describes a rumpless game female as having two unsymmetrically formed and intimately fused caudal vertebrae, followed by a knob of bone about 1 mm. in diameter, Darwin ('83) speaks of the caudal vertebrae in three rumpless fowls as being few in number and ankylosed together into a misformed mass. He also reports the inheritance of rumplessness in fowls, as does Davenport ('06).

Some other animals have been recorded as showing variation in the number of their caudal vertebrae. Bateson ('94) gives the following:

*Man:* (Normal number of caudal vertebrae, according to Flower, '85, three to four). A male with sacral and caudal vertebrae ankylosed together and of uncertain number; a female with the coccyx of three pieces ankylosed together.

*Anthropoid apes:*

*Chimpanzee:* (Normal number of caudal vertebrae, according to Flower, '85, five). One animal had six caudal vertebrae and others had two to four.

*Orang-utan*: (Normal number of caudal vertebrae, according to Flower, '85, four). One animal had three caudal vertebrae, three each had two caudal vertebrae, a fifth had the caudal vertebrae anchylosed with the sacral, and a sixth had only one caudal vertebrae.

*Sloths*: Among the sloths there was considerable variation from the normal number of caudal vertebrae.

None of the above refer to an absolutely tailless condition. We have, however, recorded by Godron ('65), a complete absence of caudal vertebrae in the dog. He examined by palpation the sacral region of a tailless female water spaniel and found at the end of the vertebral column a rounded, bony surface which he took to be the last sacral vertebra, and concluded that the coccyx was gone completely. (According to Flower, '85, the caudal vertebrae of the dog number from fifteen to twenty-three.) In his account of this female he states that, when she was mated with a tailless brother, six of the litter of seven were absolutely tailless. When she was mated with a long-tailed male water spaniel, the litter of four were all tailless like the mother. The grandfather of these dogs had a rudimentary tail 3 cm. long. Godron ('65) speaks also in a footnote of a tailless species of dog, the Dalmatian hound or brach-hound of Bourbonnais.

In all of these accounts of short-tailed and tailless animals the conditions referred to are congenital and not the result of accident.

#### MATERIAL AND METHODS

The material for this study was obtained from The Wistar Institute rat colony and consisted of rats of the species *Mus norvegicus albinus* and *Mus norvegicus* (pied). Specimens for the work were not abundant, since, during the past nine years, only five tailless rats have appeared in the colony, although forty thousand rats have been observed. Of these five, one was eaten by an older rat soon after birth, one male is at present mated in the colony, and the remaining three rats were killed and examined.

The method of examining these rats was as follows: The animal was chloroformed, its body weight and body length taken,

and its skin and viscera removed. The vertebral column with pelvic girdle attached was partly cleaned of its masses of muscle, covered with a boiling hot, 2 per cent solution of 'Gold Dust' washing powder (a 1 per cent solution was used for the youngest rat), and kept at about 95°C. until the flesh was softened so that it could be removed. This was done in tap water and, in addition to the usual instruments, a bone-scraper and a tooth-brush were used. Care was taken not to separate the vertebrae in the region of and caudal to the pelvic girdle attachment. The bones now were dried and placed in corked vials with tar camphor balls to protect them from *Anthrenus*.

#### DESCRIPTION OF THE RATS EXAMINED

Of the three tailless rats whose vertebrae were examined, one was a female and two were males. The data concerning them are presented in table 1. The normal rat of this species (*Mus norvegicus*) has six lumbar vertebrae, four sacral, and twenty-nine to thirty-one caudal.

