THE AMERICAN JOURNAL

OF

ANATOMY

VOLUME 17
1914–1915

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.

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1914–1915

No. 1. NOVEMBER

No. 2. JANUARY

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THE COMPARATIVE DISTRIBUTION OF MITOCHONDRIA IN SPINAL GANGLION CELLS OF VERTEBRATES[1]

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FOURTEEN FIGURES (THREE PLATES)

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INTRODUCTION

This paper constitutes part of a program of research, outlined some years ago, the object of which is to lay a sound foundation for the study of functional changes in nerve cells. In the first place, I confined my attention to a single type of nerve cell and brought the most refined methods of general cytology to bear upon an analysis of its cytoplasmic constituents ('12); I then began a study of the same cytoplasmic constituents in development, paying particular attention to the histogenesis of neurofibrils ('14 a); and I now wish to present some observations, based upon a study of the comparative distribution of nerve cell granulations, which I believe throw some light on the structural relations and physiological significance of both the mitochondria and the Nissl substance. They indicate that mitochondria are fundamental constituents of spinal ganglion cells, which probably play a part in metabolism; and that the Nissl substance occurs in living spinal ganglion cells as a dif-

[1] Aided by the Carnegie Institution.

THE AMERICAN JOURNAL OF ANATOMY, VOL. 17, NO. 1
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fuse, continuous deposit instead of in the form of granules and flakes of various size and shape. I have received valuable help from my father in this work, for which I am very thankful.

LITERATURE

I have prepared the following summary so that what we know of the comparative distribution of mitochondria in vertebrate nerve cells may be seen at a glance. In it, the forms in which mitochondria are described by me for the first time, are given in italics, the others being recorded in plain type:

Mammalia:	Primates	*Homo*[2]
		Macacus rhesus
	Rodentia	Guinea-pig (Nageotte '09 a and '10; Laignel-Lavastine and Jonnesco '11)
		Lepus (Levi '96; Held '97 a; Nageotte '09 b; Schirokogoroff '13)
		Mus norvegicus albinus
	Carnivora	Felis (Altmann '90; Lobenhoffer '06) Canis (Lobenhoffer '06)
Aves:	Gallinae	Gallus (Duesberg '10)
		Columba (Cowdry '12)
Reptilia:	Ophidia	*Eutaenia sirtalis*
	Chelonia	Testudo graeca (Busana '12)
		Pseudemys hieroglyphica
Amphibia:	Anura	Rana (Altmann '90); Tadpole (Duesberg '12)
		Rana palustris
	Urodela	*Necturus maculatus*
Pisces:	Teleostei	Salmo (Furst '02; Lobenhoffer '06)
Cyclostomata:	Petromyzontes	Petromyzon marinus and Ammocoetes branchialis (Mawas '10)

The evidence which these investigators have presented will now be considered.

Guinea-pig

Nageotte ('09 a, p. 472) demonstrated certain bacilli-like bodies in peripheral nerve fibers fixed in Tellyesniczky's bichromate acetic fluid and stained with Altmann's anilin fuchsin. He found them also after preliminary fixation in 10 per cent formalin followed by Tellyesniczky's fluid and staining in the same way.

[2] Collin ('13, p. 1123) has recorded the occurrence of mitochondria in the neuroglia cells of the spinal cord of man.

He claimed that they were mitochondria on account of their morphology. He did not specify either the tissue or the animal selected, but I gather from a second paper ('10, p. 41) that it was the sciatic nerve of a guinea pig which he used. In this second paper he employed an osmic bichromate mixture (presumably that of Altmann) and succeeded in staining bodies of somewhat different form and cytoplasmic distribution which, to my mind, resemble mitochondria much more closely.

Laignel-Lavastine and Victor Jonnesco ('11, p. 699) observed granules, rods and rows of granules in the Purkinje cells of the cerebellum. The technique consisted of fixation in 12 per cent formalin, followed by treatment with Weigert's neuroglia mordant, staining with hematoxylin and by the methods of Altmann and Benda. They employed, in addition, Regaud's formol-bichromate-hematoxylin technique and concluded that the structures thus revealed were mitochondria.

Lepus

Levi ('96, p. 3) studied granules which he called 'fucsinofili (rossi)' in spinal ganglion cells by means of Galleotti's modification of Altmann's method, which consists of using methyl green as a differentiator in place of picric acid. He observed rod-like bodies which took the anilin fuchsin deeply and could be distinguished with ease from the green colored Nissl bodies. These structures occurred in the axone hillock where the Nissl substance is absent. Their staining reactions, form and distribution are sufficient basis on which to conclude that they are mitochondria.

Held ('97 a, p. 293) observed bodies, which he called neurosomes, in spinal ganglion cells (plate 11, figs. 1 and 2) and in Purkinje cells (plate 11, figs. 7–9). In a second contribution ('97 b, p. 307) he records the observation of similar bodies in the anterior horn cells of the lumbar region of the spinal cord treated by an iron hematoxylin method devised by himself (plate 12, fig. 7). I have already shown ('12, p. 497) that these bodies are mitochondria.

Nageotte ('09 b, p. 825) applied the Altmann method, the Benda method and iron hematoxylin as advocated by Meves to the nervous system. By means of all three he demonstrated bodies in anterior horn cells, Purkinje cells, neuroglia cells and ependymal cells which he believed to be mitochondria on the basis of their characteristic morphology and distribution.

Schirokogoroff ('13, p. 522) observed structures which he concluded to be mitochondria on account of their filamentous and rod-like shape, cytoplasmic distribution and independence of the Nissl bodies, in spinal ganglion cells, in the cells of the spinal cord, medulla, brain and retina by fixation in Regaud's fluid and staining according to the directions of Altmann, Heidenhain and Benda.

I have confirmed these observations by making preparations of mitochondria in Purkinje cells by Bensley's anilin fuchsin methyl green method.

Felis

Altmann ('90, p. 52) described and figured granules (bioblasts) in nerve cells by means of his well-known method of technique. An analysis of his descriptions and figures shows that some of these bodies are mitochondria. In plate 11, figure 3 they are illustrated, stained brilliantly with anilin fuchsin after fixation in his osmic acid mixture, in the Purkinje cells of the cerebellum (p. 52). They occur in the form of granules which tend to be arranged in rows and they are present in the axone as well as in the dendrites, which excludes the possibility of confusion with the Nissl substance. His figure (plate 13, fig. 1) and his description (p. 53) of granules in the cells of the granule layer of the cerebellum are not sufficiently precise to justify the identification of the bodies as mitochondria; but his illustrations (plate 14, figs. 1–2) of rod-like and filamentous structures in the wall of the cerebral vesicle and neural tube of a cat embryo undoubtedly relate to mitochondria.

Lobenhoffer ('06, p. 491) found granules and rods, similar to those described by Altmann, in the cells of the spinal cord, brain and retina by Schridde's modification of Altmann's technique.

He clearly showed their independence of the Nissl substance by counterstaining with toluidin blue, so that in the same cell the granules of Altmann were colored red and the Nissl substance blue. The fixation and staining reactions of these Altmann granules, together with their rod-like form and cytoplasmic distribution, show that they are in truth mitochondria.

Canis

Lobenhoffer ('06, p. 491) also found bodies, which he likewise termed Altmann's granules, in the cells of the spinal cord, brain and retina of the dog. I have no hesitation whatever in calling these granules mitochondria since they correspond very closely with mitochondria which I have observed in the cells of the Gasserian ganglion of the dog by Bensley's anilin fuchsin methyl green technique.

Gallus

Duesberg ('10, p. 612) recorded chondriosomes (mitochondria) in adult ganglion cells (spinal?) by the Benda method. He states, however, on the same page, that no elements stainable by the Benda method occur in the adult nerve fiber.

I have found, by the application of Bensley's anilin fuchsin methyl green method, that mitochondria are very abundant in the cell bodies and medullated processes of adult spinal ganglion cells of the fowl.

Columba

I have already demonstrated, in a previous paper ('12, p. 497), that mitochondria occur in adult spinal ganglion cells of the pigeon.

Testudo graeca

Busana ('12, p. 621) studied granules and rods, which he styled mitochondria, in the cells of the spinal ganglia, cord, medulla, optic lobes and cerebellum by means of the Regaud method, as modified by Luna, and the Benda method. They presented different characteristics in the large and in the small

cells. In the former he observed them in the form of granules, and, in the latter, as granules and very tiny rods. This differentiation apparently (p. 620) applies to the large and small cells of the spinal ganglion as well as to those found in other parts of the brain. There can be no question that the bodies which he described as mitochondria are mitochondria.

Rana

Altmann ('90) also observed and figured (plate 11, fig. 2) structures in the spinal ganglion cells of the frog which may be identified with mitochondria for reasons similar to those already given in detail in the case of the cat.

Duesberg ('12, p. 809) made some observations on plastosomes (mitochondria) in the ganglion cells of tadpoles, but is unwilling to arrive at any conclusion regarding them.

Petromyzon marinus Lin. and Ammocoetes branchialis Bloch

Mawas ('10, p. 126) investigated the structure of the spinal ganglion cells of these two forms by the Regaud method. His description apparently applied to the former. He found that in the adult the nerve cells may be divided into two groups, the large ones and the small ones. In the small cells he found granules and filaments, distributed throughout the cytoplasm, electively stained in black with the hematoxylin. The larger cells differ in that the cytoplasm is less intensely stained and that there are present in addition a number of vesicles which are considerably larger than the granules and filaments. These vesicles seem to be more numerous in the region of the nucleus but they extend into the dendritic processes as well. Where they are absent the granules and filaments take their place. He concluded that the granules and filaments of the small cells are mitochondria and that the vesicles are allied to them.

It is doubtful whether the descriptions of Furst ('02), Motta-Coco and Lombardo ('03) and Motta-Coco ('04) relate to mitochondria.

Furst ('02, p. 389) described peculiar rings, threads and knots in the ganglion cells of salmon embryos, but he studied, in addition (p. 391), carp, Trutta fario, Coregonus laveretus, etc. He states that in the first place he only used Perenyi's fluid, but that later he made use of sublimate acetic and other mixtures without obtaining such good preparations as he did with Perenyi's fluid. He says (p. 389) that these structures occurred in the cytoplasm of cranial and spinal ganglion cells, but not in the ganglion cells of the brain and spinal cord, which is rather ambiguous. There is considerable difference of opinion among investigators with regard to the nature of these bodies. Van der Stricht ('09, p. 21) thinks that they are mitochondria, while Duesberg ('12, p. 806) states his conviction that they do not belong to the category of plastosomes (mitochondria). Although I acknowledge that mitochondria sometimes occur in the form of rings, threads and knots, I consider that since the fixatives which he employed are not adapted for the demonstration of mitochondria, the absence of these strange structures in the ganglion cells of the brain and spinal cord, together with a careful scrutiny of his figures are insurmountable obstacles against the conclusion that he was dealing with mitochondria.

Motta-Coco and Lombardo ('03, p. 637) described certain bodies which they called 'granulazioni fucsinofili' in the spinal ganglion cells of the rabbit and frog. They state (p. 640) that the spinal ganglion cells contain in their cytoplasm and nuclei a certain number of fuchsinophile granules of variable dimensions, which are situated in the interfibrillar spaces and in the achromatic network of the nucleus. They employed Flemming's fluid, Muller's fluid and a solution of chromic acid and formalin, as fixatives. They stained with methylene blue, safranin and eosin and by Levi's method of coloring with fuchsin and differentiating in alcoholic picric acid. One gains the impression that they believe their 'granulazioni fucsinofili' to be the same structures as those described by Levi ('96, p. 3). Motta-Coco extended the work in a second paper ('04). Duesberg ('12, p. 809) asserts that the plastochondrial (mitochondrial) nature of these bodies is very doubtful, and I am in accord with him, particu-

larly so on account of Motta-Coco and Lombardo's statement that the granulazioni fucsinofili occur within the nuclei. Nevertheless, the bodies which they find in the cytoplasm may be mitochondria; those described by Levi certainly are.

It may well be asked why the observations dealing with mitochondria in nerve cells are so scattered and so few? The reasons are technical, psychological and theoretical.

The fixatives in general use for the study of the nervous system exercise a destructive action on mitochondria. Mitochondria are completely dissolved, for instance, by the acetic acid in Carnoy's 6:3:1 fluid. It is on account of this property that the acetic acid in the mitochondrial fixatives devised by Benda, Meves, Bensley and others is reduced to a maximum of a few drops only. Formalin generally destroys them unless its action is modified by the addition of some other ingredient. Regaud combines potassium bichromate with it for this purpose. Alcohol and corrosive sublimate are also bad fixatives for mitochondria. And conversely the chemicals best adapted for the preservation of mitochondria, like osmic acid and potassium bichromate, are but little used in neurological technique because of their poor penetration. The concentration of attention upon the nucleus and nuclear changes made this condition of affairs worse because the very chemicals which destroy mitochondria give the clearest nuclear detail.

The observations which first laid the basis for our present conception of the significance of mitochondria were those of Benda ('99) on sex cells. The unfortunate lack of correlation between neurologists and psychiatrists, on the one hand, and general cytologists, on the other, has resulted in the former ignoring the significance of these observations of Benda, followed as they were by the important work of Meves, Duesberg and others, for more than a decade. The position assumed by hematologists is quite analogous, because we are only now, largely through the efforts of cytologists, beginning to hear something of mitochondria in blood cells.

Meves ('08, p. 845) advanced a theory which has exercised a dominating influence upon the trend of mitochondrial investi-

gation. It was that with the specialization of the embryo into different organs and tissues, primitively similar cells assume special functions which find expression in characteristic structures or differentiations. All these products, no matter how heterogeneous they may be, arise through the metamorphosis of one and the same elementary plasma constituent, the chondriosomes (mitochondria). Thus the neurofibrils are, according to his conception, to be classified as products of mitochondria. Hoven's ('10, p. 475) work on the transformation of mitochondria into neurofibrils in the developing nerve cell has generally been accepted (Firket '11, p. 545; Arnold '12, p. 289, and others) as a ratification of Meves' hypothesis, although Duesberg ('12, p. 745), under whose direction Hoven did his work, does not claim that he demonstrated his point conclusively. Since it was supposed that the mitochondria became transformed into neurofibrils, it was natural to believe that mitochondria are absent in the adult nerve cell after neurofibril formation has ceased. Thus we find that Meves ('10, p. 655), Duesberg (10, p. 612) and Hoven ('10, p. 478) have expressed their opinion that there are no mitochondria in fully developed nerve cells. Consequently investigators have looked for them and failed to find them. I repeated Hoven's work and found that the facts do not justify his conclusion that mitochondria become transformed into neurofibrils (Cowdry '14 a, p. 414). Now that a reaction is taking place against Meves' hypothesis (vide Gurewitsch '13, p. 126; Levi, '13, p. 550; Cowdry '14 a, p. 409, et al.) we may look forward to the study of mitochondria in nerve cells receiving the attention which it certainly merits.

MATERIAL AND METHODS

This investigation has been limited to spinal ganglion cells. Spinal ganglia were selected because they are easy to obtain, fluids penetrate them rapidly and because I believe that they constitute suitable material for experimental studies. All the animals, with one exception (Homo), were adults. The sex of the animals and the dates of the observations are recorded. Sizes were estimated by inserting, in the ocular, a micrometer

disc, each space on which had been estimated, with the combination used (Zeiss apochromatic objective 1.5 mm. and compensating ocular 4), to be equivalent to 1.5μ. Cells, the greatest diameter of which measured 30μ or less, were classified as small, the others being referred to as large.

In the study of fresh tissues the greatest care was taken to obtain isotonic media. Where possible the cells were observed in their native tissue juices without the addition of any foreign fluid. Occasionally, however, it was found necessary to employ some sodium chloride, Ringer's or Locke's solution. I used janus green (M. L. B.) and diethylsafranin, which I made myself ('14 b) from diethylsafraninazodimethylanilin, extensively as vital dyes for mitochondria; and nilblau B extra (B. A. S. F.), methylene blue medicinale (M. L. B.) and new methylene blues GB, N, NSS, NSSF, NX, R and RRR (Cassella), which Dr. H. M. Evans kindly gave to me, for the lipoid globules and Nissl substance.

The standard mitochondrial methods of Bensley ('11, p. 309), Altmann ('90, p. 27), Benda ('02, p. 752), Meves ('08, p. 832) and Regaud ('11, p. 3) were employed in the study of fixed tissues. The technique as originally outlined by these investigators was adhered to with as little deviation as possible. The temperature and time were not kept constant. Bergamot oil was often substituted for xylol as a clearing agent, but only after experimentation had shown that doing so did not alter the specificity of the stain. The duration of impregnation in paraffin at 60°C. ± varied from one-half to two hours according to the size of the piece of tissue. Solubilities were tested by fixation in fluids containing different amounts of acetic acid, alcohol, corrosive sublimate and formalin, because it is well known that these substances exercise a destructive influence on mitochondria. This was done by fixation in Zenker's fluid with and without acetic acid, Carnoy's fluid, absolute alcohol and formalin.

The observations were carefully controlled by comparing the unstained cells with vitally stained ones and permanent preparations. Where possible a number of animals of the same species were studied in order to eliminate individual variations.

OBSERVATIONS

The evidence which I have gathered together for the identification of mitochondria in the spinal ganglion cells of vertebrates[3] is based on a consideration of their morphology, distribution, staining reactions and solubilities. It is set forth in table 1. The spinal ganglion cells of man, for example, contain rod-like bodies; which occur in the axone and axone hillock, as well as

TABLE 1

ANIMAL	ROD-LIKE	AXONE AND HILLOCK	JANUS GREEN	DIETHYL SAFRANIN	BENSLEY'S METHOD	ALTMANN'S METHOD	BENDA'S METHOD	MEVES' METHOD	REGAUD'S METHOD	2 % OSMIC ACID	ZENKER	ZENKER LESS ACETIC	CARNOY 6:3:1	ABSOLUTE ALCOHOL	10 % FORMALIN	MITOCHONDRIA
Homo.....	+	+			+	+	+	+	+	+	−	+				present, fig. 1, m
Macacus....	+	+	+		+	+	+	+	+		?	+	?			present, fig. 2, m
Guinea-pig.	+	+	+		+	+	+	+	+	+	?	+	−	−	−	present, fig. 3, m
Mus.....	+	+	+		+	+	+	+	+		−	+	−			present, fig. 4, m
Pigeon.....	+	+	+		+	+	+	+		+	−	+	−	−	−	present, fig. 5, m
Eutaenia...	+	+	+	+	+	+	?		+							present, fig. 6, m
Pseudemys.	+	+	+	+	+	+	+	+	+		−	?	−	−	−	present, fig. 7, m
Rana.....	+	+	+	+	+	+	+	+	+		?	+	−		−	present, fig. 8, m
Necturus...	+	+	+		+	+	+	?	?							present, fig. 9, m

in the cell body; which stain characteristically by the mitochondrial methods of Bensley, Altmann and others; which are fixed by the action of 2 per cent osmic acid and are destroyed by the acetic acid in Zenker's fluid. They may, therefore, be termed mitochondria and are represented in figure 1, *m*. In the table '+' signifies a positive reaction, '−' a negative one. '?' a doubtful one and, where there is no record, it is to be understood that the test has not been applied.

[3] Mitochondria also occur in the nerve cells of invertebrates. I have demonstrated them with both janus green and the anilin fuchsin methyl green method in the nerve cells of Callinectes hastatus, Cancer borealis, Limulus polyphemus, Fulgur canaliculatus and Nereis virens; and with janus green alone in Eshna, Loligo pealii, Homarus americanus, Venus mercenaria and Mytilus edulis. They are rather more variable in number, size and staining reactions in invertebrate than in vertebrate nerve cells.

Mitochondria evidently occur in the spinal ganglion cells of all the forms which I have studied. Their appearance, in specimens prepared by Bensley's method, is illustrated by the figures on plates 1 and 2. The figures show the form relations very well, but the color values could not be reproduced on account of the expense. The following description applies only to those parts of the nerve cell found within the spinal ganglion. The peripheral and central processes have, of necessity, been ignored.

The morphology of the mitochondria is remarkably constant. They vary, in all the animals investigated, from granules (0.25–0.75μ, measured in Bensley preparations) to rods (1–2μ) and filaments (2–4μ). Sometimes the granules are arranged in rows. The rods may be dumb-bell shaped or pear-shaped and the filaments occasionally exhibit varicosities. I am unable to distinguish between the spinal ganglion cells of any of these animals on the basis of their mitochondrial content alone. Morphologically the mitochondria in the spinal ganglion cells of man are identical with those of the monkey, guinea-pig, white rat, etc.

The arrangement of mitochondria within the cell is subject to but slight variation. They are generally distributed evenly throughout the cell body. Occasionally they are more numerous in the region bordering on the axone hillock. This was observed in the human cell illustrated in figure 1. In the large cells the mitochondria are generally found between the flakes of Nissl substance, while in the small cells they are imbedded in it (figs. 10 a, 12 and 13). In some of the small cells the mitochondria are confined to the central cytoplasm (fig. 13 b). This condition is rare. They tend to be oriented parallel to the cell wall so that they are placed more or less concentrically about the nucleus. The guinea-pig cell shown in figure 3 illustrates this very nicely. In the medullated processes they are always arranged with their long axes parallel to the length of the process. I have not studied their relations in non-medullated fibers.

The microchemical reactions of mitochondria are likewise very constant. Janus green and diethylsafranin gave very disappointing results, as compared with their action on blood cells,

for instance. I find that they stain the mitochondria in embryonic nerve cells of vertebrates (chick), and in fully differentiated nerve cells of invertebrates (edible crab, Callinectes hastatus) much more brilliantly than the mitochondria in the spinal ganglion cells of the adult vertebrates which I have examined.

The relative amount of mitochondria is apparently constant in the nerve cells of the different animals although there is a certain amount of variation among the spinal ganglion cells of the same animal, which could not be accurately determined on account of the difficulty in enumerating them. The amount of mitochondria illustrated in the figures would be more uniform than it is were it not for this individual variation. Where the mitochondria are few in number lipoid granules are abundant and vice versa.

The relation of mitochondria to *lipoid globules*[4] was studied in detail in the guinea-pig, but a reciprocal relationship between the relative amounts of the two was observed in all the animals investigated. It is easy enough to distinguish typical mitochondria (fig. 2, *m*) from typical lipoid (*l*) but it is impossible to determine whether the intermediate stages (*i*) are true mitochondria or true lipoid. Coincident with the change in shape from mitochondria to lipoid there is a progressive increase in the resistance to acetic acid. The mitochondrial methods show no difference between the fixation and staining properties of mitochondria and lipoid, but the lipoid globules are not so readily destroyed by fixation in Carnoy's fluid and Zenker. By varying the concentration of the acetic acid in Zenker's fluid a series of gradations may be obtained in spinal ganglion cells of the pigeon between lipoid and no mitochondria, on the one hand, and lipoid plus the normal amount of mitochondria, on the other. Both mitochondria and lipoid stain with nilblau B extra in the cells of all the animals, but the actual transformation of the one into the other could not be followed. Probably

[4] I have applied the term 'lipoid' (derived from the Greek, λίπος, fat) to these globules, loosely, without meaning to convey any exact knowlege of their composition. It is possible that their composition may differ in the various animals which I have studied.

this is due to the difficulty of keeping the nerve cell under observation, in approximately normal environment, for a sufficient length of time.

The lipoid globules are of about the same size in the different animals. They are always spherical and vary from 1 to 5μ in diameter (measurements made on unstained cells in approximately isotonic media). Their arrangement in the cell is perfectly typical. They occur in clumps in any part of the cytoplasm. They sometimes extend into the processes, but rarely attain a size of more than 1μ when thus situated. They vary in amount in different cells of the same animal, sometimes being absent and sometimes present in abundance. The large and the small cells show them in equal number. Their presence is not accompanied by any sign of pathological change.

The *Nissl substance*, also, occurs in the spinal ganglion cells of man, monkey, guinea-pig, white rat, pigeon, snake, turtle, frog and necturus. Its morphology is more variable than that of either the mitochondria or the lipoid. It presents constant differences in the large and in the small cells (30μ or less, measured in fixed tissues) of man, monkey, guinea-pig and white rat. I am not yet prepared to make definite statements about the other animals. In the cells, which have been fixed, it occurs in irregular aggregates of variable size and shape, which are absent in the axone hillock and are larger about the periphery than in the more central parts of the cell; but in small cells it is generally (though not invariably) present as a diffuse, continuous, amorphous deposit. All gradations exist between these extremes. Figure 10 shows some cells occurring in a single section of a guinea pig's spinal ganglion prepared by Bensley's method. At the top the diffuse condition is seen, at the bottom, well formed Nissl bodies. It is not due to the distance of the cells from the surface, and resultant variations in the rate of penetration of the fixative, because the cell shown in figure 12 is in actual contact with the one illustrated in figure 10 d. They both typify the condition mentioned although the fixative acted on them both in the same way and at the same time. Fixation in 2 per cent osmic acid and in Altmann's osmic bichromate

mixture gives similar results. When sublimate acetic, picro-sulphuric or Carnoy's fluid are used, in place of the acetic osmic bichromate mixture of Bensley, the Nissl substance occurs in the form of discrete, well formed masses in both types of cells. This is shown in figure 11, drawn from a spinal ganglion cell of a guinea-pig fixed in Carnoy's 6:3:1 fluid and stained with anilin fuchsin methyl green (compare figs. 10 and 11). Attempts to devise a fixative which would give a uniform Nissl substance in the large cells failed. The diffuse Nissl substance in the small cells stains with variable intensity with basic dyes (methyl green, fig. 13, *a* and *b*).

All my attempts to see formed Nissl bodies in unstained cells were unavailing. The least toxic methylene blues (methylene blue medicinale and the new methylene blues GB, N, NSS, NSSF, NX, R, and RRR), applied to spinal ganglion cells of the frog (chosen because with a cold-blooded animal the warm stage may be dispensed with), give first a diffuse coloration of the cytoplasm followed by the appearance of irregular masses of stained material, which look something like the Nissl bodies of fixed tissues. I was unable to determine whether these blue stained bodies represent pre-existent structures in the cytoplasm which are invisible in the unstained cells by virtue of their low refractive index.

Pigment was seen only in the spinal ganglion cells of Necturus and Rana. It has a bright orange color in Necturus and occurs in a variety of forms. Sometimes as highly colored masses (0.5–1μ measured in fresh, unstained cells) which tend to fuse together in a conglomerate way (1–6μ). It may occur as spherules of variable size (0.5–10μ) and intensity of coloration; sickle-shaped bodies (4.5μ long by 0.5μ wide), threads of variable length and diffuse masses may also be made out. It varies in amount in different cells of the same animal and in different animals. The pigment in Rana is bright orange in color. It occurs in the shape of globules and angular masses of considerable range in size (0.5–1.5μ) either distributed evenly throughout the cell or else gathered together in clumps. It is absent

in the processes. No sickle-shaped bodies were seen. The pigment is just as abundant in the small cells as in the large ones. No relationship was observed between the pigment and mitochondria, although I would not deny that such may exist.

DISCUSSION

The analysis of the literature and the observations which I have recorded show that mitochondria occur in the nerve cells of representative examples of the chief vertebrate groups. Their properties are so constant that the spinal ganglion cells of man cannot be distinguished from those of any of the other forms, which I have studied, on the basis of their mitochondrial content.

A similar condition prevails in the developing nerve cell, in which I have found mitochondria in stages from chick embryos, before the differentiation of any somites, to adult fowls. My series consists, in addition to the embryos already described (Cowdry '14 a, p. 397), of preparations of four-day embryos, of chicks just hatched and adult fowls. Unfortunately the later stages are not very close together, but the series, as a whole, is sufficiently complete to show that the relative amount, microchemical properties and arrangement of mitochondria are approximately constant, although their morphology changes progressively. In the spinal ganglion cells of a 35-somite chick, for example, their average length is from 3 to 5μ, but in the spinal ganglion cells of an adult fowl they are seldom more than 1μ in length. These measurements were made on fixed and stained material.

I believe that this constancy of mitochondria in the phylogeny and ontogeny of the nerve cell is significant, for it may serve as a clue to their function. Their presence, the constancy of their microchemical properties, relative amount and distribution within the cell in the different stages of evolution and development may, perhaps, be explained on the supposition that their function is common to these same stages. It must be, therefore, a fundamental and a basic function, inseparably connected with the life of the cell and subject, of course, to qualitative as well as

quantitative fluctuations. Mitochondria occur in all parts of the nerve cell, in the axone as well as in the dendrites, for these basic chemical reactions to which I refer are common to the whole protoplasm. Herein the mitochondria differ from the Nissl substance, which we must look upon as a more specialized cell organ. While I regret and deplore the absence of experimental evidence, I nevertheless feel myself justified in entertaining, on these grounds, for the time being at least, the hypothesis that mitochondria are concerned with the metabolism of the nerve cell.

This conception is supported by evidence from analogy concerning mitochondria in other than nerve cells. They may almost (but not quite) be regarded as coexistent with protoplasm. They occur, with few exceptions, in the cells of plants (Guilliermond '12, p. 412; Maximow '13, p. 242, and many others) as well as in those of animals. They are transmitted from one generation to another through the medium of the egg, and, in some cases, of the sperm also (Meves '13, p. 225). On cell division, whether it be by mitosis or amitosis, they are distributed in approximately equal amounts to the two daughter cells (Romeis '13, p. 17). Cells in which mitochondria do not occur are less numerous but no less instructive. All attempts to demonstrate them in bacteria (Guilliermond '11, p. 200), in the most superficial cells of the epidermis (Firket '11, p. 544) and in the circulating red blood cells of man (Cowdry '14 b, p. 17) have failed. Moreover, Guilliermond ('12, p. 379) states that he cannot demonstrate mitochondria in the later stages of the life cycle of barley, wheat, maize, bean and pea. Now bacteria are primitive organisms in which the occurrence of a nucleus is disputed, and the epidermal cells and blood corpuscles are, like the cereals mentioned by Guilliermond, terminal stages in cytomorphosis. We may conclude, therefore, that mitochondria are present in the majority of actively functioning cells, that they decrease progressively with a diminution in cell activities and that they are absent in the most primitive organisms. In other words, that, so far as we know, the ground

substance, alone, of the constituents of the cytoplasm is more fundamental. These considerations, coupled with the fact that the mitochondria, wherever they occur, show a certain uniformity with respect to their morphology and microchemical properties, to my mind, support the view that they play a part in metabolism.

We do not know in what way they may be associated with metabolic processes in nerve cells, but I believe that Fauré-Fremiet, Mayer and Schaeffer ('10, p. 95) have furnished us with a clue. They found, by a detailed study of solubilities, fixatives and stains, that mitochondria are chemically a lipoid albumin complex. This is of course a vague statement, because there are many different sorts of lipoid and a multitude of albumins, but it is nevertheless of importance. The reciprocal relations which I have described between mitochondria and lipoid, the observations of Mawas referred to on page 6, those of M. R. Lewis and W. H. Lewis ('14, p. 332) on tissue cultures, and, above all, the artificial imitation of mitochondria by Löwschin support this view. Löwschin ('13, p. 203) made the so-called "Myelinformen" of lecithin in water, different salt and albumin solutions (resulting in the formation of lecithalbumin) which showed masses with the same morphology as mitochondria. He observed granules, rods, threads and rows of granules. The granules divided directly and the threads longitudinally. He was able to influence their form by changing the physicochemical properties of their environment. They were soluble in acetic acid and could be fixed by formalin, osmic and chromic acid. In a later paper ('14, p. 269) he discovered that particles of lecithin and cholesterin suspended in glycerin-gelatin, when fixed, stained like mitochondria by the various mitochondrial methods. Kingsbury ('11, p. 316), also, has emphasized the similarity which obtains in the microchemical reactions of mitochondria and lipoid. I find, moreover, that janus green, in addition to being a vital dye for mitochondria, stains both lecithin and egg albumin (Kahlbaum), the latter more intensely.

Notwithstanding the astonishing general similarity of mitochondria in diverse types of cells slight, but perplexing, varia-

tions do occur in their morphology, resistance to acetic acid and other fixatives and in their staining properties which require to be explained. It is here, I believe, that a knowledge of their constitution helps us, if we postulate slight variations in the relative amounts of lipoid and albumin. Not only the amount but also the properties of mitochondria vary with changes in metabolism. This is true in spermatogenesis where Regaud ('08, p. 661) has detected a progressive increase in the resistance of mitochondria to acetic acid. We may here be dealing with an increased consumption of albumin which would tend to increase the relative amount of lipoid in the mitochondria and in this way increase their resistance to acetic acid. The failure of Jordan ('11, p. 59) and Wildman ('13, p. 427) to observe mitochondria in the early stages of spermatogenesis may thus be explained because they used fixatives with a constant percentage of acetic acid, which perhaps destroyed the mitochondria of low resistance and left the others. It is possible that the formation of lipoid in nerve cells is a process essentially similar but carried to an extreme. In any case the importance of being able to see in the living nerve cell and in many others deposits of the nature of mitochondria, by means of the vital dye janus green can scarcely be overestimated.

The Nissl substance is, in a sense, a more specialized constituent of nerve cells. Recent investigation has shown that material very closely allied to it occurs in many types of cells, muscle cells and gland cells for example. Substances of this sort are grouped together and called 'chromidia.' Bensley's demonstration ('11, p. 359) that the chromidial substance is present in the living acinus cells of the pancreas as a continuous deposit which would lead one to believe that Held and others (vide Barker '99, p. 131) may be right in their assertions, which have been too frequently ignored, that the Nissl bodies, instead of being pre-formed elements in the living nerve cell, result from the coagulation (or precipitation) of a substance present in a diffuse, amorphous state. I have recorded some observations which apparently support this notion, so far as the spinal ganglion cells of vertebrates are concerned, my experience with other types of nerve cells being too limited to justify any assertions.

I have failed to observe Nissl bodies in unstained spinal ganglion cells teased out in isotonic media, and the vital dyes which I have used (Methylene blue medicinale (M. L. B.) and New Methylene blue GB, N, NSS, NSSF, NX, R and RRR) give first a diffuse staining of the ground substance followed by the appearance of typically blue stained Nissl bodies, which look very much like coagula. I would be inclined to interpret the gradations which I have observed in fixed preparations between the diffuse Nissl substance in the small spinal ganglion cells of the guinea-pig and the well formed Nissl bodies in the large ones (fig. 10, a, b, c and d) as due to a difference in the coagulability of a Nissl substance originally present in the diffuse state in all. The fact that by the use of other fixatives, which are perhaps more energetic coagulants or precipitants, formed Nissl bodies may also be seen in the small cells (fig. 11, a) supports this view.

On this supposition the fact that the Nissl bodies are larger in the peripheral cytoplasm would easily be explained because the action of the fixative is more powerful there. The 'chromophile cells' (Barker '99, p. 123) may be interpreted as cells in which the coagulability of the diffuse Nissl substance is reduced. I do not desire to draw a sharp line of demarcation between the terms 'coagulation' and 'precipitation.' Researches on a differentiation between functional groups of nerve cells, on the basis of the appearance of their Nissl bodies as seen in fixed preparations (Malone '10, etc.) stand as genuine contributions, even though they be interpreted on a hypothetical difference in coagulability due to a quantitative or a qualitative change in the Nissl substance or both. For if the diffuse Nissl substance is present in different concentration in certain types of cells, the coagula resulting from similar fixation may be different. Or it may be that the Nissl substance actually differs in kind. The great mass of work which has been done on nerve cell physiology, with the Nissl bodies as indicators (Dolley '13, etc.), is best interpreted on the first supposition, of a quantitative change; for here, coincident with the reduction in the Nissl substance in fatigue, the Nissl bodies become smaller and present a more dif-

fuse appearance (chromatolysis). In other words, with the discharge of function the concentration of the diffuse Nissl substance is lessened and its coagulability decreased so that the aggregates become smaller and smaller until finally no coagula result. The amount of chromatolysis would be inversely proportional to the concentration of the Nissl substance and its degree of coagulability.

RESULTS

1. Mitochondria occur in the spinal ganglion cells of man, monkey, guinea-pig, white rat, pigeon, snake, turtle, frog and necturus, in which they are characterized by the constancy of their morphology, distribution, relative amount and microchemical properties.

2. There is a reciprocal relation between the amount of mitochondria and lipoid granules in the spinal ganglion cells of these vertebrates.

3. The coagulability of the Nissl substance, on fixation, increases progressively in the gradation which exists between the small and the large spinal ganglion cells of man, monkey, guinea-pig and white rat.

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EXPLANATION OF PLATES

All the figures were drawn by me with Zeiss apochromatic objective 1.5 mm., aperture 1.30, compensating ocular 4 and camera lucida. They were reduced one-fourth in reproduction so that their magnification as they now appear on the plates is 960 diameters. Only spinal ganglion cells, cut about 4μ in thickness, are represented. I wish to acknowledge many helpful suggestions from Mr. James F. Didusch.

ABBREVIATIONS

c., canalicular apparatus
i., intermediate between (*m*) and (*l*)
l., lipoid
s., sheath cells
m., mitochondria
n., Nissl substance

PLATE 1

EXPLANATION OF FIGURES

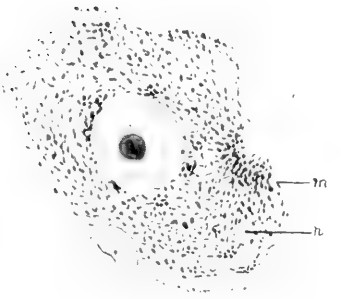
1 to 4 These figures are intended to illustrate the generality of the occurrence of mitochondria in the spinal ganglion cells of different vertebrates. All the drawings have been made from specimens prepared by Bensley's method, by which the mitochondria and lipoid are colored red and the Nissl substance green. Unfortunately the colors could not be represented.

1 Homo, colored, female, 2 years (?). Anatomical diagnosis: miliary tuberculosis, tuberculous pneumonia and terminal acute bronchopneumonia; Johns Hopkins Hospital autopsy no. 4095; fixed 4 hours after death; (this material was obtained through the courtesy of Dr. Whipple of the Johns Hopkins Hospital).

2 Macacus rhesus, female, adult; chloroformed March 11, 1914.

3 Guinea-pig, female, 332 grams; decapitated March 24, 1914.

4 Mus norvegicus albinus, female, length (snout to root of tail) 19 cms.; decapitated February 27, 1914.



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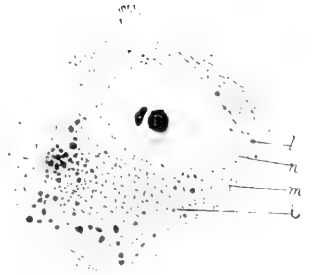

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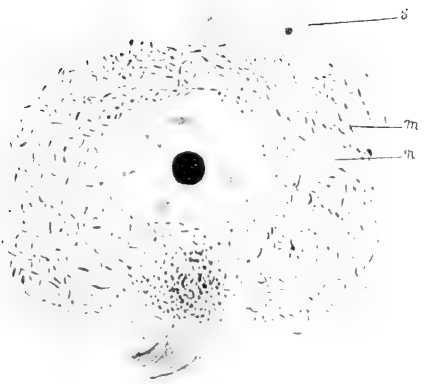

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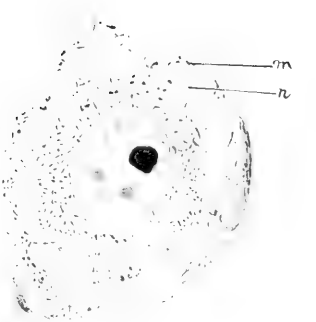

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E. V. C. del.

PLATE 2

EXPLANATION OF FIGURES

5 to 9 The preparations illustrated on this plate are also designed to show the comparative distribution of mitochondria in the spinal ganglion cells of vertebrates. All of them have been drawn from preparations made by Bensley's method. Plates 1 and 2 should be compared.

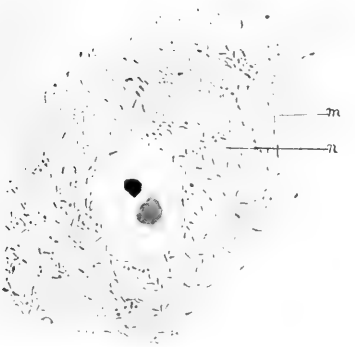
5 Pigeon, adult; decapitated, April 18, 1913.

6 Eutaenia sirtalis (garter snake), male, total length 62 cms.; decapitated April 21, 1914.

7 Rana palustris, adult; decapitated, January 22, 1914.

8 Pseudemys hieroglyphica (terrapin), male, length 17 cms.; decapitated, March 7, 1914.

9 Necturus maculatus, female, length (snout to anus) 23 cms.; decapitated, March 26, 1914.



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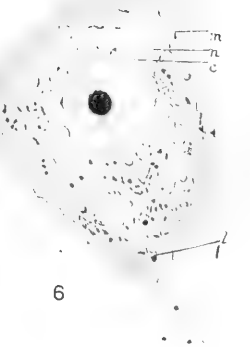

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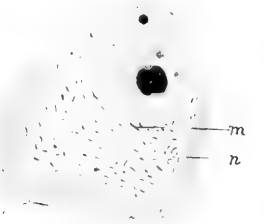

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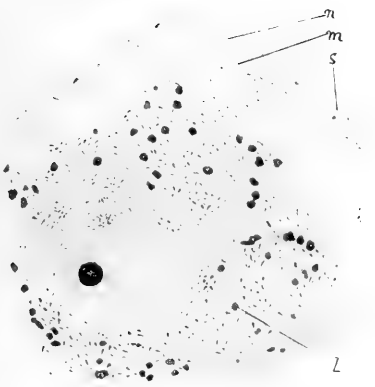

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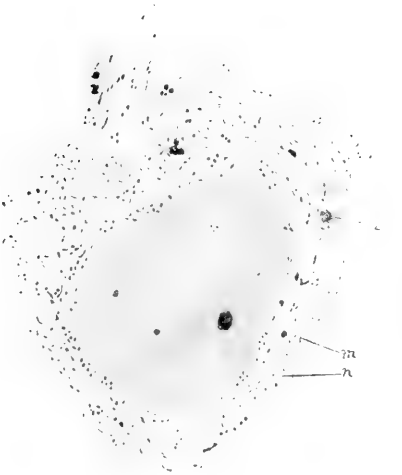

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PLATE 3

EXPLANATION OF FIGURES

10 to 14 These figures illustrate the difference in the coagulability of the Nissl substance in the large and small spinal ganglion cells of the guinea-pig and monkey. All, except figure 11, have been drawn from specimens prepared by Bensley's method. Figure 11 is from a guinea-pig's spinal ganglion, fixed in Carnoy's 6:3:1 fluid and stained with anilin fuchsin methyl green (p. 15).

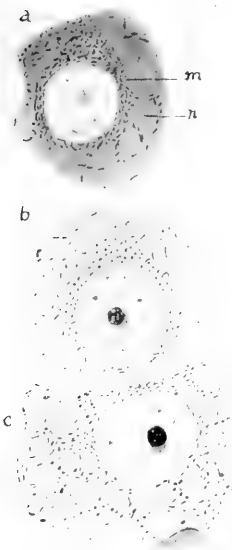
10 Four cells from a single section of a guinea-pig's spinal ganglion, prepared by Bensley's method, showing the graduation between the diffuse Nissl substance (*n*.) in the small cells (*a* and *b*) and the sharply defined Nissl bodies in the large ones (*c* and *d*). The mitochondria (*m*) are the same in both types of cells (p. 14).

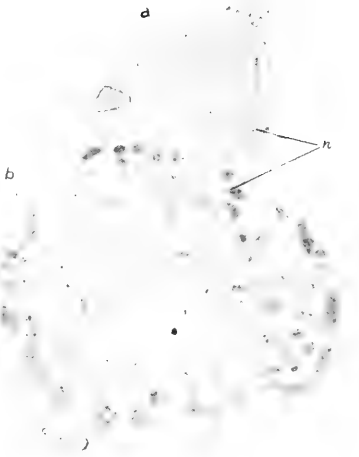
11 Two cells from the spinal ganglion of a guinea-pig fixed in Carnoy's 6:3:1 fluid and stained with anilin fuchsin methyl green. Here the Nissl substance (*n*) is present in the form of discrete granules in both the large and the small cells. The mitochondria are absent, having been destroyed by the fixative (p. 15).

12 From a small spinal ganglion cell in direct contact with the large cell illustrated in figure 10 d. The Nissl substance within it is present as a diffuse deposit (p. 14).

13 Two small spinal ganglion cells of a monkey (same animal as fig. 2) to show that the diffuse Nissl substance often stains with variable intensity. It is much lighter in (*a*) than in (*b*) (p. 15).

14 Spinal ganglion cell, from the same preparation, also showing the diffuse Nissl substance (*n*) axone (p. 15).





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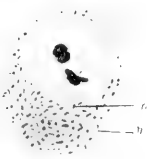

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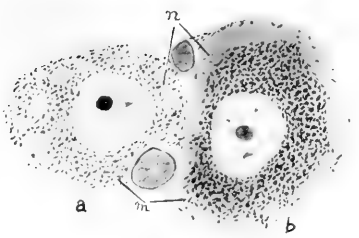

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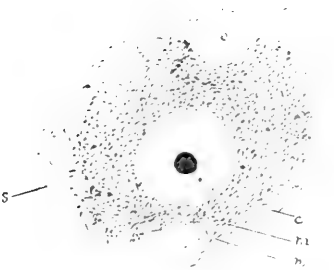

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E. V. C. del.

THE ANATOMY OF A 17.8 MM. HUMAN EMBRYO[1]

F. W. THYNG

From the Department of Comparative Anatomy of Harvard Medical School

EIGHT FIGURES

CONTENTS

[1] This work has been aided by a grant from the Elizabeth Thompson Science Fund.

INTRODUCTION

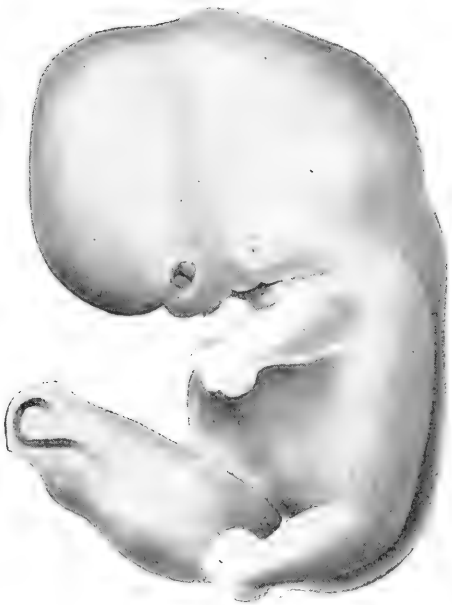
This work was undertaken at the suggestion of Prof. C. S. Minot, and carried on for the most part in his laboratory during the years 1906 and 1907 when the author held an Austin Teaching Fellowship in Histology and Embryology at the Harvard Medical School. Its completion, unfortunately delayed by other work, has been accomplished in the Anatomical Department of the University and Bellevue Hospital Medical College. During the progress of the work many helpful suggestions have been received from Professors Minot and F. T. Lewis of Harvard, and Prof. H. D. Senior of Bellevue, for which I am very grateful. I also desire to express my gratitude to Mr. W. T. Oliver of Lynn, Massachusetts, for the careful manner in which he has reproduced in finished form my original drawings.

The reconstructions upon which this work is based, were made from transverse sections of Embryo 839 of the Harvard Embryological Collection, chiefly by the modified graphic reconstruction method of His. The shading usually has been inferred from a study of the sections, but in a few instances wax models were made of regions requiring a fuller interpretation.

This embryo (extra-uterine) measured in formalin 17.8 mm., greatest length, with a neck breech of 16.7 mm. The greatest length in 80 per cent alcohol was 13.6 mm. In previous papers in which this embryo was referred to (Thyng '08, and Lewis and Thyng '08), the latter measurement was given.

EXTERNAL FEATURES

The external features of this embryo are seen in profile view in text figure 1, a reproduction of figure 104 in Minot's ('10) "Laboratory text-book of embryology," also in part in plates 3 and 5. The neck-bend is approximately a right angle; the cephalic flexure is also very nearly a right angled bend, so that the oral aperture is in close proximity to the cardiac region. The dorsal flexure has disappeared almost entirely, only a slight elevation persisting to mark its earlier position. Above this



Text fig. 1 Human embryo of 17.8 mm. (H. E. C. 839). × 5 diams. (after Minot).[2]

elevation there is a shallow depression, said to disappear in the course of development.

A distinct groove, extending transversely between the medial angles of the developing eyes, separates the forehead from the root of the nose. The maxillary process of either side has joined the adjacent lateral and median nasal processes, obliterating the naso-optic grooves. The nares are open, but separated by a rather low, broad septum. A triangular space still intervenes between the globular processes so that the median region of the upper lip is not well differentiated. The median groove between the ventral ends of the mandibular arches has been obliterated, but differentiation between the chin and lip regions has not

[2] A reproduction of figure 104, page 153 of "Laboratory Textbook of Embryology," Charles Sedgwick Minot, edition of 1910, published by P. Blakiston's Son and Company, Philadelphia.

occurred. The line of fusion between the first and second branchial arches is marked ventrally by a transverse groove, dorsal to which is seen the fossa conchae. The grooves between the other branchial arches have disappeared.

The limb buds extend nearly perpendicularly to the longitudinal axis of the body. The upper project slightly beyond the ventral border of the body, and show a differentiation of arm, forearm, and clearly outlined digits. The latter protrude slightly beyond the border of the hand-plate. Upon the lower limb buds are slight indications of developing toes.

Circular thickenings of the epidermis on the lateral body walls mark the developing mammae. In section these thickenings appear slightly convex on the surface, and project into the underlying mesenchyma. The umbilical cord as it leaves the body wall bends towards the right.

DIGESTIVE SYSTEM

Oral cavity. The primitive oral cavity appears in median sagittal section in plate 1, but is represented more fully in plate 4, a portion of the tongue having been cut away. It is a short, dorso-ventrally compressed passage consisting of a roof and a floor, the epithelia of which meet laterally at the angle of the mouth. The external aperture of the primitive oral cavity, the rima oris, (*R.or.*) is indicated as seen from the exterior in plates 2, 3 and 5; as seen in median sagittal section in plates 1 and 4. It is a narrow, horizontal expanded orifice, concave dorsally where it is bounded by the fused maxillary and median nasal processes, convex ventrally where it is formed by the united mandibular processes.

Dorsally the oral cavity communicates with the pharynx, the division between the two being marked in the median line on the roof by the stalk of the hypophysis (*Hyp.*) While the roof, as thus bounded, is of considerable extent, the floor is very limited, consisting of merely the anlage of the lower lip and teeth.

The lip-grooves are just beginning to indent the oral epithelium. The lower lip-groove is seen in sagittal section in plate 1.

Into the anterior part of the roof of the primitive oral cavity open the large primitive choanae (*Ch.pr.*) of the olfactory vesicles, separated from one another by a primitive nasal septum. The roof also presents on either side of the median line, a prominent longitudinal ridge, the palate process (*Pr.pl.*). This process of the right maxillary arch is clearly shown in plate 4 where a portion of the tongue has been removed. It begins at the intermaxillary process (*Pr.i.m.*) and extends dorsally, lateral to the choana (*Ch.pr.*) along the primitive oral cavity and the cephalic part of the pharynx. Its free ventral border nearly reaches the floor of the pharynx in the region of the alveolo-lingual groove. The roof of the oral cavity between the palate processes thus forms a high arch which receives the dorsum of the developing tongue. It is evident that the primitive oral cavity now comprises a portion which will be cut off later by the union of the palate processes and nasal septum, to form in part the nasal cavities of the adult.

The hypophysis (*Hyp.*), alluded to above, consists of a distal spade-like portion, connected to the oral epithelium in the median line by a slender stalk with reduced lumen. It is represented in side view in plate 4, in median sagittal section in plate 1. Its flattened body impinges upon the ventral surface of the infundibulum, on either side of which it projects dorsally as a short, blunt process. On its cephalic surface there is a distinct ridge or fold, continuous with the anterior surface of the stalk.

Pharynx. A left lateral view of the entodermal wall of the pharynx is seen in plates 2 and 5. The interior, as it appears in median sagittal section, is represented in plate 1. It is a broad, dorso-ventrally compressed canal which narrows rapidly in passing caudally to divide into trachea (*Tr.*) and oesophagus (*Oe.*). The epithelium of the roof and floor of the pharynx meet to form an external ridge, which extends from near the angle of the mouth to the lateral border of the oesophagus. Corresponding with the ridge there is an internal furrow.

The tongue (plate 1) is a comparatively broad elevation of the floor of the pharynx, composed of a large cephalic part (*t'*) intimately fused with a smaller caudo-lateral division (*t''*), the root. A surface view of the dorsum of the tongue would show the line of

fusion of these two parts to be a V-shaped groove with the apex of the V pointing caudad. In plate 1, the apex and right limb of this groove, sulcus terminalis, are represented, the apex marking the place of origin of the median thyreoid gland. Laterally the tongue is bounded by deep alveolo-lingual grooves which converge cephalad so as to separate its tip from the subjacent mandible.

According to Kallius ('01) the anterior anlage of the tongue is derived chiefly from the dorsum of the ventral ends of the mandibular processes, the so-called lingual folds, the tuberculum impar of His contributing only a small part thereto. Hammar, however, believes ('01), that the tuberculum impar is a transitory structure, and that the tip and body of the tongue are formed by a considerable area of the floor of the oral (pharyngeal) cavity. In regard to the development of the root of the tongue there is some disagreement in that His derived it ('85) from the ventral ends of the second and third visceral arches, while Born ('83) and Hammar ('01) limit it to the second arch.

Posterior to the root of the tongue, and fused with it, there is a broad, bilobate elevation (*Ep.*) which represents the epiglottis. It is a derivative of the third visceral arch (Born '83, and Hammar '01).

The first pharyngeal pouch (*Ph.P.1*, plate 5), the cavity of which ultimately, will form the tuba auditiva and cavum tympani, is seen at this stage to be an extensive, lateral, pointed, evagination of the pharyngeal wall, extending somewhat dorsally toward the depression of the primary meatus acusticus externus. It presents three surfaces, dorsal, cephalo-ventral, and caudo-ventral. The dorsal surface, which cannot be seen in the drawing, is triangular in outline; medially it passes over into the dorso-lateral wall of the pharynx. Dorsal to it is the cochlear division of the otocyst. The caudal boundary of this surface is the posterior tympanal ridge. The cephalic boundary is marked by a ridge overlying the tubo-tympanal sulcus of Moldenhauer ('77). This ridge extends from the tip of the first pharyngeal pouch in a cephalic and medial direction to the oral epithelium between the hypophysis and the angle of the mouth. The cephalo-ventral surface of the first pharyngeal pouch is concave. A dorso-ven-

tral ridge, representing the entodermal part of the first closing-plate, separates it from the caudo-ventral surface. Upon the caudo-ventral surface near the tip of the pouch there is a slight groove. This, the 'tensor groove' of Hammar ('02), marks the place of formation of the tensor tendon.

The second pharyngeal pouch (*Ph.P.2*, plate 2) appears on either side as a low evagination from the lateral pharyngeal wall. It is situated just caudal to the first pouch and projects towards the cephalic aspect of the glossopharyngeal nerve. According to Hammar ('03) this evagination represents only the dorsal part of the primary pouch. A deeply staining cyst is present in this embryo on either side of the pharynx, which evidently belongs to either the ectodermal or entodermal part of the second branchial groove. The left cyst is situated just lateral to the left glossopharyngeal nerve, while the right is just cephalad of the right glossopharyngeal nerve and in close relation to the second pouch. Piersol ('88) found that in rabbit embryos there were formed, in the development of the second pharyngeal pouches, two epithelial tubes on either side, one from the entoderm and the other from the ectoderm, both of which subsequently atrophied. The former, however, persisted longer than the latter. Hammar ('03) described and figured a structure in human embryos protruding above the margin of the tonsilar pouch. In early stages this was connected with the ectoderm, and hence he concluded that it was of ectodermal origin. Fox ('08) did not find any ectodermal remnant in this region of the pig embryo, but described and figured a long filiform process continuous with each of the second pharyngeal pouches.

The third pharyngeal pouches (plates 2 and 6) have lost their connection with the pharynx. They are now represented each by a compact cylinder (*Thy.*, plates 2 and 6) in the side of the neck, and which contains only a slight lumen. The cylinders converge caudally toward the median line and end approximately at the level of the aortic arch (*Arc.ao.*). The right and left cylinders become, eventually, the corresponding lobes of the thymus gland with the exception of the cephalic extremities which are

compact epithelial masses (not marked off in the figures) differing in structure from the rest of the anlagen. The cephalic portion of each cylinder is closely applied to the lateral wall of the common carotid artery, and is the part described by Katschenko ('87) as the nodulus thymicus, and by Fox ('08) as the carotid gland. The recent work of Hammar ('11) substantiates the view that this part eventually becomes separated from the thymic cylinder and (coming to lie at the caudo-dorsal border of the lateral part of the thyreoid gland) forms with its fellow of the other side the caudal pair of para-thyreoids. The cephalic ends of the thymic cylinders also show two hollow projections, a medial one, extending toward the pharynx and ending blindly, dorsal to the common carotid artery; and a more lateral process extending cephalad and ending blindly alongside the ventro-medial surface of the vagus nerve where the latter is crossed on its lateral side by the hypoglossal. The former or medial of these processes is evidently the remains of the thymo-pharyngeal duct. The lateral process seems to be the remains of the cervical sinus fused with the third pharyngeal pouch, as maintained for corresponding structures by Katschenko ('87), Fox ('08), and Hammar ('11), and not an outgrowth from the thymus as conjectured by Prenant ('94) and Bell ('05). Katschenko from a study of this structure in the pig, maintained that it formed a considerable portion of the head of the thymus, a view since corroborated by Prenant ('94) who, however, considers it of entodermal origin. Fox ('08) found that in the cat it apparently atrophied early in development, and that in later stages of the rabbit it had largely, if not entirely disappeared. He is inclined to think that, when it does persist, it does not form an integral part of the thymus, but merely an associated structure.

The fourth pharyngeal pouch of either side, exclusive of the so-called ultimobranchial (postbranchial) body (Greil '05) is represented by a solid epithelial mass (*P-thyr.* IV) seen in plates 2 and 6. These masses, which represent the cephalic pair of parathyreoids, are situated dorsal to the lateral lobe of the thyreoid and are entirely separate from the pharynx. That on the right

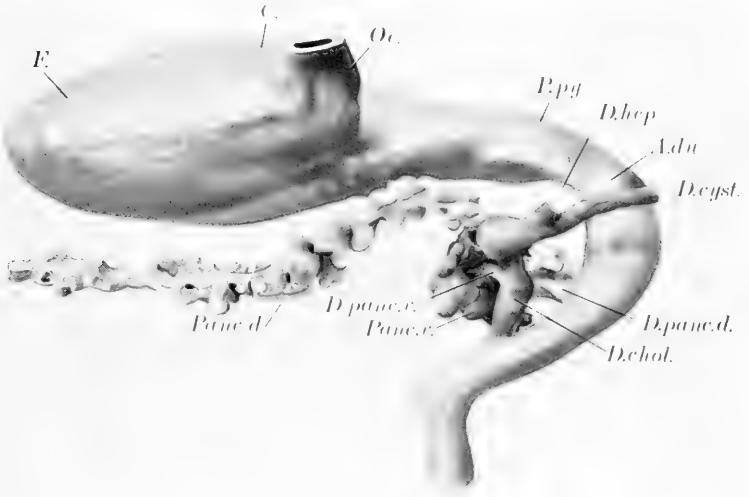
side is bilobate and somewhat removed from the proliferating entoderm of the thyreoid, but on the left the two are intimately connected.

The thyreoid gland (plates 2 and 6) is distinctly U-shaped, with the concavity of the U directed cephalad. The level of the slender connecting bar of the median thyreoid (*Thyr.m.*) is seen in plates 1 and 6. The arms of the U, derivatives in part of the ultimobranchial bodies (which are still discernible although intimately connected with the median thyreoid) are widened considerably dorso-ventrally, and terminate at a level corresponding with the cephalic ends of the thymic cylinders. Numerous proliferating cords of cells make its surface somewhat irregular. Its connection on the left with the parathyreoid anlage of the fourth pharyngeal pouch has been referred to above.

Salivary glands. The parotid and submaxillary glands are shown in plates 2 and 5. The parotid gland is represented by a small, solid cord of cells (*Gl.p.*) partly constricted off from the ridge leading caudad from the angle of the mouth and overlying the sulcus buccalis. The submaxillary gland is represented by a solid cord of cells (*Gl.s.*) but is larger than the parotid anlage. It projects from the caudal part of the floor of the alveolo-lingual groove into the underlying mesenchyma which for some space around the gland consists of closely packed cells for the development of a capsule. Its extremity is broader than the stalk, and shows slight indications of proliferating buds. The anlagen of the sublingual glands have not developed at this stage. That they are the latest of the salivary glands to develop, has been noted by His ('85), Chievitz ('85), Hammar ('01), Paulet ('10) and others.

Oesophagus. The oesophagus is comparatively long at this stage. The entodermal part of the tube only is figured in plates 1 and 2. This consists of an epithelium, containing four or more layers of nuclei. Numerous irregular cavities, as seen by Schultze ('97), and others, are found within the epithelium. They occur in scattered situations, and are separated from the surrounding mesenchyma usually by a single layer of columnar or cuboidal cells. In no case were they found to connect with the lumen of the

oesophagus. They are apparently vacuoles as maintained by Forssner ('07), and Johnson ('10). A granular coagulum was invariably found within them, but Kreuter ('05) who has studied these structures concludes that a degeneration of cells does not occur. For a general account of these structures see Lewis ('12).



Text fig. 2 Wax-plate reconstruction of the stomach, the duodenum and the pancreas; the model is represented somewhat ventrally, from the right side. *A. du.*, antrum duodenale; *C.*, corpus gastri; *D. chol.*, ductus choledochus; *D. cyst.*, ductus cysticus; *D. hep.*, ductus hepaticus; *D. panc. d.*, ductus pancreatis dorsalis; *D. panc. v.*, ductus pancreatis ventralis; *F.*, fundus gastri; *Oe.*, oesophagus; *Panc. d.*, pancreas dorsale; *Panc. v.*, pancreas ventrale; *P. py.*, pars pylorica gastri (H. E. C. 839). × 55 diams.

Stomach. The stomach has assumed practically the adult position. It is represented by the entodermal lining only, in plate 1 and text figure 2 (*Ga.*, *C*). Its primitive dorsal border representing the greater curvature has revolved to the left, while its ventral border, now identified by the lesser curvature, faces toward the right. Its entodermal lining is an epithelium containing four or more layers of nuclei. It presents at the cardiac end a prominent, dorsally directed pouch, the fundus (*F.*), which according to Keith and Jones ('01) develops as a localized outgrowth. That

this outgrowth is from the left side of the primitive stomach is evident, a relation evidenced in the adult by the reflection line of the lieno-gastric ligament. The body (*C.*) extends caudad and ventrally, passing into the attenuated pars pylorica (*P.py.*) which ends at a dilated portion of the duodenum (*A.du.*), the duodenal antrum of Retzius ('57). This, according to Lewis ('12) always marks the position of the pylorus. The external surface of the epithelium is for the most part smooth, but the internal surface is indented by slight grooves representing the beginning of the gastric pits.

Intestine. Most of the small intestine and all of the large are shown in plates 1 and 2 (*Duo.*, *Int.t.*, *Int.cr.*, *Int.r.*). The duodenal division of the small intestine (*Duo.*) leading from the pyloric end of the stomach passes transversely across the median line from the left to right. Here it bends dorsally and receives the duct of the dorsal pancreas (*D.panc.d.*), and the bile duct (*D.chol.*) (text fig. 2). The small intestine (*Int.t.*) then extends in a caudal and ventral direction, a little to the right of the median line, into the umbilical cord. In the umbilical cord it is bent twice in the sagittal plane at approximately 90°. On the left side of the second or cephalic bend (*pl.2*) it is continuous with the yolk-stalk (*D.vit.*). The portion of the small intestine beyond the second bend returns toward the body, cephalad of the part described, and terminates at the caecum.

The caecum forms a considerable dilatation and ends in the vermiform process (*Pr.ver.*) which projects ventrally and to the left.

The colon (*Int.cr.*) extends from the caecum, dorsally in the median plane, crossing to the left of the duodenum. Opposite the caudal extremity of the Wolffian body it turns caudally, and at an arbitrary point becomes the rectum (*Int.r.*). It is evident that the primitive U-shaped loop of intestine has undergone in this embryo a rotation of approximately 180°.

The epithelium of the duodenum a short distance caudad to the bile duct presents on its left side one prominent diverticulum, directed cephalad. Indications of similar outgrowths occur at twelve other places along the portion of the small intestine within

the umbilical coelom. The lumen of the duodenum, beginning somewhat cephalad of the duct of the dorsal pancreas and extending caudad to the duodenal diverticulum above mentioned, is subdivided into two or three parts by a proliferation of the epithelium in a manner similar to that already described by Tandler ('00) in a human embryo of 14.5 mm. This observation has been confirmed by Forssner ('07), Johnson ('10) and Lewis ('12). Beyond this proliferation the lumen of the small intestine is either a small cylindrical passage or a slight slit.

The yolk-stalk has a lumen for a short distance beyond its connection with the epithelium of the intestine. It then becomes a cord of degenerating cells, showing here and there traces of a lumen. Whether the yolk-stalk is still connected with a rudimentary yolk-sac could not be determined as these parts were cut away in the embryo.

The lumen of the colon as it leaves the caecum, is a very small cylindrical passage, but as it nears the rectum, it becomes a transversely directed slit (corresponding to the shape of the intestine which is compressed dorso-ventrally).

The cephalic portion of the rectum is a little larger in diameter than the colon, while the terminal part is more attenuated. The latter is circular in transverse section and the lumen is reduced to a very small cylindrical passage, which, however, does not connect with the shallow external depression (*An.*) between the protruding genital folds.

Liver. The liver (see plates 1, 2 and 4, Hepar) occupies the greater part of the cephalic and ventral regions of the abdominal cavity. The right lobe is much the larger, and extends from the cephalic end of the abdominal cavity on the right, caudad to a point on a level with the crossing of the duodenum by the colon. It is joined to the dorsal abdominal wall on the right of the dorsal mesogastrium, at the ventral region of the right suprarenal gland, by the plica venae cavae of Ravn ('89) (caval mesentery).

The caudate lobe is located between the caval mesentery and the lesser omentum. It projects somewhat toward the left into the bursa omentalis. The position of the quadrate lobe

can be determined from plate 2. It is situated to the right of the vesica fellea (*Ves.fel.*), lying between this and the umbilical vein (*V.um.s.*, plate 4).

The hepatic duct (*D.hep.*, plate 2, and text figure 2) takes origin from the hepatic trabeculae of the medial surface of the right lobe, ventral to the entrance of the portal vein (*V.P.*). It extends dorsally and to the left for a short distance and then caudad, uniting with the small cystic duct (*D.cyst*) to form the ductus choledochus (*D.chol.*).

The cystic duct has a slender lumen, and leads in a nearly dorso-ventral direction from a small, distal dilatation, the gall-bladder (*Ves.fel.*, plate 2). The latter is closely applied to the ventro-medial surface of the right lobe of the liver.

The common bile duct is considerably larger in diameter than either the hepatic or cystic ducts. It has a well-defined lumen, and extends caudad through the lesser omentum in an S-shaped course to open into the duodenum. This it does upon its left side, a short distance beyond the pyloric end of the stomach. The epithelium of the gall-bladder and of the cystic and common bile ducts, is devoid of knob-like buds and diverticula, met with in these situations in other embryos.

Pancreas. The pancreas of this embryo has been described, and pictured in a previous paper (Thyng '08), but for the sake of completeness it will be described briefly in this connection. It consists of two parts, a dorsal and a ventral pancreas (*Panc.d.* and *v.*, text figure 2). The dorsal pancreas is considerably the larger, and extends distally into the mesogastrium. It is essentially a long, irregular, hollow mass of epithelium with proliferating branches of varying length which in turn often give off hollow buds. Its duct (*D.panc.d.*) is larger than that of the ventral pancreas, contains a well-defined lumen, and opens into the left side of the duodenum nearer the stomach than the bile duct.

The ventral pancreas is in close relation with the proximal part of the dorsal anlage, the two having anastomosed ventral to the portal vein, and on the left of the common bile duct. Like the

dorsal pancreas, the ventral also shows a branching condition of its epithelium. The duct of the ventral pancreas (*D.panc.v.*) is short, and opens into the bile duct (*D.chol.*) near its entrance into the duodenum.

RESPIRATORY SYSTEM

The olfactory pits are described with the sense organs.

The entodermal lining of the larynx and trachea are represented as seen in ventral view in plate 6; from the left side in plate 2; and in the median sagittal section in plate 1. In plate 1 the anlage of the right lung is seen from the left side.

Larynx. The larynx, which opens from the pharynx by a T-shaped aperture, is placed immediately caudal to the epiglottis. The pedicle of the T or interarytaenoid notch, extends dorso-ventrally between the arytaenoid protuberances, and is bounded laterally by the aryepiglottic folds. A median raphé (*R.*) extends dorso-ventrally across the larynx, in a somewhat caudal direction, so as to close it temporarily.

Trachea. The trachea passes caudally to bifurcate into the two bronchi. The root of the left bronchus is shown in section in plate 1 (*Br.s.*) but, the greater portion of this bronchus and the corresponding lung have been removed.

Lungs. The entodermal outpocketings of the right lung and its pulmonary vessels (*A.pul.* and *V.pul.d.*) are represented in plate 1, as seen through the mediastinum. An eparterial (tracheal) bronchus is present on the right. It is situated dorsal to the right pulmonary artery, and a branch of the latter passes cephalad of this bronchus. The oesophagus (*Oe.*) passes between the developing lungs.

Pleural cavity. Each pleural cavity is closed off completely from the pericardial by the pleuro-pericardial membrane, but still communicates with the abdominal cavity. The aperture on the left is very small.

A blind prolongation of the right pleural cavity begins medially and dorsally to the anlage of the root of the right lung. From here it extends caudally and somewhat ventrally along the right

side of the oesophagus to the diaphragm. It then passes between the oesophagus and diaphragm and ends at a situation approximately corresponding to the level of the most caudal extension of the right pleural cavity. This diverticulum is ventral to the main pleural cavity and evidently corresponds to the space, termed by Broman ('04) the infracardial bursa. The infracardial bursa in this case differs from that described by Broman in that the primitive connection with the pleural cavity has been retained. The retention of this connection is of interest in that such a recess of the right pleural cavity exists normally in animals possessing an infracardial lobe of the right lung, and may be expected to occur occasionally in man.

UROGENITAL SYSTEM

The parts of the urogenital system are shown in plate 2 in which the urogenital sinus, the left Wolffian body and duct, the left metanephros and ureter, the left genital ridge, and Müllerian duct are shown from the left side.

Wolffian ridge and body. The Wolffian ridge extends almost the entire length of the abdominal cavity, its anterior three-fourths being occupied by the Wolffian body. The ridge tapers off bluntly at its cephalic end; caudad of the area of mesonephritic tubules (*T.W.*) it dwindles to a slight elevation upon the abdominal wall in which the Wolffian duct (*D.W.*) passes to the urogenital sinus (*S.u.-g.*). Under the influence of the rapidly growing liver and suprarenal glands the ridge has moved laterally from its primitively dorso-medial position.

Wolffian duct. The Wolffian duct (*D.W.*) passes through the ventro-lateral region of the Wolffian body receiving the tributary mesonephric tubules (*T.W.*). From the caudal end of the Wolffian body it extends through the remainder of the Wolffian ridge to end by piercing the dorso-lateral wall of the urogenital sinus (*S.u.-g.*). Close to its entrance into the sinus there is a slight dorsal enlargement of the duct which suggests the first anlage of the seminal vesicle. It should be noted, however, that, according to the investigations of Pallin ('01), the vesiculus seminalis first appears at a much later stage of development.

Müllerian duct. The Müllerian duct (*D.M.*) lies parallel with the Wolffian, being a little ventral to it. Its cephalic end opens freely into the abdominal cavity. The opening which occurs near the cephalic end of the Wolffian body, shows something of a fimbriated condition (*Fim.*). In the caudal direction the Müllerian duct seems to terminate in a blind pointed end close to the Wolffian duct. The point of termination is about on a level with the junction of the middle and caudal thirds of the Wolffian body.

Bladder. The ventral segment of the cloaca is divisible into two parts, a cephalic portion (*B*) which will ultimately form the bladder and a caudal portion (*S.u.-g.*) the urogenital sinus.

The region of the bladder anlage adjoining the urogenital sinus is expanded on either side to produce a dorso-lateral ridge. Into each ridge at the caudal extremity of the bladder anlage opens the corresponding ureter. The portion of the bladder remote from the urogenital sinus is continued as the allantois into the caudal wall of the umbilical cord. The proximal portion of the allantois consists of a solid cord of cells, the urachus, but distally isolated portions of the original lumen are found.

Urogenital sinus. The cephalic region of the urogenital sinus receives the Wolffian ducts. The orifices of these are placed on either side, a short distance from the median line. The remainder of the sinus extends into the median caudal part of the genital tubercle (*Pa.gen.*) as a laterally compressed structure. In profile view this part of the sinus appears somewhat triangular in outline. Its dorsal region encloses a cavity, but its latero-ventral walls are approximated to form an incomplete raphé, the urogenital membrane (*Mem.u.-g.*). This membrane is broken down in its extreme caudal part so that the urogenital sinus opens to the exterior.

The lower part of the sinus becomes the vestibule in the female, while in the male it forms the main portion of the urethra. The female urethra and the proximal part of the male urethra being formed by a canal differentiated later between the bladder anlage and the urogenital sinus.

Metanephros. The metanephros (*Met.*, pl. 2; see also pl. 1) is situated dorsal to the middle part of the Wolffian body, extending

approximately from a level of the twelfth thoracic to the second lumbar nerve, and appears externally as an oval, lobulated body. It consists of approximately eight branches arising from the cephalic extremity of the ureter, the renal pelvis. Each branch ends in a bilobated ampulla which is surrounded by condensed 'nephrogenic tissue.' The ureter as it leaves the developing metanephros, extends at first caudally and medially in the ventral part of the dorsal body wall. It then passes ventrally in the lateral parieties and opens into the extreme lateral wall of the anlage of the bladder. The ureteral orifices are slightly cephalad of those by which the Wolffian ducts communicate with the urogenital sinus.

Genital ridges. The ovaries are two compact, longitudinal protuberances projecting from the medial borders of the Wolffian bodies. They taper at either extremity, more gradually at the cephalic than the caudal. The cephalic end of the left ovary (*G.R.*) is partly hidden from view in plate 2 by the Wolffian body, but it does not extend quite to the cephalic pole of the latter. The caudal end of the ovary is on a level with that of the mesonephros by which it is hidden in the drawing. The cephalic region of each ovary is deeply marked by infolding of the germinal epithelium.

DUCTLESS GLANDS

The right suprarenal is shown in plate 4, the spleen in plate 2. The thyreoid, thymus, and parathyreoid glands are described in connection with the pharynx (pp. 38-40) and shown in plates 2 and 6.

Suprarenal gland. The suprarenal glands (*Gl.s.-r.*) are somewhat oval bodies of considerable size, developing in the ventral region of the dorsal body wall between the Wolffian body and the dorsal aorta. Their cephalic extremities lie at the level of the ninth thoracic nerves, i.e., a short distance cephalad of the caudal extension of the pleural cavities, of which they form, in part, the caudo-lateral wall. Their caudal extremities lie on a level with the twelfth thoracic nerves, i.e., slightly beyond the cephalic extremity of the Wolffian body.

Each gland has developed in the path of the cephalic part of the subcardinal vein of that side, and the vein has been subdivided to form the sinusoidal channels now found within it. Sinusoids in the suprarenal glands of mammals were demonstrated histologically by Minot ('00).

The cells composing the cortex are arranged in peripheral layers, surrounding a loose reticular core. These comprise what is usually termed the interrenal part of the suprarenal glands, from their resemblance to the interrenal bodies of Selachians. These interrenal bodies are being invaded on their medial surfaces by numerous scattered clumps of deeply staining cells, derived from the sympathetic ganglia, often termed the sympatho-chromaffine organs.

A deeply staining, oval mass of cells is present on either side of the inferior mesenteric artery, ventro-lateral to the aorta. Caudally these bodies reach the level of the proximal part of the common iliac arteries, while cephalad they are continued by scattered, smaller groups of cells to the caudal extremities of the suprarenal glands. They have a rich blood supply, and are intimately related to the sympathetic system in their locality. They unquestionably correspond to the aortic bodies discovered by Zuckerkandl ('01).

It seems very probable to the author that each of these main groups of cells has been partially isolated from the suprarenal gland of its side by the development of the large dorso-ventral segment of the supra-ureteral venous channel (plate 4) which now intervenes between them. Further evidence of this interpretation is the fact that a closer relation between the two exists on the left side in this embryo, where the vein in question is much smaller than its companion on the right.

Spleen. The spleen (*Lien.*) is clearly recognizable as a small, protuberance of the mesogastrium, containing dense mesenchyma. The tissue directly ventral to this protuberance, is permeated by a vascular network, supplied by the splenic artery, and drained by the splenic vein (*V.li.*, plate 4).

NERVOUS SYSTEM

Brain

The surface of the brain is represented from the left in plate 2, and from the right in plate 3. The brain is shown in median sagittal section from the right side in plates 1 and 4. By the cephalic flexure (*Flex.ceph.*) which occurs in the mesencephalon (*Mesen.*) the fore-brain is bent at an acute angle to the hind-brain. The cervical flexure (*Flex.cerv.*) is nearly a right angle.

Telencephalon. The telencephalon (*Telen.*) is sharply marked off dorso-laterally from the diencephalon (*Dien.*) by a deep external groove and a corresponding internal ridge or fold, the velum transversum. This fold forms the caudal boundary of the interventricular foramen (*Fo.int.*). From this fold the line of demarcation extends ventrally just behind the optic evaginations to the postoptic recess (*R.po.-op.*). The telencephalon thus bounded contains a median cavity, the anterior part of third ventricle. The latter communicates with the lateral ventricles by comparatively large crescentic openings, the interventricular foramina (*Fo.int.*). The median cavity is bounded anteriorly by the lamina terminalis. The large oval hemispheres represent the dorsal zones of the telencephalon. Each protrudes considerably beyond the lamina terminalis and presents orally two internal depressions with corresponding external swellings. These are the developing anterior and posterior olfactory lobes (*Rhin.*). Caudal and dorsal to the olfactory area the wall of each hemisphere is much thickened to form the corpus striatum (*C.str.*) which appears as a prominent swelling on the ventral surface in front of the praeoptic recess (*R.p.-op.*). Externally the position of the corpus striatum is marked by a shallow depression, the developing lateral fossa. The praeoptic recess is a slight groove passing transversely across the lamina terminalis into the optic stalk of either side. It represents the cephalic extension of the sulcus limitans, His ('92), Johnston ('09) and others. The ventral zones of the telencephalon comprise the area between the praeoptic and the postoptic recesses, which marks the place of later development of the optic chiasma. In regard to the area of

evagination of the optic vesicles it is somewhat questionable whether this belongs primarily with the tel- or di-encephalon.

Diencephalon. The boundary between the diencephalon and the mesencephalon (*Mesen.*) is a slight constriction extending nearly transversely across the brain toward the tuberculum posterius (*Tub.p.*). An ill defined furrow, sulcus limitans, extends cephalo-caudad along the internal surface of the diencephalon separating the dorsal zone above from the ventral zone or hypothalamus below. The roof of the diencephalon is thin; near its caudal limit there is a slight evagination which represents the first appearance of the epiphysis (*Corp.pin.*).

The ventral part of the dorsal zone caudad of the interventricular foramen is thickened, forming on the medial surface a low ridge, the developing optic thalamus. Dorsal to the thickening, the internal surface of each dorsal zone presents a prominent concavity. The lateral wall of the hypothalamus is thickened. Caudad of the recessus postopticus (*R.po.-op.*) the cavity of the diencephalon extends into a small median evagination from the floor-plate. The evagination is the anlage of the infundibular gland (*Gl.inf.*). The thickened knob-like termination of the gland is embraced by the forked distal end of the hypophysis (*Hyp.*). It becomes the neural lobe of the adult pituitary.

Mesencephalon. The conventional boundary between the mesencephalon (*Mesen.*) and metencephalon (*Meten.*) is the constricted portion or isthmus (*Isth.*). As already stated a slight constriction extending nearly transversely across the brain toward the tuberculum posterius (*Tub.p.*) divides it from the diencephalon. The roof-plate is comparatively thin, and bears no trace of the longitudinal ridge which has been described as occurring later. The floor-plate is considerably thicker than the roof-plate. The dorsal and ventral zones are distinct. The cavity of the mesencephalon (cerebral aqueduct) is more expanded in the caudal two-thirds of the mesencephalon, a large oval concavity appearing on the internal surface of each dorsal zone. The ventral zones are considerably thickened so that in the region of the oculo-motor nerve they project ventrally below the floor-plate. Externally the surface of the mesencephalon is smooth

except for slight dorso-lateral depressions on either side indicating the area of division into anterior and posterior colliculi.

Isthmus. The isthmus (*Isth.*) usually is described as the constricted portion between the mesencephalon and the metencephalon. It was considered by His ('92), and given in the Basel nomenclature ('95) as a distinct segment of the rhombencephalon which formed a marked ring somewhat narrower dorsally than ventrally.

In the corresponding area of the brain of this embryo there is on the internal surface of each ventro-lateral wall, a distinct transverse groove. The two grooves unite ventrally in a recess or sulcus (*Sul.*) caused by a depression of the floor-plate, perceptible on the external surface as a distinct elevation (*E.i-p.*) the eminentia interpenduncularis of His ('92). This sulcus was noted by Burckhardt ('91) and considered by him to be of general occurrence. It was observed in the human embryos by His ('92), and named by him the isthmus groove. Kupffer ('03–'05) named it the sulcus intraencephalicus posterior. The author believes that the transverse grooves mentioned above are the ventral continuations of the caudal mesencephalic neuromere; also that the adjacent brain wall should be regarded as the caudal part of the mesencephalon rather than a distinct division of the rhombencephalon.

Metencephalon. The metencephalon (*Meten.*) is the division of the primitive rhombencephalon from which the pons and cerebellum are developed. Its separation from the myelencephalon (*Myelen.*) is indicated, in part, by an internal transverse ridge (*Pl.ch.p.*) and a corresponding external groove, representing the developing plica chorioidea posterior.

The roof-plate is enormously expanded so that it forms not only the roof, but the greater part of the lateral wall of the metencephalon. The remaining or cephalic part of the lateral wall is formed on either side by a thickened band which is joined caudally to the ependymal roof-plate by a thinner intermediate layer known as the rhombic lip. This band on either side represents the corresponding dorsal zone (*Z.dors.*). It extends obliquely cephalad and medially to merge into the slightly thickened roof-

plate, caudad of the isthmus. It will form the corresponding lateral portion of the cerebellum. The vermis is believed to be developed from the slightly thickened roof-plate intervening between these thickenings.

The ventral zones (*Z.vent.*) of the metencephalon, which form the pons, are deep longitudinal bands separated by a thick median raphé which represents the floor-plate. They extend caudad to the angle made by the pontal flexure where they blend with the corresponding zones of the medulla.

Myelencephalon. The myelencephalon (*Myelen.*) is the remaining portion of the brain, which arches over the cervical flexure (*Flex.cerv.*) and joins the spinal cord. The roof-plate is exceedingly thin. It is widest in the region of the plica choroidea posterior where it forms the roof and the dorsal half of the lateral wall. Its caudal extension gradually tapers out to pass into the narrow roof-plate of the spinal cord. It becomes the caudal part of the posterior medullary velum.

The dorsal zones are thick. In the region near the spinal cord they are nearly vertical, but, by becoming progressively oblique, their internal surfaces form the lateral region of the ventricular floor at the cephalic end of the myelencephalon. The ventral zones, as in the metencephalon, are thick longitudinal bands on either side of the median line. In the floor of the ventricle a median longitudinal groove extends between them. Ventral to the groove a thickened raphé, floor-plate, unites the ventral zones. As the raphé approaches the region of the spinal cord it gradually becomes thinner.

Spinal cord

The spinal cord (*Md.sp.*) is represented in median sagittal section in plates 1 and 4. The surface is shown, in part, in plates 2 and 3. It has a narrow slit-like cavity, somewhat expanded dorsally. The lateral walls of the cord have clearly marked ventral and dorsal zones which are continued into the corresponding zones of the myelencephalon.

Cranial nerves

The cranial nerves of the right side are displayed in plate 3, and those of the left are shown in plates 2 and 5. Plate 6 shows some of the cranial nerves on both sides.

Nn. olfactorii. Numerous nerve fibers (*Nn.olf.*, plate 1) extend from the dorsal and medial surfaces of the nasal epithelium, and from the vomero-nasal organ (*Org.vom.-nas.*) to the olfactory area (*Rhin.*) of the telencephalon. In plates 2 and 3 the trunk formed by these nerves is represented as a stump. Among the fibers are numerous groups of cells which are not represented in the reconstruction. These cells perhaps have migrated from the nasal epithelium along the nerve fibers.

N. opticus. Fibers are present, extending from the retinal layer of the optic vesicle along the optic stalk to the corresponding ridge (optic) of the brain.

N. oculomotorius. The oculomotor nerve (*N.oc.*, plates 2, 3 and 5) issues from the ventro-lateral wall of the mesencephalon (*Mesen.*) by numerous small rootlets. It extends ventrally and cephalad, passing lateral to the posterior cerebral artery (*A.cer.p.*), and medial to the cavernous sinus (*S.cav.*) and the ophthalmic nerve (*N.oph.*). From the ophthalmic nerve it acquires a small sensory branch. Caudad of the optic stalk it gives off a branch to the anlage of the superior rectus muscle, but its main trunk is continued to the partially differentiated anlage for the inferior and medial recti and inferior oblique muscles.

N. trochlearis. The trochlear nerve (*N.troch.*, plates 2 and 3) issues from the roof of the isthmus and extends ventrally in a sinuous course to the orbit. In its course it passes just cephalad of the superior cerebellar artery (*A.cereb.s.*, plate 2) and lateral to the anterior cerebral vein. In the orbital region it passes dorsal to the anlage of the lateral rectus muscle, and medial to the frontal ramus (*N.fr.*, pl. 3) of the ophthalmic nerve. It terminates in the anlage of the superior oblique muscle. The trochlearis receives a small sensory branch from the ophthalmic nerve (*N.oph.*, pl. 3).

N. trigeminus. The trigeminal nerve (plates 2, 3, 4 and 5) is composed of sensory and motor components. The sensory fibers arise from the large semilunar ganglion (*G.s-l.*), which lies lateral to the cavernous sinus (*S.cav.*, plates 4 and 5), and form a large trunk which enters the latero-ventral surface of the metencephalon (*Meten.*). The motor fibers issue from the metencephalon at a point slightly ventro-cephalad of the sensory root. They form a trunk of considerable size which crosses the medial surface of the semilunar ganglion to join the mandibular nerve (plate 4). The peripheral fibers leave the semilunar ganglion as three main branches, the ophthalmic (*N.oph.*), the maxillary (*N.mx.*), and the mandibular (*N.md.*).

The ophthalmic nerve passes to the orbit. Dorsal to the optic stalk it gives a branch to both the oculomotor and trochlear nerves, then divides into naso-ciliary and frontal nerves. The frontal (*N.fr.*) passes dorsal to the superior rectus and superior oblique muscles, and breaks up into several branches of which the supraorbital may be recognized by its dorsal direction. The naso-ciliary (*N.na.-cil.*) passes ventral to these muscles, and can be followed into the cephalic part of the corresponding lateral nasal process. The maxillary nerve (*N.mx.*) soon after leaving the semilunar ganglion becomes a bundle of loosely connected fibers extending into the maxillary process ventral to the optic vesicle (*Ves.op.*).

The mandibular nerve (*N.md.*) receives, in addition to the sensory fibers from the semilunar ganglion, the motor part of the trigeminal nerve. It divides into a small cephalic and a large caudal trunk. The former or buccal nerve at first extends cephalad in company with the infra-orbital branch of the stapedial artery (*A.stp.*, pl. 2). Soon leaving this the buccal nerve crosses the anlage of the parotid gland (*Gl.p.*), and passing cephalad, furnishes branches to the epithelium near the angle of the rima oris. The larger, caudal trunk of the mandibular as it crosses the tubo-tympanal ridge, divides into three branches, the auriculo-temporal, the inferior alveolar, and the lingual. The latter near its origin is joined by the chorda tympani branch (*N.ch-tymp.*, plate 3; *ch.-ty.*, plate 2) of the facial nerve.

The auriculo-temporal has two roots of origin which embrace a branch of the stapedial artery. It extends at first ventrally and laterally, and then bends dorsally, giving off twigs to the epithelium in the neighborhood of the tuberculum tragicum.

The inferior alveolar (*N.alv.inf.*) crosses the lateral border of the pharynx, and follows the lateral side of Meckel's cartilage beneath the pharyngeal floor. It soon gives off the mylohyoid branch which passes ventrally, lateral to Meckel's cartilage, to the anlage of the mylohyoid muscle. The inferior alveolar then continues cephalad in the mandibular process of the mandibular arch where it divides into a dorsal and a ventral branch. The former supplies the oral epithelium of the corresponding side, the latter or mental nerve supplies the ectoderm on the ventrolateral surface of the mandibular process.

The lingual nerve (*N.ling.*) is formed by the union of mandibular and chorda tympani fibers (*N.ch.tymp.*) medial to Meckel's cartilage. It extends cephalad for a distance between the cartilage and the alveolo-lingual ridge where the submaxillary gland (*Gl.smx.*) has developed. Here it passes into the anlage of the submaxillary ganglion (plate 3). From the ganglion it issues as several bundles which curve medially around the alveolo-lingual ridge into the lateral part of the tongue. In this situation branches extend cephalad between the ridge and the hypoglossal nerve and, after repeated subdivisions, are ultimately distributed to the epithelium of the tongue.

N. abducens. The abducens (*N.ab.*) issues from the ventral wall of the metencephalon (*Meten.*) by several rootlets which are hidden in the drawings by the overlying auditory and facial nerves. A caudal aberrant root is present on either side which extends from the region of the glossopharyngeal and vagus nerves to join the abducens. Similar aberrant roots of the abducens have been observed by Elze ('07) and represented by Bremer ('08). The abducens extends ventrally and somewhat laterally towards the orbital region. It passes dorsal to the internal carotid artery, obliquely across the medial side of the cavernous sinus and ophthalmic vein. The abducens then turns

laterally between the vein and the oculomotor nerve to enter the anlage of the lateral rectus muscle.

N. facialis. The motor root of the facial nerve (plate 3) issues from the ventro-lateral wall of the metencephalon (*Meten.*) and passes to the geniculate ganglion (*G.gn.*) by which its fibers are enveloped for a short distance. The sensory root (pars intermedia, *N.int.*) arises from the geniculate ganglion and enters the metencephalon immediately caudo-lateral to the motor root. The mixed facial trunk which emerges at the caudo-ventral border of the geniculate ganglion (*G.gn.*) represents the post-trematic ramus of the first pharyngeal pouch. It takes a caudal and lateral direction. Having given off the chorda tympani (*N.ch.tymp.*, pl. 3, *ch.ty.*, pl. 2) it ends in several small branches.

The chorda tympani nerve leaves the facial trunk at an acute angle and extends cephalad, ventral to the auditory pouch, to join the lingual branch of the mandibular nerve as described above. From the geniculate ganglion a small nerve (*N.pet.s.m.*) extends at first ventrally, and then bends sharply cephalad medial to the mandibular nerve and the tubo-tympanal ridge. It is the great superficial petrosal nerve. As it bends cephalad it gives off caudally a short twig (anastomotic with the tympanic plexus) to meet the tympanic branch (*N.tym.*) of the glosso-pharyngeal nerve. The nerve arising from this junction (the anlage of the tympanic plexus) is the small superficial petrosal.

N. acusticus. The ganglion acusticum and otic vesicle are shown in plate 3. In plates 2 and 5 the otic vesicle has been removed. The ganglion acusticum is partially differentiated into cochlear and vestibular divisions. The vestibular part (*G.ves.*) lies cephalad of the utriculo-saccular division of the otic vesicle and lateral to the cochlear part of the ganglion, so that the latter is mostly hidden from view in plate 3. Four nerve trunks proceed from the vestibular division, a cephalic which divides into two rami to supply the ampullae of the superior and lateral semicircular canals; a caudal which passes medial to the utriculo-saccular division of the otic vesicle to supply the posterior semicircular canal; and two intermediate branches, the cephalic of which extends to the developing utricle, the caudal to the saccule.

From the cochlear division short fibers extend ventrally to the cochlear duct (*D.c.*). The central fibers of the ganglion acusticum (*N.acus.*) enter the caudo-ventral wall of the metencephalon slightly dorsal and caudal to the sensory root of the facialis.

N. glosso-pharyngeus. The motor fibers of the glosso-pharyngeal nerve (plates 2, 3 and 6) issue from the myelencephalon just ventral to the entering sensory roots. The latter are hidden partially from view in plate 3 by the ductus endolymphaticus (*D.end.*) and crus commune, but are exposed in plate 2.

The small ganglion superius (*G.sup.*) lies at the medial side of the ampulla of the posterior semicircular canal, separated from it by a narrow zone of the developing otic capsule. It extends slightly dorsal to the ampulla, and apparently involves only the more posterior and medial of the fibers of the nerve.

The ganglion petrosum (*G.petros.*) is a large ganglion, the caudo-lateral surface of which is closely applied to the trunk and ganglion nodosum of the vagus which somewhat overlap it. The medial surface of this ganglion is in contact with the internal carotid artery and the accompanying sympathetic fibers. From the cephalic part of the ganglion arises the tympanic nerve (*N.tym.*) which extends cephalad lateral to the internal carotid artery and nearly parallel with it. It passes dorsal to the remains of the second pharyngeal pouch and to the auditory (first pharyngeal) pouch, and lateral to the stapedial artery close to its origin. A little cephalad of the stapedial artery it is joined by a slender branch from the great superficial petrosal as described above (p. 57). The small superficial petrosal, resulting from this communication, I have been able to trace as far as a point near the caudo-ventral border of the semilunar ganglion.

Beyond the ganglion petrosum the glossopharyngeal nerve skirts closely around the caudal aspect of the remains of the second pharyngeal pouch and reaches the ganglion nodosum. At the ventral border of the lateral pharyngeal wall it receives a branch (not shown in the figure) from the superior cervical sympathetic ganglion. This branch, extending around the medial side of the internal carotid artery, joins the dorsal aspect of the

glossopharyngeal. The glossopharyngeal nerve, having passed on the cephalic aspect of the ganglion nodosum, leaves the vagus, but gives to it a communicating branch (also omitted from the reconstruction) which contributes fibers to the pharyngeal branch of the vagus. The glossopharyngeal then extends medially, bifurcating into a lateral or pharyngeal branch and a medial or lingual branch. The pharyngeal branch (plate 6) sends a twig to the developing stylo-pharyngeus muscle, and continues cephalad, for a short distance, along the pharynx. The lingual branch which is somewhat larger than the pharyngeal, also gives off a small branch to the pharynx, and then passes in a cephalomedial direction to the lateral side of the caudal part of the tongue where it splits up into many branches.

The glossopharyngeal trunk which extends peripherally from the ganglion nodosum, is usually considered as the posttrematic ramus of the second branchial cleft.

N. vagus. The vagus (*N.vag.*, plates 2, 3 and 6) is a large, mixed nerve, its main sensory component being derived from two ganglia, the jugular (*G.jug.*) and the nodosum (*G.nodos.*). The vagus acquires additional sensory fibers from an irregular series or chain of ganglionic masses (*Gg.hyp.*) situated caudad of the jugular ganglion and dorsal to the accessory nerve (*N.acc.*). These irregular clumps of cells without doubt represent hypoglossal ganglia, but the exact number of ganglia formed by them is uncertain. Prentiss ('11) in dissected pig embryos frequently found fibers from two or three of such ganglia passing ventrally to join the corresponding motor roots of the hypoglossal nerve. Such fibers, however, were not found in this embryo. The chain of ganglia (*Gg.hyp.*) on the left side (plate 2) is continuous with the first cervical ganglion which is very slender in its middle part.

Small motor rootlets issuing from the myelencephalon ventral to the entering sensory fibers, together with fibers from the accessory, furnish the motor components of the vagus. The vagus, therefore, from its sensory as well as from its motor composition, is a compound nerve, as has been maintained by many investigators.

Each vagus nerve extends caudad upon the medial side of the internal jugular vein as far as the common cardinal vein. It then occupies the angle between the oesophagus and trachea until the latter bifurcates. From here each nerve, as it continues caudad, passes dorsally to the bronchus of its own side where it gives off pulmonary branches. More caudally the nerves are in close relation to the oesophagus, forming a coarse plexus superficial to the anlage of the external muscular layer. From the oesophagus the vagi spread onto the stomach and give branches to the neighboring viscera.

An auricular branch of the vagus has not been identified on either side of this embryo. It is possibly a nerve of late development. In rare instances its absence has been noted in the adult.

A short, rather ill-defined pharyngeal branch is present on either side (but not reconstructed). Each arises from the medial side of the cephalic part of the ganglion nodosum, and extends to the pharynx, passing ventral to the proximal part of the internal carotid artery. To the formation of this nerve on either side the glossopharyngeal seems to contribute fibers as mentioned above. These branches of the vagus are apparently late in becoming formed into definite trunks.

The superior laryngeal nerve (*N.l.s.*, pl. 3; *N.laryng.s.*, pl. 6) arises at the ganglion nodosum, slightly caudad of its middle. It is represented in plate 3 as a stump, but is shown in part in plate 6. It extends ventrally and medially, dorsal to the distal part of the common carotid artery, and caudad of the detached (from pharynx) end of the thymico-pharyngeal duct, to divide into two branches, internal and external.

The internal branch passes medially over the cephalic border of the lateral lamina of the thyreoid cartilage, and divides into dorsal and ventral branches. The dorsal extends in the mesenchyma between the entodermal portions of the oesophagus and larynx. The ventral extends caudad, medial to the lateral lamina of the thyreoid cartilage. The external branch of the superior laryngeal is smaller than the internal, and can be traced caudally along the lateral aspect of the thyreoid cartilage for a short distance.

Froriep ('85) has shown conclusively that the superior laryngeal nerve is the posttrematic ramus of the third branchial cleft.

Another branch (*R.p-tr.*) of the left vagus arises from the ganglion nodosum directly caudad of the place of origin of the superior laryngeal. It is a small strand which extends caudally, at first between the vagus and the lateral lobe of the thyreoid gland, and dorsal to the thymus and left common carotid artery. Having passed the caudal extremity of the thymus gland it is in relation dorsally with the vagus, medially with the aortic arch, and laterally with the internal jugular vein. The nerve finally winds around the fourth aortic arch, and extends cephalad lateral to the recurrent of that side. It passes dorsal to the parathyreoid of the fourth pharyngeal pouch and turning cephalad, appears at first dorsal and then dorso-medial to the lateral lobe of the thyreoid gland. It ultimately becomes exhausted by giving off fibers which extend ventrally in the region of the termination of the external ramus of the superior laryngeal nerve.

The corresponding nerve on the right takes origin from the dorso-medial side of the right ganglion nodosum, where the hypoglossal nerve crosses the vagus laterally. As it extends caudad it lies close to the medial side of the vagus, and it is conceivable that in later development the two nerves might be inclosed in a common connective tissue sheath. It finally winds around the right fourth aortic arch (A. subclavia) in the concavity of the larger recurrent nerve, and returns cephalad, passing lateral to the parathyreoid of the fourth pharyngeal pouch, but having the same relation to the lateral lobe of the thyreoid as the corresponding nerve on the right. It has a shorter course in the neck than that of the right side, the right fourth aortic being situated considerably more cephalad than the left.

From their site of origin, and from their relation to the fourth aortic arches and to the parathyreoids of the fourth pouches, it can scarcely be doubted that these nerves represent the posttrematic rami of the fourth pharyngeal pouches.

These nerves are obviously comparable to the branches of the vagus identified by Froriep ('85) in cow embryos (8.7 to 8.8 mm.

in length) as the posttrematic rami of the fourth pharyngeal pouches, and figured in Taf. I, figs. I and I1, II, II5. Froriep, however, could find this nerve only in the young stages.

Lewis ('06) found in the 12 mm. pig embryo a small nerve running beside the postbranchial body which he thought might be comparable to the nerve described by Froriep as the posttrematic ramus for the fourth pouch.

Elze ('07) identified this posttrematic branch of the vagus in a human embryo of about 7 mm., (p. 427, text figs. 7–8), but could not find the nerve in two older human embryos (II and III), measuring (greatest length) 9.5 and 11 mm. respectively. Hence, like Froriep, he concluded that in man the existence of this posttrematic ramus of the vagus is transitory.

In this embryo it is seen that these posttrematic rami become closely associated with the recurrent, especially on the right side where the pulmonary aortic arch atrophies, and the author believes that they occasionally, at least, persist in the adult. The support for this conclusion is based not only upon their presence in an embryo of this stage of development (17.8 mm.), but upon the observations of Wrisberg. Wrisberg (Henle's Anatomie des Menschen, Bd. 3, p. 441, 1868) observed in three cases a reduplication of the right recurrent nerve. The extra branch was much smaller than the normal, and it accompanied the latter upwards between oesophagus and trachea, in much the same way as occurs in this embryo.

The recurrent nerves are displayed in plate 6. The right recurrent nerve (*N.rec.*). arises from the vagus at the caudal border of the right fourth aortic arch (A. subclavia), and passes cephalad in the neck, dorsal to the parathyreoid of the fourth pharyngeal pouch (*P.-thyr.IV*). Here it gives off some oesophageal branches, and then continues to the medial side of the right lamina of the thyreoid cartilage where it becomes exhausted by giving off branches which turn ventrally to the anlagen of the laryngeal muscles.

The left recurrent nerve (*N.rec.s.*) arises from the vagus at a more caudal level than the right, viz., at the caudal border of

the pulmonary arch (ductus arteriosus). Winding around this arch ventro-dorsally, it extends cephalad. In the neck it has relations similar to those of the corresponding nerve on the right, with the exception that it passes medial to the parathyreoid IV instead of dorsal.

It has been suggested by Froriep ('85) that the recurrent nerves may well be considered as trunks formed by the fusion of branchial nerves for clefts which fail to develop. This interpretation, again advanced by Lewis in 1906, gains support from the relation of the posttrematic rami IV to the recurrent nerves in this embryo, especially on the right side where the two nerves are brought close together, the caudal aortic arches having atrophied.

One of the inferior cardiac branches of the right vagus is seen in plate 3. It (*N.c.i.*) arises from the dorso-medial wall of the vagus caudad of the place of origin of the recurrent nerve. It extends caudad in the angle between the oesophagus and the trachea, being dorsal to the vagus and medial to the internal jugular vein. It eventually becomes in part incorporated in the deep cardiac plexus, a part rejoining the vagus. Slightly caudad of the above nerve another inferior cardiac ramus from the vagus extends medially to the anlage of the deep cardiac plexus. It is hidden in the reconstruction by the overlying vagus. An inferior cardiac nerve was found on the left side corresponding to the right nerve shown in plate 3, but it has been omitted in the reconstructions. It arises from the left vagus just after it has given off its recurrent ramus, hence at a considerably more caudal level than the right inferior cardiac. It extends caudad in the angle between the oesophagus and the trachea to the deep cardiac plexus.

N. accessorius. The accessory nerve (*N.acc.*, plates 2 and 3) is formed by a series of small rootlets which emerge from the lateral surface of the spinal cord and myelencephalon. The most caudally placed root of the series issues at the level of, and in close proximity to, the dorsal root of the second cervical nerve. In this embryo the place of emergence of the most caudally placed root is similar on the two sides, and has been noted in

consequence of its variation in other human embryos. Streeter ('04) places the level at the third or fourth cervical, but finds that sometimes it is more caudally placed.

The main trunk of the nerve arches cephalad and ventrally under the hypoglossal ganglia (*Gg.hyp.*) to become incorporated with the vagus ventral to the jugular ganglion. At the cephalo-ventral border of the ganglion nodosum of the vagus, the ramus externus (*R.ex.*, plate 3) of the accessory nerve curves laterally and dorsally around the lateral side of the internal jugular vein (plate 6) to the developing sterno-cleido-mastoid muscle. From here it passes around the cephalic part of the adjacent lymph sac to reach the anlage of the trapezius muscle. In the angle between the internal jugular vein and the lymph sac (*S.jug.*) it communicates with the great auricular nerve.

N. hypoglossus. Numerous, small, hypoglossal rootlets issue from the ventral wall of the myelencephalon on either side, and each group converges to form four trunks. These, after crossing the lateral side of the vertebral artery (*A.vert.*), further join to form the two main roots of the hypoglossal nerve (*N.hyp.*, plates 2 and 3).

On the right side, in addition to the four trunks seen on the left, a vestigial root joins the fourth trunk (plate 3). Such vestigial roots of the hypoglossal nerve have been found by Bremer ('08) to occur frequently in embryos of man, pig, sheep and dog, and almost constantly in the turtle and chick.

It will be noted that the caudal of the four hypoglossal roots on the left (plate 2) passes through an arterial fenestra of the vertebral artery. A similar, but more extensive anastomosis of arterial branches with the vertebral, enclosing the hypoglossal roots has been shown by Elze ('07), (Taf. 15, fig. 2). They show how, by means of island-formation, the vertebral artery in the adult may come to pass between the roots of the hypoglossal nerve or, in rare cases, extend lateral to the entire nerve. The hypoglossal ganglia have been considered with the description of the vagus.

The hypoglossus receives a branch from the first cervical nerve (one from the second cervical in addition on the right), and ex-

tends ventrally between the vagus and the internal jugular vein. At the ventral border of the vagus, a little caudad of the termination of the linguo-facial vein, it gives off its ramus descendens. The latter extends caudad, ventral to the internal jugular vein, and joins the descendens cervicalis to form the ansa hypoglossi. The hypoglossus then continues cephalad, medial to the linguo-facial vein and the developing submaxillary gland (plate 5) to divide in the tongue into muscular branches.

Spinal nerves

Plates 1 and 4 show portions of the ventral divisions (rami anteriores) of the spinal nerves (*Nn.sp.*); plate 3 gives a lateral view of the right cervical nerves and plexus; and plates 2 and 5, a lateral view of the left cervical nerves and plexus. In plates 5 and 6 the relations of these nerves to the jugular lymph sac (*S.jug.*) are shown. It will be noted that some of the ganglia are still connected by ganglionic bridges.

Nn. cervicales. The cervical nerves divide just beyond the union of the dorsal and ventral roots into dorsal and ventral primary divisions (rami posteriores and anteriores). The dorsal rami are shown as stumps with the exception of the great occipital branch (*N.occ.m.*) from the second, a piece of which has been added. The ventral primary divisions run ventrally and caudally, and the second to the sixth inclusive, extend laterally to the vertebral artery (*A.vert.*).

The first cervical nerve has a long slender ganglion (plates 2 and 3), the dorsal part of which overlies the accessory nerve (*N.acc.*). The ganglion shows signs of atrophy in its middle part, especially on the left side. As the nerve extends ventrally it is in close contact for a distance with the vertebral artery (plates 1 and 2), which it crosses on the medial side. A short communicating branch connects the first cervical nerve with the ventral primary division of the second cervical. The fibers of this communicating branch join the hypoglossal nerve, and are thought to assist in forming the ramus descendens hypoglossi.

The ventral primary division of the second cervical nerve behaves differently on the two sides. On the left (plate 2) beyond

the communicating branch with the first cervical nerve, it passes caudad to join a branch from the third. The junction occurs dorsal to the internal jugular vein and forms the descendens cervicalis which extends ventrally between the internal jugular vein and jugular lymph sac (plates 5 and 6). The descendens cervicalis joins the ramus descendens hypoglossi to form the ansa (*An.hyp.*).

On the right side (plate 3), beyond the communicating branch with the first cervical nerve, the ventral primary divisions of the second cervical bifurcates. One branch joins the third cervical to form the descendens cervicalis, while the other passes ventrally between the vagus and internal jugular vein to join the hypoglossus. The fibers passing direct to the hypoglossus, therefore, reach the ansa by way of the ramus descendens hypoglossi.

The ventral primary division of the third cervical nerve also differs on the two sides. On the right (plate 3) distal to the communicating branch with the second cervical nerve, it divides into a cephalic and a caudal branch. The caudal branch furnishes medially the ramus descendens cervicalis; and laterally a branch which unites with one from the fourth cervical to form the supra-clavicular nerves (*Nn. s-cl.*).

On the left side the ventral division of the third cervical (plate 2) also divides into two branches. The more cephalic of these first gives off medially a branch which passes between the vagus and jugular lymph sac to the ramus descendens cervicalis. Then, continuing ventrally between the dorsal portions of the internal jugular vein and adjacent lymph sac, it furnishes (plates 5 and 6) the great auricular (*N.aur.m.*), small occipital, and cutaneous colli (*N.c.c.*) nerves. The great auricular nerve pierces the dorsal part of the cephalic segment of the saccus jugularis, and here communicates with the ramus externus of the accessory nerve. The small occipital and cutaneous colli nerves appear a little more caudally between the two cephalic segments of the lymph sac. At the place of origin of the small occipital and the cutaneous colli, the third cervical nerve has a communicating branch with the fourth cervical, probably to assist in the formation of the supraclavicular nerves (*Nn.s-cl.*) as on the

right side. The more caudal branch of the ventral ramus of the third cervical nerve extends caudad, and gradually tapers out. Possibly this branch would have joined, eventually, the phrenic nerve (*N.phr.*).

The fourth cervical nerve on either side (plates 2 and 3) contributes to the formation of the supraclavicular nerves (*Nn.s-cl.*), which extend ventrally along the cephalic border of the jugulo-cephalic vein (plate 5). It also sends a branch to the phrenicus (*N.phr.*), and to the ventral ramus of the fifth cervical. The root which the phrenic derives from this nerve (plate 2) passes between the two terminal branches of the thyreo-cervical artery.

The ventral primary division of the fifth cervical nerve gives off the upper root of the long thoracic nerve, and then joins with the sixth cervical to form the upper trunk of the brachial plexus (plate 3). Just before joining, however, the fifth cervical nerve gives off its phrenic root which, extending caudad, dorsal to the thyreo-cervical artery (plate 2), meets the branch from the fourth cervical nerve. Each phrenic nerve (*N.phr.*), thus formed, accompanies the internal mammary artery (*A.mam.i.*) dorso-ventrally around the caudal border of the terminal portion of the subclavian vein (*V.scl.*, plate 5). The nerve here leaves the artery, and at first extends in the somatopleure along the lateral side of the anterior cardinal vein, then between this vein and the pleural cavity, and finally in the pleuro-pericardial membrane, lateral to the common cardinal. The nerves ultimately reach the anlage of the diaphragm on either side of the common hepatic vein (the right phrenic being for some distance in close relation to the latter).

The ventral primary division of the sixth cervical nerve, dorsal to where it is joined by that of the fifth, gives off the middle root of the long thoracic nerve.

The ventral primary division of the seventh cervical nerve furnishes the lower or caudal root of the long thoracic nerve, and becomes the middle trunk of the brachial plexus.

The ventral primary division of the eighth cervical and that of the first thoracic nerves unite to form the lower trunk of the brachial plexus.

SENSE ORGANS

Eye

The eye-ball is distinctly outlined, and the anlagen of the eyelids form two prominent arches. The optic vesicle (*Ves.op.*), its stalk (*Op.s.*), and the lens (*L.*) of the right eye are shown in plate 3; the same structures on the left in plate 5.

The optic stalk is slender and the lens is now devoid of a cavity. Into the loose mesenchyma, occupying the vitreous chamber extend the hyaloid artery and its branches. Other blood vessels reach this chamber through the circular fissure at the margin of the lens.

Ear

The fossa conchae (text fig. 1) is a depression rather broad and shallow dorsally, but narrow and deep ventrally. The mandibular border of the fossa is formed by a fold especially prominent in its ventral part where it presents a conspicuous projection. This, the tuberculum tragicum, extends laterally and somewhat toward the fossa. The ridge at the hyoid border of the fossa is more prominent than that on the mandibular border and is bounded caudally by the retroauricular groove. At the ventral end of the ridge is a well marked protuberance which overhangs the fossa directly opposite the tuberculum tragicum. It represents the tuberculum antitragicum. The portion of the ridge dorsal to this tubercle is the developing helix. In the central part of the floor of the fossa is the low elevation which represents the tuberculum membrane tympani of Hammar ('02).

The lateral aspect of the right otic vesicle is represented in plate 3 and the medial in plate 4. The stumps of the nerves which innervate it are seen in plate 2. The utricular (*Ut.*), saccular (*Sac.*), and cochlear divisions (*D.c.*), are not sharply differentiated from one another, and the coalesced lamellae of the lateral canal have not been absorbed as yet. The ductus endolymphaticus widens into the saccus, and extends for a considerable distance dorsally. A peculiar anomaly occurs in this otocyst; a short hollow diverticulum projecting from the utricle

just dorsal to the ampulla of the lateral semicircular canal. This diverticulum joins the crus commune of the superior and posterior semicircular canals without, however, opening into it. This diverticulum does not occur upon the left side, but a similar condition in other human embryos of the Harvard Collection, has been observed by the author.

Nose

The nares are represented in plates 3 and 5, and the ectodermal lining of the olfactory vesicles in plates 1, 2 and 4.

The nares (*Na.*) are open, but separated by a broad septum, From the naris each olfactory pit (*Ves.olf.*) extends dorso-caudally as a small tube, oval in transverse section. Each tube shortly expands to form the main nasal cavity which opens into the fore part of the roof of the mouth by a large primitive choana (*Ch.pr.*).

In lateral view (plate 2) the epithelial wall of the olfactory vesicle presents cephalad a large concavity partially subdivided by a slight external ridge (unfortunately not shown in the drawing) into a shallow dorsal depression, the nasoturbinal (agger nasi), and a more extensive ventral one (*Max-turb.*), the maxilloturbinal (concha inferior). The maxilloturbinal is limited ventrally by a pronounced, somewhat dorsally curled fold of the vesicular wall, containing within the meatus nasi inferior.

At the dorsal part of the choanal extremity of the olfactory vesicle there is seen in a lateral view a slight indentation (*Eth.-turb.I*), representing the developing ethmoturbinal I (concha media). The ethmoturbinal I, although it now forms in part the lateral wall of the olfactory vesicle, has been shown by Peter ('01) to be a derivative of the septal wall. The ridge appearing between the ethmo- and maxillo-turbinal overlies the meatus nasi medius. The palatal process forms the lateral boundary of the choanal extremity of the olfactory vesicle.

On the medial wall of the olfactory vesicle (plates 1 and 4) is seen the small, tubular outgrowth (*Org.vom.-nas.*) for the vomero-nasal organ (Jacobson's), rudimentary in man.

The nasolacrimal duct (not shown in the reconstructions) begins at the medial angle of the eye in an expanded end, disconnected from the ocular epithelium, and extends medially to end near the epithelium of the meatus nasi inferior. It is a solid, irregularly branching, cord of cells, entirely surrounded by mesenchyma. Outgrowths from the ocular extremity for the superior and inferior lacrimal ducts have not developed.

VASCULAR SYSTEM

Heart

The heart in its relation to the surrounding organs is shown in plates 1 and 4. The section shown in plate 1 passes to the left of the atrial and ventricular septa, and therefore, opens the left atrium and ventricle. In plate 4 the section passes through the cavities of the right side. The ventricular trabeculae are represented somewhat diagrammatically.

The *sinus venosus* (*S.v.*, pl. 4) receives the common hepatic (*V.hep.com.*) and the right and left common cardinal veins (*Vv.card.c.d.* and *s.*), and opens into the right atrium.

Upon the dorsal wall of the *right atrium* (*At.d.*, plate 4) is the sagittally directed, sinu-atrial orifice. The latter forms a narrow slit, bounded laterally by the right and left sinus valves (*V.v.s.*). The cephalic ends of the valves converge and meet in a ridge. The continuation of this ridge, which can be traced for some distance along the cephalic part of the interior of the atrium, is the septum spurium.

The caudal extremity of the left sinus valve meets the right side of the septum primum at the caudal part of the right atrium. Just where these two structures meet ventrally there is a ridge or tubercle, which probably represents the caudal end of the future septum secundum. Born ('89) goes into no detail with regard to the earlier stages in the development of the septum secundum, which is shown completely formed in his figure 29. From the relation which the tubercle bears to the septum primum and the left sinus valve it can scarcely be doubted that it would eventually form part of the adult limbus fossae ovalis.

A large portion of the right sinus valve subsequently disappears but its caudal part will persist to form the valvulae venae cavae inferioris and sinus coronarii. On the right of the atrio-ventricular orifice a portion of the developing tricuspid valve (*V.t.*) is seen.

The principal outlet for the *right ventricle* (*Vent.d.*) is now the truncus pulmonalis (*Tr.pul.*), although the presence of the small interventricular opening (*F.int.*, pl. 1) still allows some blood to pass out by way of the aorta. The aortic septum (*S.*) is practically complete at this stage, and is seen in plate 4, separating the root of the aorta from the conus arteriosus (*Con.art.*) of the right ventricle.

The *left atrium* (*At.s.*, pl. 1) is partially separated from its fellow on the right by the septum primum. A quadrilateral opening in the septum, the ostium secundum (*O.s.*), places the two atria in direct communication. Leading into the left atrium on its dorsal side two pulmonary veins are shown (*Vv.pul.d.*). Between the left atrium and ventricle there is a marked protuberance of the fused endocardinal cushions, which represents in part the anlage of the bicuspid valve. The vessel (*V.card.com.s.*) seen in section immediately caudad of the left atrium is the part of the left common cardinal which will become the coronary sinus.

The *left ventricle* is still in communication with the right by a small opening (*F.int.*) the foramen interventriculare which is directed obliquely dorso-ventrally from left to right. This eventually becomes closed towards the right ventricle by complete fusion between the aortic and interventricular septa. When this has occurred, the aorta (*Tr.aor.*) will become the only outlet of the left ventricle. The ventricular wall immediately ventral to the anlage of the tricuspid valve (*V.t.*) separates the left ventricle from the right atrium, and has been named by Hochstetter ('98) the septum atrio-ventriculare. This septum together with that formed by the fusion of the aortic (*S.*) and interventricular septa, form the septum membranaceum ventriculorum of the adult heart.

Arteries

The arteries of the right side are shown in plate 1 from the left side. The arteries of the left side of the head and neck are shown in plate 2.

Systemic derivatives of the aortic arches. The truncus aorticus (*Tr. aor.*, plate 1) leaves the left ventricle of the heart, extends in the dorsal mesocardium across the pericardial cavity, and immediately divides into two ventral aortae. The left ventral aorta gives off the short fourth left aortic arch which becomes part of the arcus aortae, and continues cephalad as the left common carotid. It is shown in this plate as a stump, but is displayed fully in plate 2.