*Rat No. 1*, the female, was of the species *Mus norvegicus* (pied). Its parents were among some pied, pet rats of the colony. It was two years old when killed, its body weight was 171.3

TABLE 1  
*Data on tailless rats*

RAT NO.	SPECIES	SEX	AGE DAYS	BODY WEIGHT	BODY LENGTH IN MM.	PARENTS	DATE OF KILLING
1	<i>Mus norvegicus</i> (pied)	♀	730	171.3	193	among pied pet rats ♀ × ♂ 10th stock gen. inbred	12-14-'14
2	<i>Mus norvegicus albinus</i> ; half inbred; 11th generation	♂	388	190.9 (month earlier 210)	194		5-19-'14
3	<i>Mus norvegicus albinus</i> ; stock strain	♂	30	21.7	89		2- 8-'15

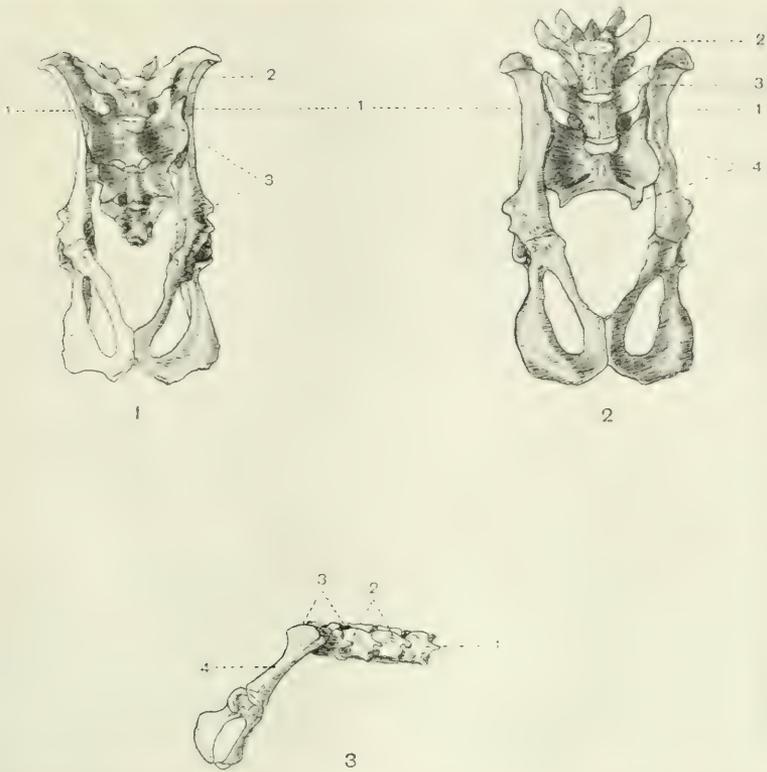


Fig. 1 Rat No. 1, ventral aspect; 1, pelvic girdle; 2, sixth lumbar vertebra; 3, three modified sacral vertebrae.

Fig. 2 Rat No. 2, ventral aspect; 1, pelvic girdle; 2, fifth lumbar vertebra; 3, sixth lumbar vertebra; 4, two modified sacral vertebrae.

Fig. 3 Rat No. 3, right lateral aspect; 1, thirteenth thoracic vertebra; 2, two first lumbar vertebrae; 3, two or three modified lumbar vertebrae; 4, pelvic girdle.

grams, and its body length was 193 mm. The arrangement of the most posterior vertebrae of this rat and their relation to the pelvic girdle may be seen in figure 1. Supposing all the lumbar vertebrae to be present, we have here, posterior to the lumbar vertebrae, only three modified sacral vertebrae. Thus one sacral vertebra and all of the caudal vertebrae are missing.

The striking feature here then is that the vertebral column ends posteriorly about midway of the long axis of the pelvic girdle, far in from the posterior end of the body.

*Rat No. 2*, a male, was a half-inbred albino (*Mus norvegicus albinus*) of the eleventh generation. Its mother was a strict inbred of the tenth generation and its father a stock male. When killed it was 388 days old, its body weight was 190.9 grams (one month earlier it had weighed 210 grams), and its body length was 194 mm. The arrangement of the most posterior vertebrae and their relation to the pelvic girdle may be seen in figure 2. Supposing all the lumbar vertebrae to be present, we have here, posterior to the lumbar vertebrae, only two modified sacral vertebrae. Thus two sacral vertebrae and all the caudal vertebrae are missing. Here again the vertebral column ends posteriorly far up the long axis of the pelvic girdle, even anterior to the middle of its axis.