The left common carotid extends cephalad between the thymic and thyreoid anlagen to the region of the larynx, where it divides into a dorsal and a ventral branch, the internal and the external carotid arteries, respectively.

The external carotid (*A.car.ex.*) represents the continuation of the ventral aorta. It is a short stem which terminates by dividing into five branches, viz.: superior thyreoid, occipital, lingual, external maxillary and posterior auricular. The superior thyreoid (*A.thyr.s.* and *d.*, plates 1 and 6) runs medially skirting the cephalic extremity of the lateral lobe of the thyreoid gland, and at once breaks up into small branches. The occipital (*A.occ.*, plates 2 and 6) takes a cephalic and dorsal direction around the lateral side of the internal jugular vein, and gives off the sternomastoid branch. The lingual (*A.ling.*, plates 2 and 6) runs medially to the tongue, giving off laterally a branch to the submaxillary gland. The external maxillary (*A.max.ex.*, plates 2 and 6) extends ventrally along the mandibular arch. The posterior auricular (*A.aur.p.*, plates 2 and 6) is a large vessel which, having given off the stylo-mastoid branch curves laterally behind the primary external acoustic meatus and passes dorsally. The ascending pharyngeal and temporal arteries have not been identified. The internal maxillary, as such, is still wanting in this embryo; it is foreshadowed by the stapedial branch of the internal carotid to be described later.

The left internal carotid (*A.car.i.*) comprises the third aortic arch, the left dorso-lateral aorta cephalad of the third arch, and a terminal portion or branch from the first aortic arch. It curves at first dorsally and cephalad, and then extends directly cephalad, dorsal to the roof of the pharynx. It passes medial to the cranial nerves and bifurcates, lateral to the diencephalon (*Dien.*) into caudal and cephalic divisions. The former is the posterior communicating artery (*A.com.p.*). This extending caudad gives off the posterior chorioidal artery, and joins the left posterior cerebral (*A.cer.p.*). The other terminal division of the internal carotid artery divides into two branches, the anterior chorioidal (*A.chr.a.*), and a stem common to the middle cerebral (*A.cer.m.*) and the anterior cerebral (*A.cer.a.*) arteries. Besides the terminal branches two other branches of the left internal carotid are shown here, the ophthalmic and the stapedial. The ophthalmic artery (*A.oph.*) arises from the internal carotid artery medial to the ophthalmic nerve (*N.oph.*). It extends cephalad and laterally, ventral to the optic stalk which it penetrates, becoming the central artery of the retina.

The stapedial artery (*A.stp.*, pl. 2) arises from the internal carotid near the middle of the pharynx, and passing through the left stapedial cartilage, runs as shown in plate 2, towards the semilunar ganglion (*G.s.-l.*). A short distance from the ganglion it divides into two branches, dorsal and ventral. The dorsal or supraorbital branch passes lateral to the semilunar ganglion into the region of the orbit. The ventral division soon bifurcates, and the resulting branches pass to the medial and lateral sides, respectively, of the mandibular nerve. The medial branch, having communicated with the lateral by a branch passing ventral to the mandibular nerve, first accompanies the buccal branch of the mandibular and later the maxillary nerve, as the infraorbital artery. The lateral branch passes between the roots of origin of the auriculo-temporal nerve and, having communicated with the medial branch, becomes the inferior alveolar which accompanies the nerve of the same name (*N.alv.inf.*).

The development and importance of the stapedial artery have been demonstrated admirably by Tandler ('02). He finds that

this artery represents a persistent portion of the dorsal part of the second aortic arch, and an intermediate segment of the first arch; the two being united by a longitudinal anastomosis. In certain mammals the stapedial artery persists throughout life, but in man an anastomosis between the external carotid artery and the mandibular ramus of the stapedial results in the formation of the adult internal maxillary artery to which the stapedial transfers its branches. In this embryo the anastomosis is yet to form; there can be no doubt, however, that it will join the lateral branch of the ventral division of the stapedial between the auriculo-temporal nerve and the branch of communication between the lateral and medial branches. As a result of such an anastomosis the lateral branch of the ventral division, together with the dorsal division (supraorbital of Tandler), would become the middle meningeal. The middle meningeal and inferior alveolar would then spring from the internal maxillary. The original main trunk of the stapedial would persist in part as the carotico-tympanic branch of the internal carotid and the superior tympanic branch of the middle meningeal. It is extremely probable that the medial branch of the original ventral division of the stapedial is represented in the adult by the accessory meningeal branch of the internal maxillary.

The right ventral aorta (plate 1) takes a cephalic direction from the truncus aorticus. It soon gives off a short dorsal branch, the fourth aortic arch, which becomes part of the right subclavian. The portion of the right ventral aorta proximal to the fourth arch becomes the innominate artery (*A.anon.*), the smaller distal continuation represents the right common carotid. The relations of the latter are essentially the same as those just given for the corresponding artery on the left. A branch, however, from the proximal part of the right internal carotid, accompanies the hypoglossal nerve. This, the hypoglossus artery (*A.hyp.d.*, plate 1) has been noted in the human embryo by Zimmerman ('89), Tandler ('02) and Ingalls ('07), and in the rabbit by Hochstetter ('90). The hypoglossus artery of the left side had disappeared in this embryo. The hypoglossus arteries represent the second pair of primitive intersegmental

arteries from the dorso-lateral aortae, later becoming temporary branches of the internal carotids.

The dorso-lateral aortae have ceased to exist as complete trunks, for their continuity has been interrupted in the segment of each intervening between the third and fourth aortic arches.

The segments cephalad of the third pair of aortic arches consist of two symmetrical vessels which enter into the formation of the internal carotid arteries, as already stated. The segments caudad of the fourth pair of arches, consist of two very unsymmetrical vessels which converge and unite, opposite the second pair of thoracic nerves, to form the dorso-median aorta (*Ao.d.m.*).

The caudal segment of the right dorso-lateral aorta is still large in the neighborhood of the fourth aortic arch, with which it participates in the formation of the right subclavian artery (*A.scl.d.*). The remainder of this segment of the right dorso-lateral aorta is reduced to a fibrous cord containing only a trace of a lumen. The caudal segment of the left dorso-lateral aorta is large and contributes to the formation of the permanent arcus aortae.

Although the intermediate segments of the dorso-lateral aortae have lost all connection with the third pair of aortic arches, they still persist as a pair of vestigial arterial tubes (*S.*) projecting from the cephalic aspect of the fourth aortic arches.

The fourth aortic arches extend from the place of origin of the common carotids, upon the right and left sides, to the vestigial tubes above mentioned. They are both short, wide trunks, of which the right occupies a more cephalic position. The right fourth aortic arch eventually forms the proximal part of the right subclavian artery. The left participates in the formation of the arcus aortae.

Pulmonary arteries. The truncus pulmonalis (*Tr.pul.*, plates 1, 2 and 4) begins in the conus arteriosus (*Con.art.*) of the right ventricle which is almost completely separated from the truncus aorticus (vestibule). Of the pulmonary arches, only the left persists in its entirety. The truncus pulmonalis is now continued directly into the left pulmonary arch which opens into the left dorso-lateral aorta in common with the left fourth arch.

The two small pulmonary arteries (*A.a.pul.*, plate 1) extend caudad from the main pulmonary trunk, one to each developing lung. The right vessel is reconstructed more completely than the left, and represents not only the right pulmonary artery, but the proximal portion of the right pulmonary arch, as has been shown by Bremer ('02). The left pulmonary artery is shown only as a stump. It represents the left pulmonary artery proper. The segment of the pulmonary arch (*D.a.*, plate 2) between the left pulmonary artery and the left dorsal aorta is the ductus arteriosus.

Derivatives of the occipital and cervical segmental arteries. The right hypoglossus (intersegmental) artery (see p. 75) and the proximal parts of the cephalic six (five on the right side) pairs of cervical intersegmental arteries have disappeared. The distal parts of these intersegmentals have now become branches of the large vertebral arteries (*A.vert.*), formed from the series of post-costal anastomoses between them. The seventh intersegmental artery (sixth on the right side) has retained its connection with the dorso-lateral aorta of its own side to form a portion of the subclavian and the proximal part of the corresponding vertebral artery. The vertebral arteries extend cephalad medial to the cervical nerves, with the exception of the first pair which they accompany towards the spinal cord. The sudden change in direction of the vertebral arteries opposite the suboccipital pair of nerves arises from the fact that the portion of these arteries between the atlas and occiput develops from the first cervical intersegmental spinal rami. The vertebral arteries pass ventral to the myelencephalon and medial to the hypoglossal and vagus nerves. Opposite the glossopharyngeal the arteries of the two sides unite to form an enormous median vessel, the basilar (*A.bas.*). The basilar artery extends cephalad in the median line between the notochord and the rhombencephalon. Between the trochlear nerves (*N.troch.*) it divides into two lateral branches, the posterior cerebral arteries. Each posterior cerebral artery (*A.cer.p.*) communicates ventrally with a branch (posterior communicating) of the internal carotid artery (*A.car.i.*) to participate in the formation of the circulus arteriosus. It also dispenses several

branches to the lateral and dorsal surfaces of the di- and mesencephalon (*Dien.* and *Mesen.*), a considerable branch to the mesencephalon passing through the rootlets of the oculomotor nerve (*N.oc.*, plate 2). The left superior cerebellar artery (*A.cereb.s.*) arising from the basilar is seen in plate 2, just caudad of the trochlear nerve. Its branches overlie the isthmus and the metencephalon. The other branches of the basilar are seen in part in plate 1, those on the left side having been cut away.

The left subclavian artery (*A.scl.*, plate 2) is a branch of the corresponding seventh cervical intersegmental artery. Beyond the origin of the vertebral artery (*A.vert.*), the thyreo-cervical (*Tr.thyr.-cerv.*) arises from the cephalic aspect of the subclavian; the costo-cervical (*Tr.cost.-cerv.*) from the dorsal; and the internal mammary (*A.mam.i.*) from the ventral aspect. The costo-cervical divides, as usual, into the deep cervical and superior intercostal; the others can be identified by their direction. The remaining portion of the subclavian trunk extends ventro-laterally into the upper limb.

On the right side (plate 1) there is a common anomaly, the sixth (not the seventh) cervical intersegmental being connected with the right dorso-lateral aorta. By means of the right dorso-lateral aorta and a short precostal anastomosis the sixth intersegmental is connected with the seventh.

Consequently the right subclavian comprises three elements (exclusive of the parts derived from the fourth aortic arch and dorso-lateral aorta). These are: (1) the root of the sixth cervical intersegmental artery; (2) a longitudinal precostal anastomosis connecting the sixth and seventh intersegmentals; and (3) a portion of the seventh intersegmental. From the first element arises the right vertebral artery (*A.vert.d.*), the root of which represents a part of the sixth cervical segmental. From the third element the right superior intercostal arises on the caudal aspect; the thyreo-cervical on the cephalic; the deep cervical on the dorsal, and the internal mammary on the medial aspect. The deep cervical is derived from the dorsal ramus of the seventh intersegmental artery. The internal mammary represents the

real continuation of the ventral ramus of the seventh cervical intersegmental. The continuation of the subclavian beyond the point of origin of the internal mammary appears to represent a lateral branch of the ventral ramus of the seventh intersegmental. By the anomaly found on the right side of this embryo, the deep cervical and superior intercostal arteries come to have separate origins from the subclavian as sometimes occur in the adult.

Parietal arteries of the trunk. From the level of the second pair of thoracic nerves, where the dorso-lateral aortae unite, the single dorso-median aorta (*Ao.d.m.*) continues throughout the trunk to end as the middle sacral (*A.s.m.*). The dorsal intersegmental arteries arise in regular pairs from the dorso-median aorta. Those of the right side are shown in plate 1, the corresponding vessels on the left having been omitted.

The dorsal intersegmental arteries curve laterally and dorsally, passing lateral to the sympathetic, and either pass dorsally to become muscular branches or continue medially as spinal rami into the vertebral canal. In the latter situation the spinal rami pass laterally to the spinal cord to form the posterior spinal arteries or join to form a bilateral ventral longitudinal anastomosis which extends from the vertebral artery (directly caudad of the myelencephalon) along the spinal cord into the tail process. Some of the spinal rami bifurcate contributing thereby a branch to each anterior and posterior spinal artery of that side.

That the embryonic right anterior spinal anastomosis eventually joins its fellow of the opposite side to form the single anterior spinal artery of the adult was first indicated by His ('86). Recently the matter has been treated in greater detail by Sterzi ('04), and Evans ('09 and '12). The latter observers conclude that fusion between the two stems does not occur, but that the single adult artery represents the selected channel persisting from the alternative paths offered by the two longitudinal vessels and the plexiform connections between them.

In the thorax and abdomen the ventral rami of the dorsal intersegmentals become the aortic intercostal and lumbar arteries, respectively. Some of the former are represented as short stumps in plate 1. The ventral rami of the first and second thoracic

pairs of dorsal intersegmentals have lost their connection with the dorso-median aorta. They are now connected with the subclavian arteries by the precostal anastomosis which forms the adult superior intercostal.

Visceral arteries. The ventral visceral arteries and the lateral visceral arteries of the right side are indicated in plate 1.

In the thorax several small, ventral visceral arteries (*A.oe.*) extend from the aorta through the mediastinum to the oesophagus. According to Broman ('08) these oesophageal arteries are of secondary formation, the primary segmental arteries having early disappeared.

The coeliac artery (*A.coel.*) leaves the aorta slightly caudad of the 11th thoracic pair of dorsal intersegmental arteries. It extends between the medial surfaces of the anlagen of the suprarenal glands, and entering the mesogastrium at once gives off the left gastric artery which turns cephalo-ventrally. The coeliac then continues in the mesogastrium (hidden in the reconstruction by the stomach, Ga.), and having given off the splenic artery it becomes the hepatic. The former artery extends in the mesogastrium to the splenic anlage; the latter enters the lesser omentum and divides into the hepatic artery proper and the gastro-duodenal. The hepatic proper can be seen in the reconstruction, entering the liver just ventral to the portal vein (*V.P.*). It gives off the small cystic branch (not shown in the reconstruction) which accompanies the cystic duct. The gastro-duodenal artery can be traced distally between the left side of the duodenum and the proximal part of the dorsal pancreas.

Tandler ('03) finds that the coeliac artery in a 17 mm. human embryo arises from the aorta slightly above the level of the 20th intersegmental (12th thoracic) artery.

The superior mesenteric artery (*A.mes.s.*) takes origin from the aorta approximately on a level with the 12th thoracic pair of dorsal intersegmental arteries. It extends ventrally between the caudal ends of the suprarenal glands (plate 4) into the dorsal mesentery. It courses to the left of the duodenum and passes dorsal to the vena omphalomesenterica. In the dorsal mesentery it gives off numerous small branches, and crosses on the right

of the large intestine. In the umbilical cord, after again crossing the gut (on the left side of the small intestine), it follows the yolk-stalk. In a 17 mm. human embryo studied by Tandler ('03) the origin of this artery is opposite the 21st intersegmental (1st lumbar) artery.

On a level with the 2nd lumbar pair of dorsal intersegmental arteries the inferior mesenteric (*A.mes.i.*) arises from the aorta. It is a small ventral visceral branch, extending ventrally and somewhat caudally between the aortic bodies (of Zuckerkandl) into the dorsal mesentery. In the dorsal mesentery it gives off several branches which ramify in the region of the colon. Tandler ('03) found this artery in a 17 mm. human embryo arising from the aorta at the level of the 23d intersegmental (3d lumbar) artery.

That the arteries of the gut migrate cephalo-caudad was demonstrated by Mall ('91 and '97), but comparison of the origins of these arteries in this embryo with those described by Tandler ('03) in an embryo of approximately the same length, indicates that the rate of migration is somewhat variable, or we may have to do merely with variations in the point of origin, such as have been demonstrated for the superior mesenteric artery.

Just caudad of the pleural cavities and on a level with the 10th thoracic pair of dorsal intersegmental arteries, there appears a left lateral visceral artery. It extends from the aorta laterally and caudad first along the medial border of, and then through, the suprarenal gland to the Wolffian body. Apparently this vessel becomes the left inferior phrenic artery which furnishes a superior suprarenal branch in the adult. Another pair of lateral visceral (mesonephric) arteries (*A.s.-r.*) presumably the middle suprarenal of the adult leaves the aorta near the origin of the superior mesenteric artery. This pair of vessels extends laterally in a cephalo-dorsal direction to the anlagen of the suprarenal glands.

Beginning just caudad of the place of origin of the superior mesenteric artery the left components of four successive pairs of lateral visceral (mesonephric) arteries are seen in the reconstruc-

tion. The cephalic two have a common stem of origin from the aorta.

The first passes through the caudal part of the suprarenal gland to the Wolffian body. It presumably becomes the inferior suprarenal branch of the left renal.

The second skirts the caudal part of the suprarenal anlage, passing dorsal to the left suprarenal vein (left subcardinal), and ends in the region medial to the metanephros of that side. It may be concluded, therefore, that this artery (including the common trunk referred to above) becomes the left renal.

The corresponding artery (renal) on the right arises from the aorta dorsal to the left renal vein (renal anastomosis) and extends to the Wolffian body, passing dorsal to the subcardinal segment of the inferior vena cava.

Broman ('08) finds that the renal arteries take origin from the 21st or 22nd aortic segment.

The third mesonephric artery is small and, hence, of little moment. The corresponding one on the right passes in a strand of mesenchyma through the inferior vena cava (i.e., the supra-ureteral channel).

The fourth (*A.sp.i.*) is of considerable caliber. It takes origin at the level of the 21st dorsal intersegmental arteries, dorsal to the termination of the left suprarenal vein, and extends caudad, lateral to the anlage of the corresponding spermatic vein, to the Wolffian body. In the Wolffian body it supplies several glomeruli, lying adjacent to the most prominent part of the genital ridge.

The corresponding artery on the right arises from the aorta dorsal to the renal anastomosis. It extends ventral to the vena cava inferior (i.e., the supra-ureteral channel) and caudad along the lateral side of the anlage of the right spermatic vein to the Wolffian body.

These arteries, from their relations, the author believes, are the embryonic representatives of the internal spermatic (ovarian) arteries of the adult.

Broman ('08) finds that usually the spermatic arteries arise from the 22nd aortic segment.

Iliac arteries. Each common iliac artery (*Aa.il.com.*) arises from the lateral wall of the aorta directly opposite the third lumbar pair of dorsal intersegmental arteries, and on a level with the 2nd lumbar nerves. At first of small caliber the common iliacs soon increase notably in diameter. The right common iliac artery is the more fully displayed in the reconstruction (plate 1). It extends laterally and caudad, passing ventral to the caudal segment of the inferior vena cava, medial to the metanephros, and dorsal to the ureter and Wolffian body (compare plate 2). This relation of the common iliac artery, which is preserved in the adult, is probably that of a dorsal intersegmental artery.

The right common iliac artery terminates in two branches, the external iliac (*A.il.ext.*), a medium sized artery which accompanies the corresponding vein, and the arteria hypogastrica (*A.hypogas.*).

The external iliac artery first extends laterally and gives off a branch directed cephalad, the inferior epigastric (not shown in the reconstruction). It then continues caudad in the proximal part of the posterior limb-bud to finally join the A. ischiadica.

The hypogastric artery is only a short trunk which divides, lateral to the ureter, into dorsal and ventral branches (the so-called anterior and posterior divisions of adult anatomy). The dorsal branch appears in the drawing only as a short stump, but probably here continues as the superior gluteal artery. The ventral branch soon divides into the large umbilical artery (*A.um.d.*) and a short trunk common to the sciatic and internal pudendal arteries (*A.isch.d.* and *A.pud.i.*). The umbilical artery extends ventrally in the lateral body wall across the lateral side of the anlage of the bladder into the caudal wall of the umbilical cord.

The sciatic (inferior gluteal and A. comitans n. ischiadica) is at first dorso-lateral to the corresponding vein (*V.isch.*, plate 4) with which it continues into the posterior limb-bud. The tap between it and the femoral has already occurred.

The internal pudendal is at first ventral to the sciatic vein but soon bends ventrally into the cephalo-lateral part of the genital papilla where it becomes the artery of the clitoris or penis, according to sex.

Veins

The general distribution of the veins of the right side of the body is shown in plate 4. Plate 5 is a left lateral, and plate 6 a ventral view of part of the anterior cardinal system.

Anterior and common cardinal system. Between the dorso-medial surfaces of the cerebral hemispheres (plate 4) appears the paired anlage of the superior sagittal sinus (*S.sag.sup.*). This vessel together with numerous other tributaries arising on the lateral surface of the tel-, di-, and mes-encephalon (*Telen.*,*Dien.*, and *Mesen.*) contributes to form the anterior cerebral vein (*V.cer.a.*) which joins the cephalic end of the cavernous sinus (*S.cav.*). The ophthalmic vein (*V.oph.*) which is represented more fully in plate 5, joins the cavernous sinus ventral to the entrance of the anterior cerebral vein.

Venules arising on the lateral surface of the metencephalon (*Meten.*) unite to form the middle cerebral vein (*V.cer.m.*) which passes around the caudal border of the semilunar ganglion (*G.s-l.*) to enter the cavernous sinus.

The cavernous sinus is medial to the semilunar ganglion, and represents a persistent portion of the primitive anterior cardinal vein. The original anterior cardinal from the cavernous sinus to the site of the future jugular foramen, has disappeared, and a temporary vein, the vena capitis lateralis (*V.cap.lat.*), has formed lateral to the otocyst and the adjacent cranial nerves. The vena capitis lateralis is joined at the caudal aspect of the otocyst by the posterior cerebral vein (*V.cer.p.*) which takes its origin from a capillary plexus overlying the caudo-lateral surface of the myelencephalon (*Myelen.*).

From the point at which it receives the posterior cerebral vein the right anterior cardinal (internal jugular) takes a direct course towards the region of the heart. Dorsal to the heart it unites with the azygos vein (*V.az.*) to form the right common cardinal (*V.card.c.d.*). The right common cardinal, which becomes the proximal part of the adult vena cava superior, empties through the sinus venosus (*S.v.*) into the right atrium (*At.d.*).

The extra-cranial part of the right anterior cardinal is represented in the adult by the right internal jugular and innominate veins, and by the part of the superior cava distal to the point of entrance of the vena azygos.

The extra-cranial part of the left anterior cardinal (plate 5) at this time is symmetrical in size and position with its fellow of the right side. Just before entering the sinus venosus the left common cardinal (*V.card.c.s.*) turns to the right and occupies the sulcus coronarius on the diaphragmatic surface of the heart. It is shown in the latter situation in plates 1 and 4.

The vertebral veins of which the right (*V.ver.d.*) is reconstructed in plate 4, take the major share in the drainage of the cervical intersegmental veins. They open on either side into the dorsal aspect of the common cardinal near the termination of the azygos and hemiazygos respectively.

The above description of the extra-cranial portions of the anterior cardinal veins includes only the main venous channels draining the sinuses of the developing dura mater. In the opinion of the author the true 'anterior cardinals' are not represented by these channels alone, but by the main trunks of the vertebral veins as well. This opinion is supported by the occurrence in a pig embryo of 7.8 mm. (Thyng '11) of a series of fenestrae in the dorsal portion of the anterior cardinal vein (fig. 2) which appears to foreshadow the segregation of the dorsally placed vertebral vein from the main ventral channel (corresponding to the anterior cardinal as described above). A vertebral vein, thus formed, would receive the cervical intersegmental veins as does the vertebral in this embryo.

The right and left linguo-facial veins (*V.ling-fac.*) (Grosser '01, Lewis '09, and others) are shown in plates 4 and 5, respectively, and the terminal parts of both are seen in plate 6. Each arises in tributaries from its own side of the tongue, mandible and face. The trunk, thus formed, enters the ventral wall of the internal jugular vein of its own side, near the p'ace where the latter is crossed medially by the hypoglossal nerve (*N.hyp.*). Earlier in development each linguo-facial vein enters the ventral wall of the anterior cardinal at a more caudal level, i.e., immedi-

ately cephalad of the pericardial cavity. Or it may open into the common or even posterior cardinal vein (Lewis '09, p. 34).

Immediately cephalad of the pericardial cavity there now opens into the ventral wall of each anterior cardinal a vein (*V.thym.-thyr.*, plate 6) arising from a venous plexus of the thymic and thyreoid anlagen. For this vein the term, vena thymico-thyreoidea, does not seem inappropriate.

The right and left thymico-thyreoid veins, it seems to the author, undoubtedly unite to form the transverse anastomosis which eventually becomes the left innominate vein. Szawlowski ('91) arrived at a similar conclusion from a study of these veins in the human embryo, as did Anikew ('09) who investigated the question in pig embryos and in a human embryo of 17.5 mm. The vena anonyma sinistra when fully formed, would thus receive tributaries from the caudal part of the thyreoid anlage, the vv. thyreoideæ inferiores and ima of the adult.

Dorsal to the vena thymico-thyreoidea the right anterior cardinal receives a vein, arising in a plexus situated at the cephalic end of the thyreoid gland, and the caudo-dorsal wall of the pharynx. It is possible that this vessel may represent the middle thyreoid vein.

The subclavian veins, of which the left is shown in plate 5, (*V.scl.*), are formed by the union of the thoraco-epigastric (*V.th.-ep.*) and brachial veins. The latter vein begins as the primitive ulnar (*V.ul.pr.*) which forms a venous loop at the circumference of the hand-plate. Each subclavian (plate 6) joins the dorso-lateral aspect of the internal jugular vein of its own side somewhat caudal to the place of entrance of the vena thymico-thyreoidea. The plexiform termination of the subclavian (plate 5), the foramina of which transmit branches of the brachial plexus, would indicate that this vein is still migrating in the cephalic direction.

The terminations of the external jugular veins (*V.jug.ex.*) are distinct in plate 6. The terminal part of the left external jugular vein (*V.jug.ex.s.*, plate 5) enters the internal jugular on a level with the caudal extremity of the jugular lymph sac (*Sac.jug.*). It is also connected, more dorsally, by a considerable

channel with the terminal part of the subclavian vein at about the same level. The connection with the internal jugular is (usually) temporary, in which case the communication with the subclavian represents the future permanent outlet of the adult vessel. Sometimes a reverse condition occurs. Distally each external jugular vein is connected through a capillary plexus, caudad of the fossa conchae (shown, in part, in plate 5) with the linguo-facial vein and its tributaries from the hyoid arch.

The cephalic vein (*V.ceph.*) of which only a part is represented here, occupies the radial border of the arm, and passes superficial to the clavicle (which is just beginning to ossify) to join the external jugular. A similar condition of this vein has been represented for a human embryo of 22.8 mm. by Lewis ('09, fig. 4), and for a human embryo of 20 mm. by Evans ('12, fig. 478). The portion of the cephalic vein superficial to the clavicle (*V.jug.-ceph.*) has been named the jugulo-cephalic vein. It usually atrophies since the cephalic commonly acquires a new connection with the axillary. The jugulo-cephalic occasionally persists in the adult, in which case the cephalic remains partially or entirely tributary to the external jugular vein.

The proximal end of the external jugular vein receives ventrally the anterior jugular vein (plate 5), proceeding from a superficial venous plexus of the neck. A similar venous connection between the external and anterior jugular veins has been represented in a human embryo of 22.8 mm. by Lewis ('09, fig. 4). Since the anterior jugular vein is in the adult normally a tributary of the external, it may be supposed that that part of the external jugular vein which now opens into the internal jugular, finally becomes the terminal part of the anterior jugular.

Posterior cardinal system. The primitive posterior cardinals in great part have lost their identity since they have been reduced to sinusoidal channels by the developing Wolffian bodies. These channels, some of which gain prominence have been reduced further or interrupted by the developing metanephros and suprarenal gland.

Minot ('98) demonstrated that the posterior cardinal veins in pig embryos become subdivided by the mesonephric tubules into

sinus-like channels, and in 1900 he introduced additional histological evidence of the presence of these channels to which he applied the name 'sinusoids.' It is also noteworthy that Hochstetter ('93) expressed doubt whether in man the azygos and hemiazygos veins could be considered in their entirety as remnants of the posterior cardinals.

Only the right posterior cardinal derivatives have been reconstructed (plate 4).

The middle sacral component (*V.s.m.*) of the right posterior cardinal begins in the tail-process and extends cephalad ventrolateral to the arteria sacralis media (*A.s.m.*, pl. 1), receiving as tributaries the right caudal intersegmental veins.

At the level of the fourth and fifth lumbar intersegmental veins the right vena sacralis media is connected to its companion vein on the left by a relatively large, transverse anastomosis (x'), passing ventral to the middle sacral artery. Other anastomoses of the middle sacral veins exist more caudally.

It is by virtue of this large transverse anastomosis (which becomes the terminal part of the left common iliac vein of the adult) that the left middle sacral and the veins of the left lower extremity, come to drain into the inferior vena cava.

Two venous channels (sub- and supra-ureteral) extend cephalad from the right middle sacral, enclosing between them an area of mesenchyma through which passes the ureter (*Ur.*). The smaller, sub-ureteral channel having at first passed dorsal to the Wolffian body, with the sinusoids of which it is intimately connected, bends medially to terminate in the caudal part of the persistent portion of the right subcardinal vein (*V.scard.d.*) described below. This channel from its relation to the ureter and right Wolffian body, corresponds perfectly with Hochstetter's 'Urnierenvene.' It is of importance as Hochstetter ('93) pointed out, in that from its cephalic portion and the tributary mesonephric sinusoids there probably arises the right spermatic vein.

The channel extending from the vena sacralis media, dorsomedial to the ureter (supra-ureteral), is much larger than the sub-ureteral. It extends cephalad, lateral to the aorta, and receives as tributaries the last two thoracic and first four lumbar

intersegmental veins. Opposite the 1st lumbar spinal nerve it bends ventrally, and is continued as a large dorso-ventral channel (compare Sabin '09, fig. 11), which terminates in the caudal extremity of the part of the subcardinal which still persists. This dorso-ventral segment of the supra-ureteral channel, extends medial to the metanephros and Wolffian body from both of which it receives tributary veins. One (or more) of these tributaries undoubtedly forms the right renal vein (or veins). The supra-ureteral channel forms a considerable portion of the definitive inferior vena cava.

The subcardinal veins are derived from Wolffian sinusoids in the region ventral to the mesonephric arteries (Lewis '02).

At this stage the right subcardinal (*V.scard.d.*) begins at the renal anastomosis (*x*), which is formed by the confluence of the sub- and supra-ureteral channels from both sides of the body. From the renal anastomosis the right subcardinal vein extends cephalad, ventral to the right suprarenal gland (*Gl.s.-r.d.*), as a component of the vena cava inferior.

By the development of the suprarenal gland the cephalic part of the V. subcardinalis (which previously opened into the posterior cardinal vein on a level now represented by the cephalic extremity of the gland) has been reduced to sinusoidal channels. The cephalic end of the portion of the right subcardinal vein, now persisting, represents the anlage of the right suprarenal vein (*V.s.-r.d.*) which connects, as does the azygos, with the suprarenal sinusoids.

It should be stated here that the presence of sinusoids in the suprarenal glands of mammals and other vertebrates, has been demonstrated histologically by Minot ('00).

Also significant in this connection are the observations of Lewis ('02, p. 236) upon the veins in question in a rabbit embryo, 11 mm. in length. He says "at the upper end of the veins, on either side, cardinal and subcardinal anastomose in condensed mesenchyma probably connected with the suprarenal anlage."

The large renal anastomosis (*x*), referred to above, unites the two subcardinals. It crosses the median line, ventral to the aorta, a little caudad of the superior mesenteric artery (*A.mes.s.*) of

which a short piece has been added in plate 4. It persists as the terminal part of the left renal vein.

Simultaneously with the transformation of the subcardinal veins that part of the right subcardinal, situated ventral to the developing suprarenal gland has tapped the hepatic sinusoids as described by Lewis ('02). In this manner there has developed that segment of the vena cava inferior, intervening between the subcardinal and common hepatic (*V.hep.com.*) veins.

The entire vena cava inferior, as shown in plate 4, is composed, therefore, of four parts. These are the supra-ureteral channel; a segment of the original right subcardinal (*V.scard.d.*) intermediate between the renal anastomosis and the termination of the anlage of the right suprarenal vein (*V.s.-r.d.*); a large channel passing through the plica venae cavae formed by the tapping of the hepatic sinusoids by the right subcardinal, and the terminal part, the vena hepatica communis (*V.hep.com.*), which empties into the right atrium through the sinus venosus (*S.v.*).

The course of the vena azygos (*V.az.*) along the lateral side of the aorta has been interrupted in the region of the suprarenal gland, so that it now receives the first ten thoracic intersegmental veins (the first three indirectly, for they unite to form a common trunk, the vena intercostalis suprema dextra).

The vena azygos, a little cephalad of the termination of the vena intercostalis suprema, also receives a small ventral tributary, the cephalic remnant of an earlier mesonephric sinusoid, figured in pig embryos, by Lewis ('03), Davis ('10), and Thyng ('11). This vessel has been designated by Davis ('10) the ventro-lateral vein of the mesonephros.

At the level of the 10th thoracic intersegmental vein the vena azygos communicates with the hemiazygos by a transverse anastomosis, passing dorsal to the aorta. Slightly cephalad of this anastomosis tributaries from the suprarenal gland (referred to above) join the azygos.

Although the left posterior cardinal system has not been reconstructed, the following observations are here recorded for the sake of completeness.

The sub-ureteral channel (Urnierenvene of Hochstetter) has been obliterated caudally. The supra-ureteral channel is not only much smaller than its companion on the right, but is subdivided caudally by numerous mesenchymal septa, indicative of commencing atrophy.

The left subcardinal vein, caudad of the renal anastomosis has degenerated, but its cephalic extremity is connected with the suprarenal sinusoids as on the right. It will ultimately form the left suprarenal vein, a tributary to the left renal.

The vena hemiazygos still opens into the left common cardinal. It is connected with the azygos by the anastomosis at the level of the 10th thoracic spinal nerve, mentioned above.

Portal system. The left umbilical vein (*V.um.s.*) is represented in plate 4. In the liver it communicates with the hepatic sinusoids, its blood passing chiefly through an especially large sinusoid, the ductus venosus (*D.v.*) which joins the left side of the common hepatic vein (vena cava inferioris).

The trunk (*V.vit.*) formed by the fused vitelline veins (Begg '12) passes through the coelom in a separate strand of mesentery (plate 2). In the dorsal mesentery of the duodenum it is joined by the superior mesenteric vein (*V.mes.s.*) of which only the stump is here shown. The trunk formed by this union, the vena omphalo-mesenterica (*V.omp.-mes.*), now bends cephalad, and passes dorsal to the anastomosis of the dorsal and ventral pancreatic outgrowths. After receiving the lienal vein (*V.li.*) it becomes the vena portae (*V.P.*) which enters the liver where it joins the hepatic sinusoids, and, by means of a considerable channel, the right side of the ductus venosus.

The hepatic sinusoids discharge into the vena cava inferior (common hepatic segment) by way of the hepatic veins (*Vv.hep.*).

Lymphatics

The jugular lymph sacs (*S.jug.*), comparable to those discovered by Sabin ('02) are seen in plate 6. The left also appears in plate 5. They are large, nearly symmetrical sacs somewhat constricted into segments, and are situated one on either side,

immediately lateral to the internal jugular vein. Through the sacs pass branches of the cervical plexus.

Saccus jugularis sinister. The cephalic end of the left sac communicates with the internal jugular vein through a small channel (*a*). There is also a large caudal connection between the sac and vein which cannot be seen in the reconstruction. The latter opening is in the caudo-medial wall of the sac near the temporary opening of the external jugular vein (*V.jug.ex.*) into the internal jugular. Incomplete valves, dorsally and ventrally placed, guard the opening.

The branches of the cervical plexus which pass through this sac are seen in plate 5. Through the cephalic extremity extends the great auricular (*N.aur.m.*). A little more caudally there issues a branch of the third cervical which is joined lateral to the sac by a branch of the fourth cervical nerve. The trunk thus formed, immediately gives off the small occipital, and then bends ventrally across the sac as the N. cutaneous colli.

The vasa lymphatica superficialia (*Vas.lym.sup.*), arising from the cephalo-lateral portion of the left lymph sac, are seen plainly in plate 6. They extend laterally and dorsally into the subcutaneous tissue. A prolongation from the caudal extremity of the left sac overlies the lateral aspect of the terminal part of the subclavian vein.

Saccus jugularis dexter. The cephalic end of the right lymph sac does not communicate with the internal jugular vein as does the left. Caudally at a level corresponding approximately with the large communication on the left, the right sac opens into the internal jugular. The opening in this case is a very small slit-like aperture between two valves, a lateral and a medial. The lateral valve is adjacent to the permanent termination of the external jugular vein, while the medial projects into the cavity of the internal jugular.

A prolongation of the sac overlies the lateral surface of the proximal portion of the subclavian vein as occurred on the left.

Saccus mesentericus. There is a plexus of vessels, situated immediately ventral to the aorta, which extends for the most part between the proximal parts of the superior and inferior mesen-

teric arteries. The cephalic part of this plexus lies between the subcardinal segment of the inferior vena cava and the left suprarenal vein (left subcardinal), the caudal portion lies dorsal to the renal anastomosis. In places the diameter of the plexus is equal to, or even greater than, that of the adjacent aorta. Although the lumina of the vessels are closely packed with corpuscles, it is difficult to connect the vessels with any of the definitely formed veins. Nevertheless, it would be impossible at least without reconstructions to say that such connections do not exist. From the relations of the plexus, given above, there seems little doubt that it corresponds to the anlage of the mesenteric lymph sac, discovered in rabbit embryos by Lewis ('02 and '05).

Baetjer ('08) who has investigated the development of this sac in a series of pig embryos, concludes that it originates in a series of small veins which separate from the renal anastomosis.

Cisterna chyli. A series of anastomosing venous channels is found on the right and left side, dorso-lateral to the aorta. The cephalic end of this plexus apparently connects with the azygos and hemiazygos veins respectively. Many of the channels open into the supra-ureteral venous channels, previously described. The intersegmental arteries in this locality extend dorsal to these channels, although offshoots of the latter frequently anastomose between the successive pairs of arteries, dorsal to the aorta. These vessels may represent the anlage of the cisterna chyli, for they correspond in position to the anlage as described by Sabin ('09).

Sacci lymphatici posteriores. Definite posterior lymph sacs in relation to the sciatic veins have not been found, but numerous small tributaries, entering the proximal part of the veins may foreshadow them. Sabin ('09) states that they first appear in an embryo of 20 mm., as a plexus of small veins.

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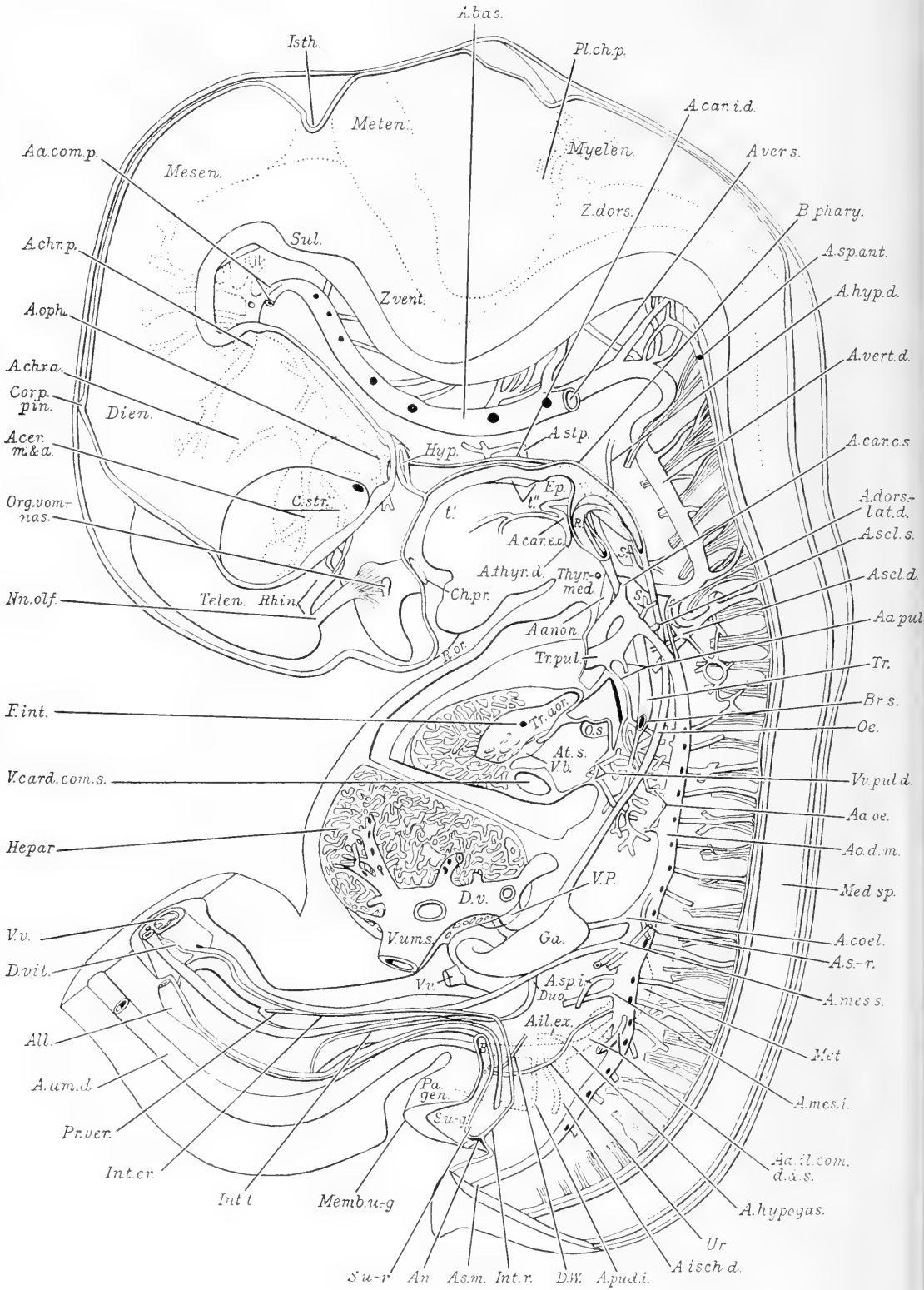
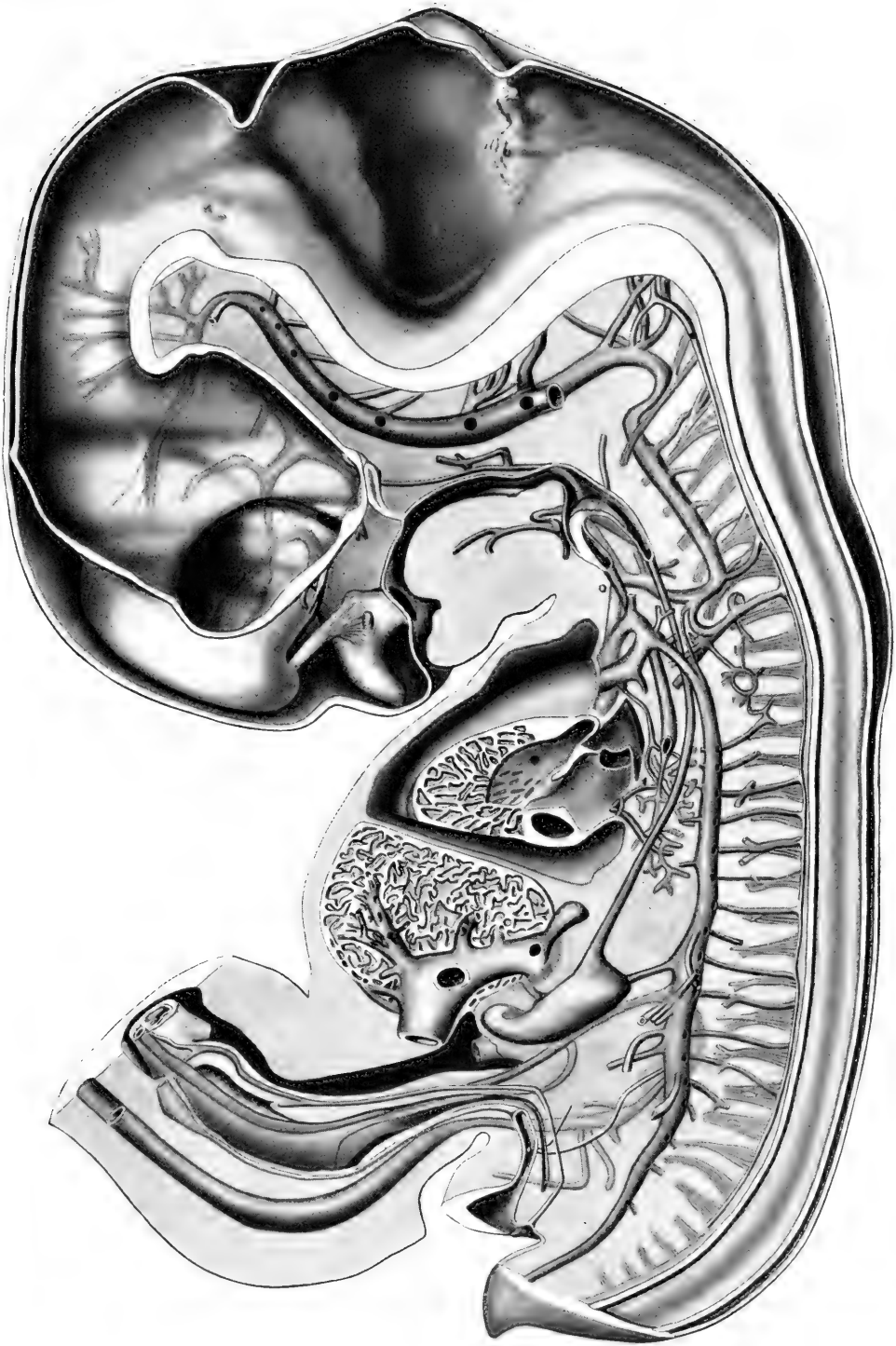
ABBREVIATIONS

All., allantois
An.hyp.(*Ans.hyp.*), ansa hypoglossi
An., anus
Ao.dors.lat., aorta dorsalis lateralis
Ao.d.m., Ao. dorsalis mediana
Arc.ao., arcus aortae
A.anon., arteria anonyma
A.aur.p., A. auricularis posterior
A.bas., A. basilaris
A.car.c.d., A. carotis communis dextra
A.car.c.s., A. carotis communis sinistra
A.car.ex., A. carotis externa
A.car.i.d., A. carotis interna dextra
A.car.i.s., A. carotis interna sinistra
A.cer.a., A. cerebri anterior
A.cer.m., A. cerebri media
A.cer.p., A. cerebri posterior
A.cereb.s., A. cerebelli superior
A.chr.a., A. chorioidea anterior
A.chr.p., A. chorioidea posterior
A.coel., A. coeliaca
Aa.com.p., Aa. communicantes posteriores
A.hyp.d., A. hypoglossa dextra
Aa.il.com.d. and s., Aa. iliacae communes, dextra et sinistra
A.il.ex., A. iliaca externa
A.isch.d., A. ischiadica dextra (A. glutea inferior et a. comitans n. ischiadici)
A.ling., A. lingualis
A.mam.i., A. mammaria interna
A.max.ex., A. maxillaris externa
A.mes.s., A. mesenterica superior
A.mes.i., A. mesenterica inferior
A.occ., A. occipitalis
Aa., Aa. oesophageae
A.oph., A. ophthalmica
A.pud.i., A. pudenda interna
Aa.pul., Aa. pulmonales
A.s.m., A. sacralis media
A.scl., A. subclavia
A.sp.i., A. spermatica interna
A.stp., A. stapedia
A.s-r., A. suprarenalis
A.thyr.d., A. thyreoidea dextra
A.thyr.s., A. thyreoidea sinistra
A.um.d., A. umbilicalis dextra
A.vert.d., A. vertebralis dextra
A.vert.s., A. vertebralis sinistra
At.d., atrium dextrum
At.s., At. sinistrum
B., vesicula urinaria
Br.s., bronchus sinister
B.phary., bursa pharyngea
Ch.pr., choana primitiva
Con.art., conus arteriosus
Corp.pin., corpus pineale
Corp.str., corpus striatum
Dien., diencephalon
D.a., ductus arteriosus
D.c., D. cochlearis
D.cyst., D. cysticus
D.end., D. endolymphaticus
D.hep., D. hepaticus
D.p.d., D. pancreatis dorsalis
D.v., D. venosus
D.vit., D. vitellinus
D.M., D. Mulleri
D.W., D. Wolffii
Duo., duodenum
Em.i-p., eminentia interpeduncularis
Ep., epiglottis
Eth.-turb.I., ethmo-turbinale I
Fim., fimbriae
Flex.ceph., flexura cephalica
Flex.cerv., flexura cervicalis
Fl.pl., floor-plate
Fo.ep., foramen epiploicum
Fo.int., foramen interventriculare (Monroi)
Fo.iv., foramen interventriculare
G.gn., ganglion geniculatum
Gg. hyp., Gg. hypoglossa
G.jug., G. jugulare
G.nodos., G. nodosum
G.petros.(*G.p.*), G. petrosum
G.s-l., G. semilunaris
G.sup., G. superius
G.ves., G. vestibularis
Ga., gaster

G.R., genital ridge
Gl.inf., glandula infundibularis
Gl.p., Gl. parotidis
Gl.smx.(*Gl.s.*), Gl. submaxillaris
Gl. s-r.d., Gl. suprarenalis dextra
Hyp., hypophysis
Int.cr., intestinum crassum
Int.r.(*Int.rec.*), intestinum rectum
Int.t., intestinum tenue
Isth., isthmus
L., lens
Md., mandible
Max.-turb., maxillo-turbinale
Md.sp., medulla spinalis
Mem. u.-g., membrana urogenitalis
Mesen., mesencephalon
Mesogas., mesogastrium
Met., metencephalon
Myelen., myelencephalon
Na., naris
N.a., aberrant nerve of myelencephalon
N.abd.(*N.ab.*), nervus abducens
N.acc., N. accessorius
N.alv.inf., N. alveolaris inferior
N.acus., N. acusticus
N.aur.m., N. auricularis magnus
N.c.i., N. cardiacus inferior
N.ch.tymp.(*Ch.ty.*), N. chorda tympani
N.c.c., N. cutaneous colli
N.fac., N. facialis
N.fr., N. frontalis
N.glos., N. glossopharyngeus
N.hyp., N. hypoglossus
N.int., N. intermedius
N.laryng.s.(*N.l.s.*), N. laryngeus superior
N.l.(*l.*), N. lingualis
N.md., N. mandibularis
N.mx., N. maxillaris
N.na.-cil., N. nasociliaris
N.occ.m., N. occipitalis major
N.oc., N. oculomotorius
Nn.olf., Nn. olfactorii
N.oph., N. ophthalmicus
N.pet.s.m., N. petrosus superficialis major
N.phr., N. phrenicus
N.rec.s., N. recurrens sinister
Nn.sp., Nn. spinales
Nn.s.-cl.(*Nn.s-c.*), Nn. supraclaviculares (common trunk)
N.troch., N. trochlearis
N.tym., N. tympanicus
N.vag., N. vagus
Oe., oesophagus
Op.s., optic stalk
Or.vom.-nas., organon vomero-nasale
O.s., ostium secundum
Panc.d.and v., pancreas dorsale et ventrale
Pa.gen., papilla genitalis
P.-thyr.IV., parathyreoidea IV
Ph.P.1, pharyngeal pouch 1
Ph.P.2, pharyngeal pouch 2 (region only)
Ph., pharynx
Pl.ch.p., plica chorioidea posterior
Pr.i-m., processus intermaxillaris
Pr.pl., processus palatinus
Pr.ver., processus vermiformis
R., raphé
R.des., ramus descendens (hypoglossi)
R.ex., ramus externus (accessorii)
R.p-tr., ramus posttrematicus (of fourth pharyngeal pouch)
R.po-op., recessus postopticus
R.p-op., recessus preopticus
Rhin., rhinencephalon
R.or., rima oris
Rf.pl., roof-plate
Sac., sacculus
Sac.jug.(*S.jug.*), saccus lymphaticus jugularis
S., septum aorticum (aortico-pulmonale)
S.u.-r., septum uro-rectale
S.cav., sinus cavernosus
S.sag.sup., S. sagittalis superior
S.u.-g., S. uro-genitalis
S.v., S. venosus
S (plate 1) vestigial portions of dorsolateral aortae
Sul., sulcus (mesencephali?)
T.1., ganglion thoracicale primum
Telen., telencephalon

Thy., thymus
Thyr., glandula thyreoidea
Thyr.m., gl. thyreoidea media
t′ and t″, anterior and posterior parts of the tongue
Tr., trachea
Tr.aor., truncus aorticus
Tr.cost.cerv., Tr. costocervicalis
Tr.pul., Tr. pulmonalis
Tr.thyr.-cerv., Tr. thyreocervicalis
Tub.p., tuberculum posterius
T.W., tubuli Wolffii
Umb.c., umbilical cord
Ur., ureter
Ut., utriculus
Vas.lymp.sup., vasa lymphatica superficialia
Vv.v.s., valvulae sinus venosi
V.b., valvula bicuspidalis
V.t., V. tricuspidalis
V.az., vena azygos
V.cap.lat., V. capitis lateralis
V.card.a., V. cardinalis anterior
V.card.c.d., V. cardinalis communis dextra
V.card.c.s.(*V.card.com.s*), V. cardinalis communis sinistra
V.card.p., V. cardinalis posterior
V.ceph., V. cephalica
V.cer.a., V. cerebralis anterior
V.cer.m., V. cerebralis media
V.cer.p., V. cerebralis posterior
V.fem., V. femoralis
Vv.hep., Vv. hepaticae
V.hep.com., V. hep. communis
V.isch., V. ischiadica
V.jug.-ceph., V. jugulo-cephalica
V.jug.ex., V. jugularis externa
V.jug.i., V. jugularis interna
V.li., V. lienalis
V.ling.-fac., V. linguo-facialis
V.mes.s., V. mesenterica superior
V.oph., V. ophthalmica
V.omp.-mes., V. omphalo-mesenterica
V.P., V. portae
Vv.pul.d., Vv. pulmonales dextrae
V.s.m., V. sacralis media
V.scard.d., V. subcardinalis dextra
V.scl., V. subclavia
V.s-r.d., V. suprarenalis dextra
V.th.-ep., V. thoracico-epigastrica
V.thy.-thyr., V. thymico-thyreoidea
V.ul.pr., V. ulnaris primitiva
V.um.s., V. umbilicalis sinistra
V.ver.d., V. vertebralis dextra
V.v., Vv. vitellinae (fused)
Vent.d., ventriculus dexter
Ves.fel., vesica fellea
Ves.op., vesicula optica
x, renal anastomosis (V. renalis sinistra)
x′, transverse iliac anastomosis (V. iliaca communis sinistra)
X, strand of mesentery containing the fused vitelline veins
y, branch of right jugular lymph sac
Z.dors., zona dorsalis
Z.vent., zona ventralis
1 to 8, ganglia cervicales


A.bas.
Isth.
Pl.ch.p.
Meten.
Myelen.
A.car.i.d.
Aa.com.p.
Mesen.
A.vers.
Z.dors.
B.phary.
Sul.
A.sp.ant.
A.chr.p.
Z.vent.
A.hyp.d.
A.oph.
A.vert.d.
A.chr.a.
Corp. pin.
Dien.
A.car.c.s
Acer. m.&a.
Hyp.
A.stp.
Org.vom-nas.
C.str.
Ep.
A.dors.-lat.d.
A.car.ex.
A.scl.s.
A.thyr.d.
Thyr.-med.
A.scl.d.
Nn.olf.
Telen.
Rhin.
Ch.pr.
Aa.pul
A.anon.
Tr.pul
Tr.
F.int.
Tr.aor.
Br.s.
Oc.
At.s.
V.b.
V.card.com.s.
Vv.pul.d
Aa.oe.
Hepar
Ao.d.m.
V.P.
Med.sp.
D.v.
V.v.
Vum.s.
Ga.
A.coel.
D.vit.
A.s.-r.
Vv.
A.spi-Duo.
A.mes.s.
A.il.ex.
All.
Met
A.um.d.
Pa. gen.
A.mes.i.
Pr.ver.
S.u-g.
Aa.il.com. d.a.s.
Int.cr.
Int.t
Memb.u-g
A.hypogas.
Ur
A isch.d.
Su-r
An
As.m.
Int.r.
D.W.
A.pud.i.

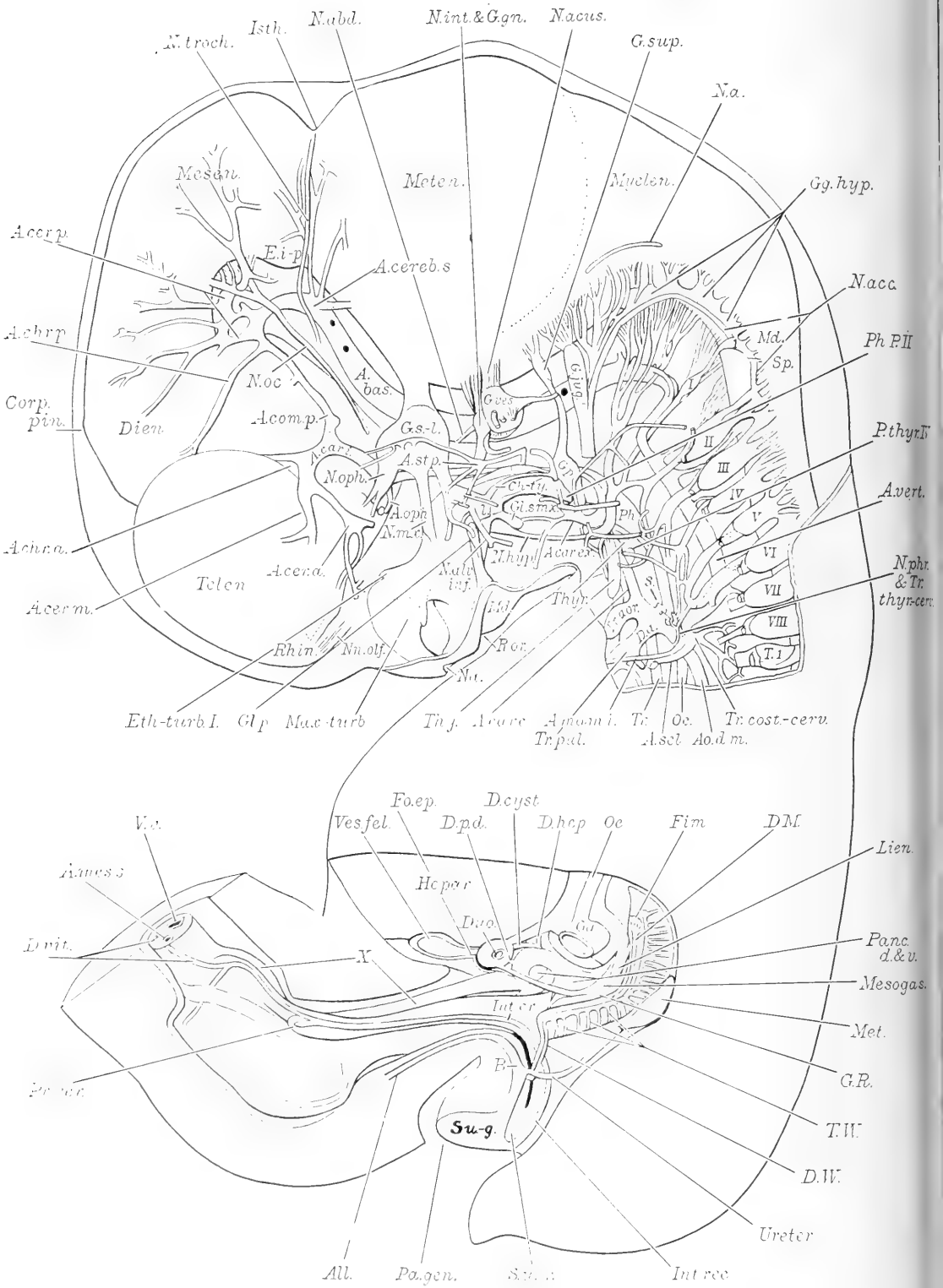


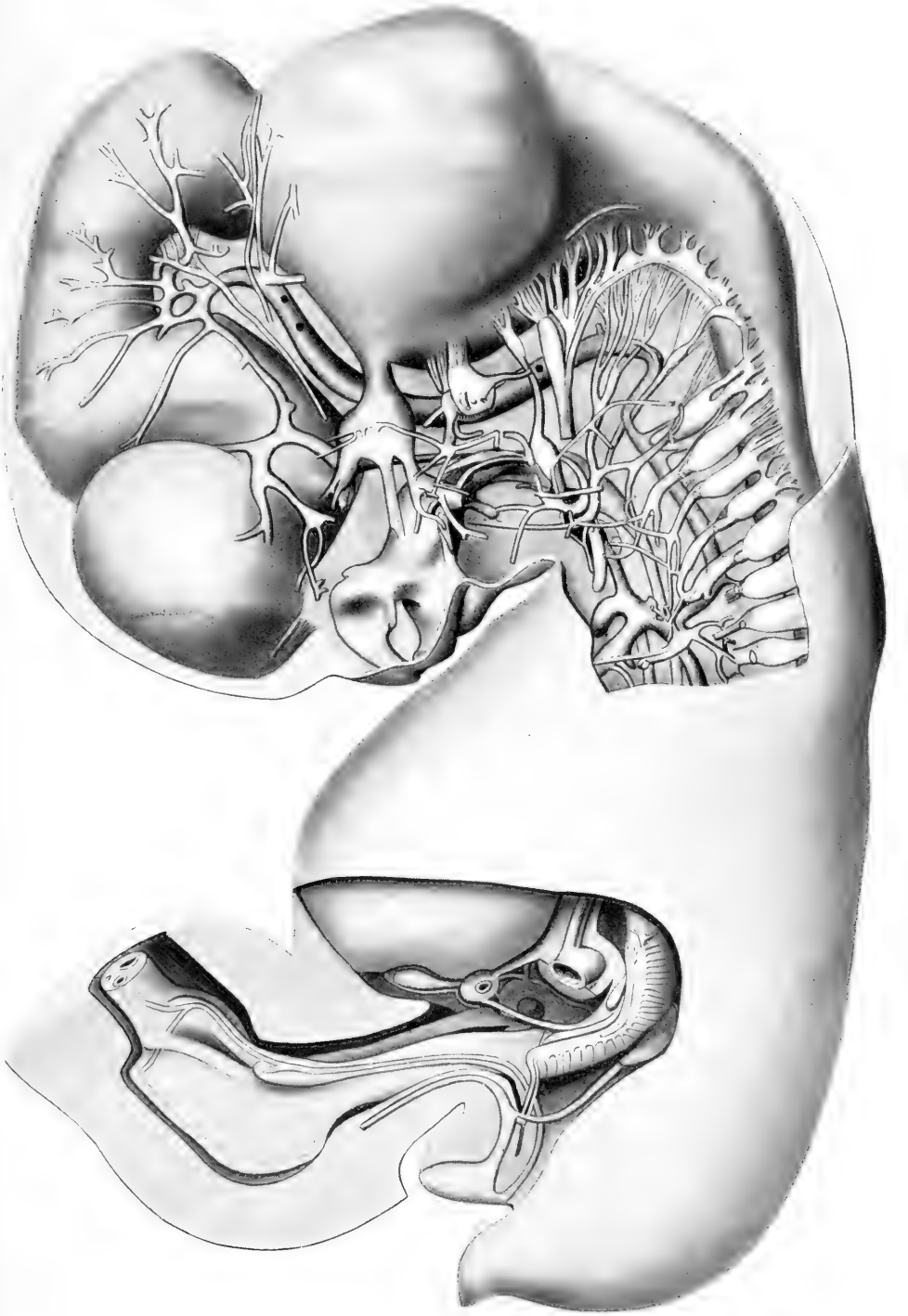
Reconstruction to illustrate chiefly the interior of the brain and the spinal cord; the digestive system and its appendages; the arterial system; the left atrium and ventricle of the heart, and in part the urogenital system of a 17.8 mm. human embryo (H. E. C. 839). × 11.2 diams.

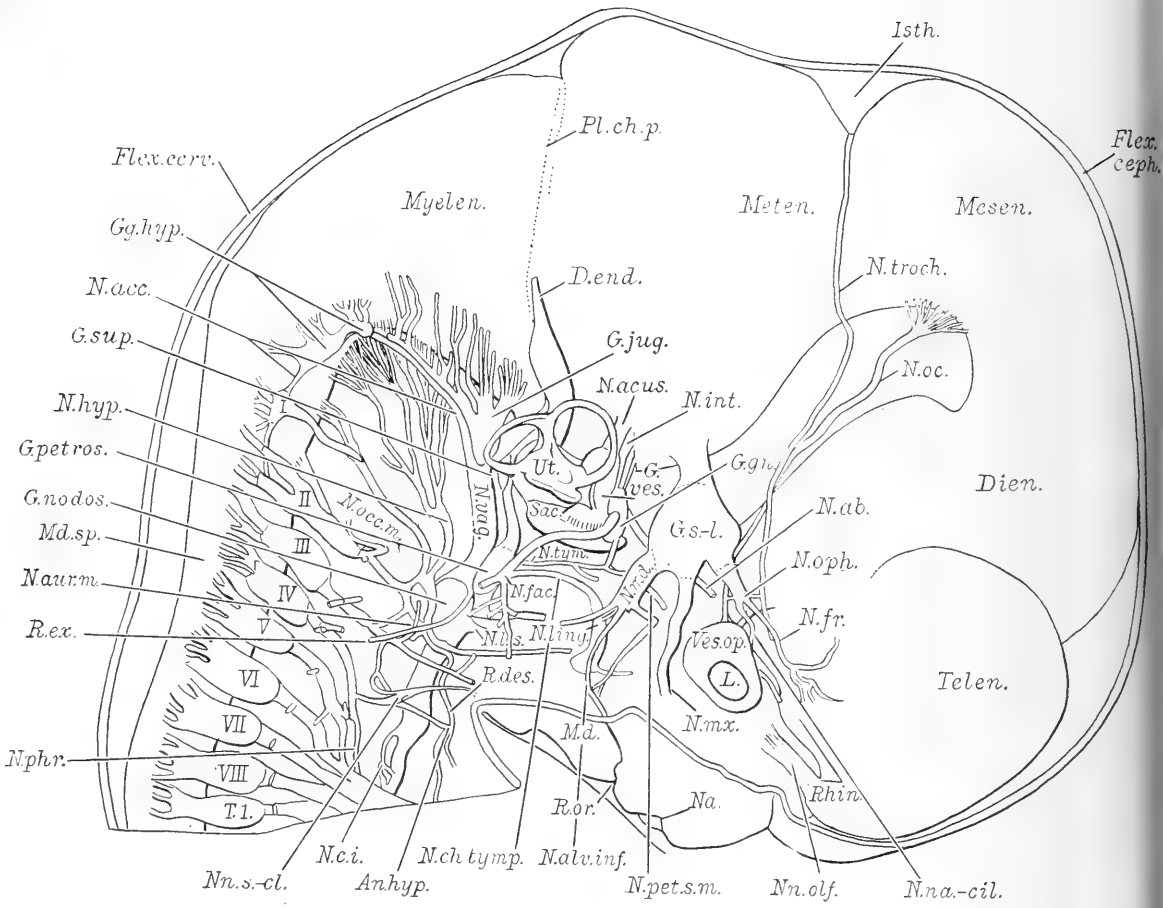
PLATE 2

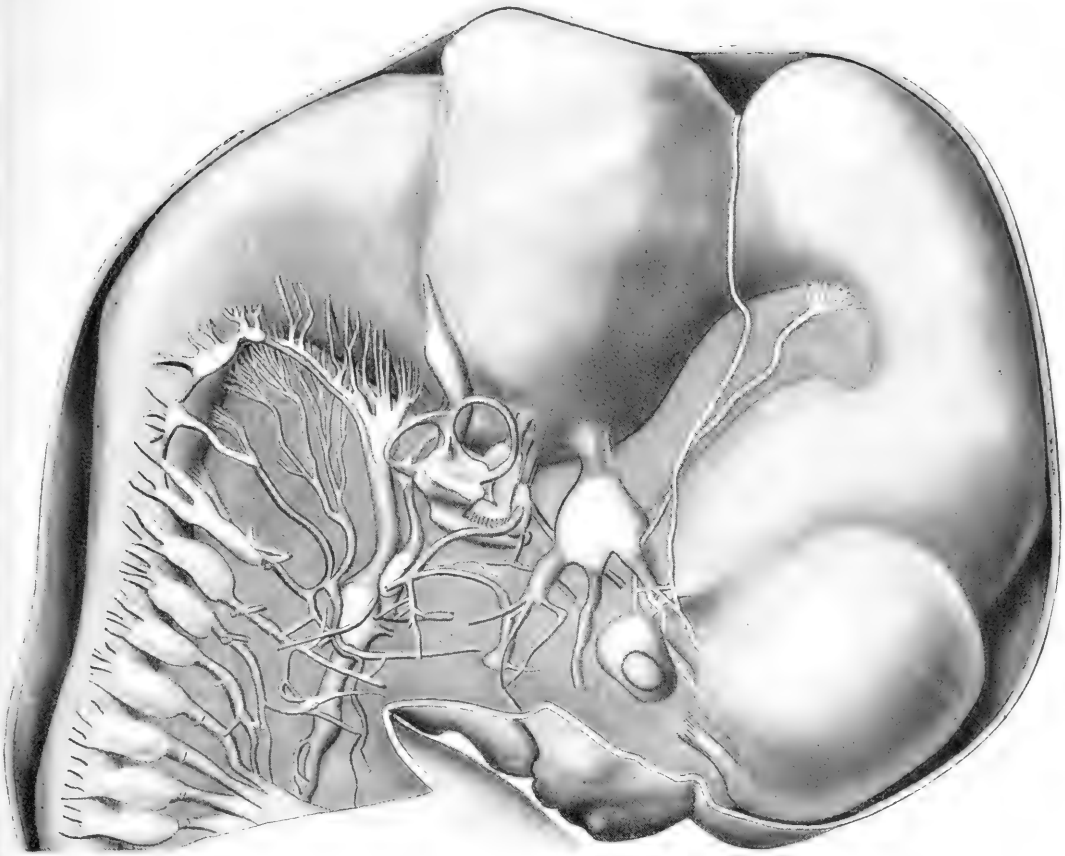
EXPLANATION OF FIGURE

This plate consists of two reconstructions. The upper shows a left lateral view of the brain and cervical cord with the nerves in situ, the aortic arch, and other arteries of the left side of the head and neck. It also represents the oral, nasal and pharyngeal epithelia; the left thymic and thyreoid anlagen; and illustrates in a measure the relation of the nerves and arteries to these epithelial structures. The lower reconstruction shows the pancreas and spleen within the mesogastrium (a portion of the stomach having been removed); the left genital ridge, and the left meso- and metanephros with their ducts opening independently into the urogenital division of the cloaca (H. E. C. 839). $\times$ 11.2 diams.

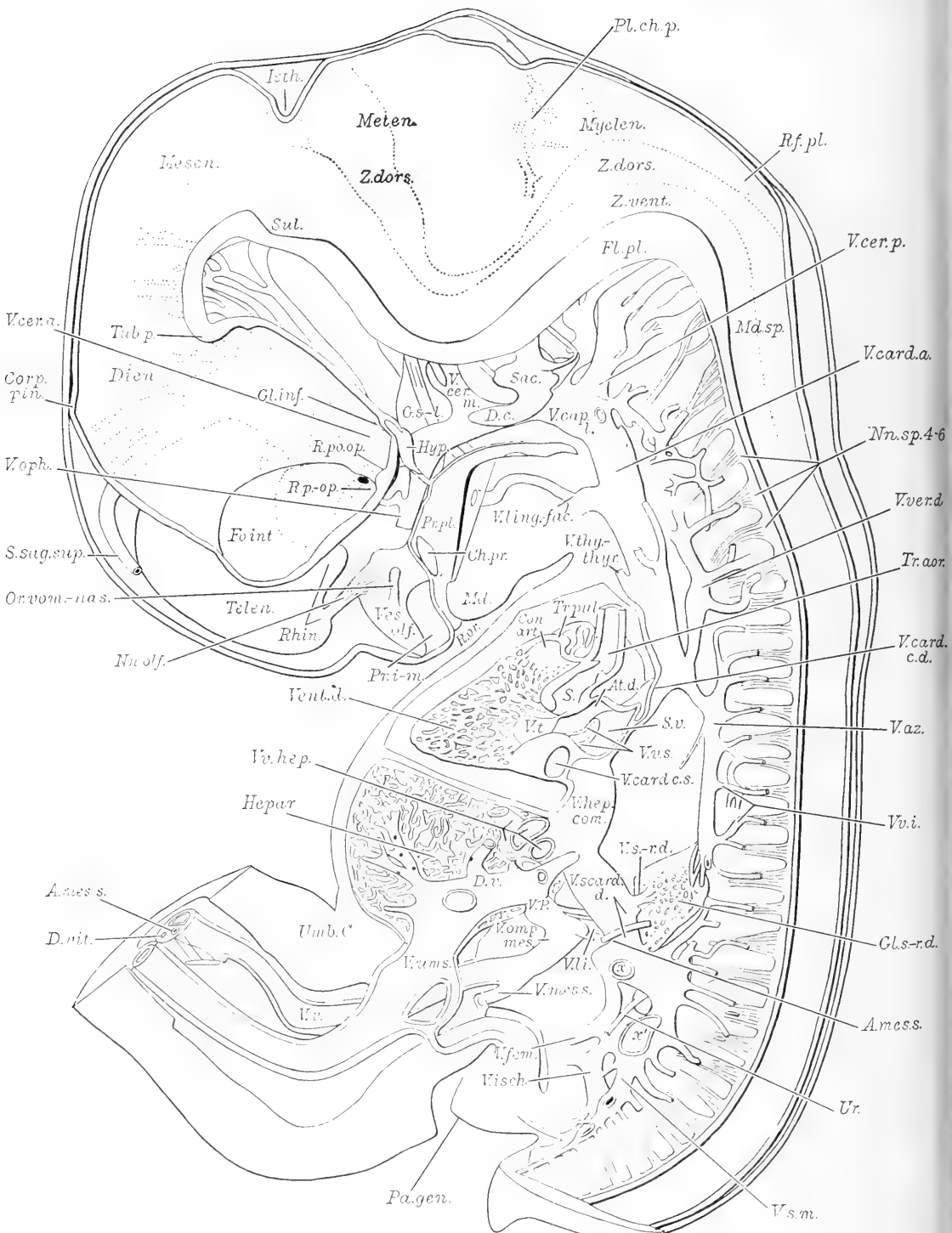

N.troch.
Isth.
N.abd.
N.int.&G.gn.
N.acus.
G.sup.
N.a.
Mesen.
Meten.
Myelen.
Gg.hyp.
A.cer.p.
E.i.p.
A.cereb.s
N.acc.
A.chr.p.
N.oc.
A. bas.
Md.
Sp.
Ph.P.II
Corp. pin.
Dien.
A.com.p.
Gs.-l.
P.thyr.V
A.car.
A.st.p.
N.oph.
A.oph.
N.m.c.
A.vert.
A.chr.a.
A.cer.a.
Telen
A.cer.m.
N.alf. inf.
Thyr.
N.phr. & Tr. thyr-cer.
Rhin.
Nm.olf.
Ror.
Nu.
Eth-turb.I.
Gl.p
Max.-turb
Thy.
A.car.c.
A.mand.i.
Tr.pal.
Tr.
A.scl.
Oe.
Ao.d.m.
Tr.cost.-cerv.
Fo.ep.
D.cyst
Vesfel.
D.p.d.
D.hep
Oe
Fim
DM.
V.c.
Lien.
Anes.s
Hepar
Duo.
Gl.
Pan.c. d.& v.
D.mit.
X
Mesogas.
Int.cr.
Met.
Pr.cr.
B.
G.R.
Su-g.
T.W.
D.W.
Ureter
All.
Pa.gen.
Int.cr.c.




Isth.
Pl.ch.p.
Flex.
ceph.
Flex.cerv.
Myelen.
Meten.
Mesen.
Gg.hyp.
N.troch.
D.end.
N.acc.
G.jug.
N.oc.
G.sup.
N.acus.
N.int.
N.hyp.
Ut.
G.
ves.
Gg.
Dien.
G.petros.
G.nodos.
N.occ.m.
N.vag.
Sac.
N.ab.
Md.sp.
G.s-l.
N.tym.
N.oph.
N.aur.m.
N.fac.
N.fr.
R.ex.
N.l.s.
N.ling.
Ves.op.
R.des.
L.
Telen.
N.mx.
M.d.
N.phr.
Na.
Rhin.
R.or.
N.c.i.
N.ch tymp.
N.alv.inf.
Nn.s.-cl.
An.hyp.
N.pet.s.m.
Nn.olf.
N.na.-cil.
I
II
III
IV
V
VI
VII
VIII
T.1.



Reconstruction showing the right side of the brain and the cervical cord; the right cranial and cervical nerves; the internal ear, and the optic vesicle of a 17.8 mm. human embryo (H. E. C. 839). × 11.9 diams.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Hepar
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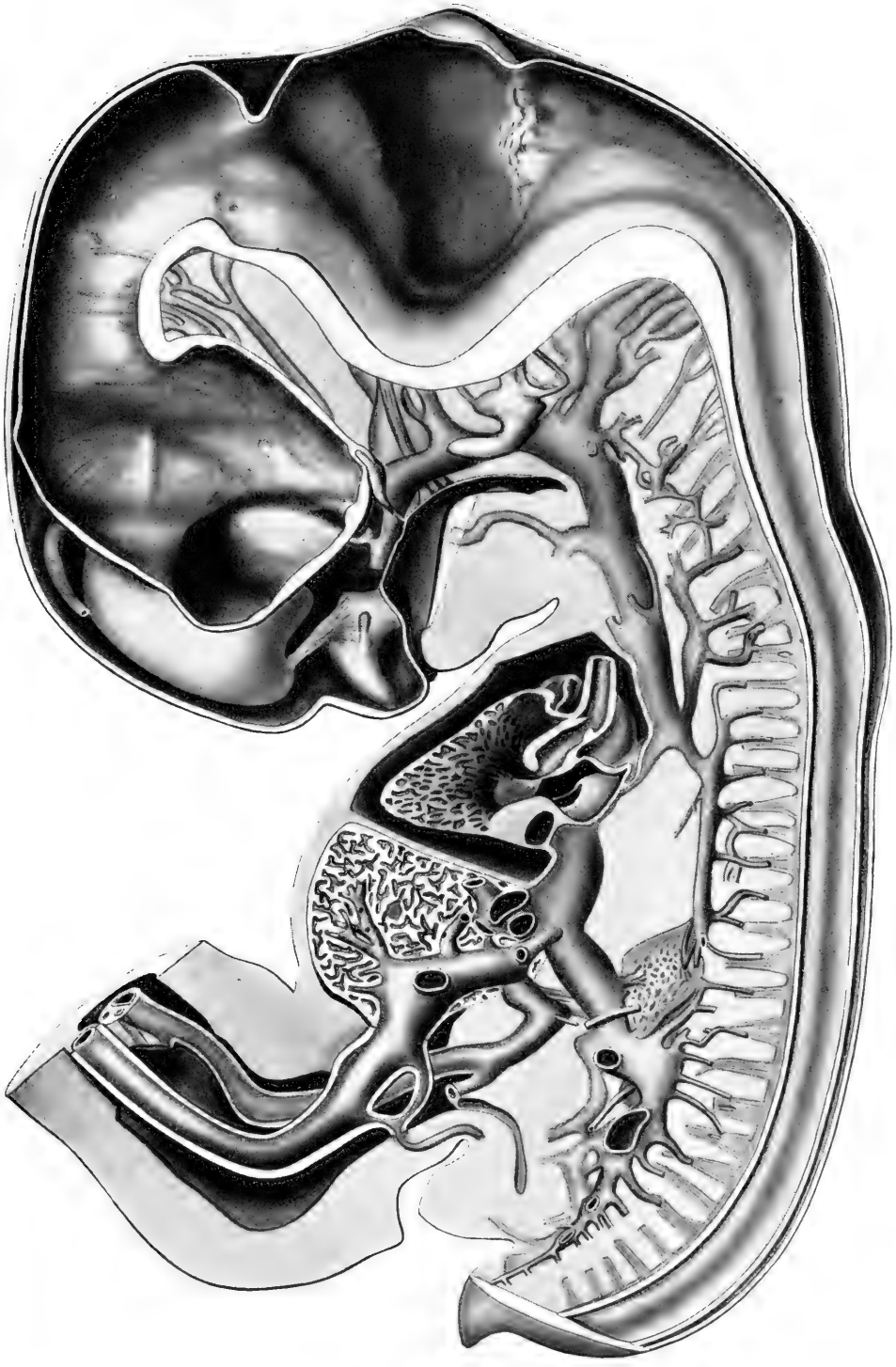
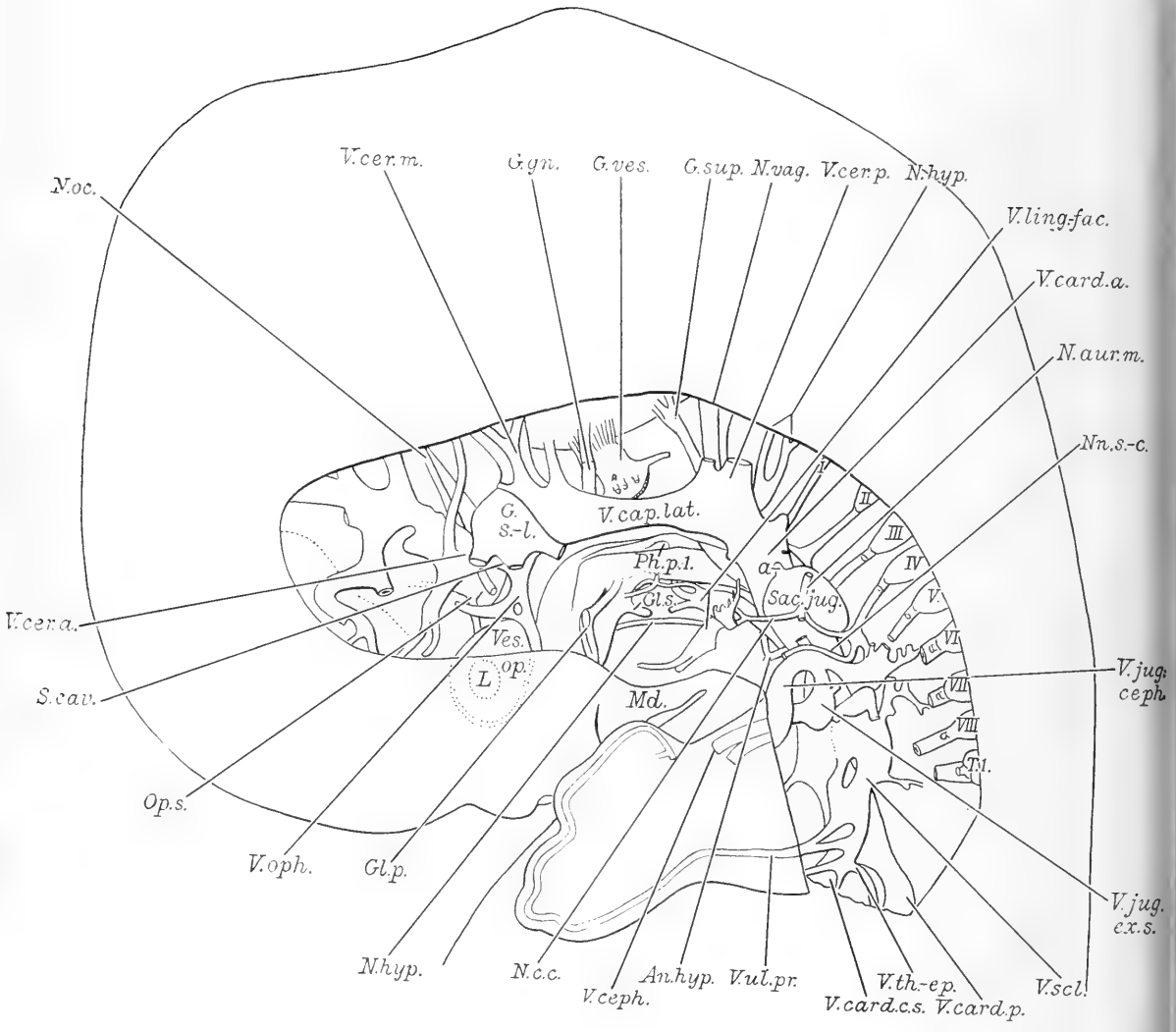


Reconstruction to show the right atrium and ventricle of the heart; the venous system of the right side; and the left umbilical vein of a 17.8 human embryo (H. E. C. 839). × 11.2 diams.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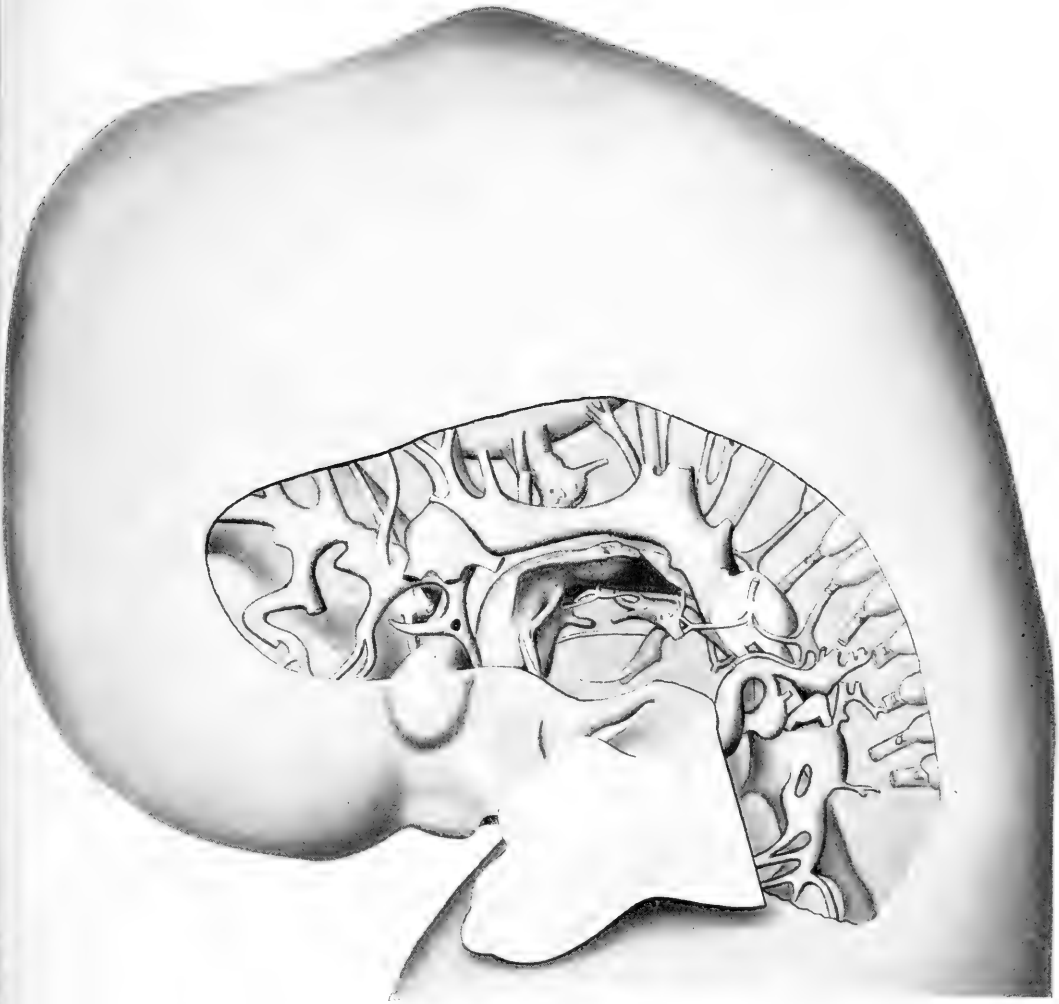
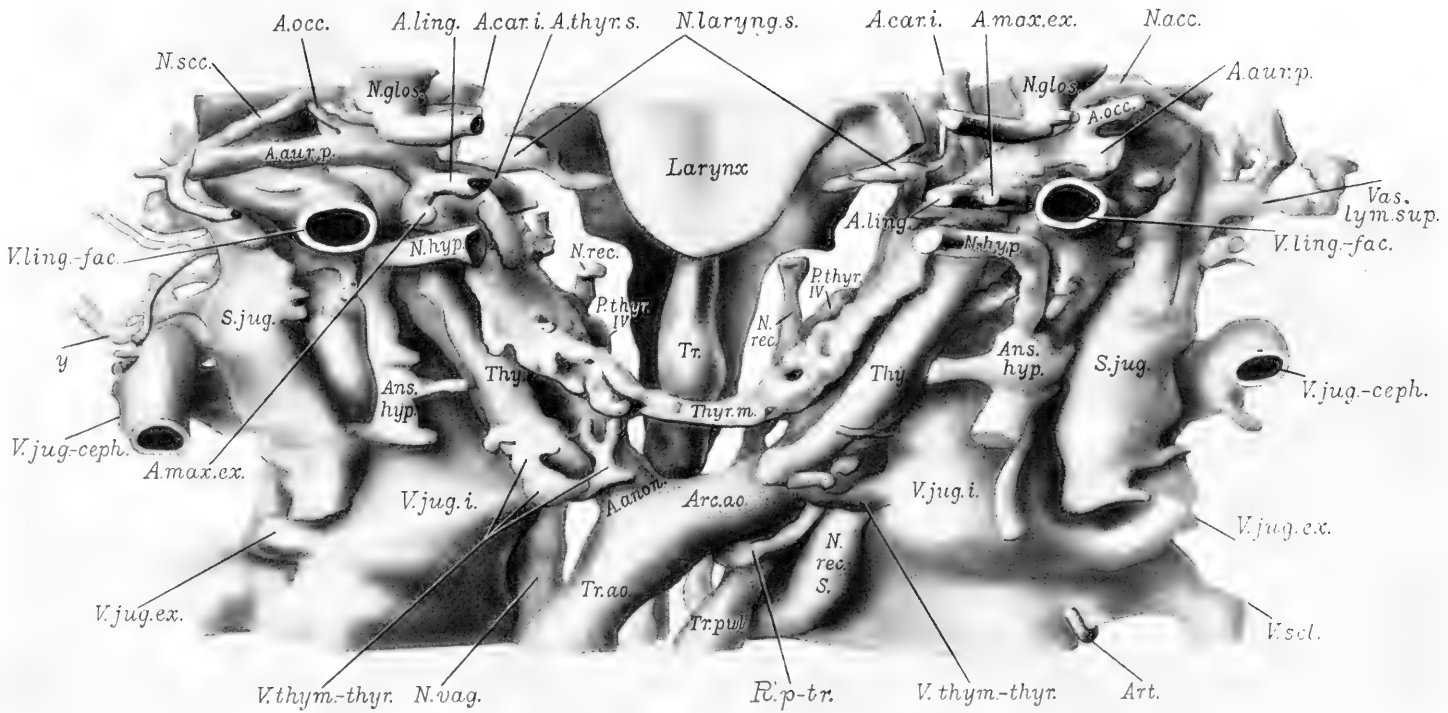


Reconstruction to show the lateral aspect of the left cephalic and cervical veins, and the left jugular lymph sac of a 17.8 mm. human embryo (H. E. C. 839). × 11.9 diams.



Ventral aspect of a wax-plate reconstruction of the cervical region of a human embryo 17.8 mm. in length (H. E. C. 839). It shows the aortic arch; the right and left internal jugular veins; the jugular lymph sacs; the thyreoid and thymic anlagen; one pair of parathyreoids, and the related nerves.

THE STATURE AND THE ERUPTION OF THE PERMANENT TEETH OF AMERICAN, GERMAN-AMERICAN AND FILIPINO CHILDREN. DEDUCTIONS FROM THE MEASUREMENTS AND EXAMINATION OF 1445 PUBLIC SCHOOL CHILDREN IN ANN ARBOR, MICHIGAN, AND 776 IN MANILA, P. I.

ROBERT BENNETT BEAN

From the Anatomical Laboratory, the Tulane University of Louisiana

FIVE DIAGRAMS

CONTENTS

Data:	2221	school children
		630 Filipino boys
	776	146 Filipino girls
		322 German boys
	628	306 German girls
		407 American boys
	817	410 American girls
	2221	Total

INTRODUCTION

The records on which this study is based were made in 1906, 1907 and 1908 and have lain fallow for more than five years, because of excessive teaching duties and lack of library facilities. However, after an extensive survey of the literature I have come to the conclusion that no other study of its kind has ever been made, except that of Hrdlička on the North American Indians (31), and it was through the initiative of Dr. Hrdlička that this work was undertaken. Observations on the teeth of many people have been made from time to time but no extensive detailed observations have ever before been published, not even of Europeans, and no absolute standards have been established as to the time of eruption or extent of decay in males and females and from the standpoint of race. This study is an attempt in that direction, but leaves much to be desired, especially as the number of Filipinos examined at each age in the early years is but small.

MATERIALS

During the school term of 1906–07 I examined and measured every available child in the public schools of Ann Arbor. This was made possible through the coöperation of Dr. McMurrich, and the superintendent of schools and the teachers who showed commendable zeal in furthering the work. The teeth of the children were examined by Dr. Bunting who also assisted me in making the records. Two groups of children were segregated, one of American parentage solely, the other of German, or Ameri-

can and German parentage. The titles American and German will be used for the two groups. When taken together the German and American children will be called European. Children were grouped as German if two grandparents or if either parent came from Germany, and whenever the name was German and the parentage was not given the child was included in the German group, although there were only a few records of this kind. There is a large colony of Germans both in the town of Ann Arbor and in the adjacent country and the majority of the Germans included in the records are of pure German extraction. A large number of children of the teaching staff at the University of Michigan are included in the American group and a large number of rural children are included in the German group. The whole lot of children may be called sub-urban.

The Manila school children were examined by myself in 1907–08 at the Normal and Trade schools, where pupils from all parts of the Philippine Archipelago come to be educated as teachers and mechanics, carpenters, etc. The young children attend classes taught by the older ones at the Normal School and are necessarily from Manila and vicinity. The majority of the children are mestizos, mixed Spanish, Chinese and Filipino, and represent the littoral population of the archipelago fairly well. A few individuals are probably of pure Spanish extraction. No attempt was made to segregate the mestizos or those of Chinese or Spanish extraction from the presumably pure Filipinos because any such attempt is necessarily imperfect and will be so until we understand better than we do at present the workings of Mendelian heredity in man.

METHODS

The age of the children was obtained from each individual and verified as far as possible through the teachers. In Manila the school registers were also examined and the age verified in this way. Discrepancies occur in spite of all efforts to avoid them, but I believe nearly all the ages are exact, and the few that may not be are approximately correct. The year and the month of

birth were obtained in all except a few instances and the child reckoned to be a certain age if it is anywhere within six months of that age. For instance, if a child was born in January 1902 and the records were made in July 1908, the child was recorded as six years old, but if the records were made in July 1907 the child was recorded as five years old, and if the records were made in August, 1908, the child was recorded as seven years old. This method is practically the same as obtaining from the child the nearest birthday, although in giving that the child is apt to miscalculate.

The stature was obtained by using a stationary vertical graduated scale with sliding arm to come down on the child's head. This was pressed firmly and several readings were made for each record, the child standing erect with its back against the scale. The children were measured without shoes when feasible, otherwise allowance was made for the height of the shoe heel. The average or 'mean' stature is used although the 'median' and the 'mode' will be presented.

Many other measurements were made, including sitting stature, weight, head and face dimensions, with observations of the hair and eyes, but these are reserved for future publication. The teeth were examined for eruption, decay, absence, or irregularity. For detailed observations see the table of records, which shows the condition of eruption and decay of each tooth in each individual, and will be published either separately or on file in The Wistar Institute of Anatomy in Philadelphia.

The standard deviation, coefficient of correlation, probable error, etc., are not given, because the characteristics of the individual rather than the mass are desired. However, the average, the mean, the mode, and the extremes have been computed, and these with other factors are utilized in an attempt to determine individual characteristics.

At the end the morphologic type, the sex, and the race are used to assist in explaining individual differences, such as why one child has a certain tooth erupted 5 years earlier than another child has the same kind of tooth erupted, which occurs with great frequency.

THE STATURE

1. Stature of the groups compared

It is to be noted that all the boys and girls of each group have about the same stature at the age of seven years, and the stature varies more both before and after this age. At five years the Igorot boys are 97 cm. tall, the Filipino boys 105.8 cm. tall, the German boys are 107.5 cm. tall, the German girls 110.8 cm. tall, and the American girls and boys each 112.5 cm. tall. The difference between the smallest and tallest group at five years is 15.5 cm., whereas the difference at seven years is only 4 cm. There are so few individuals at the age of five years in each group, the greatest number in any group is five individuals, that the differences given are of slight importance.

After the age of seven years the differences become significant. The Igorots are still much below the average of any other group, and the Filipino boys are also low in stature, but between 12 and 15 years very nearly equal to the German boys. There is a constant decrease in the increment of growth in the Filipino girls, which decrease from the age of seven years onward is greater than that of any other group.

The growth of the Filipino girls almost ceases between 14 and 15 years, and there is apparently no growth after the age of 18 years. The stature of the Filipino boys is less than that of the Filipino girls until the age of 12 years is reached at which time the boys pass the girls in a rapid growth that decreases suddenly at the age of 17, although there is a slight increment of growth in the Filipino boys even after the age of 20 is passed.

The stature of the German girls is less than that of the German boys before the age of 11, greater between the ages of 12 and 14, after which it is again less, but very slightly so. There is a sudden check in the growth of the German girls at 14 but after 15 the growth again accelerates. The growth of the German boys is fairly uniform throughout and there is no evidence of cessation at the age of 16. The growth of the German boys is, however, slightly more rapid after the age of 12 than before. There is also a slight acceleration of growth in the German boys up

to the age of 8 and a slight retardation between the ages of 8 and 12.

The stature of the American boys and girls crosses and recrosses up to the age of 10 years, that of the girls in general is less before this age. Between the ages of 10 and 11 the stature of the girls becomes greater than that of the boys until the age of 14 is reached when the growth of the girls ceases, to continue slowly after the age of 15, at which time the stature of the girls becomes less than that of the boys, and it remains so thereafter. The growth of the American girls from 15 to 17 years of age is retarded at a slightly slower rate than that of the Filipino boys, and there is a fairly uniform parallel growth of the two except for the sudden cessation in growth of the American girls from 14 to 15 years, the American girls at all ages being taller than the Filipino boys. The growth of the American and German boys is fairly parallel, the American boys slightly taller at each age except 8 when the German boys are taller. The American boys' growth is retarded from 5 to 7, accelerated from 7 to 10, again retarded from 10 to 13.

The stature of the American and German girls is fairly parallel until the age of 15, the German less than the American, but after the age of 15 the stature of the German girls exceeds that of the American girls.

In general the stature of the Filipino is less than that of the German and the stature of the German less than that of the American. The growth of the Filipino girls and boys and of the American girls has its final retardation earlier than that of the German girls and boys and American boys, the last three giving no evidence of this when the records cease at 18 years of age.

The stature of the Filipinos beyond the age of 20 is greater by 5.2 cm. than that of the adult Filipinos of Taytay (3), a village near Manila. The boys belong to the well-to-do class. The Taytayans are poor which may account for this difference. The Taytayans are also less mixed with the Spanish and Chinese than the school boys which is another factor in accounting for the difference in stature.

The stature of the Filipino boys is 11 cm. greater than that of the girls beyond the age of 20, and this is in excess of the usual sexual difference, which is often not more than 5 cm. This may be due to a greater inheritance of the father's stature by the boys and of the mothers' stature by the girls because the fathers of many of the children are Spanish and Chinese and the mothers are Filipinos, and the stature of the Spanish and Chinese is greater than that of the Filipinos. This may be a confirmation of Pearson's findings that males inherit stature from the father and females from the mother.

There are so few Filipinos below the age of 16 for each sex that the average has only an approximate value. There is also greater variability in stature at the ages of 12, 13 and 14 among the Filipinos, than either before or after. For instance at the age of 10, the extremes are only 15 cm. apart, and at the age of 15 only 25 cm. apart, whereas at 12, 13 and 14 years, they are 30, 35 and 35 cm. apart respectively. It is noted that the German and American children exhibit a variability that is greater with advancing years. The reverse is true of the Filipinos.

2. Review of literature and discussion

When the stature of the Ann Arbor boys of American parentage is compared with the stature of the Boston boys (13,691, Bowditch (11)) of American parentage it is found that the Ann Arbor boys are about 5 cm. taller at the ages of 5 and 6, and this difference decreases until the age of 15 is reached after which the boys of both places have about the same stature. This is true also of the Nebraska boys (5,476, Hastings (26)) when compared with the Ann Arbor boys, and the difference with them at the early ages is even more marked in favor of the Ann Arbor boys. It is evident that the boys of Ann Arbor attain their early stature quicker than the boys of Boston. This is probably due to the fact that the children of Ann Arbor have a more favorable environment than the children of Boston. Bowditch found that the boys of the private schools in Boston were taller than the boys of the public schools, and taller than the boys of the laboring

classes in England. It is generally recognized that the children of the well-to-do grow more rapidly than the children of the poor, that is, early in life. There may be some influence due to the selected parentage and not alone due to environment. A great number of the boys examined at Ann Arbor were children of members of the Faculty of the University of Michigan, or children of students, and this might have some influence.

The German boys of Ann Arbor (324, Bean) are also taller than the German boys of Boston (752, Bowditch), although this difference is only about 2 or 3 cm. until the age of 15 is reached when both groups become equal in stature. This difference may be due to environment alone because the Germans of Boston and Ann Arbor probably come from a similar stock.

The same difference that exists between the American boys of Ann Arbor and of Boston exists to a limited extent between the girls of the two places. The girls of Ann Arbor (409, Bean) are about 5 cm. taller at the age of 5 and 6 years than the Boston girls (10,874, Bowditch) of the same age and this difference decreases until the age of 15 is reached, at which time the stature is only about 2 cm. apart. The Ann Arbor girls pass the Ann Arbor boys in stature a half year earlier than this takes place in Boston (Ann Arbor, 10, Boston $10\frac{1}{2}$) and the boys do not again reach the stature of the girls in Ann Arbor until a half year later than in Boston (Ann Arbor 15 years, Boston $14\frac{1}{2}$ years).

Peckhan (44) reports the results of measuring 5,136 girls, and 5,117 boys of Milwaukee, in which the boys, both German boys and American, were taller than the Boston boys from 13 years onward (Bowditch 13,691 boys, 10,874 girls), with a rapid increase of stature from 16 to 19 years. The girls at all ages both German and American were taller than the Boston girls. The Milwaukee children differ from the Ann Arbor children in that the former have a late rapid growth whereas the latter have an early rapid growth. Is the late rapid growth of the Milwaukee children due to the Norse (long head) stock and is the early rapid growth of the Ann Arbor children due to the large south German (broad head) element? Or is it a matter of environment?

Schwerz (47) reports the results of measuring 960 boys and 818 girls of Schaffhausen, Switzerland, in which the Swiss boys are 4 cm. less in stature at all ages than the Ann Arbor American boys, and the increase in rate of growth is at about the same periods, 9, 14 and 17 years. The Ann Arbor German boys have 1 or 2 cm. greater stature than the Swiss boys until the age of 14 years after which the German boys attain a stature which is 4 cm. greater at 15 years and 7 cm. greater at 16 years, this, too, in spite of the fact that the Swiss boys are six months older at each period than the Ann Arbor German boys. The differences that exist between the Swiss boys and the Ann Arbor German boys also exist to about the same extent between Swiss girls and Ann Arbor girls. The periods of rapid growth are about the same in the two groups.

The cephalic index, face index, etc. of the two groups are about the same thus signifying that the two groups, Swiss and Ann Arbor Germans, are of the same stock, the Alpine or middle European, rather than of the Nordic or Mediterranean stocks. Schwerz compares the Berlin German children with the Turin Italian children in both the poorer classes and the well-to-do classes, with the result that the Berlin children are taller at each age in both classes and both sexes. The well-to-do boys of Berlin have about the same stature at all ages as the American boys at Ann Arbor and the boys of the poor in Berlin are only slightly less tall at each age (1 to 2 cm.) than the Ann Arbor German boys. The well-to-do girls of Berlin are slightly taller at each age than the American girls of Ann Arbor, and the poor girls of Berlin are only slightly less tall (1 to 2 cm.) than the Ann Arbor German girls. The Turin boys and girls are in all groups and at all ages from 5 to 10 cm. less in stature than the Berlin children. Thus the three stocks of Europe, Nordic, Alpine and Mediterranean, are represented by the Berlin, Schaffhausen and Turin children respectively (?) and it would appear that the Milwaukee children are Nordic, the Ann Arbor American children are Nordic and Alpine, and the Ann Arbor German children are Alpine. The effect of nutrition or environment cannot be

stated for the several groups but no doubt its influence accounts for some variation.

When the Filipino boys measured by Bobbitt (10) are compared with those I measured, the difference in stature is in favor of the latter to the extent of about 2 cm., after the age of 11 years; at 10 and 11 the two groups are equal, and before that Bobbitt's Filipino children are on the average 1 cm. taller down to and including the age of 6.

I measured only 11 Filipino girls below the age of 12 years, therefore they need not be considered. From 12 years onward, however, the girls measured exceed those measured by Bobbitt from 1 cm. at 12 and 13 years, to 5 cm. at 20 years and over. Not only this but Bobbitt gives the age from one birthday to the next as of the preceding birthday, whereas I give the age as of the six months before and after the birthday. For instance, he gives all children from 10 to 11 years of age as 10 years of age, whereas I give all children 9 years 6 months to 10 years 5 months as 10 years of age. His Filipino children are not only smaller but in each group are six months older than the same group given by me.

The children measured by Bobbitt were of three sorts. The oldest were about 75 per cent from the provinces, the youngest were largely from Manila and the intermediates were about equally divided between the two places. The majority of the children I measured were from the provinces, except those below the age of 10 years, who were all from Manila. Bobbitt did not include children who had evidence of mestizo blood (Spanish and Chinese), whereas I included all the children as they became available, regardless of race or color. A few were crosses of the American white and Filipino: the differences in stature may be explained by the greater amount of foreign blood in the children I measured, although when the children from the provinces are in the majority the stature is taller than when the children from the city of Manila are in the majority. It appears that European stock and rural environment may both increase the stature of the Filipino.

3. Periods of acceleration and retardation in growth

Growth occurs in waves. A period of acceleration is followed by a period of retardation. The periods occur at different times in the different sexes and races.

The first period of acceleration of which we have any record occurs between the ages of 6 and 10 years, after which there is a retardation followed by another acceleration about the age of 13 to 15. These periods may be of interest in relation to the eruption and decay of the teeth.

Disregarding the first and second periods of rapid growth, of which we have no record here, it may be seen that the third period of growth begins at about the age of 7 years and continues until about the age of 10, being most rapid from 7 to 8, and at the age of 10 years the third period of growth begins.

The third and fourth periods of rapid growth are earlier for the girls than for the boys and the fourth period of rapid growth is shorter for the girls than for the boys. The result is that the girls become larger than the boys between the ages of 7 and 15, and remain so until the fourth acceleration sets in for the boys, when they outstrip the girls in stature and remain taller thereafter.

The stature of the Filipinos is less than that of the Germans and Americans and this becomes more evident after the age of 14 years.

The stature of the Igorot boys is less than that of the Filipino boys and girls except at the ages of 7 to 8 when it is greater than that of the Filipino boys, and at the age of 20 when it is greater than that of the Filipino girls.

The Igorot boys attain their growth later than either the Filipino girls or the Filipino boys, but earlier than the German or American boys and girls.

The four groups may be arranged in order of precocity of acceleration periods as follows: 1, Filipino; 2, Igorot; 3, German; 4, American. The acceleration periods of the German begin earlier than those of the American but the ultimate stature is

reached later in the German. This follows a law, general in nature, that the precocity or rapidity of development is inverse to the ultimate size.

The precocity of the group is inverse to the ultimate stature; the Americans, the tallest, the Filipinos, the smallest, with the Igorots and Germans intermediate: the Germans taller than the Igorots.

The Filipinos may have an early rapid development which is from the European standpoint premature, and a late maturity that is incomplete, at least it looks as if growth is continued up to a later age in the Filipinos than in the Europeans, but the extent of development is less.

The Ann Arbor American children, the Milwaukee and the Berlin children are similar in stature and in periods of growth. The Ann Arbor German children, the Boston German children and the Swiss children are likewise similar in stature and in periods of growth. The Swiss children, however, show considerable retardation in growth from the age of 15 onwards, whereas the American German children show no retardation until the age of 17 is reached. All the six groups are different from the Turin children of South Europe. In the latter there is an early rapid growth followed by retardation, and a later rapid growth followed by retardation that is earlier than in the other groups. In this they resemble the Filipinos.

The urban children of Boston are less rapid in their early development than the sub-urban children of Ann Arbor and the urban children of Milwaukee are more rapid in their late development that the sub-urban children of Ann Arbor. The early development of the Ann Arbor and Milwaukee children is about the same and the later development of the Ann Arbor and Boston children is about the same.

Factors in race and climate or heredity and environment both probably cause these differences. Neither can be excluded.

THE TEETH

The consideration of the teeth will be taken up under the following headings: 1. The eruption of the permanent teeth; 2. The alternation of growth in stature and eruption of the permanent teeth; 3. The eruption of the individual permanent teeth; 4. Review of the literature and discussion.

The youngest children whose teeth I examined were five years old, and at that age all the temporary teeth have erupted, therefore the time of the eruption of the temporary teeth cannot be given. The decay of the temporary teeth occurs partly after the age of five years, and this will be considered, in addition to the eruption and decay of the permanent teeth.

1. The eruption of the permanent teeth

This is determined by taking the average number of teeth erupted or erupting at each age, as well as the 'median,' the 'mode' and the 'extremes,' after which the periods of acceleration and retardation in the eruption of the permanent teeth will be given.

The eruption of the permanent teeth begins about the age of 5 years, slightly earlier among the girls and slightly later among the boys of the German and American groups, but considerably earlier than this among the Filipinos of both sexes. The Germans and Americans of both sexes have almost exactly the same number of permanent teeth at the ages of 6, 7 and 8 years, after which the girls have a greater number of teeth than the boys until the age of 15 is reached, when the number of teeth in the two sexes is again about the same.

The girls of each group are more precocious in the eruption of the permanent teeth than the boys, they have a greater number of permanent teeth at each age, and the difference is greatest between the ages of 8 and 15 years, for at the age of 11 years the American girls have an average of 21.3 permanent teeth present, and the American boys have an average of only 17.3. The difference between the German boys and girls at this age is not so great, and they have only 16.7 and 19.5 teeth present for the boys and girls respectively. The difference between the Filipino boys

and girls is still less, and they have a greater number of teeth present than either the German or Americans at the age of 11 years, 24.3 and 27.0 for the boys and girls respectively.

The Filipinos are much more precocious than either the Germans or Americans in the eruption of the permanent teeth, and there is little difference between the boys and girls in the number of teeth present at each age. The Americans are a little more precocious than the Germans in the eruption of the permanent teeth, and the difference between the boys and girls is greater among the Americans than among the Germans and Filipinos.

About the age of 15 years the children of both sexes in all the groups have nearly the same number of teeth, and after this age the German and American children maintain the same number of teeth up to the time at which the records cease (18 years), but the Filipino children continue to gain in the number of their teeth to the age of 20 and beyond, although the majority of the Filipinos have acquired the complete set of 32 teeth at the age of 20 years. This is due to the early eruption of the third molars among the Filipinos (begins at 13) and their later eruption among the Germans and Americans (begins at or after 16).

Sixty odd negro boys and girls, mostly mulattoes, were examined at Ann Arbor, and it is found that the negro boys and the American boys are almost parallel in the time of the eruption of their teeth, and the negro girls are only slightly more precocious than the American girls.

2. The alternation of growth in stature and the eruption of the permanent teeth

The eruption of the permanent teeth does not occur with the same rapidity at each age, but there are waves of eruption of the teeth like the waves of growth, although the two are not synchronous. The periods of acceleration in the eruption of the permanent teeth occur about the ages of 7 and 11 years, and the periods of retardation follow those of acceleration. It will be seen that the periods of acceleration in the eruption of the permanent teeth initiate or precede the periods of rapid increase in

stature. The eruption of the teeth through the gums is but evidence of their previous rapid development, and there is doubtless a period of rest throughout the body between the periods of rapid development of the teeth and the periods of rapid growth in stature.

Others have repeatedly demonstrated that the six months following birth is a period of rapid increase in length of the infant, which is followed by the eruption of the temporary teeth, all of which are through the gums by the age of three years. After this there is a period of rest which is followed by a second period of growth in stature about the age of five years, succeeding which the permanent teeth begin to develop, and this development is most rapid about the age of 7 years. This is followed by the third period of rapid growth in stature about the age of 8 years after which comes a period of rest and then the second acceleration in the eruption of the permanent teeth occurs between 10 and 11 years. The final rapid growth in stature comes after this at about 12 years of age, and immediately precedes puberty. Following puberty the increase in stature and the eruption of the teeth are delayed, especially in the girls. However, the third period of the acceleration in the eruption of the teeth (second period of acceleration in the eruption of the permanent teeth), the girls are about one year earlier than the boys, and the period of growth following this is about two years earlier in the girls than in the boys; (see The law of alternation in development, p. 137).

Résumé. After this brief consideration of the average or 'mean,' time of eruption of the permanent teeth, the 'median,' the 'mode' and the 'extremes,' as well as the periods of acceleration and retardation in the eruption, it is to be noted that the second teeth begin to erupt about 5 years of age, slightly earlier among the girls than among the boys and earlier among the Filipinos than among the Germans and the Americans, and latest of all among the Germans.

The girls are more precocious in the periods of acceleration of the eruption of the teeth than the boys, as well as in the average number of teeth, the 'median' and 'extremes' at any age. The

Filipinos are likewise more precocious than the Americans, who are slightly more precocious than the Germans.

There is an alternation in the periods of acceleration and retardation in the eruption of the permanent teeth, and there is also an alternation in the periods of acceleration of the growth in stature and the periods of acceleration in the eruption of the permanent teeth.

3. The time of eruption of each individual tooth

This is to be determined for each sex and race by means of the beginning of eruption, the end of eruption, and the average per cent of the teeth erupted at specified ages during which time the teeth are erupting in all the groups. The beginning of eruption may be utilized: that is the time at which the tooth first erupts in any individual in a race-sex group; the end of eruption, that is the time at which the tooth last erupts in any race-sex group; the 'median,' the time half way between the beginning of eruption and the end of eruption, may be taken as the time of eruption; or the time when 50 per cent of the teeth of any type have erupted in a sex-race group may be taken as the time of eruption. For purposes of comparing the groups with each other the average number of teeth erupted at the ages in which all the comparable groups have the teeth erupting at the same time may be utilized. This may be called the average per cent. As, for instance, the right upper second molar is erupting in all the groups during the ages of 12, 13 and 14 years, and the average per cent erupted at these ages is as follows:

	per cent		*per cent*		*per cent*
Filipino boys	85	American boys	53	German boys	51
Filipino girls	94	American girls	68	German girls	58

From this we may gather that the Filipinos are more precocious than the other two groups, the Americans are more precocious than the Germans, and the girls are more precocious than the boys in the eruption of the right upper second molar teeth.

The median incisors. The median incisors begin to erupt in some individuals before the age of five years, and the teeth are

nearly if not all through the gums at the age of 10 years. There is practically no difference in the time of eruption on the two sides, but the lower median incisors erupt about a year earlier than the upper, a little less than a year on the left side and a little more than a year on the right side.

The median incisors erupt in the following order: (1) lower right; (2) lower left; (3) upper left; (4) upper right. The left upper median incisors erupt at approximately the same time in the Germans and Americans, the left lower erupt earlier in the Americans than in the Germans, and the right upper erupt earlier in the Americans than in the Germans, although the German girls may be slightly more precocious than the American girls. The median incisors erupt earlier in the Filipinos than in either the Germans or Americans. The median incisors erupt earlier in the German and American girls than in the German and American boys.

The lateral incisors. The lateral incisors begin to erupt in some individuals as early as the age of 6 years, and the teeth are nearly if not all through the gums at the age of 13 years. There is practically no difference in the time of eruption on the two sides, but the lower lateral incisors erupt about a year earlier than the upper, a little less than a year on the left side and a little more than a year on the right side.

The lateral incisors erupt in the following order: (1) lower right; (2) lower left; (3) upper left; (4) upper right. The lateral incisors erupt earlier in the girls than in the boys. The lateral incisors erupt earlier in the Filipinos than in the Germans and Americans, although there is very little difference in time for the eruption of the lower teeth of this type.

There is so little difference in time of eruption between the Germans and Americans that they may be considered as having the lateral incisors erupted at the same time, but the slight difference indicates that the Germans are more backward than the Americans.

The canines. The canines begin to erupt in some individuals as early as the age of 8 years, and the teeth are nearly all, if not all, through the gums at the age of 15 years. There is practically

no difference in the time of eruption of the canine teeth on the two sides of the mouth but the lower canines erupt earlier than the upper. The difference in time between the eruption of the lower and upper canines is about one year in the American boys, less than a year in the German boys, and more than a year in the German and American girls. There is no order of eruption of the canines except that the lower erupt earlier than the upper.

The canines erupt earlier in the girls than in the boys, except that the upper canines erupt earlier in the Filipino boys than in the Filipino girls. The sexual differences amount to at least two years for the Filipinos, between one and two years for the Germans, and about one year for the Americans.

The upper canines erupt at about the same time in the Filipino, German and American girls, but the lower canines erupt at least three years earlier in the Filipino girls than in the German and American girls. There is almost no difference between the German and American girls in this. The canines erupt anywhere up to four years earlier in the Filipino boys than in the American or German boys, and the American boys are slightly more precocious than the German boys. The Filipinos of both sexes are precocious in the eruption of the canine teeth, the Germans are backward and the Americans are intermediate.

The median premolars. The median premolars begin to erupt in some individuals as early as the age of 8 years and the teeth are nearly if not all through the gums at the age of 15 years.

There is practically no difference in the time of eruption on the two sides of the mouth, but the upper median premolars erupt slightly earlier than the lower, although this difference is considerably less than one year.