*Rat No. 3*, a male, was an albino rat (*Mus norvegicus albinus*) of unknown parentage. It was thirty days old when killed, its body weight was 21.7 grams, and its body length was 89 mm. This rat was small and in rather poor condition. The arrangement of the most posterior vertebrae and their relation to the pelvic girdle may be seen in figure 3. We have here no sacral vertebrae, but two good lumbar vertebrae and two or three modified lumbar vertebrae. The last (sixth and perhaps fifth also) lumbar vertebra, all of the sacral vertebrae, and all of the caudal vertebrae are missing. In this case then the vertebral column is more modified than in the other two rats, for here the vertebrae reach only to the anterior part of the pelvic girdle, and the girdle is attached to the column merely by a small surface near its anterior end. This mode of attachment allows the posterior end of the girdle to hang very low down, almost at right angles to the column. In the living rat the sagging of the girdle was very noticeable, as it allowed the head of the femur to drop far down and thus gave an odd appearance to the posterior part of the rat's body.

This completes the description of the three tailless rats whose bones have been examined. As to the tailless male albino rat

which has been mentioned as at present mated in the colony, we are confident from inspection that in this animal also the vertebral column ends far forward along the long axis of the pelvic girdle. for this condition may be felt distinctly by pressing the finger on the rat's back in the pelvic girdle region.

The vertebral structure of all the tailless rats which we have examined seems to show that the deformity is not due to an accident after birth, since in each case the column ends in the pelvic region far from the posterior end of the body. We conclude, therefore, that the rats were born tailless, and even more than tailless, since they lack more than the caudal vertebrae.

#### SUMMARY

An examination of the vertebrae of three tailless rats showed that all of them lacked all of the caudal vertebrae; and besides, the first lacked one sacral vertebra; the second, two sacral vertebrae; and the third lacked one (and perhaps two) lumbar vertebra and all four of the sacral vertebrae.

In each case the vertebral column terminated in the pelvic region far anterior to the posterior end of the body, showing that the tailless condition was due not to accident after birth but to a congenital deformity of the vertebral column.

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## AN ANOMALOUS ORIGIN OF THE SUBCLAVIAN ARTERY

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THREE FIGURES

The subject containing this variation is that of a large Caucasian female, aged probably 40 or 45 years, with a good muscular development and no signs of emaciation. The right subclavian arises from the aortic arch distal to the left subclavian, and the right common carotid comes directly from the aortic arch in the position usually occupied by the anonyma, which is absent in this case. Hence, instead of arising from the anonyma, the a. subclavia dextra arose directly from the aortic arch, at a point 1 cm. distal to the origin of the normal a. subclavia sinistra. From here it took a course toward the right and cephalad, across the ventral surface of the second thoracic vertebra passing dorsal to the esophagus and trachea, and making an angle of about forty degrees with the latter. Its branches are (1) an a. vertebralis dextra which arises 4 cm. from the origin of the right subclavian; (2) an a. cervicalis profunda, arising from the dorsal aspect of the subclavian 7 mm. lateral to the vertebral; (3) an a. cervicalis ascendens coming from its cranial margin 12 mm. distal to the vertebral; (4) an a. mammaria interna, from the caudal border directly below the ascending cervical; and (5) an a. transversalis colli, in the normal position, 18 mm. distal to the vertebral. After passing dorsal to the m. scalenus anterior the anomalous vessel follows a normal course into the axila. The truncus thyreo-cervicalus, the a. thyroideus inferior, and the truncus costocervicalis were absent.

The a. subclavia sinistra arose in the usual place, but presented abnormalities in its branches similar to those on the right side. There are no truncus thyrocervicalis, an a. thyrocervicalis

inferior, or a truncus costocervicalis. The following rami arise in this region: (1) the a. vertebralis sinistra; (2) a small a. intercostalis suprema; (3) an a. cervicalis ascendens; (4) an a. mammaria interna; (5) an a. cervicalis profunda; and (6) an a. transversalis colli.