The median premolars erupt in the following order: (1) left upper; (2) right upper; (3) right lower; (4) left lower. The median premolars erupt first in the Filipinos, second in the Americans and third in the Germans, although the differences are not great between the last two. The median premolars erupt earlier in the girls than in the boys, except that apparently the upper premolars erupt slightly earlier in the Filipino boys than in the Filipino girls, but this is questionable.

The lateral premolars. The lateral premolars begin to erupt in some individuals at the age of 8 years, and they are all through the gums in all individuals at the age of 16 years. There is very little difference in time of eruption on the two sides of the mouth, especially of the lower lateral premolars, although the left upper erupts earlier than the right upper. The order of eruption is as follows: (1) left upper; (2) right upper; (3) right lower, and (4) left lower. The upper lateral premolars erupt less than a year earlier than the lower.

The lateral premolars erupt first in the Filipinos, and second in the Germans and Americans, at almost the same time for each of the latter. The lateral premolars erupt earlier in the girls than in the boys.

The first molars. The first molars begin to erupt in some individuals before the age of five years, and nearly all, if not all, the first molars are erupted in all individuals at the age of 12 years.

There is practically no difference in the time of eruption of the first molars on the two sides of the mouth, but the lower first molars erupt slightly earlier than the upper, therefore the order of eruption is (1) and (2), lower first molars, (3) and (4), upper first molars. The lower first molars erupt earlier than the upper, the interval being considerably less than a year. The first molars erupt later in the Filipinos than in the Germans or Americans who are almost exactly alike in the time of eruption of these teeth.

The first molars erupt about one year earlier in the girls of each group than in the boys.

The second molars. The second molars begin to erupt at about the age of 10 years—slightly earlier in the Filipinos—and their eruption is completed in all, or nearly all, the individuals at the age of 16.

There is practically no difference in the time of eruption of the second molars on the two sides of the mouth, but the lower second molars erupt about a year earlier than the upper, therefore the order of eruption is (1) and (2) lower second molars, and (3) and (4) upper second molars. The second molars erupt earlier

in the Filipinos than in the Germans and Americans, and earlier in the Americans than in the Germans.

The second molars erupt more than a year earlier in the girls than in the boys, except in the Filipinos, where the boys are slightly more precocious than the girls, although the lower second molars erupt earlier in the Filipino girls than in the Filipino boys.

The third molars. The third molars begin to appear in the Filipinos at the age of 13, and from 61 per cent to 83 per cent of the third molars have erupted in the Filipinos at the age of 20 and over.

The third molars have appeared in none of the German and American boys up to the age of 18 years, but they begin to appear in the American girls at the age of 16 years, and the left lower third molar has begun to erupt in the German girls also at the age of 16 years. The girls therefore appear to be more precocious in the eruption of the third molars than the boys, and the American girls more precocious than the German girls. The latter is not true among the Filipinos at the beginning of eruption, which occurs first among the Filipino boys, but as age advances up to 20 the Filipino girls become more advanced than the Filipino boys, and at the age of 20 and thereafter the girls have a greater average number of third molar teeth erupted than the boys.

There is practically no difference in the time of eruption of the third molar teeth on the two sides of the mouth, but the lower third molar erupt slightly earlier than the upper.

Résumé. On pages 128 to 132 we have considered the eruption of the teeth from the standpoint of the beginning of eruption, the end of eruption, and the average per cent. The results are as follows: The order of eruption in groups is (1) Filipino girls, (2) Filipino boys, (3) American girls, (4) German girls, (5) American boys, (6) German boys.

The order of the eruption of the individual teeth of the German and American group is (1) lower median incisors, (2) lower first molars, (3) upper first molars, (4) upper median incisors, (5) lower lateral incisors, (6) upper lateral incisors, (7, 8) upper median premolars and lower canines, (9) lower median premolars,

(10, 11) upper lateral premolars and upper canines, (12) lower lateral premolars, (13) lower second molars, (14) upper second molars, (15) lower third molars, (16) upper third molars.

The order of eruption of the individual teeth in the Filipinos is the same as the above except that the lower first molars and the lower median incisors erupt at the same time, the upper median incisors and the upper first molars change places, the lower canines erupt before any of the premolars, and the upper canines erupt at the same time as the upper median premolars.

If these results are compared with those obtained from the time at which 50 per cent of the teeth are erupted little difference will be found. The order of eruption in the sex-race groups is the same, and the order of eruption of the types of teeth is the same, but the order of eruption of a few of the individual teeth is slightly different. This is manifest chiefly in the relative time of eruption of the first molars and median incisors, and of the canines and premolars. The meaning of this is that the first molars and median incisors are intimately related in time of development, if they are not synchronous, and the same is true of the canines and premolars. In either case the canines are more precocious in the Filipinos than in the Germans and Americans.

4. Review of the literature and discussion

We may arrange the teeth in the order found in their eruption among the German and American children of Ann Arbor taking the time at which 50 per cent have erupted and with this compare records for the French by Magitot-Broca (12, 13, 36, 37, 38, 39), Mayet (40), and Cherot (14), for the German by Welcker (51), and for the English by Livy (35).

It may be said that the time of the eruption of the teeth as given by Cherot for the French is almost the same as that of the Ann Arbor German and American children, or only a little earlier, whereas those of Magitot-Broca, and Mayet for the French are earlier, and those of Welcker for the Germans are later than those for the Ann Arbor children. The records of Welcker are only slightly later than those for the Ann Arbor German boys

and those of Magitot-Broca are about the same as for the Ann Arbor American boys. The Americans and the French appear to be more precocious than the Germans. The English are precocious in the eruption of the lateral premolars, and backward in the eruption of the lateral incisors, but otherwise they conform fairly well in the eruption of the teeth to the Germans.

It is generally recognized that girls are more precocious than boys in the eruption of the teeth, and that the lower teeth erupt earlier than the upper, except the premolars, but Livy found among 4,000 children of the workers of Bolton, England, that the upper canines invariably precede the lower in the girls, whereas the lower canines invariably precede the upper in the boys. The upper canines erupt a year later than the lower among the German, American and Filipino girls that I examined, and less than a year later among the boys of the three groups.

Spokes (48) found among British children that the eruption of the canines occurs between the time of eruption of the median and lateral premolars, which is a verification of what I found among the German and American children of Ann Arbor, but among the Filipinos the canines erupt before the premolars. I found that the lower canines erupt between the time of eruption of the upper and lower median premolars, and the upper canines erupt between the time of eruption of the upper and lower lateral premolars. This may explain the contention of Owen (51) of England on the one side, who contends that the canines erupt before the premolars (canines, 7 to 9 years, premolars, 8 to 10 years), and Welcker, Sommering (51), Hyrtl (51), Henle (54), and Blumenbach (51) on the other, who contend that the median premolars erupt before the canines. It may be, however, that the canines erupt earlier among the British than among the Germans, just as the canines erupt earlier among the Filipinos than among the children of Ann Arbor, or at least this may be true for some parts of the British Empire. Livy did not find it so for the children of the laboring classes of Bolton, England.

The work of Cherot is of value because it is of recent date, and because he procured data from 20 to 30 children of each sex at each age. The time of the beginning of eruption and of the

end of eruption as he found them in the French may be compared with my records of the Ann Arbor children.

It is to be seen that the French children are earlier than the children of Ann Arbor both in the beginning of eruption and in the end of the eruption of the teeth, except in the beginning of eruption of the lower median incisors and upper canines, wherefore it is evident that the French at any age are more mature in the eruption of the teeth than the children of Ann Arbor.

Cherot believes there are four periods of acceleration in the eruption of the permanent teeth, from six to eight and a half years when some teeth are erupting all the time; from 10 to 11 years; at 12 years; and about 20.5 years during the eruption of the third molars. The periods of 10 to 11 and at 12 should be combined because they run together, thus leaving three periods, which have been associated by others with the eruption of the three series of molar teeth. The periods of dentition given by Cherot would then correspond to the periods of acceleration given on page 127.

Hrdlička (30, 31) has published the only detailed data of the examination of children's teeth that I have been able to find in the literature. He examined the teeth of the Apache and Pima Indians of North America, and from his records he concludes that the teeth of the Indians appear at about the same ages as the teeth of the whites, with the exception of the canines and second molars which apparently erupt earlier in the Indians than in the whites. I have calculated the beginning of eruption and the end of eruption of the teeth in the Indians, which is based on the stature rather than the age, because the age of the Indians was not obtained by Hrdlička. The age is calculated from the stature by Hrdlička, and he remarks that it is doubtless imperfect.

The order of eruption is similar to that of the Europeans or white peoples, and different from that of the Filipinos, because in the latter the canines erupt before the premolars, whereas in the others the canines erupt at the time of the eruption of the premolars, or as noted above: the lower canines erupt between the eruption of the upper and lower median premolars and the upper canines erupt between the eruption of the upper and lower lateral premolars.

The eruption of the teeth begins later in the Indians than in the whites, and ends earlier. The teeth of the Indians erupt more promptly than those of the whites and the canines, second and third molars erupt earlier in the Indians than in the whites.

To test the relative precocity of the Indians, I have calculated the average age of all the Indian, Filipino, German and American children and the average number of teeth at all ages in all the groups. The ratio of the age to the number of teeth erupted in the order of precocity, is shown below:

	Age: teeth		*Age: teeth*
(1) Indian girls	100: 176.5	(5) German girls	100: 167.0
(2) Filipino girls	100: 175.0	(6) Indian boys	100: 153.6
(3) Filipino boys	100: 170.3	(7) American boys	100: 153.0
(4) American girls	100: 170.0	(8) German boys	100: 150.0

The Indian girls are the most precocious girls, and the German boys are the most backward boys. The Filipino boys are the most precocious boys, and the German girls are the most backward girls. The German boys and girls are the most backward, the American boys and girls are next to the most precocious. The girls are more precocious than the boys, but the difference is not great among the Filipinos and is greatest among the Americans.

The ratio of the groups when both sexes are combined is:

	Age: teeth		*Age: teeth*
(1) Filipinos	100: 172.6	(2) Indians	100: 165.05
(3) Americans	100: 161.5	(4) Germans	100: 158.5

The Filipinos are the most precocious, the Germans are the most backward, and the Americans and Indians are between the other two, the Indians more like the Filipinos and the Americans more like the Germans. The Indian women have a greater precocity than the Filipinos, and the Indian boys are almost exactly like the German and American boys.

Boas (6, 7, 8) and Boas and Wissler (9) have made reports as to the eruption of the permanent teeth in American whites but their work has been inaccessible to me, and what I give is based on

abstracts. Boas published the following results recently with the remark that they "are not very accurate:"

TEETH	AGE IN YEARS			
	Boys		Girls	
	Boas	Bean	Boas	Bean
Inner permanent incisors	7.5	7.0	7.0	6.65
Outer permanent incisors	9.5	8.4	8.9	8.00
Bicuspids	9.8	11.1	9.0	10.6
Canines	11.2	11.6	11.3	10.5
Second molars	13.2	12.6	12.8	12.0

I do not know how the calculations were made by Boas but when compared with my results of the time when approximately 50 per cent of the teeth are erupted, his records show a retarded eruption of all the teeth except the premolars, which are precocious and erupt more than a year earlier than the premolars of the Germans and Americans of Ann Arbor. The canines in his records erupt about two years later than the 'bicuspids' (premolars?), which is very unusual. Boas and Wissler also place the end of eruption of the permanent teeth as follows: First molars, 9 years; median incisors, 12 years; bicuspids, 6 to 12 years; canines, 6 to 15 years; lateral incisors, 15 years; and second molars, 7 to 15 years. So far as I am aware, they are the only records that place the eruption of the canines after the bicuspids and the lateral incisors after both.

THE LAW OF ALTERNATION IN DEVELOPMENT

Donaldson (17–22), Jackson (32–34), Hatai (27, 28) and others have demonstrated the alternate periods of development of the parts of the body without stating a law that would apply to this phenomenon, and I have simply added to their work the results of my observations and from the combined data I have deduced a law which may be formulated tentatively somewhat as follows:

There are one or more periods of acceleration alternating with periods of retardation in the development of each structural unit or organ of the body. The periods of acceleration in the development

of one structure may be synchronous with the periods of retardation in the development of another, and the two may be called complementary structures. Each organ or structure has a critical period when it is developing most rapidly, and when it is probably most susceptible to its environment.

Jackson determined the growth in volume of the parts of the body in relation to the whole from observations on 43 human embryos and fetuses, and other similar material. The time at which the structure attains its greatest relative size is given, but this may or may not be the time of greatest relative accel-

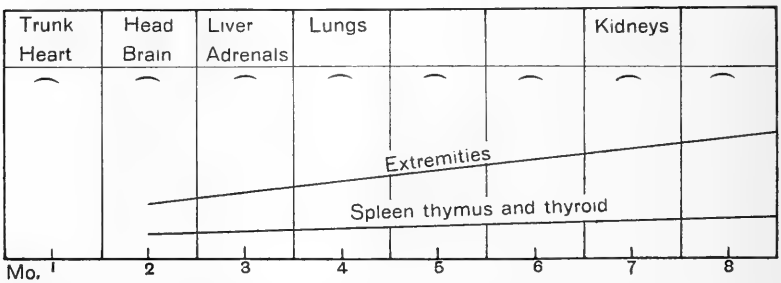


Diagram 1 Prenatal growth in man. The curved lines represent the month at which the organs named above grow most rapidly. The diagonal lines represent the approximate relative rate of growth of the organs named thereon. The number of the months is written below.

eration in growth, and it certainly is not its greatest absolute extent of growth, all of which should be considered in any study of growth.

The growth of any part of the body should be determined in at least three ways. First, the amount of growth in relation to the size of the part previous to the period of growth; second, the amount of growth in relation to the adult size of the part; and third, the amount of growth of the part in relation to the total growth of the individual. The first is important because it would give the real activity of the part at different stages of its development, yet this method is not usually adopted. Diagrams 1 and 2 will serve, however, to illustrate the relative growth of the body parts.

From these diagrams it will be seen that there are three general types of growth in the white rat as demonstrated by Hatai and Donaldson: (1) represented by the brain, which is "characterized by a very rapid growth in weight at an early period and after this period the rate of growth is much reduced;" (2) represented by the extremities, spleen, thymus and thyroid which are characterized by a relatively rapid rise at an early period followed by a straight line at an angle from the base line always much greater than that of type 1; (3) represented by the sex glands, which have an irregular growth, first slow, then rapid, then slow again.

In any consideration of development it must be remembered that general laws should apply to all forms of mammals, at least, yet inasmuch as certain parts may be pathological (the brain of man is a pathological organ in relation to other forms and to evolution) any laws made for one form may not apply to another.

The first part of the white rat to develop in prenatal growth is the trunk, including the heart, spinal cord and somites, which form 65 per cent of the total body weight in the first month of intrauterine life. Next the head develops, including the brain, skull, eyeballs and face, which forms 45 per cent of the total body volume at 2 months. The extremities maintain a uniform development throughout the prenatal period after their initial rise in the second month, and so do the spleen, thymus and thyroid, although the relative growth of the last three is small compared to the relative growth of the extremities. The individual organs have a maximum relative volume during the prenatal period of growth as follows: heart, first month; brain, second month; liver, third month; lungs, fourth month; and kidneys, seventh month.

The head in man is reciprocal in its growth to the trunk, and the trunk and the parts of the extremities are reciprocal to each other and to the extremities, as demonstrated by Godin (23, 24) and Pfitzner (45, 46), therefore these parts may be called complementary structures. The development of the heart is coincident with the development of the trunk, and the development of the head is coincident with the development of the brain, therefore the heart and brain may be called complementary

BRAIN, CORD, EYEBALLS, LUNGS, SPLEEN	THYMUS, HEART, KIDNEYS, ADRENALS	STOMACH, INTESTINE, LIVER	SEX GLANDS
Rat 1st period (7 days)	2 (20 days)	3 (6 wks.)	4 (puberty)
Man 1–2 yrs.	3–5 yrs.	7–10 yrs.	puberty

Diagram 2 Postnatal growth in the white rat

structures. The development of the heart is also reciprocal to that of the lungs. The heart develops in time before the development of the brain and the lungs, but it is complementary to both. Likewise the liver may be reciprocal to the lungs, heart and brain in its development, and so each may be reciprocal to the other, but if we examine adjacent organs in their development, such as the head and trunk, the heart and lungs, and the liver and intestine, as well also as the upper and lower teeth, their reciprocal development makes them logical complements of each other.

1 STATURE	I TEETH	2 STATURE	II TEETH	3 STATURE	III TEETH	4 STATURE	SEX	IV TEETH
1–6 mos.	1–2 yrs.	5 yrs.	7 yrs.	8 yrs.	11 yrs.	12 yrs.	puberty	20 yrs.

Diagram 3 Periods of growth in stature and eruption of the teeth

The postnatal development of the structures of the white rat as given by Jackson may be grouped into four periods, and I have roughly approximated these periods for man. The brain and lungs develop most rapidly soon after birth, the heart and kidneys a little later, followed by the development of the stomach, intestine and liver. The sex glands develop irregularly but their most rapid period of development immediately precedes puberty.

I have roughly approximated the periods of most rapid growth in stature and the most rapid development of the teeth after birth, and have placed them in diagram 3. This is a tentative scheme and awaits further observations for confirmation.

The first period of postnatal growth is the most rapid of all, and is associated with the development of the trunk and extremities. This is followed by the eruption of the temporary teeth, associated with the rapid development of the brain. This re-

minds us of the development of the trunk in the first month of prenatal life, followed by the rapid development of the head (brain). Between the arrival of the temporary teeth and the second acceleration of growth in stature (2 to 5 years), the heart and kidneys are apparently developing most rapidly. The eruption of the first permanent teeth, followed by the third acceleration of growth in stature, is related to the most rapid postnatal development of the liver, stomach and intestine. The third period of rapid tooth development followed by the fourth period of rapid growth in stature succeeds this and precedes puberty, after which the growth of the boys is retarded and the growth of girls almost ceases. There is need of more detailed

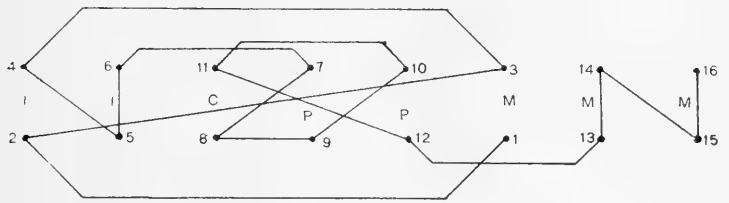


Diagram 4 The order of eruption of the permanent teeth in Europeans. One side only of the mouth is represented, the left side as viewed from in front. The dots represent the individual teeth, whose symbols are placed between them. The order of eruption of the teeth is represented by numbers and by the lines connecting the dots. *i*, incisors; *c*, canines; *p*, premolars; *m*, molars.

data relating to the postnatal periods of growth in stature, in the teeth, and in the other structures of the human body. Until this is forthcoming there can be no final statement as to the exact relations of these periods, but enough is known of both prenatal and postnatal development to justify the law of alternation in development.

This law is applicable to the eruption of the individual teeth. No two adjacent teeth erupt at the same time except the median incisors, and the lower canines and median premolars. The median line represents a barrier, therefore the places filled by the median incisors are almost like remote areas. Diagram 4 represents the order of eruption of the teeth on one side of the mouth in the Germans and Americans.

The order of eruption is represented by serial numbers and followed by the line with barbs, beginning with the lower first molar and terminating with the upper third molar. The line skips from maxilla to mandible or mandible to maxilla nine times, and five times it goes from one tooth to another in the same jaw.

There are two waves of growth, one from the median line laterally, which includes the incisors, canines and premolars, 20 teeth in all, that erupt more rapidly (from 6 to 11 years) than the other wave, which includes the first, second and third molars, only 12 teeth in all, that erupt more slowly (from 6 to 20 years or later). Four molars erupt every 6 years, the first molars at 6 years of age, the second molars at 12 years of age, and the third molars at about 18 years of age. We may illustrate the

Diagram 5 The eruption of the teeth of the Filipinos and the Europeans in two series. A, American: F, Filipino. Each tooth is represented by a dot. Under the dots are symbols for the tooth; *i*, incisor; *c*, canines; *p*, premolars; *m*, molars. The order of eruption is represented by the lines.

two waves of growth by diagram 5, showing the relative time of development of each tooth in the two waves.

When the permanent teeth begin to erupt the lower teeth erupt before the upper in the first period of acceleration in eruption (the first molars, incisors and canines); the upper teeth erupt before the lower in the second period of acceleration in eruption (the premolars); and the lower teeth again erupt before the upper in the final stages of eruption (the second and third molars).

The alternation in development seems to apply to abnormal as well as normal development, although insufficient data are as yet available to demonstrate this conclusively. If one structure is unusually precocious in the periods of acceleration in development its complementary structure will be backward in the periods of acceleration, and vice versa. The eruption of the teeth

of the Filipino boys may illustrate this. Take the lower teeth, for instance. In the median series the median incisors are precocious, hence the lateral incisors are relatively backward, hence the canines are relatively precocious, hence the median premolars are relatively backward, and hence the lateral premolars are relatively precocious. In the lateral series the first molars are relatively precocious, hence the second molars are relatively backward, and hence the third molars are relatively precocious. The alternation is also true for the lateral series of the upper teeth where the first molars are backward, the second molars are precocious and the third molars are backward, which is not only an alternation of adjacent teeth, but also of the similar teeth of opposite jaws, because the lower first molars are precocious, and the upper first molars are backward, the lower second molars are backward and the upper second molars are precocious, and the lower third molars are precocious and the upper third molars are backward.

Racial differences in growth also fall within the law of alternation in development. It is evident from this and other studies already published or at present under way that the Filipinos mature later than the Europeans in morphologic form, especially of the face, head, nose, etc., or never reach the state of the mature European, yet the Filipinos mature earlier than the Europeans (Ann Arbor Germans and Americans) in stature and in the eruption of the teeth. Does the early development of the teeth and stature in the Filipinos cause the late maturity of the head and face, and the early development of the head and face of the European cause a late development of the teeth and stature, or are the differences incidental, or caused by other factors?

A presentation of the supposed causes of alternation in development would be incomplete without suggesting the influence of mechanical factors and the internal secretions. The alternation in development of the teeth may be produced by a more rapid growth of one tooth than another due either to an initial stimulus, or to a better blood supply. The adjacent tooth may be crowded back by the precocity of the one that develops first and the substances used in the building of the latter delay the growth of

the adjacent tooth. The position or size of the blood vessels may determine the precocity of the tooth, just as the greater quantity of pure blood going to the head in the fetus may account for the early precocity of that part. There may be blood vessels to the first molars and median incisors that are larger than those to the other teeth at first, or there may be an initial stimulus to these teeth that is greater than to the other teeth. Biting and chewing in the region of the median incisors and first molars is apt to be greater at first than in the region of the other teeth. In the same way the rapid growth of the lungs soon after birth and the closure of the ductus arteriosus and foramen ovale may be explained by the shunting of the blood stream from its fetal course through the foramen ovale and ductus arteriosis to its postnatal course through the right ventricle and pulmonary artery. The activity of the lungs immediately after birth sucks the blood through the pulmonary artery and right ventricle that formerly went through the foramen ovale and the ductus arteriosus to the body, and thus allows the closure of the last two channels.

The ductless glands with their internal secretions poured into the blood stream play a part in development that is little understood, but if the normal effect of the secretions may be inferred from their abnormal effect, we may know more than we think, or understand. I cannot enter here into a review of the literature of the internal secretions, which is enormous, but I wish to present a few facts that may be relevant. We may infer from recent work that the hypophysis influences the growth of the bones, and there is some indication of the antagonistic action of the sex glands and the hypophysis, which may account for the retardation of the growth of the bones (stature) after puberty.

The growth of the sex glands is irregular after birth, according to Jackson and Hatai, and if this irregularity in their development bears any relation to the periods of acceleration in stature, we may have a causal relationship.

The thyroid influences growth, because hypothyroidism produces cretinism and hyperthyroidism produces rapid differentiation with irritability of the nervous system, when either occurs

during development. A person of 13 to 14 may appear to be 20 to 25 years of age. If later in life, hypothyroidism causes simple goiter, and hyperthyroidism causes exophthalmic goiter. Exophthalmic goiter, or hyperthyroidism occurs invariably in hyperontomorphs, and cretins are hypoöntomorphs, therefore the hyperactivity of the thyroid gland may have something to do with the hypermorphism of Europeans, and the hypoactivity of the thyroid gland may have something to do with the hypomorphism of the Filipinos and other peoples (see p. 148). Climatic conditions, habits, food, water, and animal parasites may account in part for the differences.

The sexual activity of a people may have a profound effect upon their bodily form and mental condition through the interactivities of the secretions of the sexual glands and the other glands of internal secretions. A great deal more has yet to be done on the effect of the internal secretions before definite results can be assured, but the indications are that the secret of multiple activities resides in them and the explanation of many phenomena of development may be there, as well. Factors of selection, of evolution and involution, of progression or regression, of progressive metamorphosis or of retrograde metamorphosis should be mentioned here. The third molars and canines are retrograding, more in Americans than Filipinos, more in Germans than Indians.

The process of selection in evolution cannot be passed over as a factor in the eruption of the teeth. Biting and chewing, or gnawing and grinding, are the essentials in man, rather than holding and tearing, therefore the incisors and first molars develop early and are larger, and the canines develop late and are small. It is to be presumed that in prehistoric times those individuals in whom the incisors and molars developed early would be better fitted in the struggle for existence, and their kind would be propagated to a greater extent than those who had the incisors and molars developed late. The third molars are at present undergoing retrograde metamorphosis among Europeans; they appear late, and in some cases fail to appear. It is probable that the second molars erupt in man later than they once did.

The canines are also undergoing retrograde metamorphosis as indicated by their size in prehistoric times and today. The Filipinos and Indians, in whom the canines erupt early, are more like the prehistoric men than are the Germans and Americans, in whom the canines erupt late. The same is also true of the second and third molars, which also erupt earlier among the Filipinos and Indians than among the Germans and Americans.

THE MORPHOLOGIC TYPE AND THE TEETH

In a former study of Filipino students (2) I called attention to the bad teeth of what I then called the Iberian type, but have since named the hyper-onto-morph. This type is usually small, slender, narrow nosed, and has a long slender face with pointed chin, and a long narrow head with projecting occipital region, although at times the type may be fat, the face may be short and not slender, the nose may be stubby, and the head may be rounded; but at all times the type has characteristic ears, with everted anthelix, tragus and antitragus, and rolled back helix. The type is identical with the Mediterranean race of Sergi. In studies subsequent to those on the Filipino students I made observations in Manila (4), in Taytay (3), a town near Manila, and in New Orleans (5). I have demonstrated that this type is what I call an epitheliopath, a type which is very susceptible to diseases of the structures derived from epithelium, such as the lungs, alimentary canal and central nervous system. The hyper-onto-morph is quite distinct from the round-headed, round-faced type with more or less infantile nose and bowl-shaped ears. However, no observations were made of the ear form of the school children represented in this study, hence only the cephalic and facial indices, and the relative size of the occipital and parietal regions of the head can be utilized here. This will not effect a complete segregation of the hyper-onto-morph and the hypo-onto-morph, but it will indicate that a complete segregation such as might be accomplished by the use of the ear form and other factors would give a more marked contrast between the two types

in the eruption of the teeth, in the number of good sets present, and in the average number of bad teeth. As it is, the segregation by cephalic and facial indices gives considerable difference in all three of these conditions.

The two types are segregated by putting those with a cephalic index below 80 and a facial index below 120 in one group, and those with a cephalic index of 80 and over and a facial index of 120 and over in another, and the remainder, or those with a cephalic index of 80 and over and a facial index of 120 and less, or those with a cephalic index of 80 and less and a facial index of 120 and over, in a third group. The three will be called the hyper-onto-morph, hypo-onto-morph and remainder. Below the age of 11 years the dividing figure of the cephalic index is 81 instead of 80.

1. The average number of teeth

The Filipinos from 5 to 10 years of age may be neglected because of the few individuals who come under observation. The average number of teeth erupted among the German and American boys from 5 to 10 years of age is

Hyper 9.87	Hypo 9.14	Remainder 9.45

The average number of teeth erupted in all the groups from 11 to 16 years of age, including both sexes, is

Hyper 26.6	Hypo 24.1	Remainder 25.4

The hyper- is more precocious than the hypo- from 10 to 16 years of age. It is therefore evident that the hyper-, at all ages, is more precocious in the eruption of the permanent teeth than the hypo-, and this difference amounts to one or more teeth.

2. The percentage of good sets of teeth

The percentage of good sets of teeth among the German and American boys and girls from 5 to 10 years of age is

Hyper 80.33	Hypo 80.98	Remainder 82.8

The percentage of good sets of teeth among the boys and girls of all the groups from 11 to 16 years is

Hyper 18.3	Hypo 46.8	Remainder 34.4

Among the Filipinos alone from the age of 17 to 30, both sexes, it is

Hyper 9.3	Hypo 34.3	Remainder 24.2

It is evident that the hyper- has fewer good sets of teeth at all ages than the hypo-.

3. The average number of bad teeth

The Filipinos below the age of 10 years may be disregarded. The average number of bad teeth below the age of 10 years among the German and American boys and girls is

Hyper 0.468	Hypo 0.36	Remainder 0.30

The average number of decayed teeth in all the groups from 11 to 16 years of age is

Hyper 3.35	Hypo 1.79	Remainder 2.09

The average number of decayed teeth among the Filipinos alone from 17 to 30 years of age is

Hyper 3.33	Hypo 2.53	Remainder 3.08

It is evident from a consideration of the average number of bad permanent teeth that the hyper-s have the worst teeth and the hypo-s have the best.

4. Racial and sexual differences

Racial differences may be found by calculating the relative number of hyper- and hypo- individuals in each group. Between the ages of 5 and 16 the Filipinos have 1 hyper- to 15.2 hypo-s, the Germans have 1 hyper- to 5.3 hypo-s, and the Americans have 1 hyper- to 1 hypo-. From 17 to 30 years the Filipinos have 1 hyper- to 3.8 hypo-s.

Sexual differences may be determined in the same manner. The Filipino girls have 1 hyper- to 28 hypo-s; and the Filipino boys have 1 hyper- to 4.6 hypo-s; the German girls have 1 hyper- to 7.1 hypo-s, and the German boys have 1 hyper- to 4.3 hypo-s; and the American girls have 1 hyper- to 1.2 hypo-s, and the American boys have 1 hyper- to 0.84 hypo-.

It is also noticed that the relative number of hyper-s increases with age. This is especially noticeable with the American boys for whom the ratio is 1 hyper- to 3 hypo-s at 8 years of age, and 12 hyper-s to 1 hypo- at 16 years of age.

Hyper-morphism is a condition, of greater age, of development in a certain direction, a male condition, and a condition characteristic of the American white. Hypo-morphism is a condition of less age, of development in another direction, a female condition, and a condition characteristic of the Filipino.

A number of other conditions besides the shape of the head and face characterize the hyper- and the hypo- types, such as the shape of the nose, the relative length of the extremities and of their parts, the ear form, the size of the regions of the head, etc., but it will suffice to give only one of these here, that is, the relative size of the occipital and parietal regions of the head.

The circumferences of the forehead, frontal, parietal and occipital regions of the head of the school children were taken but they will be reserved for future studies of head form that are at present under way, only the relative size of the occipital and parietal regions of the head to each other will be given here.

The points selected from which to measure the circumferences of the regions of the head are the dorsal extremities of the middle roots of the zygoma on each side of the head immediately ventral to the external ear, a place that is easily accessible and distinctly felt. The tape was passed from the point on the right side to the point on the left side around the maximum protuberance of the parietal and occipital regions of the head, and this distance recorded as the circumference of the part. An index is obtained that is called the occipito-parietal index, by multiplying the occipital circumference by 100 and dividing by the parietal circumference. This gives the occipital circumference in

terms of the parietal, the latter always being 100. A high index denotes a large occipital region, a low index the reverse.

The occipito-parietal index in the German and American children from 5 to 10 years of age is

Hyper 80.9 Hypo 79.9

and from 11 to 16 years of age is

Hyper 82.2 Hypo 79.4

The occipito-parietal index in the Filipino boys is

5 to 10 years	Hyper 88.4 (1)	Hypo 78.3
11 to 16 years	Hyper 81.9	Hypo 76.7
17 to 30 years	Hyper 83.4	Hypo 79.9

The index of the Filipinos is less than that of the Germans and Americans at the same age, and the index of the hypo- in the Filipino from 17 to 30 years of age has reached that of the Germans and Americans at the age of 5 to 10 years, but the index of the hyper- among the Filipinos at 17 to 30 years of age has passed that of the Germans and Americans at the age of 11 to 16. The index increases with age except that the hypo-s among the Germans and Americans do not change.

The hypo- has a relatively smaller occipital region in the Filipino than in the German, and a relatively larger occipital region in the American than in the German. The same is not true for the hyper-, for in them the occipital region is about the same for the American and Filipino, but relatively smaller for the German.

The sexual differences indicate that the male has a relatively larger occipital region than the female, and the female a relatively larger parietal region than the male although the difference is not great.

No other differences will be presented here, although differences in stature, weight, nose form, ear form, facial angle, cranio-facial index, etc., will be given in subsequent publications, but enough has been done to establish the hyper-onto-morph and the hypo-onto-morph as entities and to indicate their identity.

Magitot (37) called attention to the differences in the decay of the teeth in Normandy and Brittany, and attributed the difference to the type of people in the two compartments of France. In Brittany are the Celts or Gauls, who are small, with dark hair and eyes, broad head, and with good teeth; in Normandy are the Belgians or Kymries, who are tall, with fair hair and eyes, long head, and with bad teeth. The color of hair and eyes seems to be incidental, because in those I examined with bad teeth and of hyper-onto-morph form, the color of hair and eyes was of all shades. In southern Europe the long head has dark hair and eyes and in northern Europe fair hair and eyes, and in central Europe the broad head has dark hair and eyes and in eastern Europe the same form has fair hair and eyes. The morphologic type is independent of pigmentation and I believe the time of eruption of the permanent teeth and the extent of their decay are due to inherent differences in the morphologic type of the individual.

The teeth of the hyper- form are undergoing retrograde metamorphosis because they appear early and decay early. It is believed that the third molar in man is undergoing retrograde metamorphosis more rapidly than the other teeth and there are indications that the canines are also undergoing rapid retrograde metamorphosis, because the canines appear late and the third molars sometimes do not appear at all. It is strange to say that the teeth of the hyper- are undergoing retrograde metamorphosis because they appear early and decay early and the canines and molars are undergoing retrograde metamorphosis because they appear late or not at all, yet such is the inference. In the case of the hyper- there is rapid differentiation with early decay and in the canines and premolars retardation or failure to appear through backwardness. Retrograde metamorphosis, as in the human ear, seems to come about with precocity; involution probably occurring earlier and earlier with each succeeding generation, therefore the hyper- is a very much involved form. Involution in the teeth may occur in the same way until crowding causes the third molars and canines to appear late and finally to fail to appear. There may also be more than one method of evolution.

The hypo- and hyper- are both undergoing retrograde metamorphosis, the hyper- in America, the hypo- in Asia, and the Meso form carries the evolution for the future.

It is noted that the Filipinos are more precocious than the Americans in the eruption of the permanent teeth, although the Filipinos are more hypo- than the Americans, and the hypo- is more backward than the hyper- in this respect. Therefore the precocity of the Filipinos must be attributed to something other than hypermorphism. Whether it is race, climate, food, water, animal parasites, or something else, is problematic.

PHYSIOLOGICAL STANDARD

Each tooth may be considered from the standpoint of the time at which it first appears in any individual, and the time at which it last appears in any individual, as well as from the time at which 50 per cent of the individuals have the tooth erupted, and the time during which the greatest number of individuals have the tooth erupting. Suppose we take the left upper second molar tooth in the American girls. This tooth has not erupted in any American girl at 9 years of age, but 2 per cent appear at 10 years, and the latest age at which any erupt is 15 years, all are erupted at 16 years. Approximately 50 per cent have the tooth erupted at 12 years, therefore there is a shorter period for the eruption of the first 50 per cent (10 to 12 years), than for the eruption of the second 50 per cent (12 to 15 years). The greatest number of teeth erupt between 11 and 12 years (41 per cent) although there is a second period of rapid eruption between 13 and 14 years. This may be a segregation of the precocious and backward American girls.

In like manner we may obtain the same data for all the other teeth of the American girls, and of the teeth of all the other boys and girls. The upper teeth of the same type on the two sides, as the upper median incisors, erupt at so nearly the same time that they may be put together. The periods of most rapid eruption almost invariably coincides with the time at which 50 per cent of the teeth have erupted therefore the limits of the first mentioned period will cover the second. Table 1 (p. 155) has been constructed showing the results, and this table will be the physiological stand-

ard for each group of individuals in relation to the eruption of each tooth. The ages given are the ages at which the teeth normally appear. If the teeth appear earlier in any individual, that individual is precocious, and if later, backward.

The physiological standard may be utilized to greater advantage with increasing age, because the racial and sexual differences increase with age. A word as to one way in which such a table may be of use. Take a German girl of 10 years, and all the incisor teeth, the first molars, premolars and canines may be erupted, whereas a German boy may not have all these teeth erupted until 11 years of age, yet at 11 years he may be at the same physiological standard as the girl at 10 years. Should either one have all the teeth mentioned erupted at the age specified there would be evident precocity. In like manner evident backwardness may be determined.

The teeth are more convenient and more exact as a means of determining the physiological standard than stature, or weight, or the growth of the bones, or secondary sexual characters, etc., and they *may* be of greater value than any other means that can be utilized. The teeth can be seen, recognized and counted by almost anyone after a little experience, and they are either present or absent, therefore very definite.

Precocity in the eruption of the permanent teeth is a sign of hyper-morphism, and hyper-onto-morphs are epitheliopaths, who are especially susceptible to diseases of the alimentary canal, the lungs and the central nervous system, therefore precocious children should be shielded from injuries to the susceptible organs.

Backwardness in the eruption of the permanent teeth is a sign of hypo-morphism, and hypomorphs should be shielded from injuries to their susceptible organs.

THE SCHOOL GRADE AND THE TEETH

The modal grade, or the grade that has the greatest number of individuals at each age, varies little with sex but the Germans are about one year behind the Americans. This corresponds with the backwardness of the Germans in the eruption of the permanent teeth, and indicates a correlation of mental and dental develop-

ment. This is true not only of the modal grade, but also of the grades above and below the mode. At each age, from 7 to 14 inclusive, the children who are below the modal grade have an average of 0.9 of a tooth less than those in the modal grade, and the children who are above the modal grade have an average of 0.8 of a tooth more than those in the modal grade. The difference is greatest at 10 and 11 years, when the second period of the second dentition is at its height, and the difference at these two years amounts to 1.5 fewer and 1.8 more teeth for those below and above the mode respectively.

It will be recalled that there is a period of one year during which the greater number of teeth of any form erupt, and during this year in nearly every case, 50 per cent of that form of tooth has erupted. From this a physiological standard, or age of eruption of each tooth, was determined. Evidence is therefore produced to indicate that the eruption of the teeth is a better criterion than age as a standard of both physical and mental development.

This evidence is not conclusive for each individual, but only as an average or modal factor, because some individuals with fewer teeth than normal are above the modal grade, and some individuals with more teeth than normal are below the modal grade. Other factors therefore play a part and must not be overlooked. For instance, one child who was advanced in school grade beyond her age, and who had less than the normal number of teeth present, was the child of a teacher, and had evidently been pushed in school work. However, the condition of the teeth, both as to eruption and decay, may be utilized and may be of value in determining the relative development of the individual.

Maturity in the Filipinos is different from maturity in the Europeans, at least the face, body form, extremities, etc., of the Filipinos differ from the same parts in the Europeans, and the time of definite maturity of the parts is different. Each people is probably an expression of different conditions, and each represents development in a different direction, at a different rate, and to a different extent. The adult Filipino resembles the infant European in morphologic type more than it does the adult

European. The condition of the Filipino in stature and in the eruption of the permanent teeth seems to be an early precocity superseded by an early retardation in development, but in morphologic type the Filipino is retarded throughout the period of development.

RÉSUMÉ

1. The eruption of the teeth in relation to the development of the individual: the physiological standard

The time of most rapid eruption of the teeth and the time at which 50 per cent have erupted is the same, and may be called the physiological standard of the teeth. This is shown in table 1, for the Americans, Germans, and Filipinos.

TABLE 1

FIRST MOLARS	GIRLS, AMERICAN AND GERMAN, 6 YEARS	BOYS, AMERICAN AND GERMAN, 6.5 YEARS	FILIPINOS, 5? YEARS
Median incisors	6.5	7.0	5?
Lateral incisors	8.0	8.5	6?
Median premolars	10.0	11.0	8.0
Canines	10.5	11.5	7.0
Lateral premolars	10.0	11.5	9.0
Second molars	11.5	12.5	10.5

2. The eruption of the teeth in relation to stature: periods of rapid development

TABLE 2

	1 PERIOD	2 PERIOD	3 PERIOD	4 PERIOD
Boys' stature	1–6 mos.	3–5 years	7–9 years	12–14 years
Boys' teeth	6 mos.–2 yrs.	6–9 years	10–13 years	
Girls' stature	1–6 mos.	2–4 years	6.5–7.5 years	10–14 years
Girls' teeth	6 mos.–2 yrs.	6–8 years	9–11 years	

3. The eruption of the teeth in relation to race

The American white is taken as the standard. Order of eruption: (1) Filipino, (2) French, (3) American Indian, (4) American white, (5) German. The time at which 50 per cent of the teeth have erupted: French 6 months to 1 year earlier than American white. German 6 months to 2 years later than American white. American Indian is from 1 to 6 months earlier than the American white. Filipino 1 to 4 years earlier than American white.

4. The eruption of the teeth in relation to sex

American girls are from 0.2 years for the incisors, to 1.4 years for the canines, earlier than the American boys. German girls are from 0.3 years for the incisors to 1.1 years for the canines, earlier than the German boys. Filipino girls and boys are more nearly alike in the time of eruption.

5. The eruption of the teeth in relation to school grade

The modal grade is the grade which has the greatest number of individuals at each age. For instance at 7 years of age the second grade is the modal grade. At ages 7 to 14 inclusive, children above the modal grade have 0.8 more teeth, and children below the modal grade have 0.9 fewer teeth, than children at the modal grade. The difference is greatest at 10 and 11 years, i.e., during the most rapid eruption of the second part of the period of second dentition, when children above the modal grade have 1.8 more teeth, and children below the modal grade have 1.5 fewer teeth, than children at the modal grade.

6. The eruption and decay of the teeth in relation to morphologic form

Two types, extremes: (1) Hypo- equals infantile, round head, broad face and nose, large parietal and small occipital region of head. (2) Hyper- equals long head, face, nose, small parietal, large occipital region of the head (table 3):

TABLE 3

Teeth erupted:			
Below 10 years	Hyper, 9.87	Hypo, 9.14	German and
Above 10 years	Hyper, 26.6	Hypo, 24.1	American
Good sets:			
Below 10 years	Hyper, 80.3 %	Hypo, 81 %	German and
Above 10 years	Hyper, 18.3 %	Hypo, 46.8 %	American
17–30 years	Hyper, 9.3	Hypo, 34.3	Filipino
	Average number of decayed teeth		
Below 10 years	Hyper, 0.47	Hypo, 0.36	German and
Above 10 years	Hyper, 3.35	Hypo, 1.8	American
17–30 years	Hyper, 3.33	Hypo, 2.53	Filipino

Characteristics of the individuals in each group:

	5–16 years		17–30 years		Girls		Boys	
	Hyper,	Hypo	Hyper,	Hypo	Hyper,	Hypo	Hyper	Hypo
Filipinos	1	15.2	1	3.8	1	28.0	1	4.6
Germans	1	5.3			1	7.1	1	4.3
Americans	1	1.0			1	1.2	1	0.84

American boys have 1 hyper- to 3 hypo-s at 8 years, and 12 hyper-s to 1 hypo- at 16 years.

7. The law of alternation of development

There are one or more periods of acceleration alternating with periods of retardation in the development of the structures of the body. Each organ or structure has a critical period when it is developing most rapidly, and when it is probably most susceptible to its environment. The periods of acceleration in the development of one structure are synchronous with the periods of retardation in the development of another, and the two may be called complementary structures.

The relative number of hyper-onto-morphs is greatest among the Americans, least among the Filipinos, and nearly as great among the Germans as among the Americans. Hypo-morphism decreases with age, and hyper-morphism increases, so that whereas among the Filipinos there are 15.2 hypo-s to 1 hyper- between the ages of 5 and 16, there are only 3.8 hypo-s to 1 hyper- from 16 to 30 years of age. Hypo-morphism is a condition of less maturity than hyper-morphism. Apparently the Filipinos mature more slowly than the Americans and Germans in morphologic form,

although they mature earlier in stature and in the eruption of their permanent teeth, which, again, may be only another expression of compensation in the law of alternation in development.

The teeth are useful as a physiological standard for determining the relative development of the individual, physically and mentally.

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ON THE ORIGIN OF LYMPHATICS IN BUFO

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THIRTY-FIVE FIGURES[1]

The question of the origin of lymphatics continues to incite the most animated controversy in the field of anatomy in America at the present time. Consequently all observations bearing directly upon this problem are eagerly awaited and are received with enthusiasm by investigators specially interested in it. For the last two years the author has focused his attention upon the development of the lymphatic vessels in Amphibia with the hope of being able to discover an evidence to the solution of that problem which could not be controverted by any criticism, and incidentally of adding to our as yet fragmentary knowledge of the phylogenesis of the lymphatic system. Although such a hope may have been too sanguine, inasmuch as the evidence offered does not entirely reveal how much fact and how much fiction there is in the various hypotheses propounded hitherto, the observations reported here differ so decidedly from observations made in the past and at the same time fit in so readily with them that we trust much has been accomplished towards a speedy termination of the dispute. An extensive paper dealing with the genesis of the lymph hearts, ducts and sinuses in Amphibia is in the process of preparation. Some months, however, will inevitably elapse before this work is in its final form. It has seemed expedient, therefore, to publish immediately for the benefit of the writer's co-workers in lymphatic research a partial account embodying the central point of his investigations.

Early embryos of the American and the European common toads constitute the chief material of this inquiry. The first were collected near Princeton, New Jersey, the second were

[1] Cost of illustrations in part borne by the Laboratories.

THE AMERICAN JOURNAL OF ANATOMY, VOL. 17, NO. 2
JANUARY, 1915

gathered in the marshes of the Isar near Munich and prepared in Professor Rückert's laboratory at the Anatomical Institute. The specific descriptions in the following pages are based entirely upon the specimens secured in Europe and, strictly speaking, pertain only to them. The serial sections of the native toad were not used extensively because they were prepared first, that is, at a time when the writer's practice in making perfect series of yolk-laden Amphibian larvae was still in its trial and error phase. Such experimentation, particularly in regard to fixation, embedding and sectioning, was conducive of better results with the second batch of specimens, which were procured last spring[2] while the writer was engaged in study abroad. Mention of the methods of technic as well as a review of the work of other investigators relating to the formation and growth of lymphatics in Amphibia will be reserved for the later article. Bufo embryos were chosen in preference to frog embryos because their mesenchyme appears less scanty and less loose in texture, a condition which it was supposed might prove helpful in distinguishing between lymphatic anlagen and tissue spaces. Nevertheless, it will become evident presently that such a precaution was needless.

At the beginning of the inquiry the gaining of exact special information was beset with what at first seemed a very disconcerting obstacle, namely, the enormous number of yolk spherules which are closely massed in all tissues of the young larvae and cover and obscure everything except darkly staining cell nuclei. The effort required to follow the development of such material with some degree of accuracy exceeds the ingenuity and patience expended in the preparation of satisfactory serial sections. Many details of the earliest genetic changes cannot be followed with certainty until much of the yolk has vanished. But in this respect a comparison of young and somewhat older stages will yield the important fact that the yolk does not disappear uniformly from the embryonic body. In other words, the period in which the yolk substance is lost is different for the various

[2] April and May, 1913. The manuscript was completed in the spring of 1914.

tissues. Of course this does not necessarily imply that certain tissues are more active than others and use up the stored nutritive supply more rapidly. On the contrary, some may be more richly supplied at the outset. Thus organs derived directly from entoderm retain traces of yolk much longer than others. These considerations suddenly led to the inspiration that the presence of yolk might after all prove to be an advantage since it might point out the relationship of lymphatic endothelium. Such a natural and simple factor, if found and capable of being utilized as an instrument of proof, would carry far more weight than any possible evidence obtained by the use of special staining processes, injections, or other artificial procedure. A critical study of the behavior of the several tissues in this respect has convinced the writer of the soundness of this principle. Referring especially to the head region, which in vertebrate embryos is ever in advance of other parts of the organism in degree of development, it was observed that the mesenchymal cells dispose of their yolk content much earlier than do blood corpuscles and vascular endothelia, both haemal and lymphatic. To be more specific, in 6 and 7 mm. embryos of the European common toad the cephalic mesenchyme is virtually destitute of yolk, while the lining of the lymphatic anlagen contains large globules even in specimens measuring 9 mm. in length. To the author's mind these facts indicate fundamental differences between the two tissues and show that in Amphibia, at least, the lymphatic intima arises not by direct differentiation of mesenchymal elements. The idea of similarity of origin but diversity due to function cannot be urged against this argument, for at the time when the yolk-filled rudiments of the large cranial lymph channels appear the embryonic connective tissue of the same region has lost most of its yolk granules.

Before entering into an interpretation of the appended figures illustrating the source of certain lymph vessels, a brief description of a later embryonic phase, when an effective system of lymphatics is already in existence, is essential to the proper understanding of the narrative. This can best be done by referring the reader to Hoyer's figure 417 in the seventh edition of Wieder-

scheim's "Vergleichende Anatomie der Wirbeltiere," or to his original article which depicts such a condition in a frog tadpole. An enormous lymph sinus, probably tantamount to the several cephalic subcutaneous sacs of the adult, is seen to occupy almost entirely the ventral and lateral territory of the head. On each side this reservoir passes backward as a short slender duct which opens into the anterior lymph heart, situated in the region of the fore-limb bud. A second set of vessels, draining the posterior portions of the body, unite cranially to form a single trunk, which communicates with the duct coming from the head at its point of entrance into the lymph heart. Besides the chief systemic vessels, numerous smaller subsidiary channels and plexuses spring from them; but these will not be considered in the present paper and may therefore be disregarded.

The inception of the large ventral cephalic lymph sinus will be discussed fully, for in the writer's opinion it offers a very clear case of the derivation of lymphatic endothelium from the lining of blood vascular channels. This lymph vessel is an especially favorable object for study not only on account of its size, but because it originates in the immediate vicinity of veins located in a broad uninterrupted expanse of mesenchyme which is loosely woven yet sufficiently abundant in number of cells to facilitate an examination and comparison of the rôle played by these structures during the formation of it. Figure 1, inserted for the purpose of orientation, represents a cross-section of the head of an 11 mm. embryo and illustrates the situation of this resevoir (*l.*) relative to other organs. The position also of the external jugulars (*j.d.* and *j.s.*)[3] should be carefully noted, for the five succeeding plates portray events that take place proximately around these veins. Beginning with the stage of the almost completed lymph sinus, the territory ventral to the mouth cavity (*m.c.*) in consecutively younger embryos was scrutinized with the oil immersion lens. The important revelations are set forth in the camera lucida sketches reproduced on plates 1 to 6 inclusive. To duplicate as nearly as is feasible the course by which the data were obtained, the descriptions commence with

[3] Goette called the external jugular (Gruby and Ecker) the inferior jugular.

that of a fairly definitive stage in the organization of the sinus and then treat of progressively earlier conditions, which may subsequently be summarized in a more logical manner.

In figure 1 the ventral cephalic lymph sinus (*l.*) of an 11 mm. embryo is shown in section as a wide clear chamber, the periphery of which is sharply defined and the cavity is not interrupted by partitions. Disregarding the disparity in magnifications, with it should be compared the one represented in figure 13, from a 9 mm. embryo, where it (*l.*) is seen to be less broad dorso-ventrally and to be crossed wholly or part way by tissue strands (*t.* and *s.*). Imagined in its entirety, these trabeculae are very numerous and exhibit several variations. Some are proportionately thick (*t.*, figs. 13 and 14) and are composed of a core of mesenchyme (*m.*) covered with endothelium; others are more tenuous, barely stretching across the lumen; and still others exist as spurs (*s.*) of varying lengths which project into it from the surface. Furthermore, there are scattered freely throughout the cavity small clumps of lightly staining débris (*d.*) whose appearance would suggest their being cellular shreds or fragments of former trabeculae or partitions, which had perchance become separated from the walls of the sinus during its formation. Such an explanation presents itself as the most credible one. Pictured in figure 14 individual yolk spherules are conspicuous here and there in the endothelial cytoplasm generally in the neighborhood of a nucleus. At this stage the lining cells also possess all of the intrinsic qualities of typical endothelia and accordingly it is an easy matter to distinguish them from the stellate mesenchymal cells.

Figure 12 delineates a little more than the right half of a section through the sinus (*l.*) in an 8 mm. embryo and plainly indicates its plexiform character at this period. This is confirmed by an enlarged graphic reconstruction[4] which shows it to be a network of interanastomosing vessels arranged one layer deep in a slightly curved plane, and which, viewed from the ventral surface, brings to mind a coarsely and irregularly meshed sieve. Followed

[4] Wax models and graphic reconstructions of crucial stages in the development of the lymph hearts, ducts and sinuses of the toad will be pictured in the later contribution.

throughout its total extent, the sinus is found to be nowhere in connection with the veins, although it closely approaches the external jugular of each side at its extreme anterior limit; nor has it as yet established junction with the lymph hearts.

Plate 4 is representative of conditions observed in a 7 mm. embryo. The upper sketch, figure 10, drawn from a section of the region eventually occupied by the completed sinus, shows this vessel as a double or bilateral rudiment (*l.*) which does not communicate from side to side, but each half is isolated from the other and consists of several individual anlagen which are developed ventro-laterally of the external jugulars (*j.d.* and *j.s.*). In the lower picture, figure 11, is sketched a highly magnified area on the left including the vein (*j.d.*) and three lymphatic anlagen (*l.*, *l.*) in cross section. One of these is solid, being laden with yolk globules, and the larger two are hollow but possess walls which are dense and firm. With reference to yolk content, the drawing, a faithful copy of the actual state of affairs, impresses the distinction between mesenchyme and lymphatics so forcibly that further words to the same effect are superfluous. Indeed, there are occasional mesenchymal elements which do contain yolk, but then it is usually in the form of minute granules and is distributed thinly in the protoplasm. Other distinguishing marks are not so apparent. In all probability the lymphatic endothelial nuclei are on the whole somewhat smaller and more compact than those of the connective tissue at this relatively late stage, but manifold exceptions are encountered among them and consequently the observer would hesitate to emphasize such a difference unduly.

Turning to the next previous stages, 6 and 5 mm. embryos, more interesting phenomena were witnessed, which are in part reproduced on plates 3 and 2. Figure 7 was drawn from a transverse section of a 6 mm. specimen to exhibit the features in the environment of the left external jugular (*j.s.*). At this level the larger one of the two sinus anlagen (*l.*) contains a small slit-like lumen, the confines of which are thick and are packed with yolk spherules. This lymphatic followed from end to end was discovered to be applied to the lining of the vein at its ante-

rior extremity and then to accompany the vein back a considerable distance, sometimes lying hard against it, at other times slightly aloof from it, as displayed in the sketch, and finally becoming a solid attenuated cell cord that ends freely in the mesenchyme. The smaller sinus anlage, shown in the same sketch (fig. 7), stands in similar relations to the vein, but it is less extensive than the other, less wide in diameter and discloses fewer vacuoles. The two anlagen are strictly independent of one another. Beyond their terminations several shorter anlagen are met with which are essentially like those described.

A circumscribed area on the right side of another 6 mm. larvae is portrayed in the second drawing, figure 8. Medially a sinus rudiment (*l*.1.) is inseparably attached to the intima of the external jugular (*j.d.*); at least no visible boundary line can be detected between the adjoining walls of the two structures. Moreover, the lumen of the lymphatic is bisected by a thin cytoplasmic filament which passes from the inner combined venous and lymphatic wall to the free outer wall. After extending through a number of sections in this manner, the anlage becomes detached from the vein and pursues its way parallel to it alone through the mesenchyme until it bends downward and comes in touch with another lymphatic undergoing development on the ventral side of the vein. This is clearly set forth in figure 9, where the anlage labelled *l*.3 is approximated by anlage *l*.1, which is identical with or, more correctly stated, a continuation of the one (*l*.1) situated on the dextral wall of the blood channel in figure 8. Eight sections, each 6 micra in thickness, intervene between the two levels. The reader perceives that the lymphatic rudiment (*l*.1) not only severs connection with the vein in this short stretch, but it becomes broader and at the hinder level (fig. 9) lacks a lumen in consequence of the large yolk corpuscles that crowd every available nook and corner in it. This condition is true of the greater part of its course. The rudiment *l*.3 (fig. 9) is of brief length, appears behind the level represented in figure 8 and possesses a large cavity proportionately, which, separated from that of the bloodvessel (*j.d.*) merely by a thin partition, resembles strikingly the extra-intimal spaces existing

in mammalian embryos of certain ages. Another anlage (*l.2*, fig. 8) begins anteriorly as a distinct thickening of the lining of the external jugular (*j.d.*), but after two or three sections it breaks loose from the vein and proceeds posteriorly as a compact yolk-stuffed endothelial column (*l.2*, fig. 9) surrounded by mesenchymal cells. Slightly farther back the same anlage has acquired a lumen. After a variable course, in which it lies occasionally against the venous intima and now and then buds collateral sprouts, it terminates near the posterior niveau of the thyroid anlage, just in front of the heart.

The earliest initial stages in the genesis of the ventral cephalic lymph sinus occur in 5 mm. embryos. The sketches on plate 2 illustrating its inception are representative of the phases observed. Figures 2, 3 and 4 must be considered together since they treat of the same anlage the origin and character of which they plainly depict. In figure 2[5] attention should first be directed to the endothelium of the external jugular (*j.s.*) which is very undulating and nodular, each node or protuberance consisting of a cell and a cluster of yolk corpuscles. Then, one cannot but be impressed by the knoblike appendage (*l.*) which protrudes from the ventral venous wall into the open tissue reticulum. This endothelial projection is the anterior end of a developing lymphatic channel and, as the figure suggests, arises indisputably by proliferation from the lining of the bloodvessel. Its nucleus in the section (fig. 2) does not differ materially from mesenchymal nuclei except for the depressions in its contour which are caused by the crowding of the yolk bodies against it. A large typical stellate tissue cell is shown in the lower left corner of the picture (fig. 2). Like a number of such cells, its protoplasm at this stage incloses a few scattered yolk granules. Figure 3 represents the next successive section in which the lymphatic anlage (*l.*) is joined to the vein (*j.s.*) solely by delicate cytoplasmic threads.

[5] Near the left hand margin of figure 2, the lumen of the external jugular appears to be crossed by an endothelial partition. But this is not the fact. The sketch is of a section taken immediately behind the level in which a medial branch leaves the vein at a very acute angle. Hence the strand of endothelium represents the point at which the walls of trunk and tributary meet, and the two cavities are the lumina of these vessels respectively.

Three sections back, the diameter of the external jugular (*j.s.*, fig. 4) has markedly contracted while that of the incipient lymph channel has increased. This rudiment is solid from end to end, being stuffed with yolk, which attribute sharply demarcates it from the embryonic connective tissue. To repeat what has been said but a moment ago, the visible differences between lymphatic endothelial nuclei and mesenchymal nuclei are too trivial at this early genetic period to warrant our emphasizing them as positive differential characteristics.

One of the earliest sinus anlagen observed by the writer is shown in section in figures 5 and 6. It is of very brief extent, being only as long as the total thickness of four or five sections. In the first (fig. 5) of these two sketches it appears as a compact protuberance (*l.*) on the intima of the right external jugular (*j.d.*). The structure labelled *b.c.* in the same drawing and which at first glance might be mistaken for another initial lymphatic is a blood cell closely pressed against the lining of the vein. In very young toad larvae blood cells like vascular walls are abundantly supplied with yolk. Overlooking one section we come to figure 6. Here two features should be noted in particular: firstly, the blind slit-like space within the lymphatic anlage, and secondly, the appearance of a boundary between anlage and vein which however is still imperfect since the line of division extends only partway. The cavity of the anlage is not in connection with that of the vein.

From the data so clearly displayed in the camera lucida sketches reinforced by much similar evidence at the writer's command, the following generalization or coherent account of the genesis of the ventral cephalic lymph sinus can be constructed. In 5 mm. embryos, a stage in which the mesenchyme and the vascular endothelia of the head already differ to a noteworthy degree in the fact that the former is more meagerly furnished with yolk, knot-like thickenings occur at unequal intervals on the lining of the external jugular veins in the direction of their long axis. These thickenings, potentially lymphatics, are few in number and arise unquestionably as proliferations of the venous intimal cells and like them are provided with many yolk spherules which are

generally large and lie closely crowded in the cytoplasm. Dependent apparently on the degree of development, these cell aggregations present somewhat different characteristics Some have the form of a bulging compact local swelling of the venous wall and can be pursued through a few sections only. Others are quite as short in extent but manifest one or several small crevice-like cavities which it appears never open into the lumen of the parent vein. Still others, older doubtlessly, exist as comparatively long yolk-stuffed sprouts, one end of which adheres to the bloodvessel and the other lies some distance aloof among the thinly scattered large mesenchymal cells. Such growths, at this time, are solid cell cords for the greater part of their course, but between the masses of yolk ill-defined and irregular vacuities now and then occur which intimate an intracellular origin. Solely superficial differences, if any, can be detected between the nuclei of the lymphatic endothelial buds and those of the mesenchyme. The former seem to be denser in chromatic substance, although this is by no means diagnostic, for in this regard much variation has been found among nuclei according to the mitotic phase active at the time of fixation. A difference which may be more pronounced, yet a difference which after all is neither causal nor specific but is secondary, is the more frequent occurrence in endothelial nuclei of an indented periphery due evidently to the pressure of the yolk spheroids upon the nuclear membrane. As has been and will be emphasized again, the character which visibly differentiates the endothelial from the mesenchymal cell in the early genetic stages of the cephalic region is the presence of much yolk. From this it is obvious that were both tissues devoid of yolk it would scarcely be possible to follow the incipient lymphatics through the meshwork of mesenchyme; and even though all the details of their formation were known the seasoned observer would suffer considerable perplexity in localizing and tracing them. On the contrary, with a large quantity of yolk present in one of these two tissues and absent in the other, the lymphatic anlagen, after they have been once recognized as such, can be pursued from section to section with exceeding ease. In the sections stained with hematoxylin and orange G the contrast

between the brilliant brownish yellow color of the yolk-rich tissue and the blue of the yolk-poor is very striking, and in the colored sketches an attempt has been made to express the distinction as strongly.

In succeeding stages, 6 and 7 mm. embryos, the anlagen of the lymph sinus have in the main received a lumen. Their confines are still thick and heavy and the contour of the cavity is irregular on account of the many large protruding yolk spheres packed in the cytoplasm of the endothelium. Frequently protoplasmic bridges or trabeculae traverse the cavity from side to side and nuclei have been observed to be included in them. Such a condition, coupled with the fact that yolk globules occasionally lie freely in the lumen of an anlage, would point to the derivation of that lumen from intracellular vacuoles by their enlargement and coalescence. By this time all of the initial lymphatics have grown much in length and have also expanded proportionately in caliber. Some of them still retain their original junction with the intima of the vein; others have lost this connection and exist in the mesenchyme as longer or shorter discrete endothelial and yolk-lined fusiform spaces. Other such spaces cling to the parent vein almost throughout their entire extent and hence bear an extraordinary resemblance to the extravenous spaces of mammalian embryos. Blood corpuscles have not been seen in the cavities of these structures; yet this finding is not as significant as it might seem, for as the sketches show, even in the external jugular veins of the specimens studied there is a paucity of them as a result apparently of fixation currents and contractions.

The transformation of the discontinuous bilateral lymphatic rudiments into one broad continuous sinus is a very rapid one. During the stages previous to older 7 mm. embryos the anlagen had developed chiefly in a longitudinal direction, that is, along the cranial portion of the external jugulars, but now they begin to germinate collateral sprouts in an oblique direction towards the mid-ventral line, especially at the level of the thyroid anlage. These sprouts again proliferate buds, the tips of which grow out, soon collide and fuse with one another, and their cavities become

confluent. Others continue to grow medially until they meet those coming from the opposite side. In this manner a very complicated plexus of interanastomosing lymph vessels is established in the ventral territory of the head. This plexus, as reconstructions of it show, is remarkable for its regularity and symmetry, roughly suggesting the form and extent of the finished lymph resevoir. Such a phase has been reached in the younger 8 mm. embryos. The channels constantly distend and in effect the walls of adjacent ones are rapidly approximated so that the mesenchyme filling the meshes of the lymphatic network is diminished in amount. In older 8 mm. embryos the sides of contiguous vessels have met and begin to break down and disappear at the place of coincidence; in this way the plexiform character of the developing sinus is progressively obliterated. Transverse sections at this stage show many mesenchymal and endothelial strands and partitions crossing the cavity of the sinus dorso-ventrally and giving to it a multi-locular appearance, which compares favorably with a similar transient phase in the embryonic history of the thoracic duct in the pig. Such trabeculae are still conspicuous in 9 mm. specimens, but later these last vestiges of boundaries between originally independent channels vanish and leave the sinus a vast uninterrupted lymph chamber.

During these genetic processes, the yolk content of the lymphatic anlagen has suffered marked diminution, and coexistent with this change the intimal cell has passed through a gradual metamorphosis from the stage of an undifferentiated generalized cell to that of a specialized endothelial-like cell. In 10 mm. larvae a casual yolk spherule may still be found in the confines of the sinus, and the lining cells have assumed the features typical of all well-formed endothelia. From now on the prime alteration which the lymph sinus undergoes is the establishment of cont nuity between it and the other components of the lymphatic channel system; other minor changes are chiefly in the nature of growth. But such considerations are beyond the scope and purpose of this paper, and a discussion of them will be postponed until a later date.

To forestall the criticism of insufficient data, a few typical and decisive stages in the genesis of two other lymphatic vessels will be briefly detailed. These stages shall primarily show the occurrence of discontinuity in a developing lymph duct just as the developing sinus has shown in the first instance the origin of its endothelium from venous intima. The channels to be considered are the lymph ducts situated laterally, a pair on each side, and extended through the entire length of tail and trunk to open into the anterior lymph hearts (*cf.* Hoyer's fig. 417, 7th edition, Wiederscheim's "Vergleichende Anatomie der Wirbeltiere"). For a more precise conception of their place in the anatomy of the embryo the reader is directed to figure 15, which pictures a little more than the sinistro-dorsal quarter of a section through the mid-trunk region of a 9 mm. embryo. The lymph ducts, *l.s.* and *l.i.*, are seen to be located between epidermis and myotome (*m.s.*), the first (*l.s.*) near the superior or dorsal border of this structure, and the second (*l.i.*) near the inferior or ventral border. To make more comprehensive the succeeding sketches, the relative position and direction of two or three neighboring organs should be pointed out. The Wolffian or pronephric duct (*w.*) is intercalated between the lower margin of the muscle segment and the dorsal peritoneum or roof of the coelom. Along its free walls run two veins which in reality are the medial and lateral divisions of but one, the postcardinal. Regarded in their longitudinal aspect, the postcardinal divisions are bound together by numerous cross-anastomoses, which pass over and under the pronephric duct at unequal but frequent intervals and hence produce a cylindrical vascular network which closely invests this duct.[6] The postcardinal vein of each side also give off in regular sequence the intersegmental veins (*i.v.*), every one of which supplies chiefly a myotome.

[6] According to Goette ('75) the postcardinal veins in Anura lie medial to the pronephric ducts. But the writer can not subscribe to this statement unconditionally, for it was observed in young toad embryos that the postcardinals resemble more nearly those of Urodeles (Hochstetter) where they surround the pronephric ducts as vascular sheaths. It is true, the portion of the cylindrical postcardinal network lying medially in Bufo is on the whole larger and doubtlessly constitutes the main channel. But this channel and the portion of the postcardinal

In 6 and 7 mm. larvae, the collateral tributaries of consecutive intersegmental veins tend to anastomose with one another and give rise to a longitudinal channel near the upper border of the muscle somites. It is along this vessel that the dorsal or superior lateral lymph duct originates. It is formed by the fusion of several discontinuous anlagen which evidently are engendered by the attendant vein. Figures 16 to 25 inclusive, drawn from sections of a 6 mm. embryo, reveal in detail the character of such an anlage situated in the posterior half of the trunk, thus far removed from the anterior lymph heart. A comparison of these sketches with figure 15 will show that topographically this rudiment lies in the pathway ultimately occupied by the completed duct. It begins blindly and it ends blindly. It is relatively long, extending through forty-six sections. It is closely applied to the venous wall throughout by far the major part of its course. In figure 16 its anterior tip (*l.*) is indicated some distance dorsad of the intersegmental vein (*i.v.*), but seven sections distally the vein has approached the lymphatic (*l.*, fig. 17) by bending upward and backward. After two additional sections the vessels are in contact (fig. 18), and it is impossible to discern a boundary line between their adjoining walls. During the remainder of its course the lymphatic anlage remains attached to the venous intima. Four sections back of the level represented in figure 18, it (*l.*, fig. 19) appears on the side of the vein (*i.v.*) as a lump solidly packed with yolk spheres, and as such it continues for five or six sections. In figure 20 the lumen of the anlage (*l.*) is bisected by a broad protoplasmic partition containing a nucleus and a yolk corpuscle. In the ten following sections, one of which is illustrated in figure 21, a similar condition prevails. Passing over fourteen further sections, we meet with a sprout (fig. 22) which is given off dorsally by the lymphatic anlage (*l.*), but

complex situated laterally are not straight uniform longitudinal channels throughout their course, since either one at times bends around the pronephric duct, fuses with the other on the opposite side for a short distance, then again becomes independent and resumes its former position. Thus the separation of the postcardinal vein into a medial and a lateral division is a more or less arbitrary one, here instituted for the sake of convenience and clearness in the descriptions.

strangely enough it squeezes between vein (*i.v.*) and myotome (*m.s.*). Three sections caudad an endothelial nucleus curiously protrudes into the lumen of the lymphatic (fig. 23). Seven sections beyond this level the anlage has become compressed and in transverse section appears as a crescent shaped cavity (*l.*, fig. 24) clinging to the venous lining. Three additional sections bring us just beyond its end (fig. 25); in fact, it terminates at the large endothelial nucleus on the ventral venous wall. In this (fig. 25) and the following levels there is no indication of the lymphatic anlage. Between it and the anterior lymph sac one hundred and sixty sections intervene, but in this interval two similar anlagen occur which, however, are of much shorter length.

In another younger 6 mm. embryo the longitudinal venous channel of the same locality, in which the anlage just discussed has its being, pursues its way alone and without a dependent structure adhering to its walls, save for a distance of three sections where a small lymphatic rudiment (*l.*) is intimately associated with it (*i.v.*), as indicated in the sketch, figure 26. Its appearance brings to mind some of the initial rudiments of the cephalic lymph sinus, for instance, one (*l.*3) shown in figure 9.

In the foregoing description of two genetic stages of the superior lateral lymph duct no mention was made of the mesenchyme. It requires but cursory notice, for in the youngest toad larvae it is extremely sparse between trunk myotomes and epidermis; only after the larvae have attained the length of 8 mm. does the territory between these two structures expand and the mesenchyme invade it more plentifully. On the other hand, in the area in which the formation of the inferior lateral lymph duct takes place, that is lateral to the Wolffian duct and the postcardinal, the mesenchyme is considerable even in 6 and 7 mm. embryos. Moreover, it loses its yolk content much later than does that in the head; yolk bodies may be found in its cells long after the rudiments of the lymphatic duct had their inception. Thus it is evident that, though the trunk mesenchyme proportionately contains less yolk than do the endothelia, the presence or absence of this substance can not strictly be used as a differential character in the development of the lymph duct

rudiments in the trunk region. It is clear also that were the cephalic mesenchyme more amply furnished with yolk from the start and would lose it more tardily, the discovery of the fundamental moment in lymphatic development in toad embryos would have been by far more difficult and tedious and the evidence less striking. Yet the judicious reader cannot contend for the direct mesenchymal origin of the lateral lymph ducts of the trunk after he has carefully inspected the figures illustrating their anlagen; nor can he, though this remark be irrelevant at this point, conscientiously claim for them continuity in development.

The genesis of the inferior lateral lymph duct accompanying the postcardinal vein (cf. fig. 15) evinces the actuality of discontinuity in a large developing lymphatic vessel probably more forcibly than does the genesis of the two vessels already considered. Figure 27 was drawn from a section of a 6 mm. embryo through the posterior portion of the anterior lymph heart. From the ventro-lateral aspect of the heart (*l.h.*) a vessel (*l.*), the foremost anlage of the lymph duct, is given off which at first turns downward between pronephric duct (*w.*) and epidermis (*ep.*) and then backward. After continuing in this direction for eight sections, it ends blindly, the termination being shown in figure 28 (*l.*). Between this and the anterior limit of the next succeeding anlage there is an interval of twenty-five sections in which lymphatic rudiments or anything resembling them are absent, at least are not in evidence. The territory between pronephric duct, lateral postcardinal division and epidermis is in possession of apparently only mesenchymal cells as pictured in the sketched section, figure 29, which typifies the character of this region. After such an interval the blunt tip of a discrete endothelial-lined space suddenly springs into view (*l.*, fig. 30). The next section (fig. 31) reveals plainly other salient features of this rudiment (*l.*). It composes a well-defined and closed cavity, the confines of which are strong and firm, and like those of the vein (*p.l.*) contain many yolk globules; in brief, there is no feature, except perhaps its large rotund nuclei, which could cause it to be confused with the mesenchyme. But the nuclei do not offer a serious hazard in the matter, for we have seen that the nuclei of the initial lymph

sinus anlagen, as well, resemble mesenchymal nuclei indistinguishably. What lends interest to the case is that the anlage is exceedingly short, being limited to six sections, though its caliber is proportionately broad. It ends as suddenly as it begins (compare figs. 32 and 33 with 30). In its abrupt course it lies adjacent to the vein (*p.l.*), and at its posterior end (*l.*, figs. 32 and 33) the two vessels are in contact but their lumina do not communicate. Proceeding distally from this level, nothing is encountered which might suggest a lymphatic anlage until twenty-eight sections have been passed over, where another similar endothelial-lined space exists in a similar position.

Figures 34 and 35 illustrate a rudiment of the inferior lateral lymph duct on the right side of another 6 mm. embryo. More than fifty sections intervene between the anterior lymph heart and the blind anlage (*l.*) pictured here. In all notable qualities it is like the anlage described and figured last, except that it is longer, extending through eighteen sections, and is larger in circumference (fig. 34). Near its posterior limit it becomes much compressed and flattened out against the venous intima to which it is apparently firmly adherent (*l.*, fig. 35). The terminal portion, however, is not attached to the vein (*p.l.*) but lies slightly removed in the mesenchyme.

The discontinuous anlagen described above are not isolated cases; several such anlagen may be found in all 6 and 7 mm. toad larvae, which are the critical genetic stages of the lateral lymph ducts. In older 7 mm. larvae these discrete lymph vessel rudiments have elongated and by further increment and by coalescence are creating continuous channels. Yet it is exceedingly interesting to note that the tips or ends of consecutive anlagen do not always strike each other squarely; the writer has frequently observed the posterior tip of one and the anterior of another a considerable distance apart in the same section. In other words, the anlagen in their growth and elongation had shoved past one another without immediately meeting. Eventually they become confluent by the gemmation of lateral sprouts or the dilatation of their lumina. This doubtlessly explains the irregularity and sinuosity of their course at an early period after

the establishment of continuity. The writer would show figures portraying these phases, but the number of illustrations already far exceeds his intentions; moreover, such conditions are concerned more especially with their later development and will receive consideration in the subsequent contribution. At that time the profuse plexus or network will also be considered which in 8 and 9 mm. embryos is developed between the two lateral lymph ducts and from which ultimately the definitive lateral subcutaneous lymph vessels of the trunk are, so to speak, crystallized.

The history of science, in fact the history of lymphatic research alone, has so often shown the fallacy of theorizing from insufficient or problematical data and premises, that the writer feels little inclination to base upon a simple finding an hypothesis that shall attempt to harmonize or properly valuate the work of other investigators at variance with his own. Only after observation has been corroborated repeatedly or from several viewpoints and the demonstration of fact is final can a law be formulated which is sound, comprehensive and stripped of all opinions and prejudices. Suggestions thrown out, however, to give direction to inquiry are ever seasonable and little hesitation is felt in expressing poignant ones. The purpose, then, of the theoretical considerations in the following paragraphs is neither to defend nor to refute any one view of lymphatic development; nor is it the aim to effect a compromise between conflicting views or to promulgate a new one. Consistent with the conditions in Bufo, such considerations are offered as plausible possibilities, the truth or error of which subsequent researches on other vertebrate embryos will determine.

If the interpretation of the structures described in this treatise is the only possible one as the author believes it to be; if the lymphatic system of Amphibia is homologous to that of other vertebrate animals as we expect it to be; and if the morphological dogma; like structure, like origin, is infallible, as all biologists tacitly assume it to be, then the view of the *direct* mesenchymal origin of lymphatics seems to be untenable But not only is this theory affected; the opposing one also must certainly be radically

modified. If we are to have agreement in our accepted belief of the mode of lymphatic genesis, the two chief antagonistic schools of investigators are compelled to re-examine their material with a new unbiased attitude, both those who maintain that lymph ducts are formed *in situ* from mesenchyme and discontinuously, and those who contend for the view of the venous origin of such vessels, of their continuity in development and of their centrifugal growth and spreading from a few definite foci in the body. The idea that strikes the writer primarily is that most workers have been deceived as to the time when lymphatic anlagen first make their appearance; it is conceivable how structures which have been described as the incipient anlagen of a lymph duct may already represent a much later phase. The characteristics which the lymphatic rudiments, discussed in this paper, manifest, such as solidity or imperfect vacuolation and discontinuity, though venous in origin, irrefutably show that the injection method would have been utterly incapable of revealing them at this time. Might not the same contention apply to the study of lymphatics in other vertebrate embryos, and might not anlagen exist long before injections could attest their existence. Is most of the other work on lymphatic development exempt from similar criticism? In their suppositions investigators have been more or less led astray by appearances, and in no other field of inquiry perhaps do appearances intimate so little of the truth. In a previous paper on the development of the thoracic duct in the pig embryo, the writer has described and figured very definite spaces which lie in the vicinity of the cardinal veins or their tributaries and in the path of the future definitive duct, and which he believed to be lined by cells mesenchymal in derivation. This conclusion was reached because the intimal cells of the thoracic duct anlagen at their inception visibly resembled the embryonic connective tissue elements very closely in certain seemingly important qualities. In finding that the character which marks incipient endothelium from mesenchyme particularly in the head region of young toad larvae is its abundance of yolk while other cell attributes appear identical, the present inquiry has convinced him of other potent possibilities.

Are the discontinuous thoracic duct rudiments, like those of the ventral cephalic sinus in Amphibia, derived from endothelial proliferations which have severed their connections with the parent veins and have acquired lumina, subsequently to meet and become confluent with one another to create a continuous channel? Is the relation of these isolated cavities to some of the confused group of Mayer-Lewis anlagen a much more intimate one than has been supposed by most investigators?

Recently the discussion has centered largely around an injected and sectioned pig embryo, series no. 23a, of the Johns Hopkins University Embryological Collection. Professor Sabin originally held[7] that the developing thoracic duct in this specimen was completely filled with the injecta. Some time later the writer was given the privilege of examining and describing this particular embryo. He pointed out[8] the existence of a long, blind, dilated space which follows closely upon the injected portion of the duct and is sharply demarcated from the neighboring veins as well as from the indefinite connective tissue interstices, a contrast admirably brought out in the photographs published at that time. Sabin now argues[9] that the injection in this case was not a perfect one. She accepts the space as a part of the thoracic duct anlage, but assumes, firstly, that it is united with the injected vessel by a very frail connection, which, if at all possible, must be extremely difficult to distinguish from the surrounding tissue reticulum, and secondly, that the pressure of the injection was entirely inadequate to force the fluid through the narrow passage. Leaving aside a consideration of the contradictory evidence which has been explicitly expressed in previous papers,[10] the writer would ask Professor Sabin, whether her explanation of discontinuities in an inchoate lymphatic duct is the most likely one, in view of the observed conditions in Amphibia. The view of the

[7] In a report before the American Association of Anatomists, Ithaca, 1910.

[8] Kampmeier; Anat. Rec., vol. 6, no. 5, June, 1912.

[9] Sabin; Anat. Rec., vol. 6, no. 7, August, 1912, and Johns Hopkins Hospital Reports, new series, no. 5.

[10] Kampmeier; Am. Jour. Anat., vol. 13, no. 4, September, 1912, and Anat Rec., vol. 6, no, 5. 1912.

origin of lymphatic channels from a few very definite centers in the organism has been too dogmatically asserted, and those investigators who have strongly adhered to it now find it difficult to consider exceptions, which, there is a probability, may prove the rule. In an analysis of the researches on the genesis of the lympathics we do not find a single really valid objection, based on correct interpretation of observations, to discredit the view of the origin of a lymph duct from a number of points, that is, from mutliple anlagen, which in toad embryos are proliferated from the intima of the vein which the definitive duct accompanies. The present findings, in Bufo, then, will permit of a partial acceptance of both the "centrifugal growth theory" and the "discontinuous, *in situ* mesenchymal origin" of lymph ducts. That the lymphatic endothelium, here considered, arises from venous endothelium has been shown beyond the shadow of a doubt, and the writer consistently, though tentatively, abandons the hypothesis of its derivation in other vertebrates from mesenchymal cells, unless their lympathic system is shown to behave differently, which is scarcely conceivable. But the other tenets of the 'outgrowth theory,' such as continuity and centralization in lymphatic development, he can not accept, for the present observations reinforce strongly the diametrically opposite doctrines of discontinuity and multiple origin. The ventral cephalic lymph sinus is a product of the walls of the external jugular veins; its anlagen arise not at one point but individually at intervals along the entire extent of the cranial division of these veins. Even after the lymph sinus has become a single chamber by the coalescence of its rudiments, it remains as a blind reservoir until a relatively late stage when it joins the lymph hearts. The lateral lymphatic ducts of the trunk, too, do not spring from one center but are formed and acquire continuity by the fusion of a number of anlagen, which originate along the postcardinals and their dorsal tributaries, the intersegmental veins. To satisfy the 'outgrowth theory,' the lymphatic vessels mentioned, the sinus and at least the anterior half of the lateral ducts, should develop as continuous growths from the anterior lymph hearts,

which correspond, as the writer will show in the later paper, to the two anterior centers, the jugular lymph sacs, in Mammalia.

Finally, a certain peculiarity in the development of lymphatic channels in Bufo has suggested a possible homology between the incipient anlagen here observed and peri-venous lymphatic spaces first discovered and described by Huntington and McClure in cat embryos.[11] Opponents of this view of lymphatic formation maintain that such spaces are artificial, due to shrinkage, though adequate proof to uphold this contention is not forthcoming. As has been shown in the figures, there occur in certain stages of the toad larvae hollow lymphatic anlagen which hug the vein closely and consequently resemble extra-intimal spaces, but which have been shown to be really intra-intimal in nature since their lumen arises as a vacuolation of the endothelial proliferation. Might not a similar condition be found to prevail in mammalian embryos were the genesis of extra-intimal spaces followed back far enough?

In the writer's judgment, it is only by carrying out extensive and minute cytological studies in all classes of vertebrate embryos, to determine specific cell character and behavior of both mesenchyme and endothelium during the very early genetic stages, that we can arrive most surely and quickly at a uniform and comprehensive conception of lymphatic development and can measure the amount of truth and the number of fallacies inherent in the several respective theories hitherto advanced. This may prove to be an arduous task inasmuch as a natural and peculiar diagnostic trait, like that of yolk content, to distinguish incipient lymphatic intima from mesenchyme, probably does not exist in embryos other than Amphibian, but by the invention and application of different staining methods the results would perhaps be quite as surprising.

[11] Huntington and McClure; Am. Jour. Anat., vol. 6, 1907, Anat. Rec., no. 3.

APPENDIX

At the time when the foregoing observations had already been made, a reprint of a preliminary report by S. Fedorowicz on the development of the lymph vessels in the larvae of Anura fell into the writer's hands. (Untersuchungen über die Entwicklung der Lymphgefässe bei Anurenlarven. Vorläufige Mitteilung. Extrait du Bulletin de l'Academie des Sciences de Cracovie, June, 1913. S. Fedorowicz). Although his observations pertain to the development of the posterior lymph hearts in Bufo, he expresses a view essentially similar to that advanced in this treatise. The fact that two investigators have arrived at the same conclusions independently of each other gives additional weight to their work. Since Fedorowicz has the priority, the writer gladly submits this contribution in corroboration of his work.

PLATES

All of the sections from which the following illustrations were made are stained with hematoxylin and orange G and are six microns in thickness. Figures 2 to 9, 11, and 16 to 26 inclusive are camera lucida sketches drawn with a 2 mm. oil immersion objective and a no. 8 ocular. Figures 1, 10, 12 to 15, and 27 to 35 were produced with the aid of the Edinger Projection Apparatus. All of the figures were drawn as nearly as was possible at one plane of focus or optical section.

PLATE 1

EXPLANATION OF FIGURE

1 Transverse section of a 11 mm. toad embryo through the head at the level of the eye. Series 35, slide 1, row 9, section 5. × 150. Reduced to × 75.

REFERENCES

l., ventral cephalic lymph sinus
l.l., *l.l.*, lateral extensions or wings of the sinus which are joined to it further forward
j.d., *j.s.*, right and left external jugular veins
m.c., mouth cavity

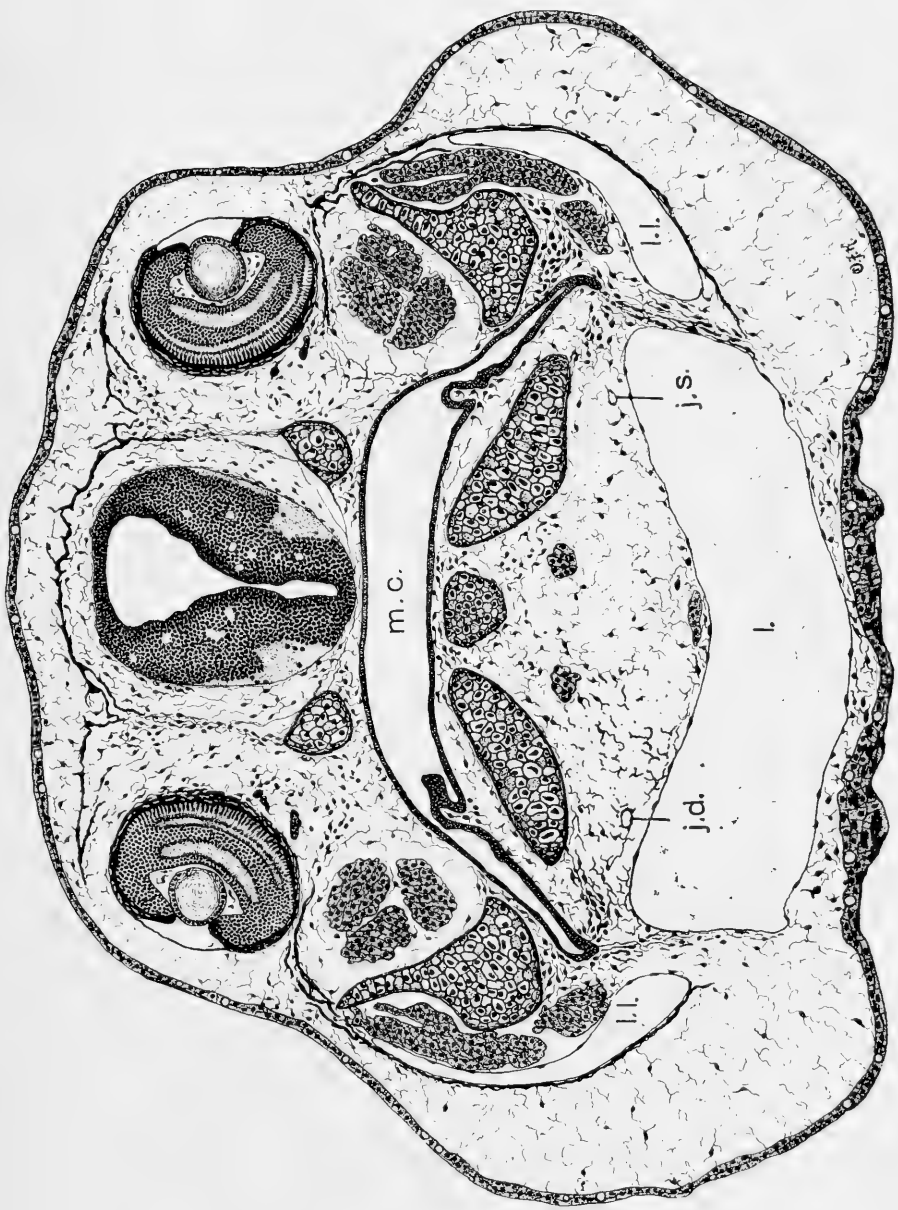

l.l.
j.s.
m.c.
l.
j.d.
l.l.

PLATE 2

EXPLANATION OF FIGURES

2 to 6 Five transverse sections of a 5 mm. toad embryo through a small portion of the ventral region of the head to show the initial anlagen of the lymph sinus. Series 25. × 1100.

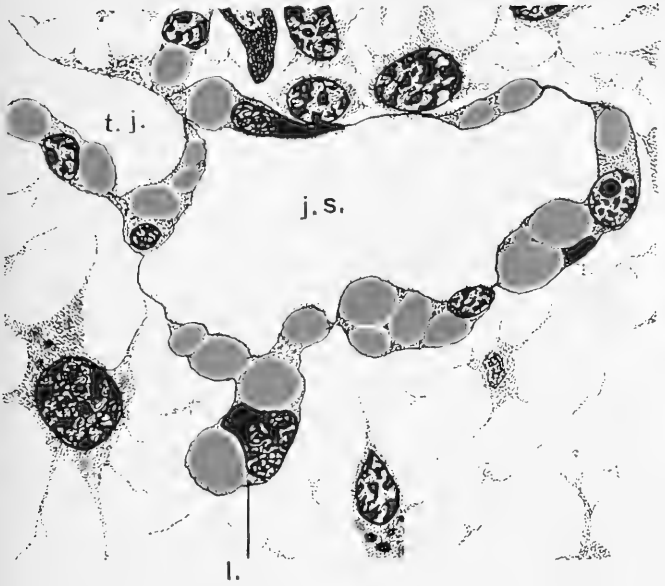
REFERENCES

l., *l.*, sinus anlagen
j.d., *j.s.*, right and left external jugular veins
t.j., jugular tributary (see footnote 5, page 168)
b.c., blood corpuscle

2 and 3 Successive sections from the left side. Slide 1, row 4, sections 19 and 20.

4 From the same side, three sections caudad. Slide 1, row 4, section 23. Compare the lymphatic rudiment (1) with the mesenchyme in regard to yolk, and note its solidity.

5 and 6 Two alternate sections from the right side. Slide 1, row 4, sections 18 and 20. Observe the cavity of the lymphatic (1) in the second sketch.



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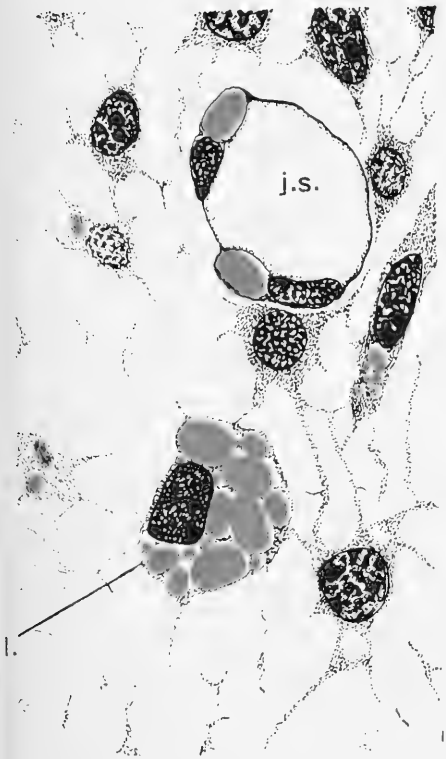

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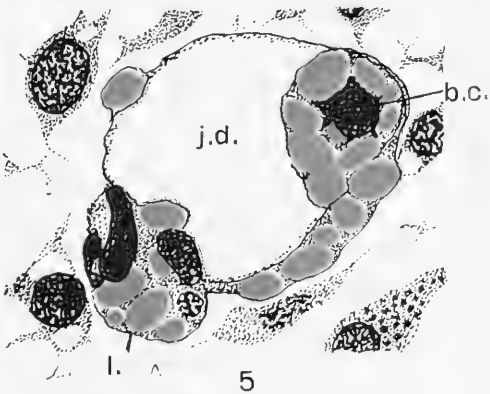

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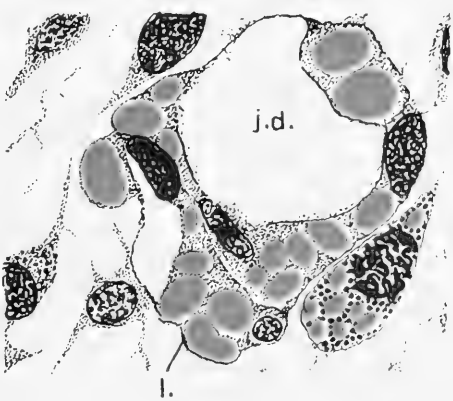

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PLATE 3

EXPLANATION OF FIGURES

7 Transverse section of a 6 mm. toad embryo through the sinistro-ventral region of the head. Series 54, slide 1, row 4, section 8. × 1100.

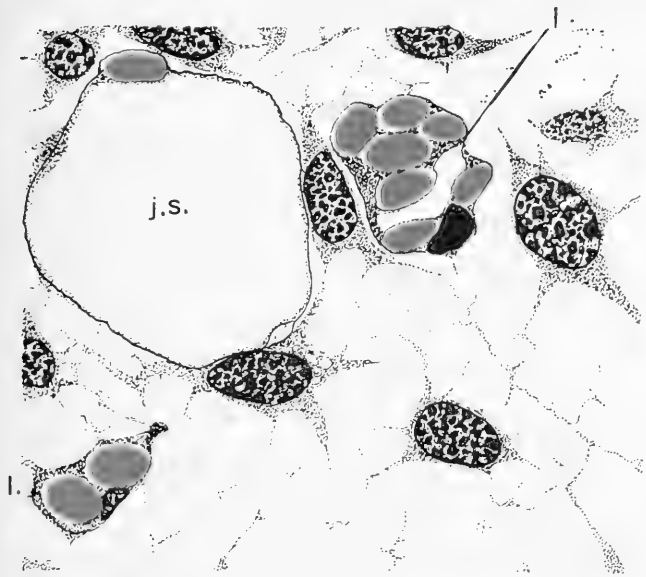
REFERENCES

l., sinus anlagen
j.s., left external jugular vein

8 and 9 Transverse sections of another 6 mm. toad embryo through the dextro-ventral region of the head. Series 53, slide 1, row 5, section 13, and row 6, section 4. × 1100. Eight sections intervene between these two levels.

REFERENCES

*l.*1, *l.*2, *l.*3, sinus anlagen
j.d., right external jugular vein
t.j., wall of a tributary cut tangentially



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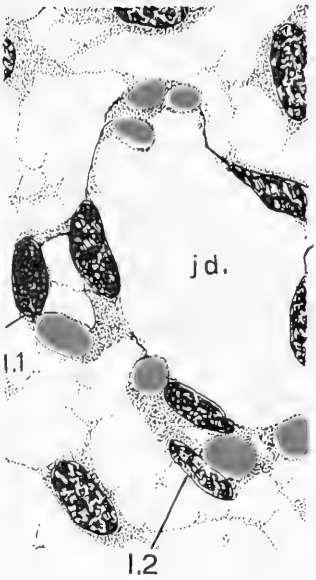

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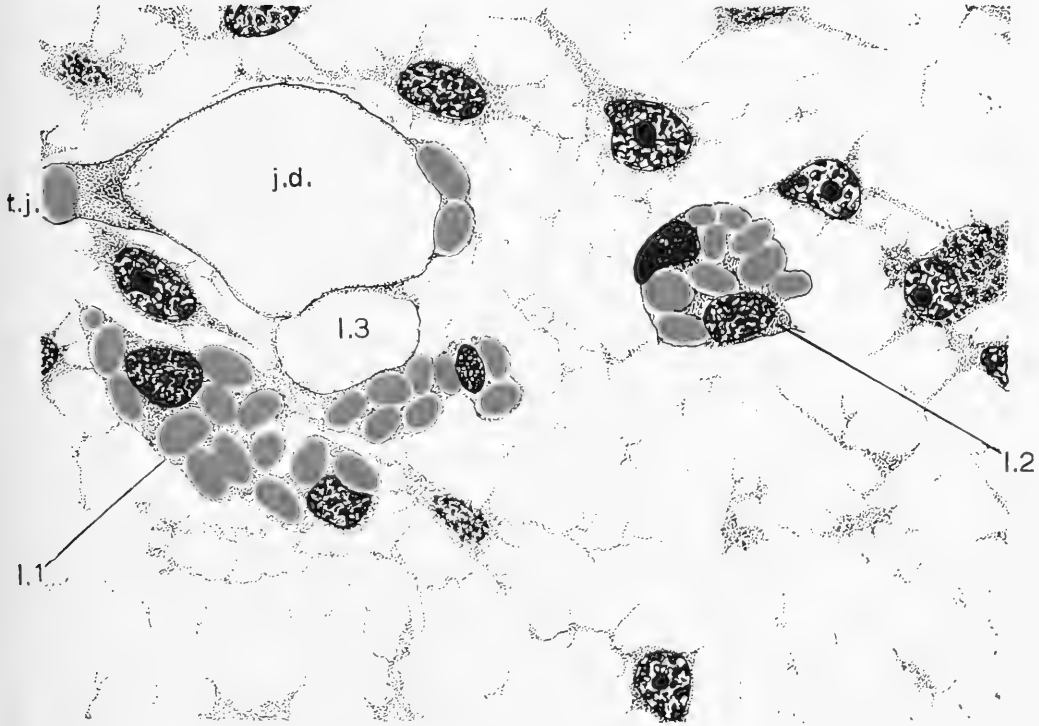

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PLATE 4

EXPLANATION OF FIGURES

10 Transverse section of a 7 mm. toad embryo through the ventral region of the head. Series 52, slide 1, row 4, section 5. × 300.

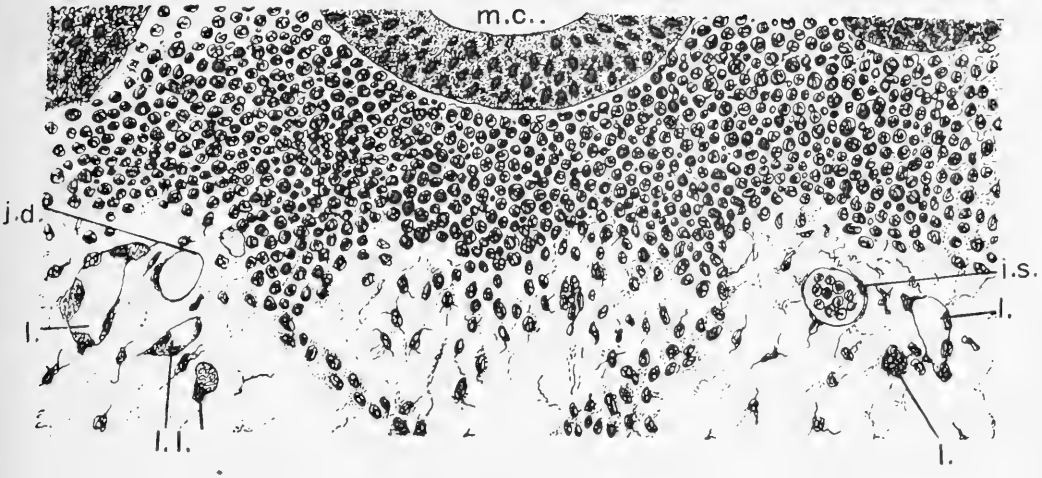
11 A small area near the lower left corner of the same section (fig. 10). × 1100.

REFERENCES

j.d., *j.s.*, right and left external jugular veins

l., *l.*, sinus anlagen

m.c., mouth cavity



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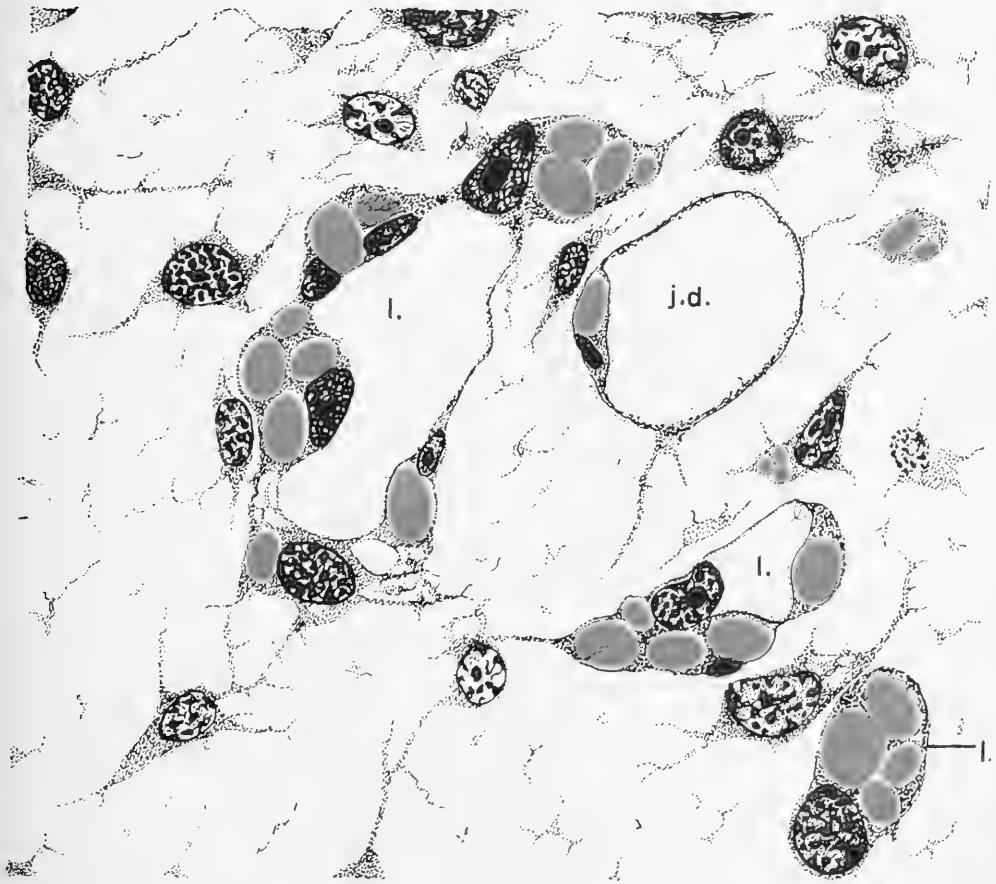


11

PLATE 5

EXPLANATION OF FIGURES

12 Transverse section of an 8 mm. toad embryo through a little more than the right half of the territory ventral to the mouth cavity (cf. fig. 1). Series 27, slide 1, row 5, section 8. × 500.

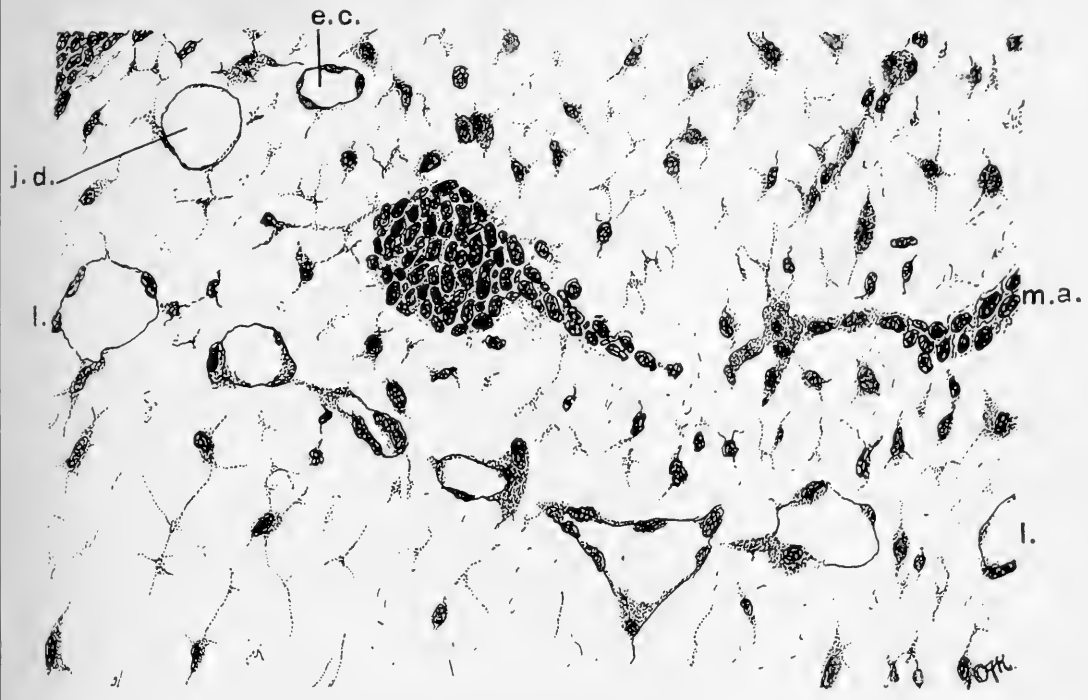
REFERENCES

l. to *l.*, plexiform anlage of sinus
j.d., right external jugular vein
e.c., right external carotid artery
m.a., muscle anlagen

13 Transverse section of a 9 mm. toad embryo through the ventral region of the head. Series 2, slide 1, row 8, section 5. × 300.

REFERENCES

l., multilocular anlage of sinus
t., *s.*, tissue strands and trabeculae, remnants of walls between originally independent channels
j.d., *j.s.*, right and left external jugular veins
m.c., mouth cavity



12

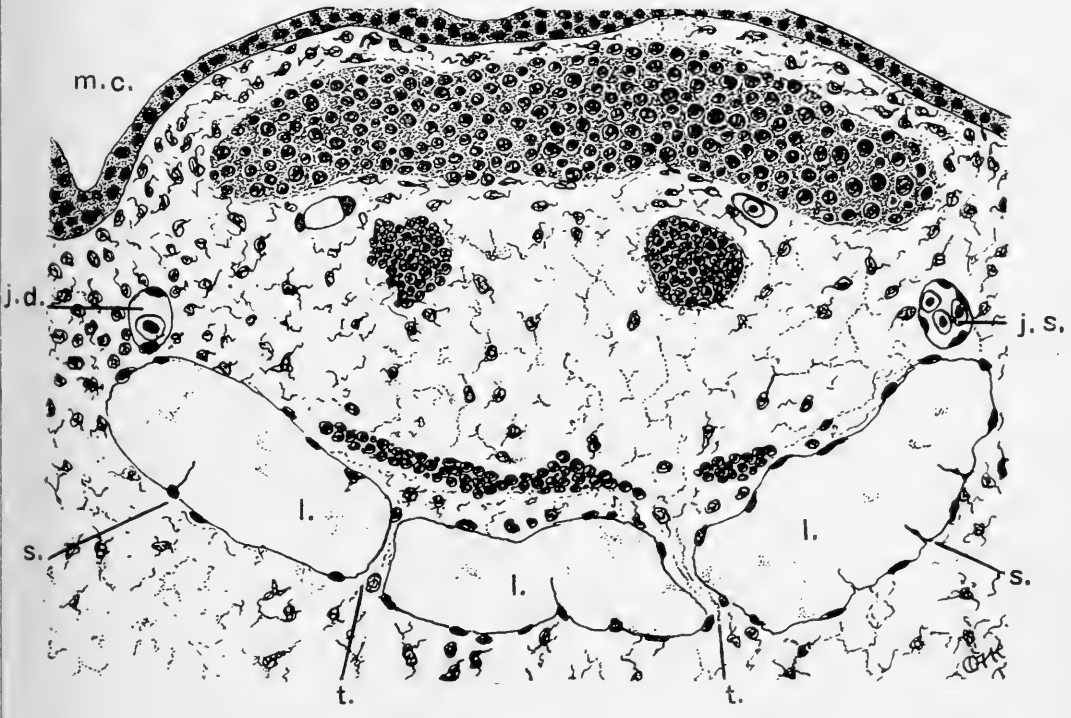


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PLATE 6

EXPLANATION OF FIGURE

14 A small portion of the section shown in figure 13. × 1100.

REFERENCES

l., lumen of sinus
t., s., tissue spurs and trabeculae
d., cellular débris, probably vestiges of former partitions
m., mesenchymal cell

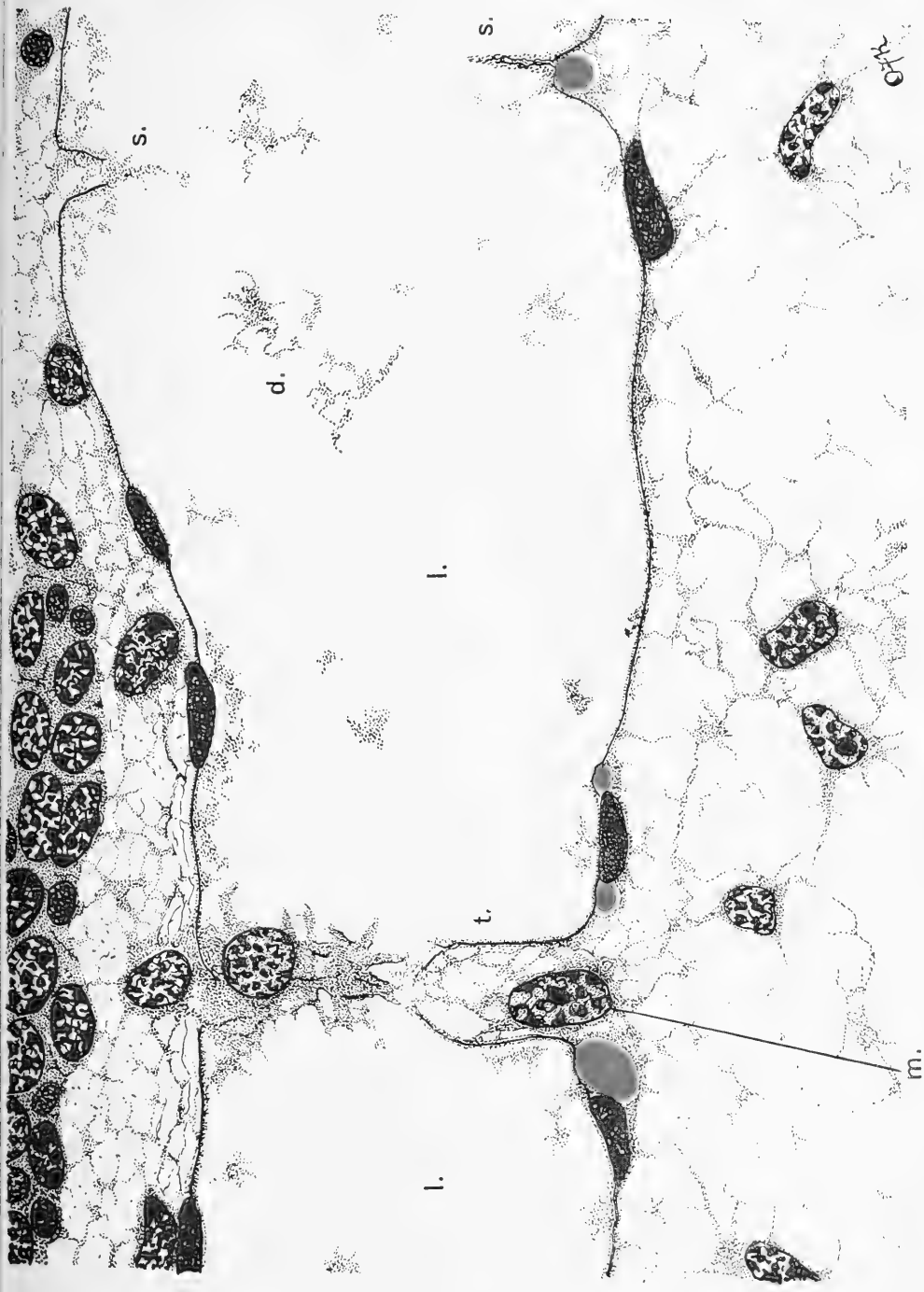

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PLATE 7

EXPLANATION OF FIGURES

15 Transverse section of a 9 mm. toad embryo through the middle of the trunk. Series 2, slide 3, row 7, section 2. × 150.

REFERENCES

l.s., *l.i.*, superior and inferior lateral lymphatic ducts
i.v., intersegmental vein
p.m., *p.l.*, medial and lateral divisions of the postcardinal vein
w., Wolffian or pronephric duct
m.s., muscle segment

16 A small area of a section of a 7 mm. toad embryo through the left mid trunk region. Series 52, slide 3, row 4, section 16. × 1100.

REFERENCES

l., anterior tip of a blind anlage of the developing superior lateral lymph duct
i.v., intersegmental vein
ep., epidermis
m.s., myotome

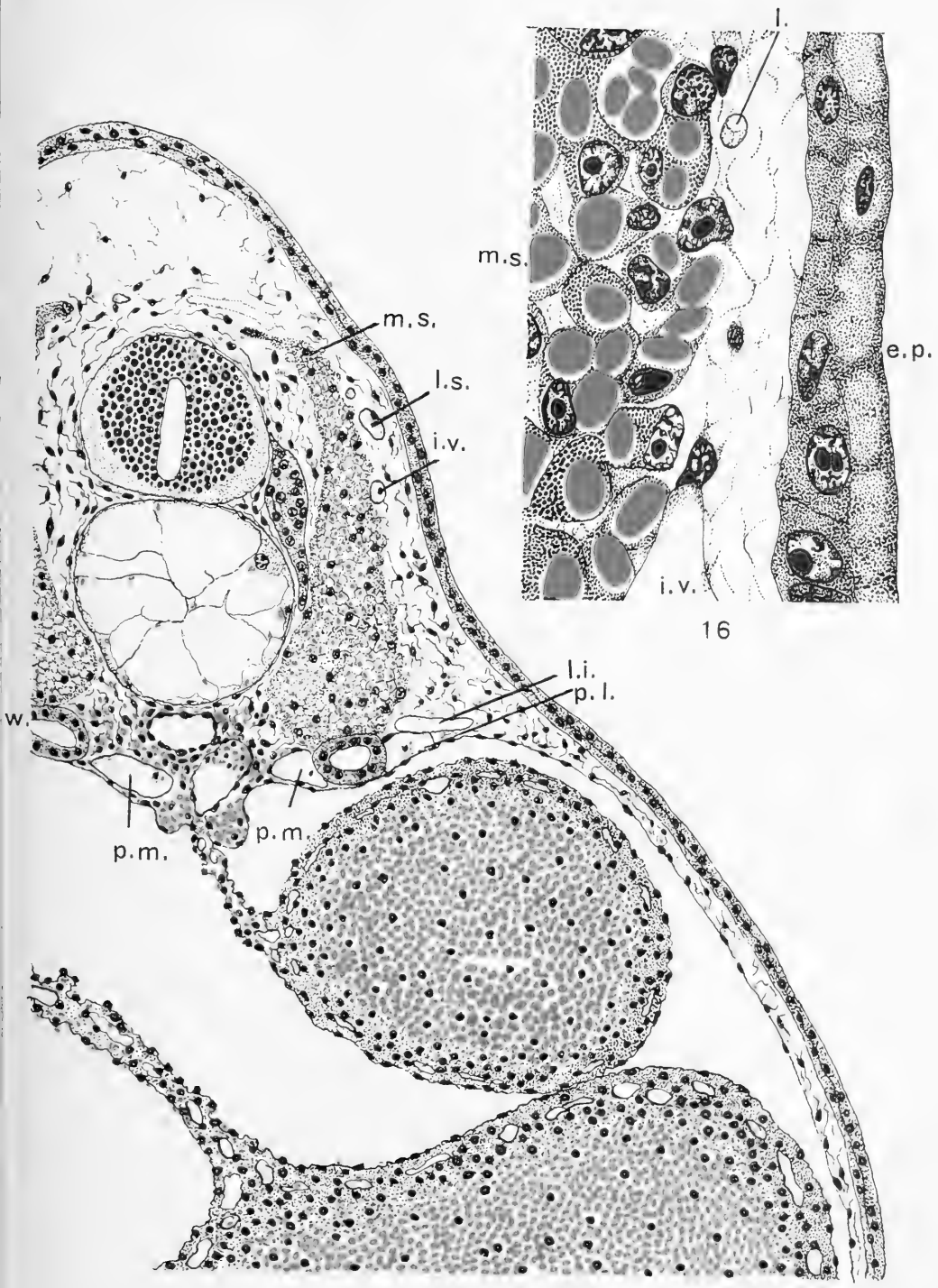

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PLATE 8

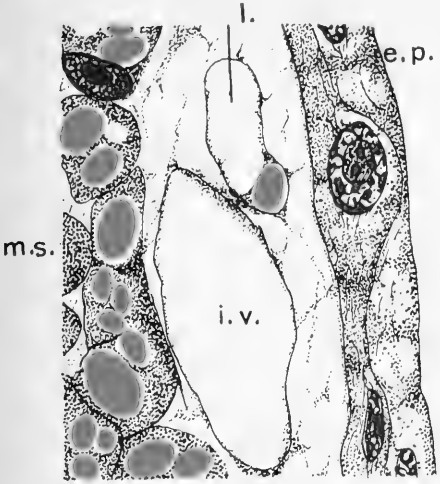
EXPLANATION OF FIGURES

17, 18, 19, 20, 21 and 22 Transverse sections to show different levels of the lymphatic anlage the tip of which is pictured in figure 16. Series 52, slide 3, row 4, section 24; row 5, sections 2, 6, 9, 11; row 6, section 1. × 1100.