The aa. thyreoidea inferiores were absent and the a. thyreoidea ima was also missing. However, the a. thyreoidea superior on the right side was unusually large and divided into two branches as high as the level of the hyoid bone.

The right lobe of the thyroid gland was also much larger than the left and was formed by two partly distinct lobes. It is possible, but unlikely, that the upper of these two dextral lobes represents the pyramidal process, which in this case has been developed as an extra dextral lobe. A well-developed levator glandulae thyreoidea was present, connecting the capsule of the gland to the hyoid bone.

The a. carotis communis dextra is longer than usual for it also takes the place of the anonyma. As shown in figures 1 2, it follows the normal course of these two arteries, passing anteriorly and to the right as far as the right margin of the trachea and then turns almost vertically cephalad. As there is no a. thyreoidea ima, the only vessel given off below the a. thyreoidea superior is a small branch arising 13 mm. superior to the aortic arch on the ventral surface. This supplies the pericardium and the remnants of the thymus.

The n. recurrens laryngis forms a short loop above the subclavian and does not hook around it, as is usually the case. As is well known, the nn. recurrentes hook about the fourth aortic arches and as these develop into the anonyma and subclavian on the right side, and the aortic arch on the left, the nerves are dragged down to a lower level, thus establishing the recurrent condition. The fact that the right recurrent nerve does not hook around the subclavian artery may indicate that the latter was not developed from the fourth arch. An interesting recent article on the embryological development of such variations may be found in connection with a report of a similar anomaly by Cobey ('14).

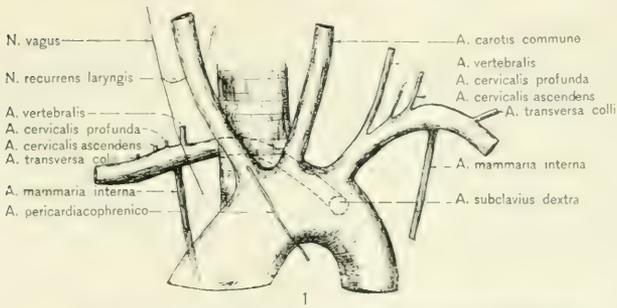


Fig. 1 Diagrammatic sketch of the actual arrangement.

Fig. 2 Trachea and esophagus pulled forward, heart and aortic arch thrown toward the left; 1, transverse portion of the aortic arch; 2, descending aorta; 3, trachea and esophagus; 4, *a. carotis communis* dextra; 5, *a. carotis communis* sinistra; 6 *a. subclavius* sinistra; 7, *a. subclavius* dextra; 8, *a. mammaria* interna dextra; 9, *v. azygos* (retracted); 10, *v. cava* superior.

There was no noticeable aortic impression on the vertebral column, but the anomalous subclavian (which arose from the aortic arch pointing directly toward the vertebral column and which then turned to the right at an angle of nearly ninety degrees to cross the body of the second thoracic vertebra) produced a distinct groove on the left and also a flattening across the ventral aspect of the body of that vertebra. However, if the current in the artery was impeded because of its position and origin the obstruction was not sufficient to affect the development of the right arm, for both arms seemed equally well-developed

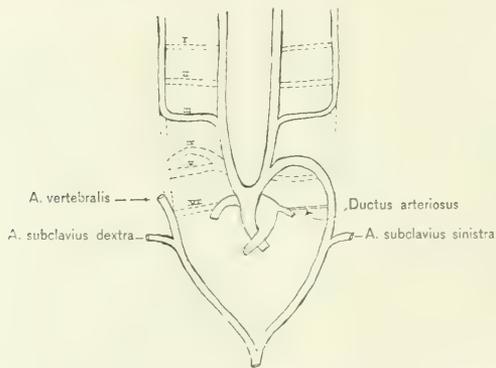


Fig. 3 Diagram indicating possible development of this anomaly (modified from Piersol).

and were covered with a good panniculus adiposus. Strangely enough, the right ulna was 1 cm., and the right radius 0.9 cm. longer than the corresponding bones of the left arm although the humeri were the same length. Although the arteria volaris superficialis originated abnormally from the radial artery about midway between the origin of the radial and the wrist no other vascular anomalies were present in the arm. The mm. palmares longi and extensores carpi ulnaris were completely absent.