REFERENCES

l., anlage of the superior lateral lymph duct

i.v., intersegmental vein
ep., epidermis
m.s., myotome



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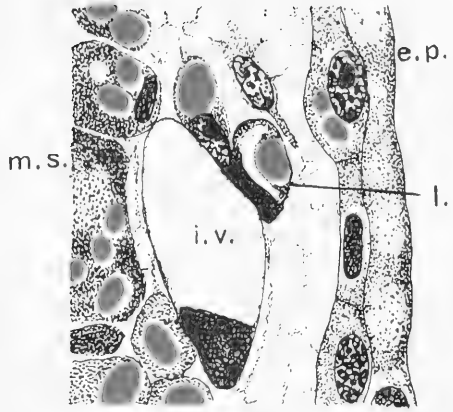

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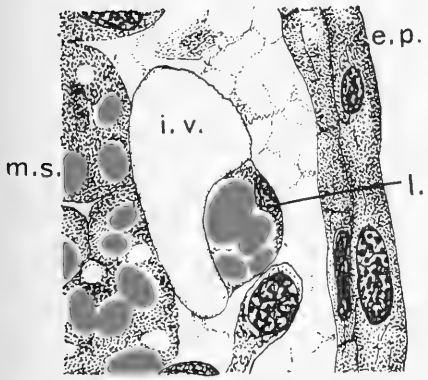

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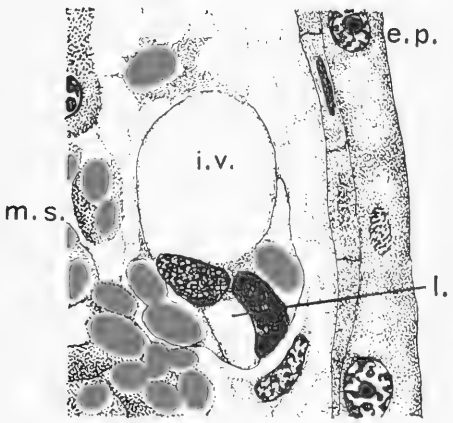

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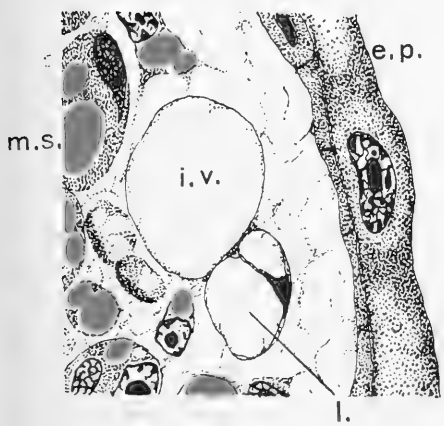

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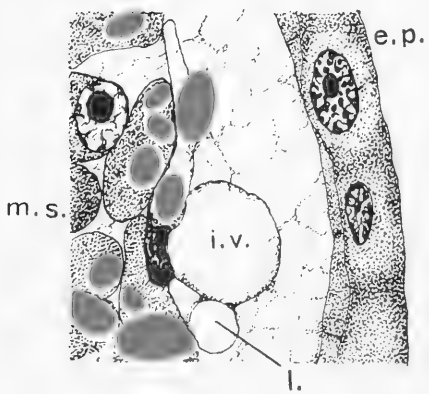

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PLATE 9

EXPLANATION OF FIGURES

23, 24 and 25 Transverse sections to show three additional levels of the lymphatic rudiment illustrated in the sketches on plate 8. Series 52, slide 3, row 6, sections 4, 11, 13. × 1100.

26 Transverse section of a 6 mm. toad embryo through approximately the same region (cf. figs. 17–25) to show an earlier condition, evidently, of the superior lateral lymph duct. Series 53, slide 4, row 5, section 17. × 1100.

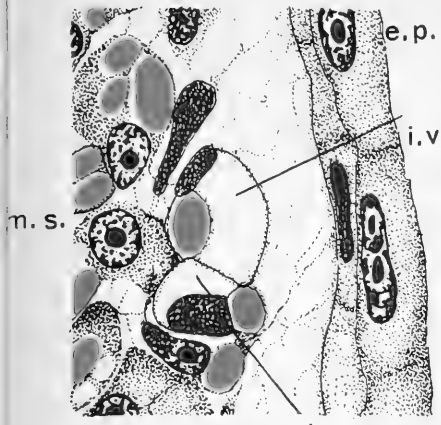
REFERENCES

l., anlage of superior lateral lymph duct
i.v., intersegmental vein
ep., epidermis
m.s., muscle segment

27 Transverse section of a 6 mm. toad embryo at the level of the left anterior lymph heart. Series 53, slide 3, row 3, section 13. × 1100.

REFERENCES

l.h., posterior portion of the anterior lymph heart
l., anterior extent of the developing inferior lateral lymph duct
i.v., intersegmental vein
p.m., *p.l.*, medial and lateral divisions of the postcardinal
b.c., blood corpuscles
c.c., coelomic cavity
ep., epidermis
m.s., muscle segment



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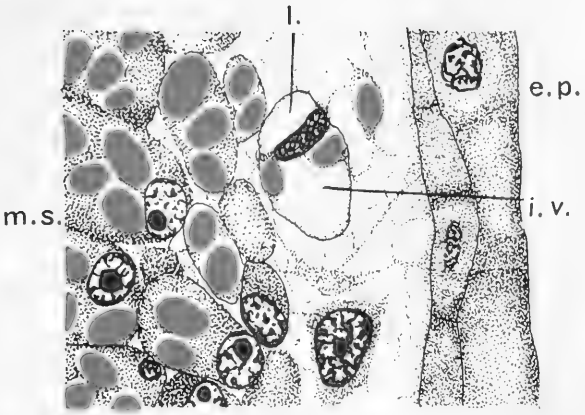

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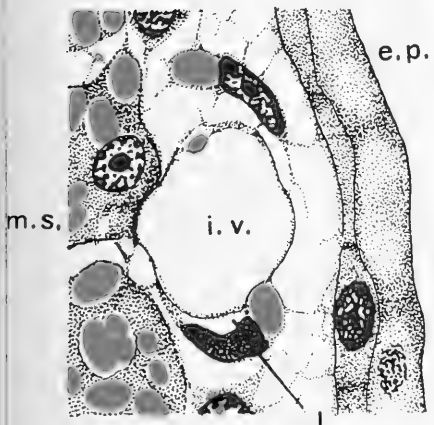

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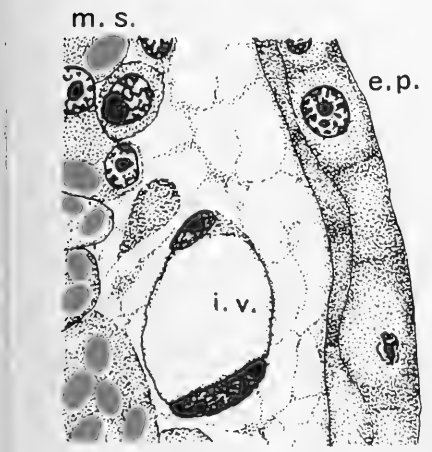

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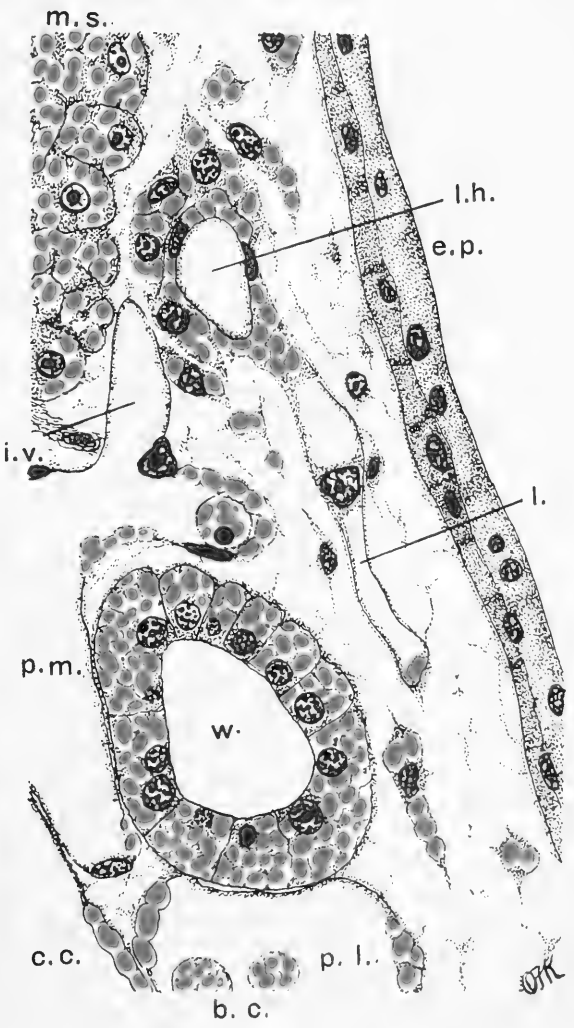


27

PLATE 10

EXPLANATION OF FIGURES

28 Transverse section a short distance behind the left lymph heart indicated in figure 27. Series 53, slide 3, row 4, section 3. × 1100.

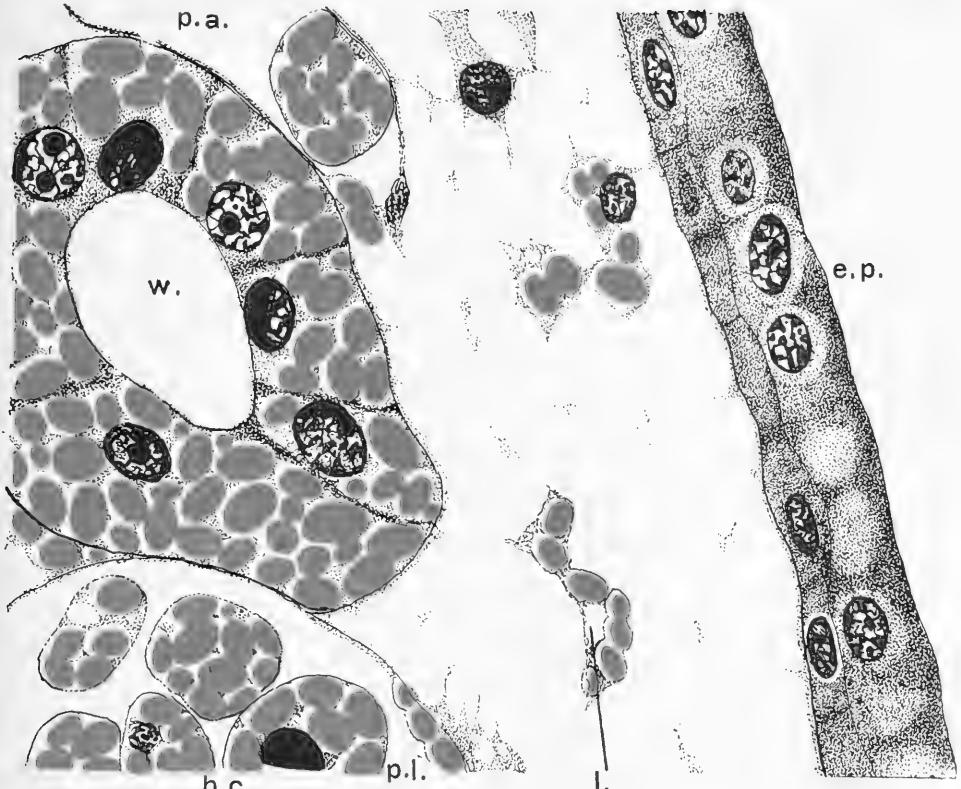
REFERENCES

l., extreme posterior tip of the anterior anlage of the inferior lateral lymph duct, shown in figure 27 as a ventral sprout of the lymph heart
p.l., lateral division of the postcardinal
p.a., dorsal anastomosis connecting the two postcardinal divisions
b.c., blood corpuscles
ep., epidermis
w., pronephric duct

REFERENCES

29 Eighteen sections caudad of the level represented by figure 28. × 1100. Series 53, slide 3, row 5, section 4. × 1100.

m., mesenchymal cells; no indication of a lymphatic anlage
p.a., ventral postcardinal anastomosis; other explanations the same as above



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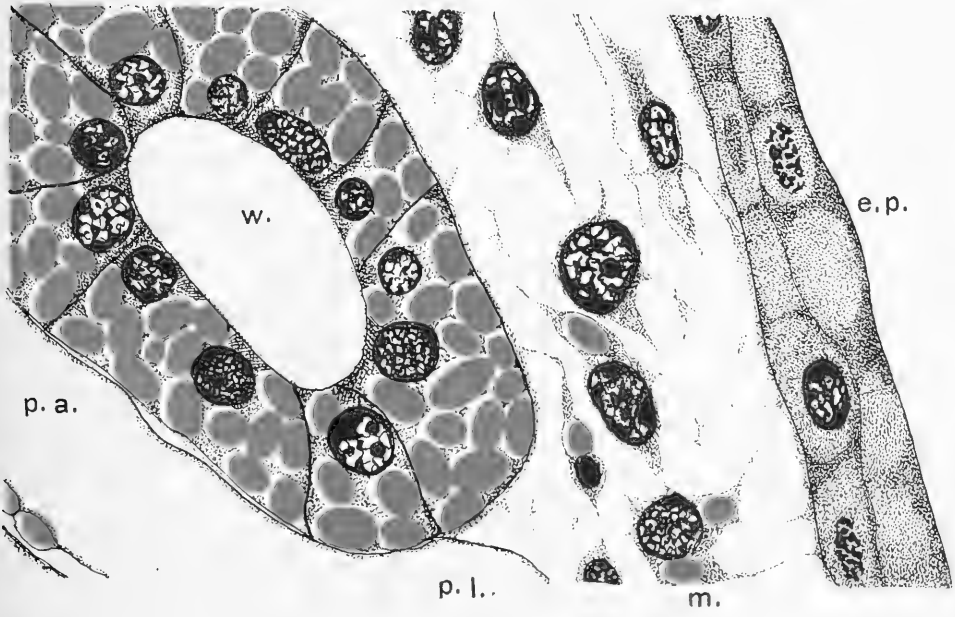

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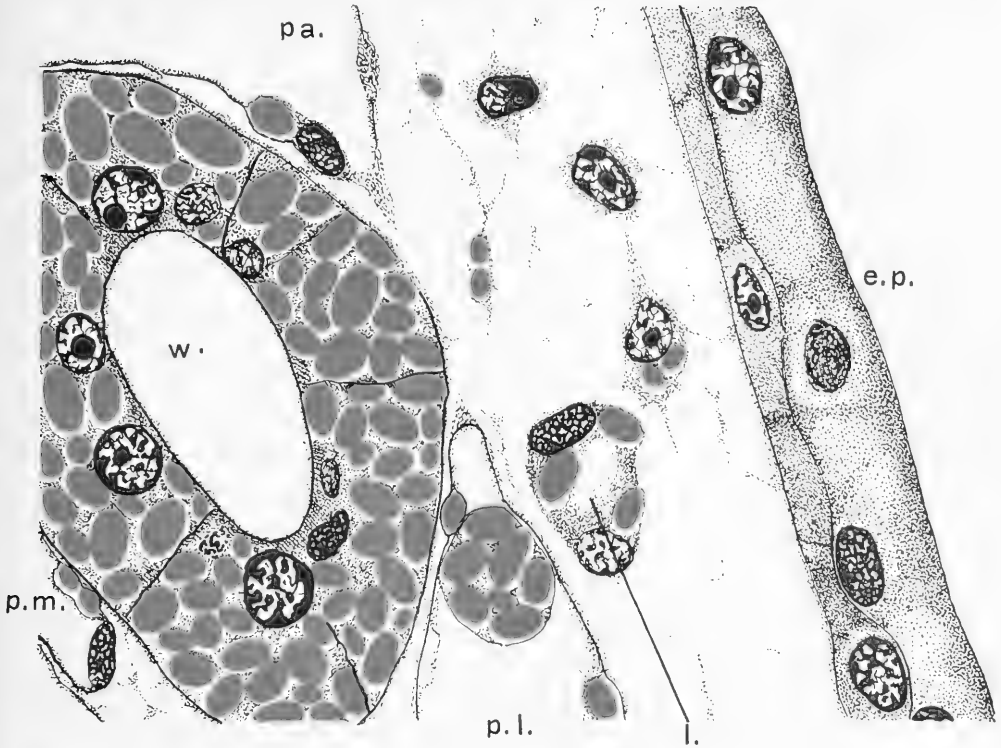
PLATE 11

EXPLANATION OF FIGURES

30 and 31 Successive sections after an interval of nine sections behind that pictured in figure 29. Series 53, slide 3, row 5, section 12. × 1100.

REFERENCES

l., discontinuous anlage of the inferior lateral lymph duct; figure 30 shows the anterior tip
p.m., *p.l.*, medial and lateral postcardinal divisions
p.a., dorsal anastomosis between postcardinal divisions
w., pronephric duct
ep., epidermis



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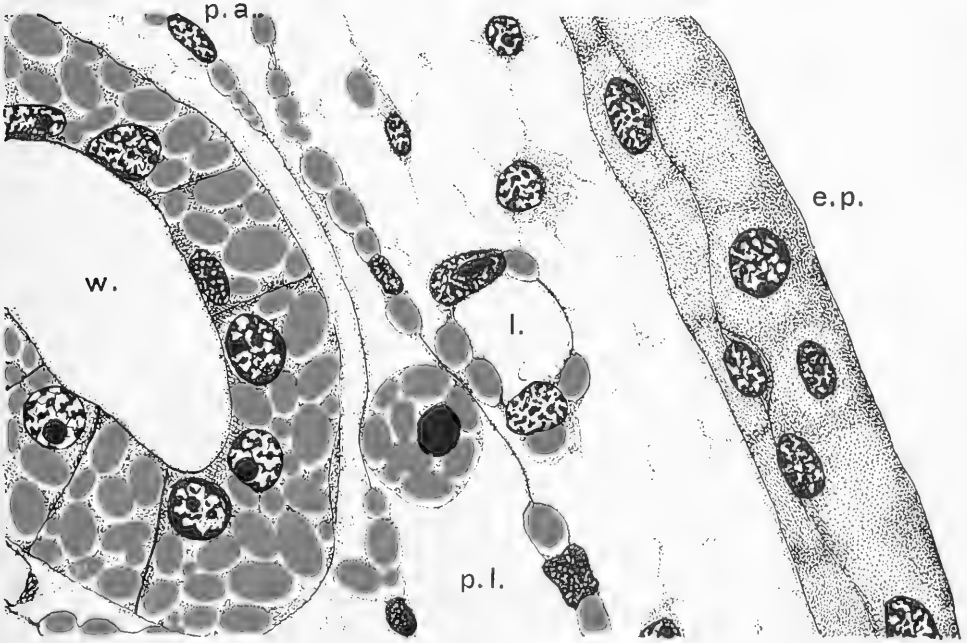

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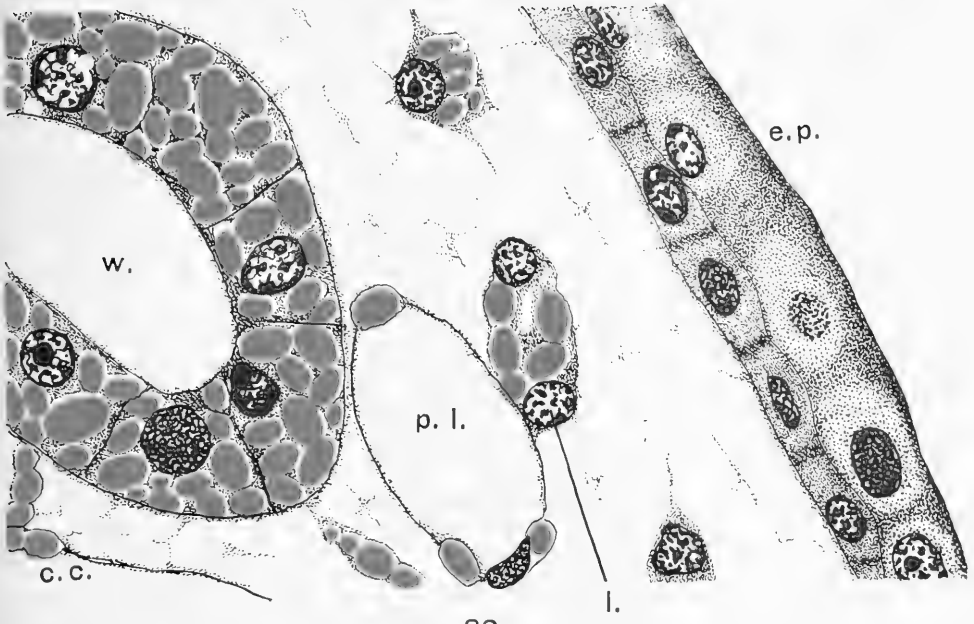
PLATE 12

EXPLANATION OF FIGURES

32 and 33 Consecutive sections after an interval of four sections posterior to those represented on plate 12. Series 53, slide 3, row 5, section 16; row 6, section 1. × 1100.

REFERENCES

l., discontinuous anlage of the inferior lateral lymph duct. Figure 33 shows the extreme posterior tip
p.l., lateral postcardinal division
c.c., coelomic cavity
w., pronephric duct
ep., epidermis
p.a., dorsal anastomosis between postcardinal divisions



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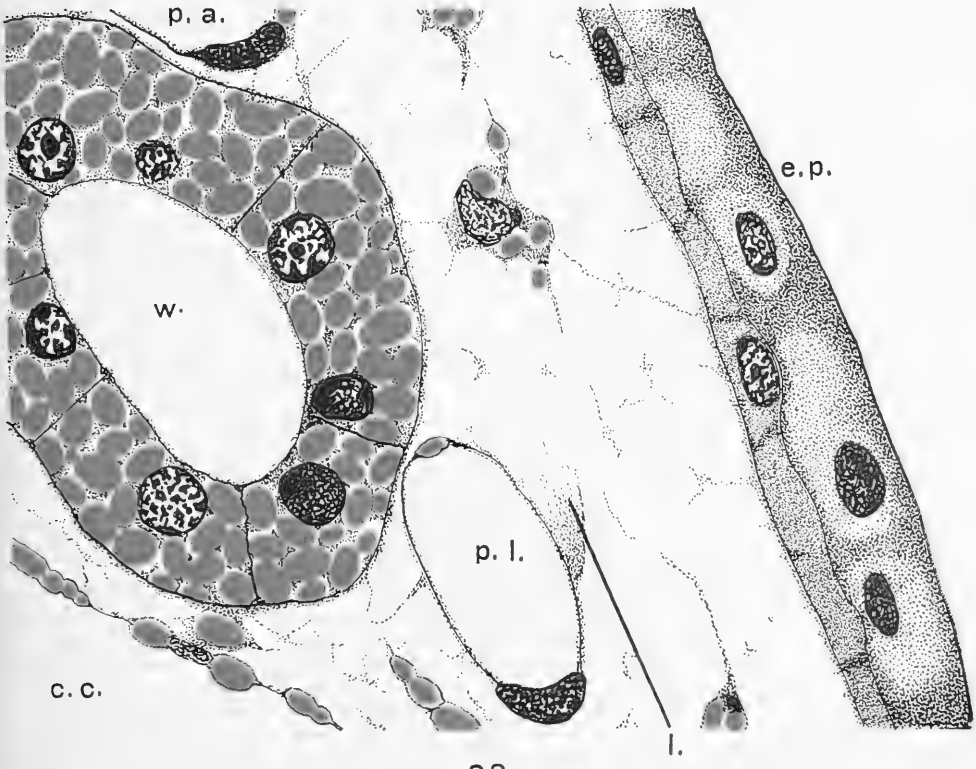

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PLATE 13

EXPLANATION OF FIGURES

34 and 35 Transverse sections of another 6 mm. toad embryo through the right side of the mid trunk region. Series 54, slide 3, row 3, sections 4 and 10. × 1100.

REFERENCES

l., discontinuous anlage of the right inferior lateral lymph duct. The last figure shows the anlage clinging to the venous wall

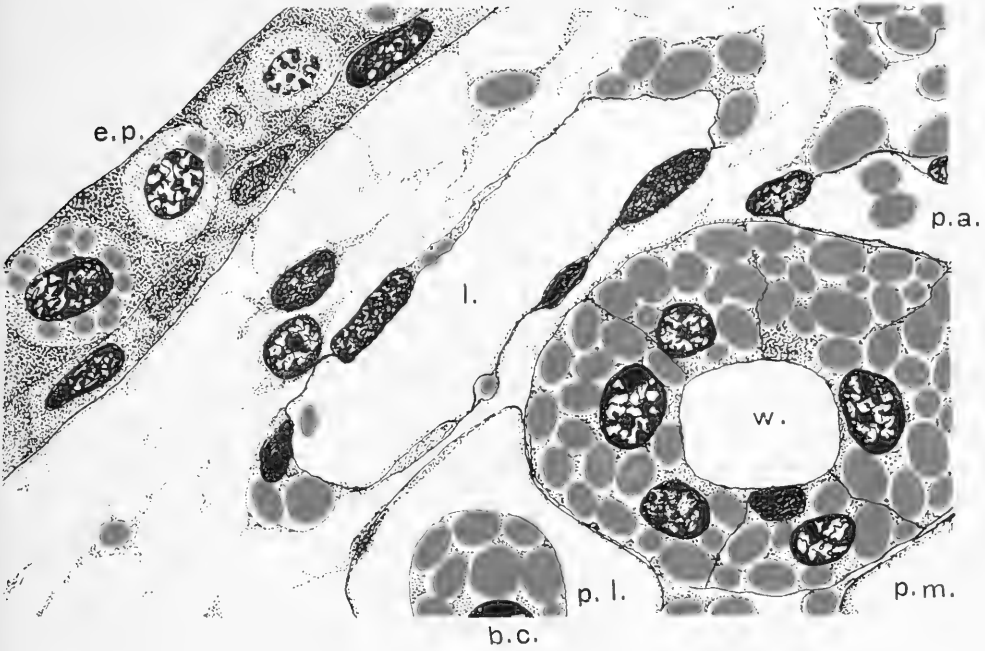
p.m., *p.l.*, medial and lateral postcardinal divisions

p.a., dorsal postcardinal anastomosis

b.c., blood cells

ep., epidermis

w., pronephric duct



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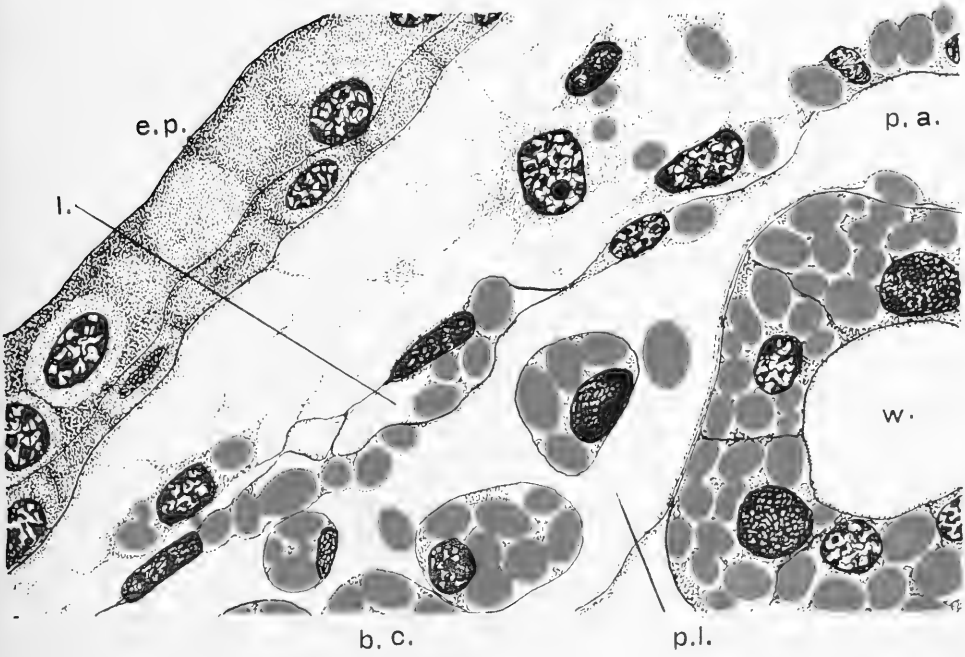


35

A STATISTICAL STUDY OF THE THORACIC DUCT IN MAN[1]

HENRY K. DAVIS

From the Anatomical Laboratory of the Cornell University Medical College, Ithaca, New York

THIRTY-TWO FIGURES

INTRODUCTION

It has long been known that the thoracic duct in man presents a certain amount of variation and in addition to the usually described ducts various marked anomalous conditions have been noted. This investigation was undertaken to determine the percentage of occurrence of the different variations of the thoracic duct. An attempt has also been made to explain these variations from an embryological standpoint. The various types of duct that might develop from the primitive embryological network have been indicated and the ducts here described together with those described by other investigators have been divided into corresponding groups.

MATERIAL AND METHODS

This paper is based upon the records of the dissection of 22 cadavers in the Anatomical Laboratory of the Cornell University Medical College, Ithaca, New York. Forty-two cadavers, on which autopsies had been performed, were examined, but many of them had to be discarded on account of injury to the duct at the post mortem. In 11 of these however, the duct was found complete and records were taken of the ducts in these bodies. The other 11 records were taken from bodies which were dissected by the medical students. During the course of

[1] From a thesis presented to the faculty of the Graduate School of Cornell University for the degree of Master of Arts, June, 1914.

the dissection of the abdomen, thorax, and base of the neck, I supervised the students' work so that no injury would occur to the duct and made the dissection of the duct myself.

The thoracic duct in each case was injected with a carmine gelatin mass (Lee '05). At first, I attempted to make the injection from the cephalic portion of the duct but could not make the injection mass flow caudad on account of the valves. By experimenting, I found that by exposing the duct just cephalic to where it pierces the diaphragm and making the injection from this point, that the injecting mass flowed freely caudad as well as cephalad. This seems to indicate that the valves are much more efficient in the cephalic than in the caudal portion of the duct. Before the injection, the innominate, vertebral, subclavian, and internal jugular veins were clamped. This insured a good filling of the duct. After the injecting mass had been allowed to cool and gelatinize, a careful dissection and a natural sized drawing of the duct was made.

EMBRYOLOGY

According to Sabin ('09) the thoracic duct in human embryos begins in the abdominal cavity at the cisterna chyli as two ducts. These pass cephalad through the thorax, the right duct crossing, at about the level of the 4th thoracic vertebra, dorsal to the aorta to join its fellow of the opposite side. There is thus formed on the left side a single trunk that connects with the jugular portion of the thoracic duct. The jugular portion of the thoracic duct is a caudal outgrowth of the jugular lymph sac on the left side. Sabin, however, has not been able to find the jugular portion, that is, the caudal outgrowth of the right jugular lymph sac, connecting with the thoracic duct. She was able to trace it to the root of the lung but could find no connection of it with the thoracic duct in this region. For this reason the duct as described by Sabin is not a complete bilaterally symmetrical duct. In the embryo there is a distinct right and left duct. These two ducts are connected by numerous cross anastomoses thus forming a plexus of lymphatic vessels along the course

of the aorta. Sabin ('09) reports the first appearance of the cisterna chyli in 23 mm. human embryos. Here it is a definite sac opposite the 3rd and 4th lumbar vertebrae. The thoracic duct is first found in human embryos of 24 mm. In human embryos of 30 mm. the thoracic duct is complete.

Sabin ('02) in her study of the thoracic duct in pig embryos found it to be essentially the same as she found in human embryos.

Lewis ('06) found the thoracic duct in the rabbit embryo to be practically the same as Sabin found in the human and pig embryos.

Huntington (11) in his study of the lymphatic vessels of the cat states that the thoracic duct is "potentially bilaterally symmetrical" and he pictures a bilaterally symmetrical duct in figure 29, plate 22.

It is interesting also to note that Sala ('99–'00) and Pensa ('08–'09) picture bilaterally symmetrical thoracic ducts in birds

DIVISION OF THE THORACIC DUCTS INTO GROUPS

Assuming that the embryonic thoracic duct is bilaterally symmetrical and that the duct in the adult is produced by the persistence and growth of a part of the embryonic duct and the disappearance of other parts, one might expect to find variations in the adult thoracic duct. These variations depend then upon which portions of the embryonic thoracic ducts atrophy and disappear and which continue to develop. These possible varieties of the thoracic duct may be divided into the following types.

Type 1. To this type of thoracic duct belong those ducts which would retain more or less the early embryological conditions and would consist of a completely bilaterally symmetrical duct connected by numerous cross anastomoses (fig. 1).

Type 2. In this type of thoracic duct we would have caudad the persistence of the original double thoracic duct of the embryo. There would be a right and left duct, which starting in the abdominal cavity would pass cephalad through the thorax and at about the level of the 4th thoracic vertebra, the right duct would cross by persistence of one of the embryonic cross anastomosing

branches to join the duct of the left side. A single trunk would be thus formed which would empty into the venous system of the left side. The cephalic portion of the right duct would fail to connect with the thoracic duct and would remain as the right lymphatic duct (fig. 2).

Type 3. In this type of thoracic duct we would have caudad the persistence of the original double thoracic duct of the embryo. There would be a right and a left duct, which starting in the abdominal cavity, would pass cephalad through the thorax and the left duct would cross by persistence of one of the embryonic cross anastomosing branches to join the duct of the right side. A single trunk would thus be formed which would empty into the venous system of the right side. The cephalic portion of the left duct would fail to connect with the thoracic duct and would remain as a left lymphatic duct which would be comparable to the usual right lymphatic duct (fig. 3).

Type 4. In this type of thoracic duct, we would have cephalad the persistence of the original double thoracic duct of the embryo. There would be complete atrophy of the caudal portion of the left duct and the cephalic portion of the left duct would join the right duct through the persistence of one of the embryonic cross anastomosing branches (fig. 4).

Type 5. In this type of thoracic duct, we would have cephalad the persistence of the original double thoracic duct of the embryo. The caudal portion of the right duct would be completely atrophied and the cephalic portion of the right duct would join the left duct through the persistence of one of the embryonic cross anastomosing branches (fig. 5).

Type 6. In this type of thoracic duct, we would have the persistence of the cephalic portion of the left duct and the caudal portion of the right duct. These two segments would be joined together by the persistence of one of the embryonic cross anastomosing branches. The caudal portion of the left duct would be completely atrophied and the cephalic portion of the right duct would persist as the right lymphatic duct (fig. 6).

Type 7. In this type of thoracic duct, we would have the persistence of the cephalic portion of the right duct and the

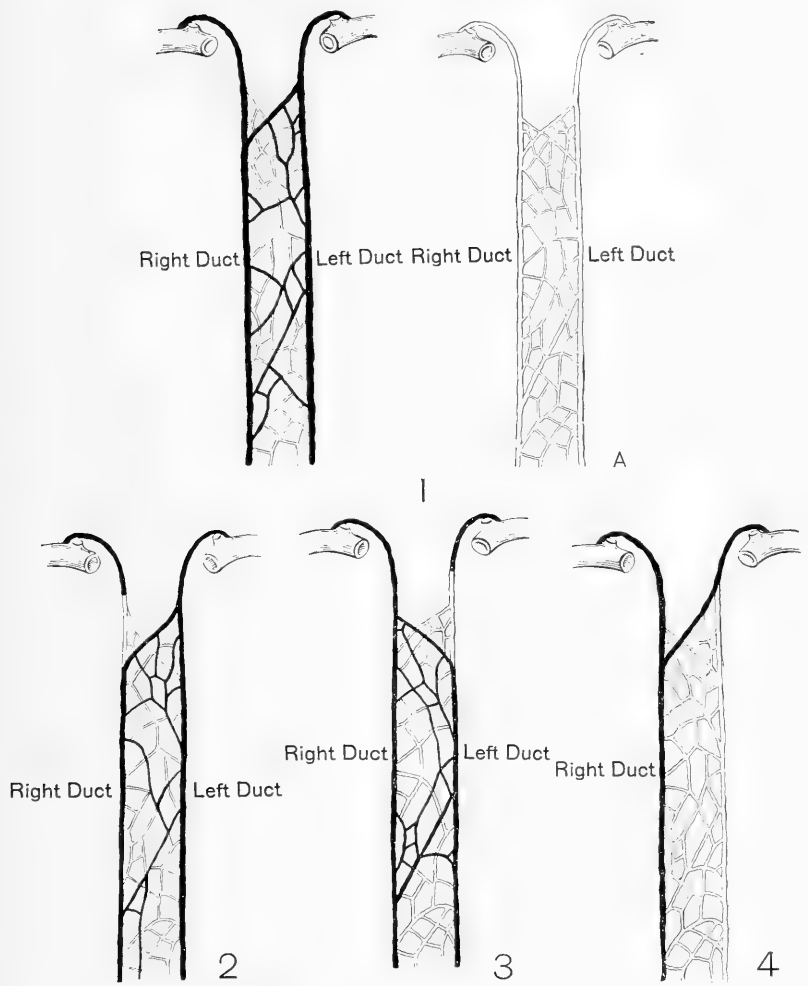


Fig. A Schematic representation of the embryonic lymph channels.

Fig. 1 Type 1; schematic representation of the embryonic lymph channels which might persist.

Fig. 2 Type 2; schematic representation of the embryonic lymph channels which might persist.

Fig. 3 Type 3; schematic representation of the embryonic lymph channels which might persist.

Fig. 4 Type 4; schematic representation of the embryonic lymph channels which might persist.

caudal portion of the left duct. These two segments would be joined together through the persistence of one of the embryonic cross anastomosing branches. The caudal portion of the right duct would be completely atrophied and the cephalic portion of the left duct would not connect with the thoracic duct and it would persist as a left lymphatic duct (fig. 7).

Type 8. In this type of thoracic duct, we would have the complete persistence of the right embryonic duct. The caudal portion of the left duct would be completely atrophied and the cephalic portion of the left duct would not be connected with the thoracic duct and would persist only as the left lymphatic duct which would be comparable to the usual right lymphatic duct (fig. 8).

Type 9. In this type of thoracic duct, we would have the complete persistence of the left embryonic duct. The caudal portion of the right duct would be completely atrophied and the cephalic portion of the right duct would not connect with the thoracic duct and would persist only as the right lymphatic duct (fig. 9).

It should be noted that Types 2 and 3, 4 and 5, 6 and 7, 8 and 9 are respectively the reverse of one another in that those channels which persist in one atrophy in the other and vice versa.

Group I

Winslow ('66), Cruickshank ('90), Sömmering ('92), and Hommel ('37) describe bilaterally symmetrical thoracic ducts. The thoracic ducts start in the abdominal cavity as two ducts which pass cephalad through the thorax, one opening into the venous system of the left side and the other into the venous system of the right side. The right duct lies to the right of the aorta and the left duct on the left side of the aorta. These two ducts are joined together by numerous cross anastomoses. I found no ducts of this type. It is clearly evident that the thoracic ducts described by the above investigators belong to Type 1 (fig. 1).

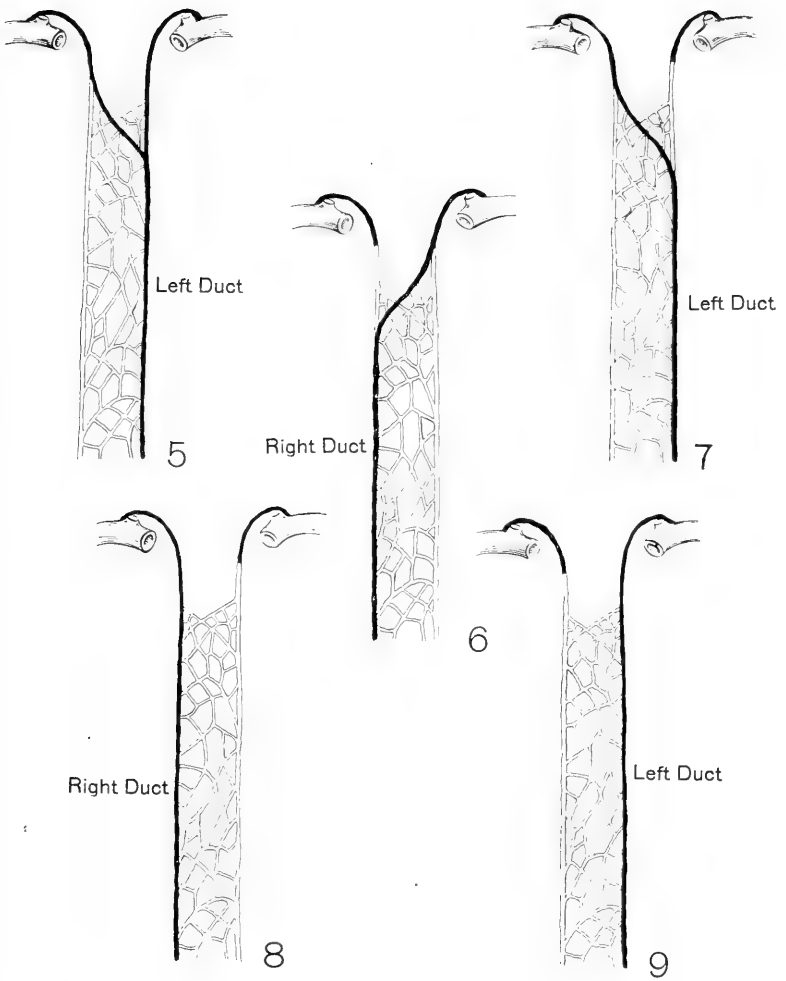


Fig. 5 Type 5; schematic representation of the embryonic lymph channels which might persist.

Fig. 6 Type 6; schematic representation of the embryonic lymph channels which might persist.

Fig. 7 Type 7; schematic representation of the embryonic lymph channels which might persist.

Fig. 8 Type 8; schematic representation of the embryonic lymph channels which might persist.

Fig. 9 Type 9; schematic representation of the embryonic lymph channels which might persist.

Group II

The thoracic ducts of this group (cases 1–6, figs. 10–15) begin in the abdominal cavity as two ducts which extend cephalad through the thorax. The right duct lies to the right of the aorta and the left duct to the left of the aorta. The right duct crosses in the thorax at the level of the 4th thoracic vertebra dorsal to the aorta to join the left duct forming a single trunk which empties into the venous system on the left side at the base of the neck. The two ducts are connected by cross anastomosing channels. This type of duct occurred in 6 cases out of 22, or in 27.27 per cent. This form of duct corresponds to the thoracic duct represented in Type 2. In proportion to the completeness of the persistence of embryonic conditions these ducts have been divided into three divisions, A, B and C. Lower ('80) and Nuhn ('49) describe a similar thoracic duct.

Division A. The thoracic duct of this division (fig. 10) begins in the abdominal cavity as two ducts which pass cephalad through the thorax. The right duct at the upper level of the 5th thoracic vertebra begins to incline to the left and passing dorsal to the aorta reaches the left side and at the level of the lower third of the body of the 2nd thoracic vertebra joins the left duct forming a single trunk which continues cephalad to open into the left subclavian vein a short distance from its junction with the internal jugular vein. The right duct lies to the right of the aorta and is situated between the aorta and the vena azygos major. The left duct lies to the left of the aorta. The two ducts are of equal calibre and are connected by numerous cross anastomoses. Three of these are especially well developed (1) a cephalic one which connects the cephalic end of the right duct with the left; (2) one which is situated opposite the body of the 9th thoracic vertebra nearly transverse in direction; (3) and one starting in the abdominal cavity on the right side and passing cephalad and to the left to join the left duct opposite the body of the 11th thoracic vertebra. There is no cisterna chyli. It is represented in this case by a plexus of lymphatic vessels. This type of duct occurred in 1 case out of 22, or in 4.545 per cent.

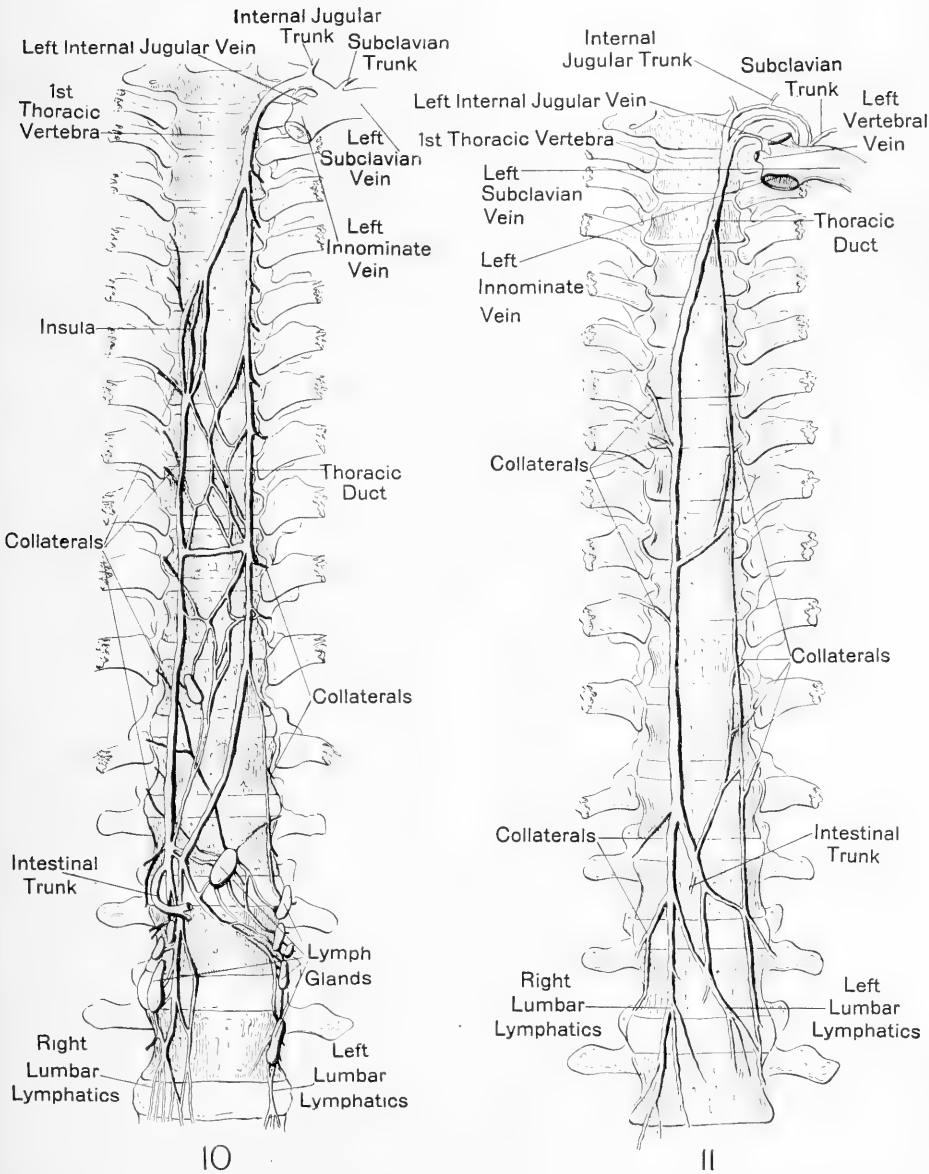


Fig. 10 Type 1; thoracic duct in a male white subject, age 35. Note the double duct and the abundant cross anastomoses.

Fig. 11 Type 1; thoracic duct in a male white subject, age 51. Note the double duct and the reduction in size of the left duct.

Division B. The thoracic duct of this division (fig. 11) begins in the abdominal cavity as two ducts which pass cephalad through the thorax. The right duct at the upper level of the 5th thoracic vertebra begins to incline to the left and passing dorsal to the aorta reaches the left side and joins the left duct at the level of the lower third of the body of the 2nd thoracic vertebra forming a single trunk which passes cephalad. This trunk at the lower level of the 7th cervical vertebra divides into three branches which do not become united again before opening into the venous system. The most cephalic branch opens into the left subclavian vein a short distance distal to its junction with the left internal jugular vein. The intermediate branch opens into the angulus venosus formed by the junction of the left internal jugular and left subclavian veins and the most caudal branch opens into the left vertebral vein a short distance medial to its junction with the left innominate vein. The right duct lies to the right of the aorta and is placed between the aorta and the vena azygos major. The left duct lies to the left of the aorta. The two ducts are of unequal size, the right duct being of much greater caliber than the left duct. The cross anastomosing channels in this case are not as numerous as in case 1 (fig. 10). The chief anastomosis is the cephalic one which joins the cephalic end of the right duct with the left duct. The reduction in caliber of the left duct and the decrease in the number of cross anastomoses point to a stage in the atrophy of the left duct. There is no cisterna chyli present. It is represented by a plexus of lymphatic vessels. This type of duct occurred in 1 case out of 22, or in 4.545 per cent (fig. 2).

Division C. In cases 3 and 4 (figs. 12–13), in cases 5 and 6 (figs. 14–15) there is a partial doubling of the caudal portion of the thoracic duct. In each case the caudal portion of the right duct is complete but the left duct has partially atrophied.

In case 3 (fig. 12) the thoracic duct begins in the abdominal cavity as two ducts which pass cephalad into the thorax. The right duct lies to the right of the aorta and is placed between the aorta and vena azygos major. It begins to incline to the left opposite the inferior level of the body of the 4th thoracic

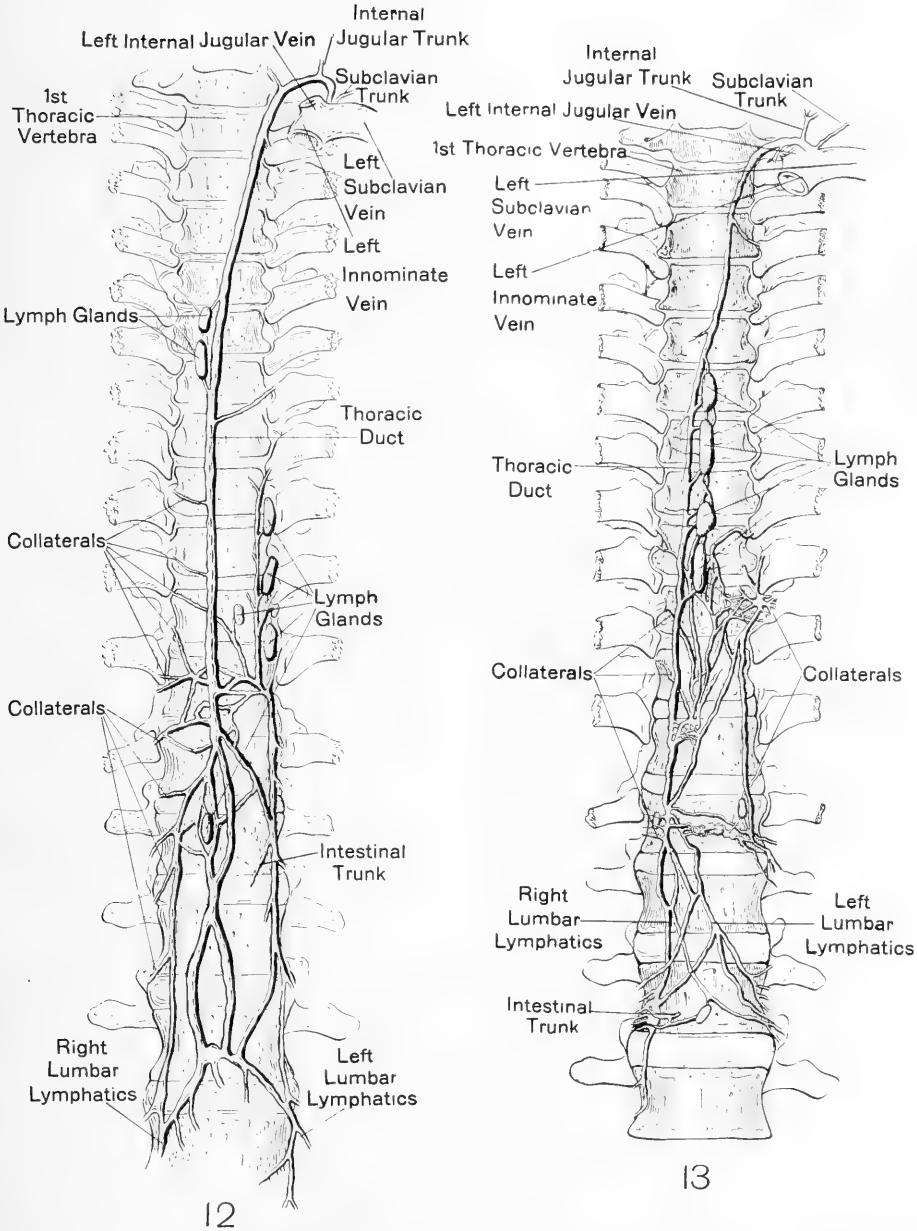


Fig. 12 Type 1; thoracic duct in a male white subject, age 72. Note the incomplete duct of the left side.

Fig. 13 Type 1; thoracic duct in a female white subject, age 69. Note the incomplete duct of the left side and the abundant cross anastomoses between it and the duct on the right side.

vertebra and crossing dorsal to the aorta reaches the left side where it continues its course cephalad to open into the angulus venosus formed by the junction of the left internal jugular and left subclavian veins. The right duct lies to the right of the aorta. It passes up into the thorax from the abdominal cavity and ends at the lower level of the 7th thoracic vertebra. The portion cephalad of this has atrophied. The two ducts are joined together by numerous cross anastomoses. There is no cisterna chyli present. It is represented by a plexus of lymphatic vessels.

In figures 13, 14 and 15, the right duct corresponds to the right duct described in case 3 (fig. 12) with the exception that it empties into the left subclavian vein instead of the angulus venosus. The left ducts are essentially the same as in case 3 (fig. 12). In case 4 (fig. 13) there is no cisterna chyli but there is a lymphatic plexus. In case 5 (fig. 14) there are two cysternae chyli. The right duct is a direct continuation of the right cisterna chyli. The left cisterna chyli is connected to both the right and left ducts. The left duct, however, is not a direct continuation from the left cisterna chyli. In case 6 (fig. 15) there is a single cisterna chyli. The right duct is a direct continuation of this cisterna. The left duct is also connected with it. In this case there is a division of the right duct into two branches. This bifurcation takes place at the lower level of the body of the 6th thoracic vertebra and the two branches unite again to form a single trunk at the upper level of the body of the 5th thoracic vertebra. In these 4 cases the lymphatics on the left side of the aorta including the left duct, drain in a caudal direction. Lymph glands are associated with the thoracic duct in cases 3, 4 and 5 (figs. 12–14). A further account of these will be given in dealing with the variations.

The arrangement of the two ducts in these 4 cases points to an originally double thoracic duct, as in case 2 (fig. 11). There is represented in these 4 cases another more advanced stage in the atrophy of the left duct. In case 2 there was a reduction in the size of the left duct, while in these 4 cases there is a reduction in size and a complete atrophy of a portion of the left duct. This type of duct occurred in 4 cases out of the 22, or in 18.18 per cent.

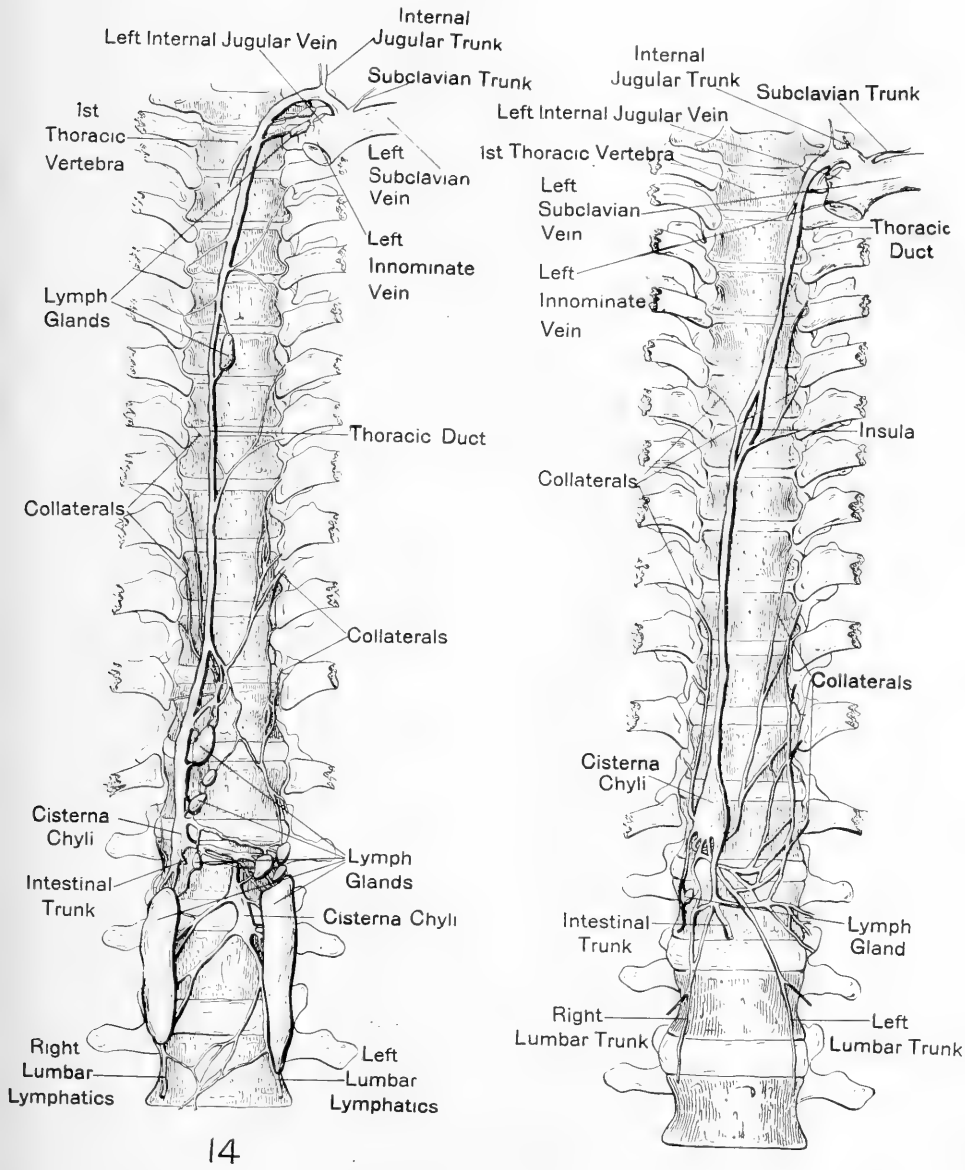


Fig. 14 Type 1; thoracic duct in male white subject, age 56. Note the two cisterna chyli and the incomplete duct on the left side.

Fig. 15 Type 1; thoracic duct in a male white subject, age 67. Note the incomplete duct on the left side.

Group III

Breschet ('36) describes a thoracic duct as seen by Haller which was double in its caudal portion. The thoracic duct in this case start in the abdominal cavity as two ducts, which pass cephalad into the thorax, one lying on each side of the aorta. In the cephalic portion of the thorax, the left duct crosses over to the right side and the right and left ducts both open into the left angulus venosus. This form of duct belongs to Type 3 (fig. 3). I found no ducts of this type among my own cases and could find no other cases described in the literature.

Group IV

Butler ('03), Lauth ('35), Patruban ('44), Diemerbroeck ('85), Cousin ('98), and Walther (described by Haller '46), describe a thoracic duct which starts in the abdominal cavity as a single trunk and passing cephalad into the thorax on the right side of the aorta divides into two branches at about the level of the 4th thoracic vertebra. The right branch opens into the angulus venosus on the right side and the left branch passing dorsal to the aorta opens into the angulus venosus of the left side. The type of duct described by these investigators belongs clearly to Type 4 (fig. 4). I found no ducts of this type among my own cases.

Group V

The thoracic duct in this type starts in the abdominal cavity as a single duct and passing cephalad into the thorax on the left side of the aorta divides into two branches. The right branch opens into the right angulus venosus and the left branch into the left angulus venosus. I have been unable to find ducts of this type described in literature and there were none among my own cases.