The veins also exhibited slight abnormalities in the region under discussion. The vena azygos was unusually large, having a diameter of nearly 1 cm. where it joined the aorta. The v. hemiazygos crossed the vertebral column at the level of the

8th and the v. hemiazygos accessorius at the level of the 6th thoracic vertebra. It anastomosed with the v. intercostalis suprema, which joined the v. anonyma sinistra dorsal to the internal mammary and together they drained the first seven intercostal spaces.

The accompanying diagram (fig. 3), modified from Piersol, indicates the probable developmental explanation of this anomaly.

In conclusion, I wish to express my thanks to Professor Meyer for his assistance in the preparation of these notes.

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## APPLICATION OF THE CAJAL METHOD TO TISSUE PREVIOUSLY SECTIONED

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While engaged in a study of the mammalian brain-stem it became necessary for me to prepare serial sections of certain regions by the Cajal method. In order to obtain a series of a large portion of the brain-stem, and in order to stain any individual section by the method of Nissl instead of that of Cajal, the problem was presented of applying the Cajal method to mounted sections. Since the Cajal method can be applied to tissue fixed in strong alcohol (the best fixative also in case of the Nissl method) the problem appeared to resolve itself into the establishment of certain conditions, mechanical rather than chemical, which would insure a uniform and sharp reduction of the silver in the cell-bodies and cell processes. Not only has this expectation proved justified but it has been possible to obtain satisfactory Cajal preparations from sections previously stained by the Nissl method; the value of applying the Nissl and Cajal methods successively to the same section needs no comment. My problem involves the demonstration of all portions of the neurones rather than that of the internal structure of the cell-body, and although the internal structure of many cells is well shown I have made no effort to adapt conditions to obtain this end. Of course the method should be varied according to the animal, region of the nervous system, or the especial picture desired. I shall now describe a method which fulfilled the requirements of my problem, namely, the correlation of the internal structure of the cell body (Nissl) with the connections of the cell processes (Cajal).

I shall assume that the tissue to be prepared is an adult human brain-stem. The entire brain-stem is placed in two liters of 95 per cent alcohol and kept in the ice-box, but the tissue should not freeze. After two hours change alcohol and leave in cold overnight; thereafter the alcohol should be changed once every day. After two or three days the tissue should be cut into pieces 1 cm. in thickness, and should remain in 95 per cent alcohol two or three days longer (always at low temperature). Dehydrate several days in absolute alcohol; clear in chloroform of good quality for two days or longer, changing twice. The pieces of tissue are then placed in a mixture of equal parts of chloroform (fresh) and 42° paraffin at 35°C. for at least twelve hours and at 50°C. for four hours. To remove chloroform place in 42° paraffin at 50° C. for at least twelve hours (four to six changes). Embed in 50 to 55° paraffin. The essentials of this technique, which is the best

one for the Nissl method also, are the avoidance of acid, quick and thorough removal of water (keeping tissue in the cold until dehydrated) and thorough impregnation with paraffin. Prolonged stay in alcohol and prolonged heating are necessary, and poor results are due not to these causes but, on the contrary, to maceration in weak alcohol and to incomplete dehydration and impregnation.

The sections are then cut; for the purpose of following the course of cell-processes  $24 \mu$  is not too thick. The sections to be prepared by the Cajal method are mounted on large slides ( $2 \times 3$  inches), while every sixth and every seventh section is mounted on a separate slide (one section on each slide) to be stained by the Nissl method. Of the two Nissl preparations one is retained permanently, while the other, after being studied and drawn, is restrained by the Cajal method. Mounting the sections demands certain precautions: The slides are covered with a thin film of fresh egg albumen, without glycerine or preservative, flooded with distilled water, and the albumen uniformly distributed by rubbing with a brush. The slides are then placed on a warm bar or water bath and the sections allowed to flatten out. Drain off excess of water and after the slide begins to cool (about ten seconds) express the water from beneath each section by means of a small brush. The brush must be moist but not wet, and must be rotated so that it passes over the section as a roller. The direction of the movement is indicated by the nature of the section; in the case of large sections it is often necessary to begin at the center of each section and work outward radially. The water pressed out must be removed from the slide. If the sections are not too warm and if the brush passes over the sections as a roller, a fair amount of pressure may be employed; but at first the pressure should be light and increased after most of the water has been expelled. I recommend this method for mounting large paraffin sections of the brain (especially if the sections be rather thick), and have never observed any bad results. The sections dry rapidly. The essentials in mounting are to obtain a uniform mixture of distilled water and fresh egg albumen, and to express all water from beneath the sections by rolling them with a brush. The brush should be about 4 mm. in diameter and about 1 cm. long.

When the sections are dry the paraffin is cautiously melted on a hot bar and the slides placed successively in xylol, absolute alcohol and 95 per cent alcohol; in any of these reagents the slides may remain for days without injury (this applies also to the Nissl method), but they must not be placed, even for a short time, in weak alcohol or water, and of course all traces of acid must be avoided. From 95 per cent alcohol the slides are placed (separated by intervals of at least five minutes) in a 1.5 per cent solution of silver nitrate in distilled water; this solution is kept at 55 to 60°C. A beaker holding 100 to 150 cc. is satisfactory, and this amount of solution will serve for about eight  $2 \times 3$  inch slides; if used too long the solution becomes discolored and after reduction the slides show a diffuse reduction of the silver and poor contrast. In the silver bath the slides remain 10 to 15 minutes.

The most important step in the technique is the reduction. This is accomplished by a 1.5 per cent solution of pyrogallic acid in distilled water to which is added 5 per cent of formalin. This solution should be made up fresh, and should not be kept longer than two hours. The sublimed pyrogallic acid should be used, and I cannot recommend the crystalline form, which remains colorless in solution. Into a dish 8 cm. in diameter enough of the reducing solution is poured to make a depth of about 1 cm.; this solution must be renewed for each slide (2 × 3 inch). Before placing the slide into the reducing solution three conditions must be observed, namely, the excess of silver solution must be drained off, the hot (55 to 60°C.) sections must not be allowed to dry, and finally, the silver solution must be uniformly distributed over the slide (or at least over the sections and in their immediate neighborhood). To avoid drying one may pour some cool silver solution on the slide and allow slide to cool before draining off excess. A uniform distribution of the silver solution may be obtained by tilting the slide; with the aid of a bit of filter paper isolated drops may be removed or distributed. Remove excess of silver also from under surface of slide. As soon as this is accomplished the slide is quickly placed, in a horizontal position and with the sections on the upper surface, in the reducing solution. The slide is left in the reducing solution absolutely undisturbed for about 1¼ minute. The slide must undergo once more the silvering and reducing processes, and to accomplish this successfully proceed as follows: As soon as slide has remained the proper time in the reducing solution remove and flood it two or three times with distilled water; the object of this procedure is to remove some, but not all, of the pyrogallic acid. The slide is then placed on a hot bar or water bath at about 60°C. and flooded with a 1.5 per cent solution of silver nitrate (not previously used) and allowed to remain one to two minutes; during this process the sections usually become darker. If under the microscope the reduction appears sufficient the slide should be very quickly washed with distilled water (two seconds) before reducing (but in the great majority of cases the slide should not be washed before the second reduction); if the first reduction appears unusually successful the second silver bath should be employed half strength, followed by reduction without previous washing. After second silver bath flood slide with cold silver nitrate solution to avoid any local concentration, due to evaporation, drain off excess, distribute evenly and reduce 1¼ minute as before; usually the same solution may be used for both reductions, but should then be thrown away. The sections are washed for a few minutes or longer in several changes of distilled water (or tap water), placed in 95 per cent alcohol, absolute, xylol, and mounted under cover in balsam. Sections may remain for hours in water, alcohol or xylol

The principal defects to be guarded against are insufficient deposit of silver, unequal deposit of silver over slide (due to unequal distribution of silver solution before reduction), and most troublesome of all, diffuse deposit of silver (often in a finely granular state) which seriously injures the contrast. This last difficulty is due either to the deterioration of

the first silver bath (replace with fresh), to the deterioration of the reducing solution (not good for more than two hours), or to the presence of too much reducing solution in the sections when they undergo the second silvering (wash out somewhat more of the reducing solution before placing in silver bath). A third silvering and reduction is usually not desirable. Sections not too heavily stained may be counterstained with toluidin-blue as follows: Stain in a 1 percent aqueous solution of toluidin-blue (Grübler) for two hours or longer (or for a few minutes if heat be employed), wash quickly in several jars of 95 per cent alcohol, absolute, xylol, mount; the stain washes out in alcohol more readily than in the case of a primary toluidin-blue stain.

To restrain toluidin-blue sections by the Cajal method: Dissolve off cover, and thoroughly remove balsam in xylol followed by absolute. The sections are then washed in 95 per cent alcohol until as much of the toluidin-blue as possible has been removed; it is well to leave the sections in 95 per cent alcohol overnight. When the slides are placed in the silver solution the rest of the toluidin-blue comes out rapidly upon agitating the slide, but an excess of toluidin-blue causes the formation of a coarse precipitate. Accordingly, after washing the slides one or two minutes in hot silver nitrate solution they should be placed in a clean silver bath. Thereafter, the technique is the same as for unstained sections, except that the silver bath deteriorates more quickly.

I recommend this modification of the Cajal method for the human central nervous system, where I have employed it with success both on unstained material and on material previously stained with toluidin-blue. It has been used with success also on the brain of the lemur. In case of the rat's brain it failed, but just as poor results were obtained by silvering and reducing *en bloc*. The advantages of the method are: the possibility of obtaining a series of a large piece of tissue with uniform stain from center to periphery of section, the possibility of staining any section of the series by either the Cajal or Nissl method, and the conversion of a Nissl into a Cajal preparation. It is especially to be recommended when the tissue is very valuable or when the pieces of tissue are so large as to involve much labor in their preparation, since the partial failure in the case of one slide does not prevent the successful preparation of the rest. Finally, this method has an important advantage over other methods which demonstrate neurofibrils in previously sectioned material, since with the use of only a few simple reagents a paraffin section may be completed and in balsam within twenty to thirty minutes.

Below is a summary of the method, but I wish to emphasize the necessity of strict adherence to the details previously described.

1. Thorough fixation in cold in 95 per cent alcohol. After several days cut into pieces 1 cm. thick and leave in alcohol several days longer.
2. Thoroughly dehydrate in absolute (in cold).
3. Chloroform about two days.
4. Thorough impregnation with paraffin.

5. Sections mounted by means of water and pure egg albumen; water expressed as described.

6. Xylol, absolute, 95 per cent alcohol.

7. Silver nitrate (1.5 per cent solution in distilled water) at 55 to 60° C. for 10 to 15 minutes.

8. Drain off excess; careful distribution of solution over slide; avoid drying.

9. Reducing solution (fresh) about  $1\frac{1}{4}$  minute; slide must lie horizontally and remain undisturbed.

10. Flood two or three times with distilled water.

11. Place on hot bar at 60° C. and cover with silver solution; leave one or two minutes.

12. Drain off excess and distribute solution uniformly.

13. Reduce for second time  $1\frac{1}{4}$  minute.

14. Wash thoroughly.

15. Ninety-five per cent alcohol, absolute xylol, balsam, cover.

16. To restrain toluidin-blue preparations, dissolve out stain in 95 per cent alcohol and proceed as above, but note that first silver bath soon becomes contaminated.

In conclusion, I wish to remove any impression of being too enthusiastic over the results of this method; many slides will be unsatisfactory. But with practice nearly all slides may be rendered satisfactory for tracing cell processes, and an ever increasing number (at least for human material) show really beautiful pictures.







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