Group VI

In 14 instances in my series (figs. 16–29) the thoracic duct begins in the abdominal cavity as a single trunk which passes cephalad into the thorax and at the level of the 5th to the 3rd thoracic vertebra begins to incline to the left and finally passes to the left of the median line of the bodies of the thoracic vertebrae. The duct continues cephalad and at the level of the 2nd thoracic to the 6th cervical vertebra changes its course passing cephalad, ventrad, and to the left, and then caudad and slightly ventrad to open into the venous system at the base of the neck.

In 8 instances (figs. 16, 17, 20, 21, 23, 26, 27, 29), the thoracic duct begins to incline to the left opposite the body of the 5th thoracic vertebra; opposite the body of the 6th thoracic vertebra in 1 instance (fig. 28); opposite the body of the 4th thoracic vertebra in 4 instances (figs. 18, 19, 24, 25); and opposite the body of the 3rd thoracic vertebra in 1 instance (fig. 22). The terminal portion of the thoracic duct changes its course opposite the 2nd thoracic vertebra in 1 instance (fig. 20); opposite the 1st thoracic vertebra in 6 instances (figs. 16, 17, 19, 21, 24, 26); opposite the 7th cervical vertebra in 6 instances (figs. 18, 23, 25, 27, 28, 29); and opposite the 6th cervical vertebra in 1 instance (fig. 22).

The mode of termination is somewhat variable. In 5 instances (figs. 16, 20, 22, 27, 28) the thoracic duct terminates by a single opening into the left subclavian vein; into the left subclavian vein by 2 branches in 1 instance (fig. 17); into the left angulus venosus by a single branch in 5 instances (figs. 18, 19, 21, 24, 25); into the left angulus venosus by 2 branches in 1 instance (fig. 29;) into the left internal jugular by a single branch in 1 instance (fig. 26); and into the posterior wall of the left innominate vein by a single branch in 1 instance (fig. 23).

The thoracic ducts begin in the abdominal cavity by the confluence of the lumbar lymphatics and sometimes the intestinal trunk or by a lymphatic plexus in which the lumbar trunks are

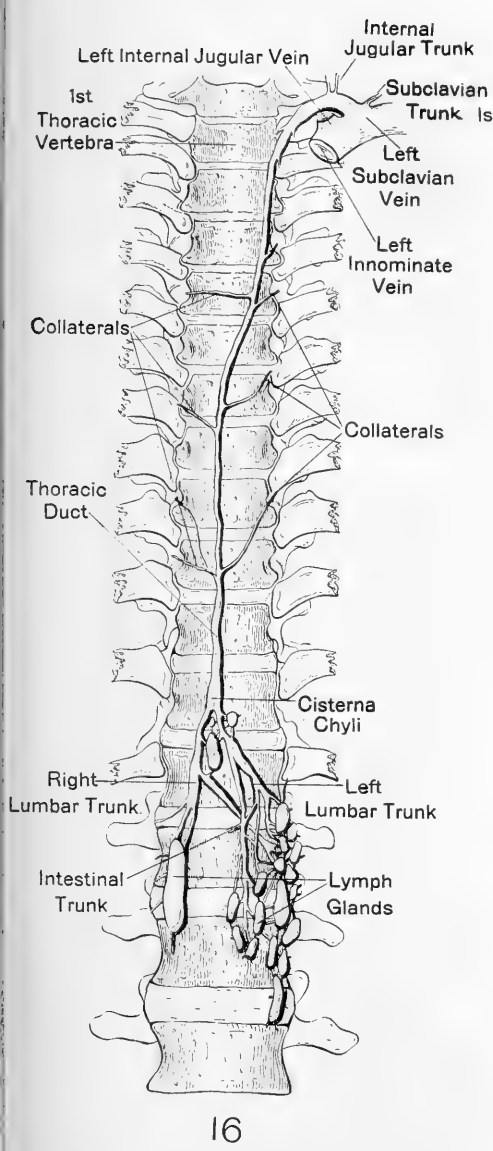
indistinct. Cases 14 and 18 represent such a plexus. In all the other cases of this type, the right and left lumbar trunks are distinct. The intestinal trunk joins the caudal extremity of the thoracic duct in 6 instances (figs. 16, 17, 19, 20, 24, 28); the right lumbar lymphatics in 3 instances (figs. 18, 25, 29); the left lumbar lymphatics in 4 instances (figs. 22, 23, 26, 27); and in both the right and left lumbar lymphatics in 1 instance (fig. 21).

The caudal extremity of the thoracic duct in 9 instances (figs. 16, 17, 18, 20, 22, 24, 26, 28, 29) presents an ampulliform dilatation, the cisterna chyli. This is absent in 5 instances, and in its place in 4 instances (figs. 21, 23, 25, 27) there is a lymphatic plexus. In 1 instance (fig. 19), there is neither a cisterna chyli nor lymphatic plexus.

In addition to the right and left lumbar and intestinal branches, the thoracic duct may receive the following branches: (1) collaterals which drain the intercostal spaces (present in all cases); (2) efferent vessels which drain the posterior mediastinal lymph nodes; (3) the left internal jugular trunk in all cases; and (4) the left subclavian trunk in cases 14, 15 and 16. The collaterals mentioned above drain, as a rule, more than one intercostal space. There is not, however, a collateral for each intercostal space. The trunks draining the posterior mediastinal nodes had been destroyed in most of the cases.

Lymph glands are associated with the thoracic duct in 11 instances (figs. 16, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29). In 9 instances (figs. 17, 18, 19, 21, 24, 25, 26, 28, 29) the duct divides into two branches which unite again after a short distance to form a single trunk. These were called 'insulae' by Haller ('75). In case 20 (fig. 29) there is a bifurcation of the terminal portion of the duct and each branch presents an ampulliform dilatation, similar to a cisterna chyli.

In the abdominal portion, the thoracic ducts of this group lie ventral to the bodies of the first 2 lumbar and 12th thoracic vertebrae and between the crura of the diaphragm or under cover of the right crus. Ventrad they are in relation with the right side of the abdominal aorta.



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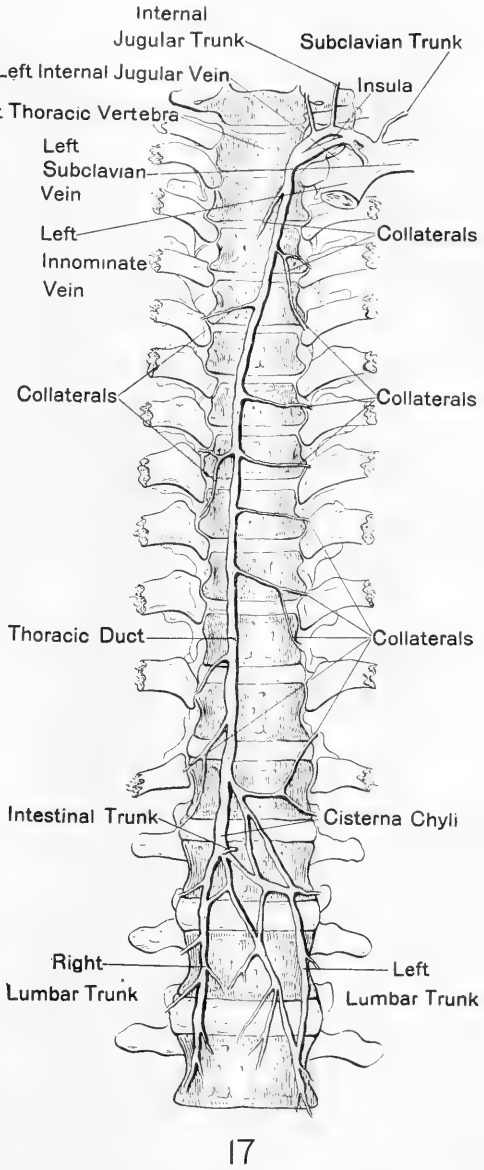


17

Fig. 16 Type 6; thoracic duct in a male white subject, age 62. Note the position of the cisterna chyli opposite the body of the 11th vertebra.

Fig. 17 Type 6; thoracic duct in a male white subject, age 22. Note the insula at the terminal portion of the duct and the opening of the duct by two branches into the left subclavian vein.

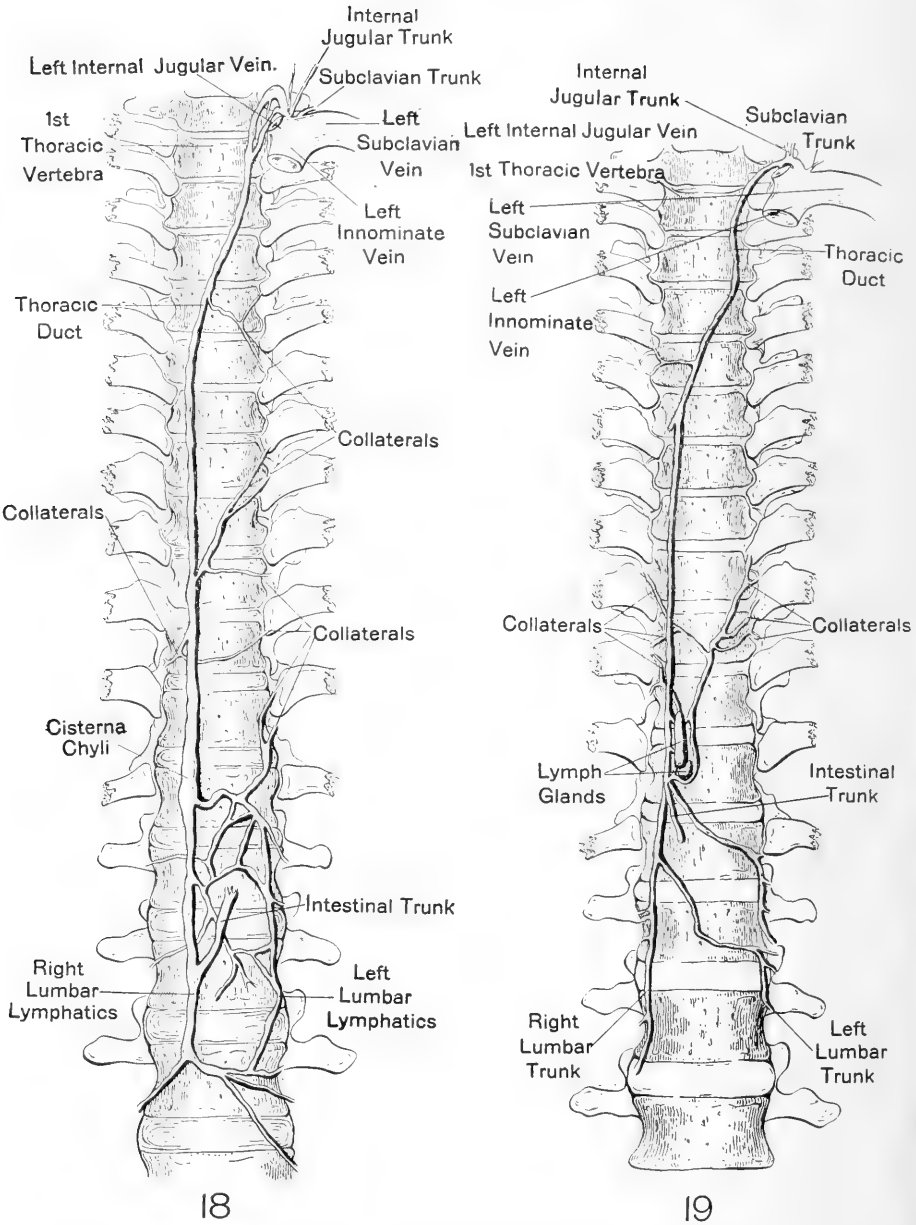
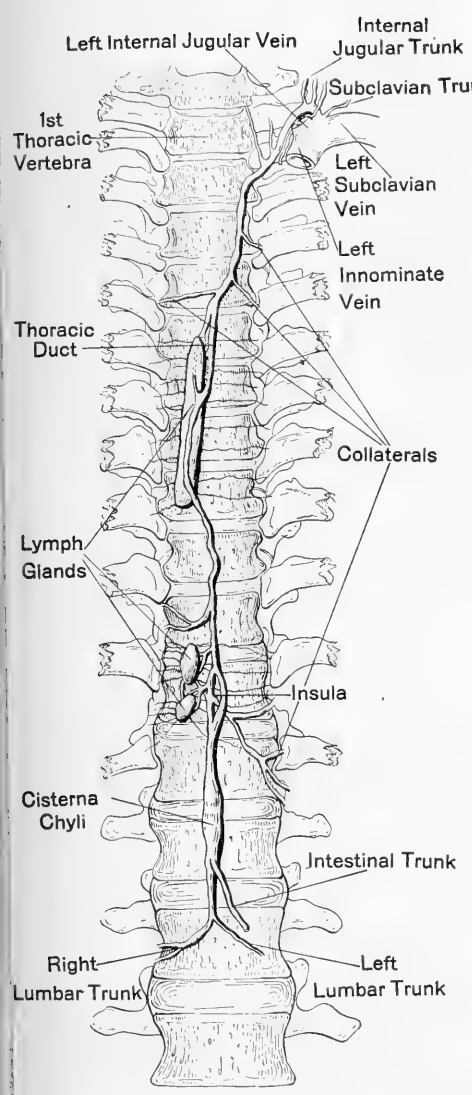


Fig. 18 Type 6; thoracic duct in a female white subject, age 39. Note the insula at the terminal portion of the duct.

Fig. 19 Type 6; thoracic duct in a female white subject, age 50. Note the caudal portion of the duct; there is no cisterna chyli or lymphatic plexus.



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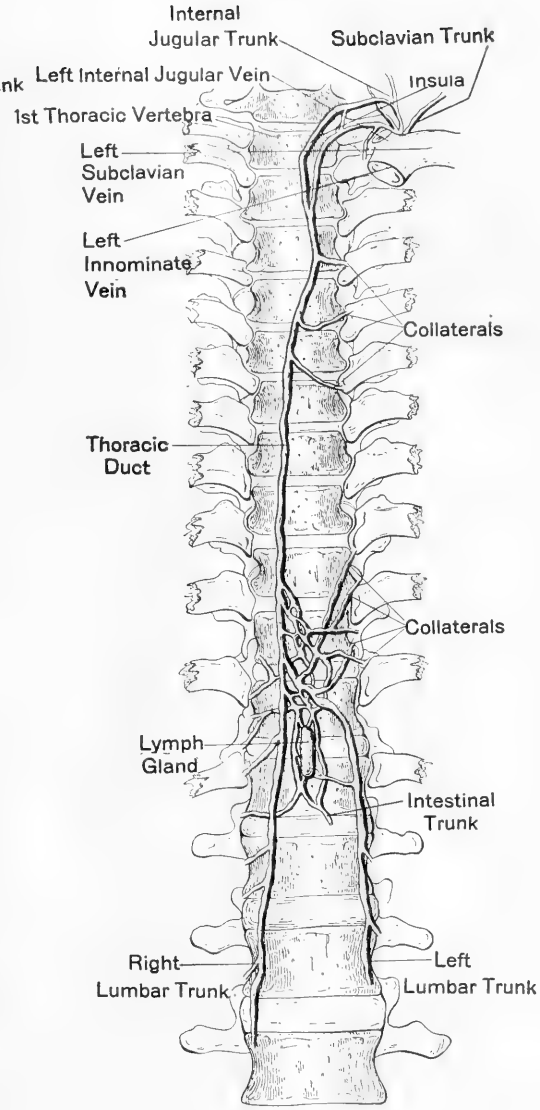

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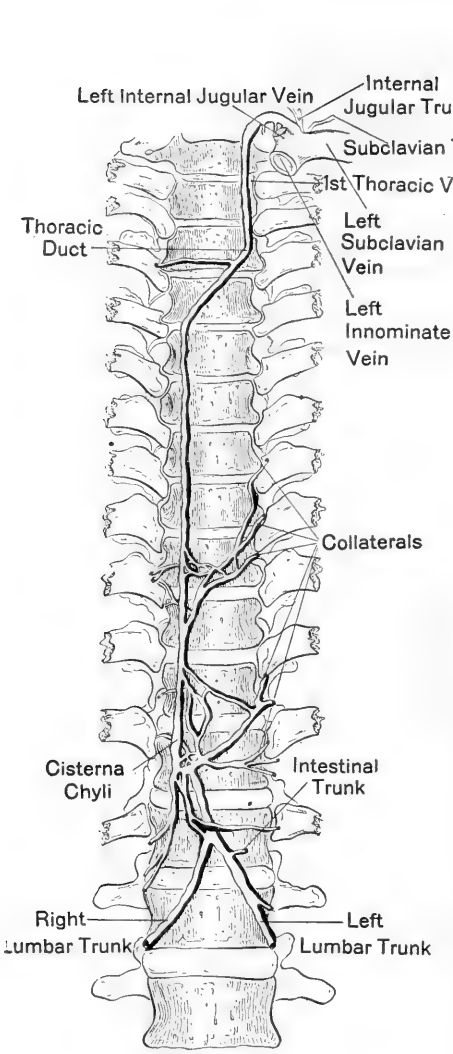
Fig. 20 Type 6; thoracic duct in a male white subject, age 57. Note the large lymph gland associated with the thoracic portion of the duct.

Fig. 21 Type 6; thoracic duct in a female white subject, age 66. Note the plexiform arrangement of the caudal portion of the duct and the insula at its terminal portion.



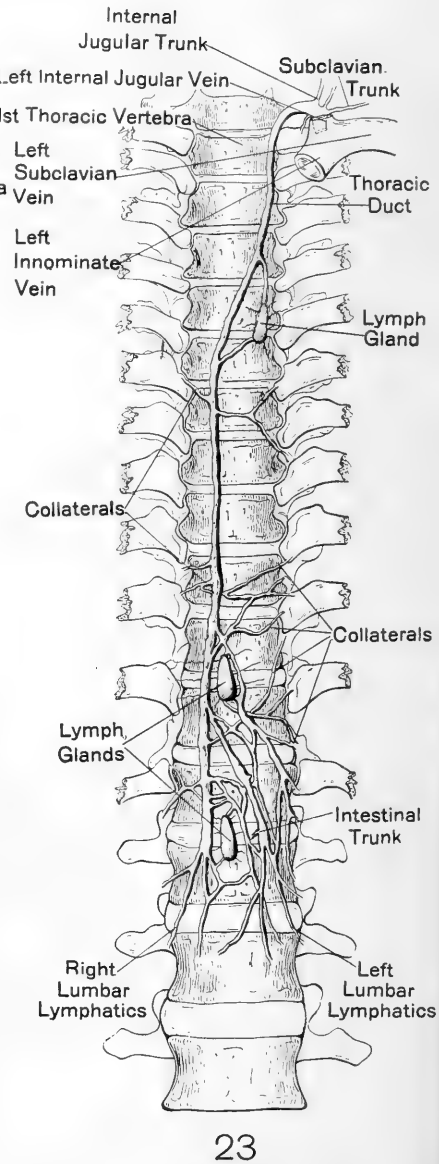
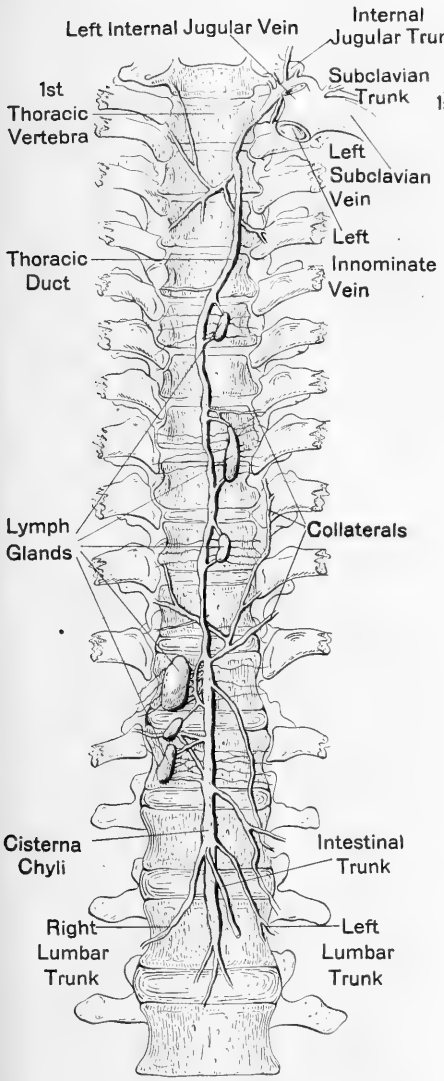


Fig. 22 Type 6; thoracic duct in a female white subject, age 42. Note the abrupt manner in which the right duct crosses over to the left side.

Fig. 23 Type 6; thoracic duct in a male white subject, age 43. Note the plexiform arrangement of the caudal portion of the duct and its termination into the posterior wall of the innominate vein.



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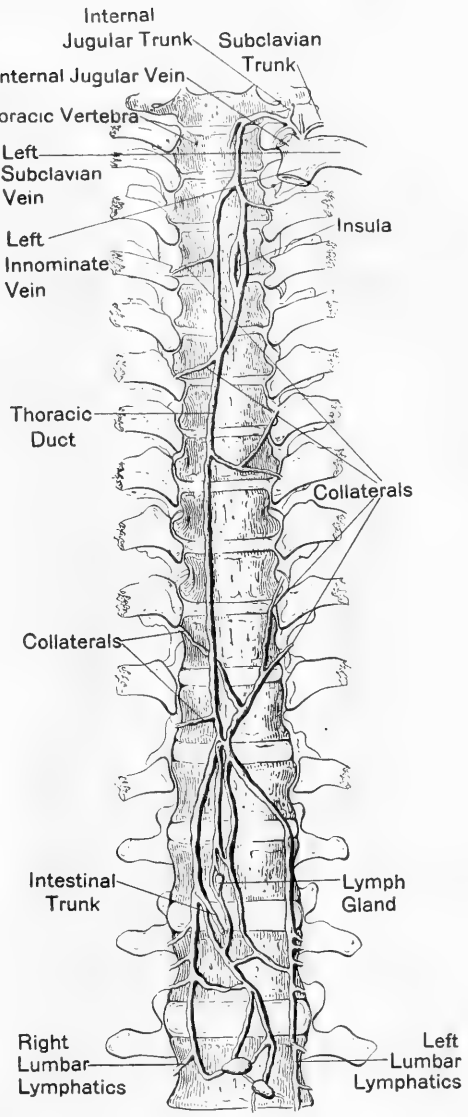

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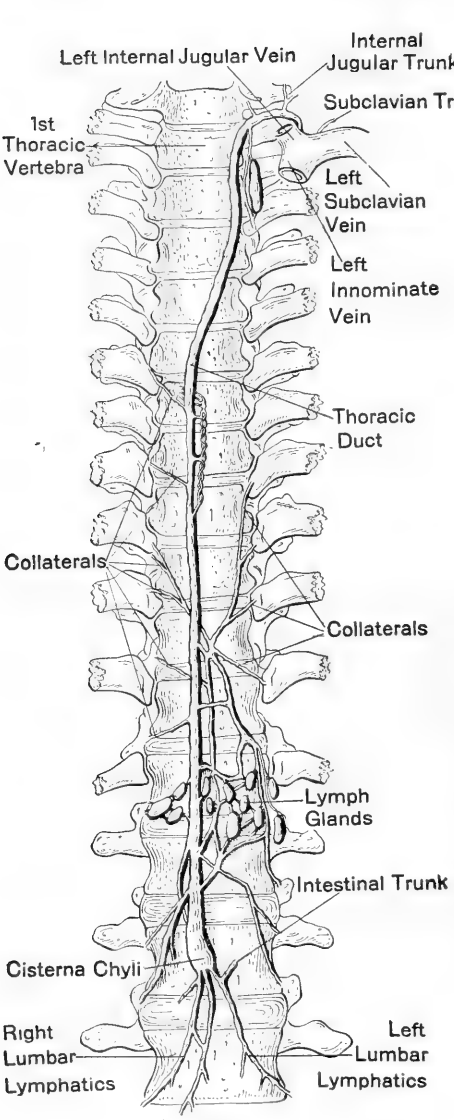
Fig. 24 Type 6; thoracic duct in an adult male white subject. Note the lymph glands along the thoracic portion of the duct and position of the cisterna chyli.

Fig. 25 Type 6; thoracic duct in a female white subject, age 51. Note the plexiform arrangement of the caudal portion of the duct and the insula associated with its thoracic portion.



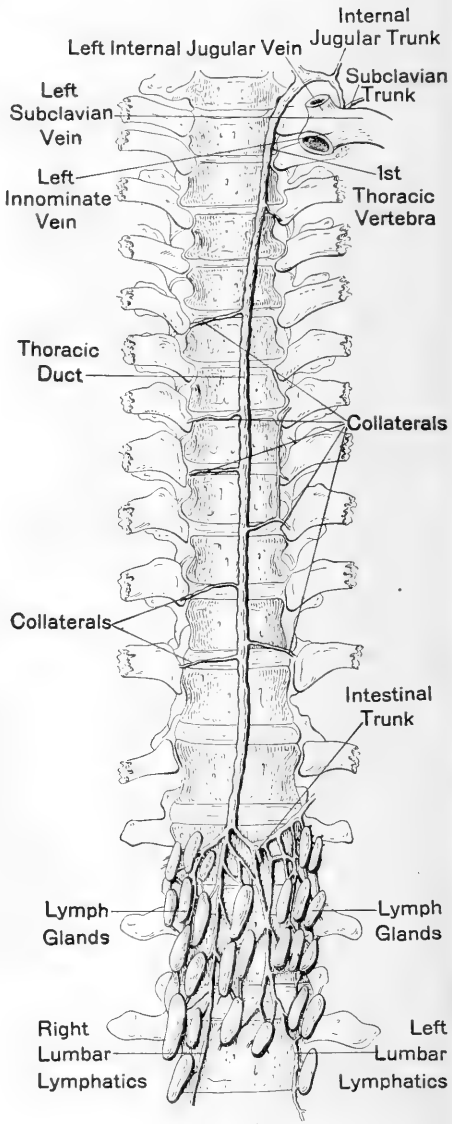


Fig. 26 Type 6; thoracic duct in a male white subject, age 21. Note the cisterna chyli and the termination of the duct into the left internal jugular vein.

Fig. 27 Type 6; thoracic duct in a male white subject, age 70. Note the lymph glands associated with the caudal portion of the duct and its termination into the left subclavian vein.

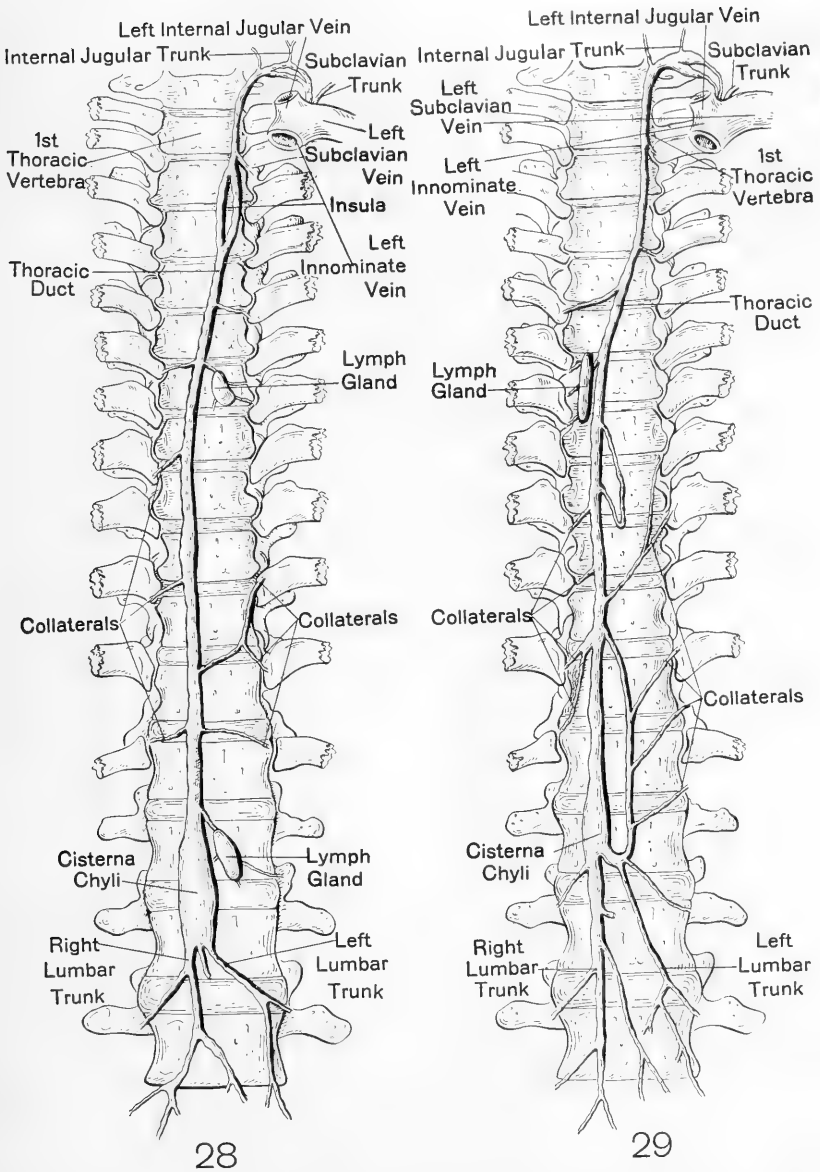


Fig. 28 Type 6; thoracic duct in a male white subject, age 60. Note the cisterna chyli and the two insulae.

Fig. 29 Type 6; thoracic duct in a male white subject, age 61. Note the cisterna chyli and the two ampulliform dilations at the terminal bend of the duct.

In their thoracic portion, they lie at first within the posterior mediastinum, but cephalad they enter the superior mediastinum. In the posterior mediastinum, they lie ventrad to the bodies of the 11th to the 5th thoracic vertebrae, and have ventral to them the pericardium, the oesophagus, and the arch of the aorta. The thoracic aorta lies to the left and to the right are the right pleura and the greater azygos vein. The caudal right intercostal arteries pass between them and the bodies of the vertebrae, as does also the terminal portion of the hemiazygos vein. In the superior mediastinum, they rest upon the caudal part of the longus colli muscle, being separated by it from the bodies of the three cephalic thoracic vertebrae. Ventrad they are in relation with the origin of the left subclavian artery and with the vertebral vein; to the left is the pleura and to the right are the oesophagus and the left recurrent laryngeal nerve.

The arch of these is in relation caudad with the apex of the left lung and with the left subclavian artery. Dorsad and to the left is the vertebral vein and to the right and ventrad are the left common carotid artery, the left internal jugular vein, and the left vagus nerve.

This type of duct belongs clearly to Type 6 of my classification. It occurred in 14 cases out of 22, or in 63.63 per cent. This type of duct is described as normal by all anatomists.

Group VII

The thoracic duct of this type begins in the abdominal cavity as a single trunk and passes cephalad into the thorax lying on the left side of the aorta. In the thorax it crosses over to the right side and opens into the right angulus venosus. I was unable to find any ducts of this type described in the literature, nor did I find any among my own cases. It seems strange that ducts of this type and also of Type 5 have not been reported, inasmuch as ducts of all the other types have been found.

Group VIII

In case 21 (fig. 30) the thoracic duct begins in the abdominal cavity from a plexus of lymphatic vessels and passes cephalad into the thorax. In its course through the thorax, it lies to the right of the aorta, placed between it and the vena azygos major. At the level of the lower third of the 1st thoracic vertebra, the duct divides into two branches which do not become reunited before emptying into the venous system of the right side. The cephalic branch ascends to the 6th cervical vertebra opposite the body of which it begins to incline to the right and divides into two branches which become united again after a course of 20 mm. to form a single trunk which opens into the right internal jugular trunk which opens into the right internal jugular vein a short distance cephalad of its junction with the right subclavian vein. Another branch is given off from this cephalic branch just after it bifurcates and which opens into the right internal jugular vein cephalad to the opening of the branch just described. The more caudal branch of the thoracic duct passes cephalo-laterad, then latero-caudad and ventrad to open into the posterior aspect of the right internal jugular vein.

The thoracic duct receives the lumbar and intestinal lymphatics in its abdominal portion, collaterals in its abdominal portion, collaterals draining the intercostal spaces in its thoracic portion, and the right internal jugular lymphatic trunk in its cervical portion.

There are no vascular peculiarities associated with the duct in this case and there is a left lymphatic duct comparable to the usual right lymphatic duct.

Watson ('72), Todd ('39), Haller ('75), Cruickshank ('90), and Fleischmann ('15) describe cases similar to this, in which the thoracic duct runs its entire course on the right side and opens into the venous system at the base of the neck on the right side. This type of thoracic duct belongs clearly to Type 8 of my classification. It occurred in 1 case out of 22 in my series, or in 4.545 per cent.

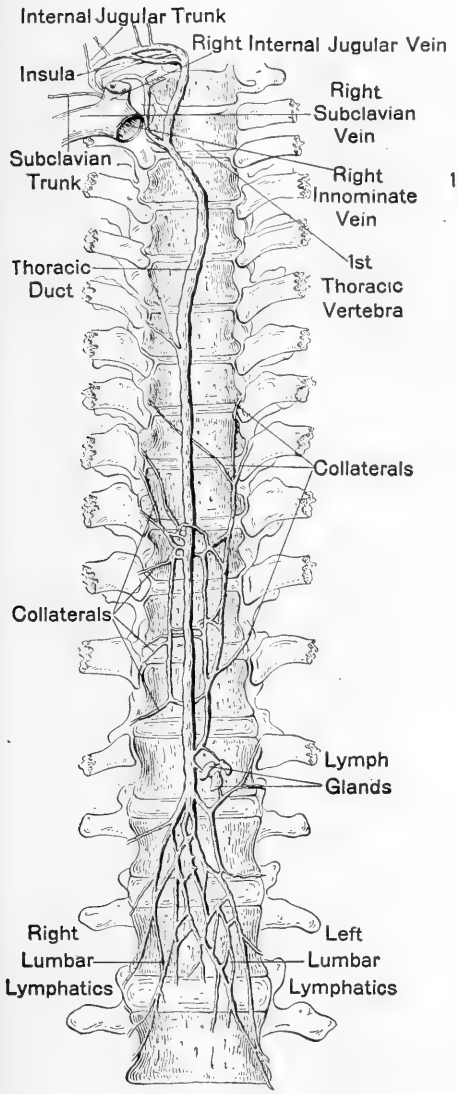
Group IX

In case 22 (fig. 31) the thoracic duct begins at the upper level of the 9th thoracic vertebra by the confluence of a plexus of lymphatic vessels. It lies on the left side of the aorta and in this position continues its direction cephalad and opposite the body of the 1st thoracic vertebra begins to incline to the left and then caudad dividing into two branches which open into the left subclavian vein. The caudal branch terminates singly but the cephalic branch divides into three branches just before its termination and opens into the left subclavian vein by three branches. This duct in its abdominal portion receives the lumbar and intestinal lymphatics, in its thoracic portion the collaterals which drain the intercostal spaces and in its cervical portion the left internal jugular and subclavian trunks.

Cameron ('02) describes a similar case in which the thoracic duct runs its entire course on the left side of the aorta. This type of duct belongs clearly to Type 9 of my classification. It occurred in 1 case out of 22 in my series, or in 4.545 per cent.

VARIATIONS

In 11 cases out of 22, or in 50 per cent, there is a cisterna chyli present (figs. 14–18, 20, 22, 24, 26, 28, 29). In one of these cases (fig. 14) there is a double cisterna chyli. There is also a double cisterna chyli in case 23 (fig. 32). This case differs from the previous one in that a cisterna chyli is placed on each lumbar trunk and the two lumbar trunks unite to form a single thoracic duct. This case has not been considered among my series because nearly the entire thoracic portion of the duct had been destroyed at the post mortem. Jossifow ('06) reports a similar case in which there was a cisterna chyli on each of the lumbar trunks. Instead of the caudal portion of the duct being dilated as a cisterna chyli it may be represented by a plexus of lymph channels. This condition was found in 10 cases out of the 22, or in 45.45 per cent (figs. 10–13, 21, 23, 25, 27, 30, 31). In 1 case out of the 22, or in 4.545 per cent, the thoracic duct is formed by the confluence of the right and left lumbar and intestinal



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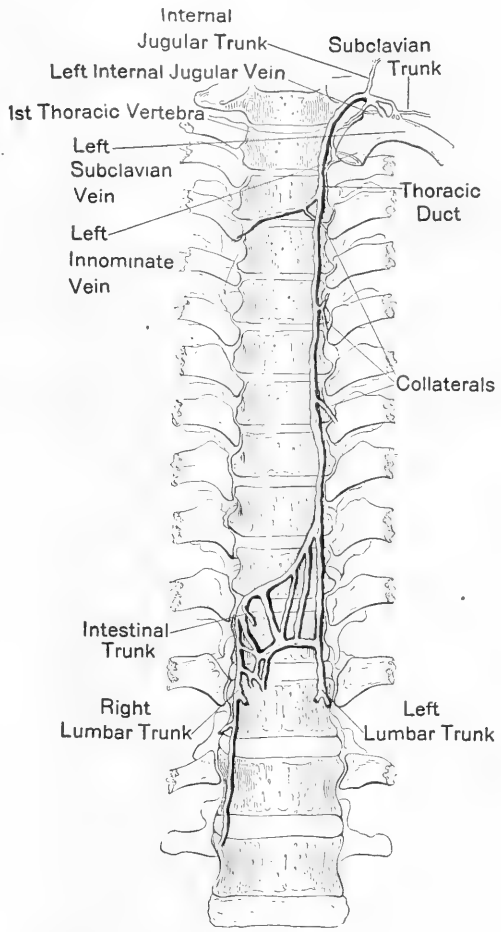

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Fig. 30 Type 8; thoracic duct in a male white subject, age 55. Note the plexiform arrangement of the caudal portion of the duct and its termination into the right internal jugular vein by three branches.

Fig. 31 Type 9; thoracic duct in a male white subject, age 71. Note the position of the duct on the left side, and its quadruple termination into the left subclavian vein.

lymphatics without the formation of a cisterna chyli and there is no lymphatic plexus (fig. 19).

The cisterna chyli is placed opposite the body of the 11th thoracic vertebra in 2 cases out of 22, or in 9.09 per cent (figs. 16 and 22); opposite the bodies of the 11th and 12th thoracic vertebrae in 2 cases out of the 22, or in 9.09 per cent (figs. 15 and 18); opposite the bodies of the 12th thoracic and 1st lumbar vertebrae in 4 cases out of 22, or 18.18 per cent (figs. 14, 17, 20, 29); opposite the bodies of the 1st and 2nd lumbar vertebrae in 3 cases out of

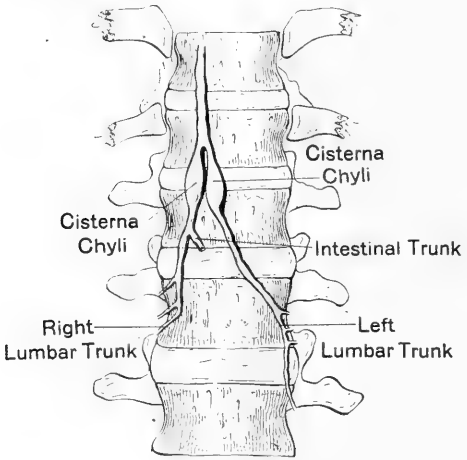

Fig. 32 Thoracic duct in an adult male white subject. Note the cisterna chyli associated with each lumbar trunk.

the 22, or in 13.635 per cent (figs. 24, 26, 28); and opposite the bodies of the 2nd and 3rd lumbar vertebrae in 1 case out of 22 or in 4.545 per cent (fig. 14).

The intestinal trunk empties into the left lumbar trunk in 7 cases out of 22, or in 31.815 per cent (figs. 11, 12, 16, 22, 23, 26, 29); into the right lumbar trunk in 5 cases out of 22, or in 22.725 per cent (figs. 13, 15, 18, 25, 29); into both the right and left lumbar trunks in 1 case out of 22, or in 4.545 per cent (fig. 21); into the cisterna chyli in 5 cases out of the 22, or in 22.725 per cent (figs. 14, 17, 20, 24, 28); into a lymph plexus in 2 cases out

of 22, or in 9.09 per cent (figs. 30, 31); and into the thoracic duct in 2 cases out of the 22, or in 9.09 per cent (figs. 10, 19).

The point at which the thoracic duct lying on the right side of the aorta begins to incline to the left is subject to some variation. The inclination begins opposite the body of the 3rd thoracic vertebra in 1 case out of 22, or in 4.545 per cent (fig. 22); opposite the body of the 4th thoracic vertebra in 5 cases out of 22, or in 22.725 per cent (figs. 14, 18, 19, 24, 25); opposite the body of the 5th thoracic vertebra in 12 cases out of 22, or in 54.54 per cent (figs. 10–13, 16, 17, 20, 21, 23, 26, 27, 29); and opposite the body of the 6th thoracic vertebra in 2 cases out of 22, or in 9.09 per cent (figs. 15, 18). In 1 case out of 22, or in 4.545 per cent the duct lying on the right side of the aorta did not cross over to the left side (fig. 30) and in 1 case out of 22, or in 4.545 per cent there was no duct on the right side of the aorta (fig. 31).

There is a division of the thoracic duct into two branches which unite again to form a single trunk. This has been termed an 'insula' by Haller. One or more insulae occurred in 13 cases out of the 22, or in 59.085 per cent (figs. 10, 12, 13, 15, 17, 18, 20, 21, 25, 26, 28, 29, 30).

In 10 cases out of the 22, or in 45.45 per cent (figs. 10, 12, 13, 14, 19, 20, 23, 24, 28, 29) lymph glands are situated along the thoracic portion of the thoracic duct. According to Sabin ('12) lymph glands develop from a lymphatic plexus and as Pensa ('08–'09) remarks, lymph glands may occur anywhere along the course of the thoracic duct. This one may expect, I think, if he recall the early embryonic plexiform arrangement of the thoracic duct.

I have observed among my cases the forms of terminations shown in table 1. Terminations of the thoracic duct similar to those described in my cases have been reported by Parsons and Sargent ('09), Wendel ('98), and Verneuil ('53), who cites Boullard's cases (tables 2 and 3).

Table 4 is a comparison of the percentages of the different modes of termination of the thoracic duct as given in tables 1 to 3. From table 4, it will be seen that my results agree quite closely with those of Boullard and with those of Parsons and Sargent.

SUMMARY

The thoracic duct may be double throughout its entire extent, one channel lying on each side of the aorta and opening into the venous system of the corresponding side. This type of duct is similar to the diagram, figure 1. Ducts of this type have been described by four authors.

The thoracic duct may be partially doubled and open into the venous system of the left side (figs. 10–15). This type of duct

TABLE 1

Termination

MODE	WHERE	CASES	PER CENT	FIGURES
Single	Left angulus venosus	5	22.725	12, 18, 21, 24, 25
Single	Left subclavian	10	45.450	10, 13–16, 19, 20, 22, 27, 28
Single	Left internal jugular	1	4.545	26
Single	Left innominate	1	4.545	23
Double	Left subclavian	1	4.545	17
Double	1 branch in l. int. jug.			
	1 branch in ang. ven.	1	4.545	29
Triple	2 branches in l. int. jug.			
	1 branch in l. vertebral	1	4.545	11
Triple	R. internal jug.	1	4.545	30
Quadruple	Left subclavian	1	4.545	31

TABLE 2

Parsons and Sargent's cases

Termination

MODE	WHERE	NO. OF CASES	PER CENT
Single	Left internal jugular	28	70.00
Single	Left angulus venosus	3	7.50
Double	Left internal jugular	4	10.25
Double	Left internal jugular and some other vein	2	5.00
Double	1 br. in l. int. jug.		
	1 br. in l. subclavian	1	2.50
Quadruple	Left int. jug.	1	2.50
Quadruple	1 br. in left int. jug.		
	3 br. in left subclavian	1	2.50

occurred in 6 instances in my series of 22, or in 27.27 per cent. This type of thoracic duct has been described by two authors.

The thoracic duct may be partially doubled and open into the venous system of the right side. This type of duct is similar to the diagram, figure 3. One author has described a duct of this type.

The thoracic duct may pass cephalad into the thorax on the right side of the aorta as a single duct and divide into two branches, one branch connecting with the venous system of the left side and the other branch with the venous system of the right side. This type of duct is similar to the duct in the diagram, figure 4. Thoracic ducts of this kind have been described by six authors.

TABLE 3

Wendel's cases

Termination

MODE	NO. OF CASES	PER CENT
Single	9	52.941
Double	3	17.647
Triple	1	5.882
Multiple	4	23.528

Boullard's cases, reported by Verneuil

Termination

Single	18	74.98
Double	3	12.49
Triple	2	8.33
Sixfold	1	4.16

TABLE 4

MODE OF TERMINATION	PARSONS AND SARGENT'S CASES:	BOULLARD'S CASES:	WENDEL'S CASES:	DAVIS' CASES:
	Per cent	*Per cent*	*Per cent*	*Per cent*
Single	77.50	74.98	52.941	77.265
Double	17.50	12.49	17.647	9.09
Triple		8.33	5.882	9.09
Quadruple	5.00			4.545
Multiple			23.528	
Sixfold		4.16		

The thoracic duct may be single and pass cephalad into the thorax on the right side of the aorta and at about the level of the 5th thoracic vertebra cross over to the left side and open into the venous system of the left side. This type of duct occurred in 14 instances in my series of 22, or in 63.63 per cent (figs. 16–29). *This is the most predominant type of thoracic duct and is described as normal by all anatomists.*

The thoracic duct may lie to the right of the aorta in its entire extent and open into the venous system of the right side. This type of duct occurred in 1 instance in my series of 22, or in 4.545 per cent (fig. 30). Ducts of this type have been described by five authors.

The thoracic duct may lie to the left of the aorta in its entire extent and open into the venous system of the left side. This type of duct occurred in 1 instance in my series of 22, or in 4.545 per cent (fig. 31). A similar thoracic duct has been described by one author.

Assuming that the thoracic duct is developmentally bilaterally symmetrical, one might expect to find in the adult some cases in which a single duct was situated on the left side of the aorta and divided in the thorax into two branches, one of which would open into the venous system of the left side and the other into the venous system of the right side. This type of duct would be similar to the diagram, figure 5. I found no duct of this type in my own series, nor could I find any described in the literature.

Again, assuming that the thoracic duct develops with bilateral symmetry, it may start in the abdominal cavity as a single duct and pass cephalad into the thorax on the left side of the aorta and at about the level of the 5th thoracic vertebra cross over to the right side and open into the venous system of the right side. This type of thoracic duct would be similar to the diagram, figure 7. I found no ducts of this type among my own cases nor could I find any described in the literature. It seems strange that no ducts of these last two types have been reported inasmuch as ducts of all the other types have been found and reported.

A cisterna chyli is present in 50 per cent of my cases (figs. 14–18, 20, 22, 24, 26, 28, 29) from which results it is evident that a cisterna chyli is not present as often as one would suspect from the descriptions of the thoracic duct in modern anatomical textbooks.

In 59.085 per cent of my cases, there is an insula associated with the thoracic duct (figs. 10, 12, 13, 15, 17, 18, 20, 21, 25, 26, 28, 29, 30). Haller ('75) considered this the normal condition.

The thoracic duct terminates singly in 77.265 per cent of my cases (figs. 10–16, 18–28); doubly, in 9.09 per cent of the cases (figs. 17, 29); triply, in 9.09 per cent of the cases (figs. 11, 30; and quadruply, in 4.545 per cent of the cases (fig. 31).

The thoracic duct terminated in the left subclavian vein in 59.085 per cent of my cases (figs. 10, 13–17, 19, 20, 22, 27, 28, 31); in the left innominate in 4.545 per cent of the cases (fig. 23); in the left angulus venosus in 22.725 per cent of the cases (figs. 12, 18, 21, 24, 25); in the left internal jugular in 4.545 per cent of the cases (fig. 26); in the right internal jugular in 4.545 per cent of the cases (fig. 30); in the left internal jugular and left angulus venosus in 4.545 per cent of the cases (fig. 29); and in the left internal jugular and left vertebral vein in 4.545 per cent of the cases (fig. 11).

In conclusion, it is a pleasure to thank Dr. Abram T. Kerr for many valuable suggestions and continued interest throughout the course of this work.

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THE HISTOGENESIS OF THE SELACHIAN LIVER

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FORTY-FIVE FIGURES (SEVEN PLATES)

CONTENTS

INTRODUCTION

The structure of the adult selachian liver is far removed from that type which is generally considered characteristic of the organ in ertebrates. This variance from the common vertebrate type is seen in the great accumulation of fat in the hepatic cells, in the comparatively slight development of the bile duct system and in the absence of lobulation of the kind generally found in higher vertebrates.[1] However, these pecularities which

[1] The histology of the adult elasmobranch liver was first briefly described by Leydig ('51) from observations on Chimaera. He published a more complete account dealing with several forms of selachians in 1853. Later descriptions of the general histology of the adult liver have been given by Shore and Jones ('89), Pilliet ('90), and Holm ('97). Deflandre ('05) has investigated the fat content of the hepatic cells, and Monti ('98) has studied the bile capillaries by the Golgi method.

THE AMERICAN JOURNAL OF ANATOMY, VOL. 17, NO. 3
MARCH, 1915

distinguish the selachian liver are not manifested until a comparatively late stage in the development of the organ. In earlier stages characters common to the liver in all vertebrates, but which are often masked or modified in higher forms, are shown with unusual clearness. It is chiefly with these more fundamental characters such as the formation and anastomoses of the hepatic cylinders, the differentiation of the minor bile ducts and the relation of the parenchymatous and vascular structures in the liver, that this paper has to do. The specific characters of the selachian liver, which have been mentioned, have been considered only incidentally.

The main material employed in this study consisted of embryos of Squalus acanthias, but specimens of Raia batis, Torpedo ocellata, Mustelus canis, Mustelus laevis and Squatina angelus have been used for supplemental and comparative work. For a large part of the Acanthias material, and for the specimens of Mustelus laevis, I am particularly indebted to the late Dr. Charles S. Minot, who not only permitted the removal of numerous series from the Harvard Embryological Collection, but also provided special material for use in this work.

DEVELOPMENT OF THE HEPATIC CYLINDERS

1. Literature

Our conception of the glandular structure of the vertebrate liver rests upon a large number of observations made mainly in the first half of the last century and culminating in the work of Eberth ('66) and Herring ('72). Since that time it has been recognized that the liver is a compound gland with a more or less regularly branching system of ducts and with a terminal network of anastomosing end pieces, and our knowledge of the details of this network has been greatly extended by the Golgi method in the hands of Retzius ('92), Hendrickson ('96) and others.

It is generally stated that the anastomotic type of liver is a modification of the compound branching gland type. Of this the best proof is the phylogenetic one, for in the lower cyclos-

tomes, the liver is indeed a true compound branching gland, as has been shown by the work of Retzius ('92), Holm ('97), Cole ('13) and others. In Petromyzon, however, anastomoses occur occasionally in the embryo (Brachet '97) and are very numerous in the adult (Renaut '97). On the side of embryology little evidence of this modification has been offered, although the statement that the liver arises in many vertebrate embryos as a branching gland, and that it takes on its adult reticular structure through the anastomoses of its end pieces, is common enough in texts. But an examination of the literature shows no more complete account of this early period of the histogenesis of the liver than that given by Remak ('55) excepting Hilton's work ('03) on the pig which has hardly received the attention that it deserves. The study of the actual course of the anastomosis of the end pieces has also been neglected, and the only clear statement to be found regarding this process is not in connection with the liver at all but in Laguesse's ('96) description of the histogenesis of the pancreas where he found numerous anastomoses at different stages.

The literature of the histogenesis of the liver in selachians is scanty and has been to a large extent reviewed in the comprehensive works of Oppel ('00) and Fiessinger ('11).

Balfour's ('76) account was the first to appear and as it has formed the basis of all later descriptions, it is given here in full:

> By stage K the hepatic diverticula have begun to bud out a number of small hollow knobs. These rapidly increase in length and number and form the so-called hepatic cylinders. They anastomose and unite together so that by stage L there is constructed a regular network. As the cylinders increase in length their lumen becomes very small but appears never to vanish.

Hammar ('93) in the course of a study devoted particularly to the development of the larger hepatic structures illustrates the development of the hepatic cylinders in two models of the liver of Torpedo embryos corresponding in development to Balfour's stages L and M. He makes no comment upon them aside from stating that the trabeculae increase in number and decrease in caliber in the period intervening between the two stages.

Brachet ('95) studying Torpedo embryos determined with accuracy the area of the hepatic diverticulum which gives rise to the hepatic tubules, and confirmed Balfour's description of the structure of the hepatic cylinders in later stages.

Holm ('97) figured and described very briefly sections of the liver of two Scyllium embryos of advanced stages.

Most of our later information concerning the embryonic hepatic parenchyma comes from the studies of Braus ('96) on embryos of Acanthias, Spinax and Scyllium. Confirming Balfour's observations in regard to early stages he noted a complete and regular anastomosis of the tubules in Acanthias embryos of 38 mm. Here the tubules were of even size and consisted in cross section of seven cells surrounding a lumen of variable size but distinctly larger than that seen in the adult. The hepatic cells of this stage were free from fat. In older embryos of Spinax and Acanthias the cells were fat laden. Braus saw no side branches nor blind endings of any hepatic capillaries.

Choronshitzky ('00), studying Torpedo, found several secondary hollow outgrowths from the hepatic pouch in his "Stadium II," which corresponds approximately to Balfour's stage K, and to the Normal-plate Nos. 22–24. In "Stadium III" which is represented by considerably older embryos, the liver pouches give rise to a number of small hollow buds, the cavities of which communicate with that of the pouch. "Die Leber macht im allgemeinen den Eindruck einer verzweigten Drüse." By "Stadium IV" the hollow buds have been transformed into much branched hepatic trabeculae which contain no traces of lumina. Choronshitzky's opinion of the mechanical influence of the blood vessels on the formation of the hepatic tubules will be discussed in a later part of this paper.

Minot ('00) in discussing the development of sinusoids, mentions the presence of the first short hepatic cylinders in an Acanthias embryo of 11.5 mm. and speaks of their anastomosis and growth in older stages. He noted the interesting retardment of development which is to be seen in later stages in the caudal tip of the liver as compared with the cardiac end.

Debeyre ('09) made use of observations upon the development of the hepatic cylinders in Acanthias to lay the ghost of the theory of the mesodermal origin of the hepatic parenchyma, which had been again raised a short time before by Géraudel ('07). He gives no complete or detailed history of the cylinders but notes, with illustrations, their general appearance in embryos 16, 22, and 30 mm. in length, respectively. In the latter he recognized the beginnning of a period of pronounced increase in the diameter of the cylinders. Debeyre noted the presence of numerous granules in the apices of the hepatic cells and bases upon this the interesting suggestion that the liver may serve as an organ of internal secretion during a part of embryonic life.

2. Early development of the hepatic tubules

In this account the structures which have been variously termed hepatic cords, trabeculae, cylinders and tubules will be spoken of as tubules as long as they remain as portions of simple or branching unanastomosed glands. The term hepatic cylinders will be employed for the same structures after the process of anastomosis has taken place.

The exact time when the anlagen of the hepatic tubules first make their appearance is somewhat variable. In general they are first to be seen in embryos from 7.5 to 9 mm. in length, being somewhat younger than Balfour's stage K[2] and corresponding to numbers 22 and 23 of the Normal plate series. Such embryos have from fifty to sixty-five segments and four or five pairs of gill pouches of which the anterior three or four may open externally. The spiral valve is in the process of formation, making at this time one or two complete turns of the intestine and the vitelline duct is reduced to a short wide canal. The form of liver anlage at this stage is represented somewhat diagrammatically in figure 1. The organ consists of a ventral median pouch from the foregut just anterior to the vitelline duct. The anterior

[2] In correlating embryos with stages of Balfour's series only the general development of the embryo has been considered and not the state of development of the organ under discussion.

part of this pouch is full and rounded and may be termed the pars hepatica mediana. From the median pouch spring two large lateral pouches which form together the pars hepatica lateralis. In stages just preceding the appearance of the tubule anlagen the lateral pouches are smooth and globose and project outward almost at right angles to the median hepatic pouch. At the time when the tubules are formed, however, the lateral pouches are flattened transversely and have entered upon a pronounced dorsal growth. Connected with the liver pouch above and in front and with the anterior wall of the yolk stalk behind is a small thick walled sac, the anlage of the gall bladder. The hepatic tubules take

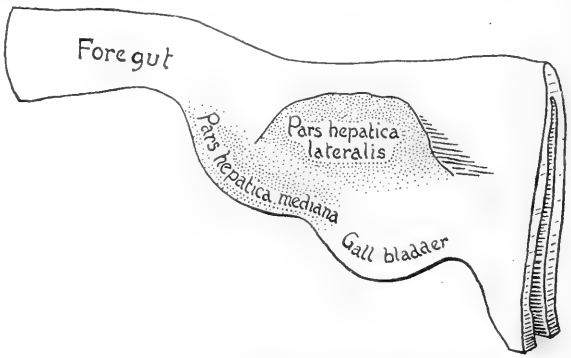


Fig. 1 Lateral view of a semi-diagrammatic reconstruction of the foregut and liver of an Acanthias embryo 9 mm. long. The areas represented in stipple give rise to hepatic tubules.

origin entirely from the pars hepatica mediana and the pars hepatica lateralis. These areas are indicated in stipple in figure 1.

The tubule anlagen arise in two forms: as slight longitudinal ridges upon the lower part of the outer surface of the lateral pouches and as very small irregularities of the dorsal margins of the same structures. When first observed the longitudinal ridges are two to four in number on either side. They extend almost the entire length of the lateral pouches and are distinctly separated by shallow lateral furrows. Sometimes these ridges may be subdivided longitudinally at their ends. Figure 17 is a view from the left side and below of the liver of an embryo 7.5

mm. in length (S.C. 14).[3] This specimen bears two ridges on the left hepatic pouch, but the right pouch is entirely smooth. The irregularities of the margin of the hepatic pouches, which also form tubules, are at first so ill defined as to be scarcely noticeable unless reconstructed. Then it is seen that the formerly straight dorsal margin has a wavy contour.

The tubule ridges rapidly increase both in size and number. New ridges appear ventral and mesial to the earlier ones and extend to the base of the lateral pouches and upon the ventral surface of the pars hepatica medialis of the median liver pouch. No new ridges appear dorsal to the first ones and I think that no new ridges arise between older ones. By the time the embryo reaches a length of 10 to 12 mm. each lateral pouch wall and lateral half of the pars hepatica medialis bears seven to ten tubule ridges. This is the total number formed on either side, and when tubules thereafter arise from the pouch directly, they do so as individual tubules and not in the form of tubule ridges. Figure 18 is a view from the left side and below of a reconstruction of the liver of an Acanthias embryo 10 mm. in length (S.C. 20) showing the later form of the tubule ridges and the beginning of the differentiation of tubules from them.

Before all the tubule ridges are formed the ones which first develop are broken up by transverse or oblique furrows into rather irregular rows of low mound like elevations. These elevations are the anlagen of the individual hepatic tubules and may be seen in figure 18 referred to above. They are semicircular or nearly so in cross section and nearly twice as long as broad, their greater length being always directed antero-posteriorly. Almost immediately the tubules begin an active outward growth and each is differentiated into a distal extremity which often is large and pouch like, and a more slender proximal stalk which is connected with the hepatic pouch and which is circular in cross section. The further growth of the tubules takes place by the formation of tubules of the second order from the distal

[3] In designating embryos the following abbreviations will be employed: H.E.C., Harvard Embryological Collection; K.U.E.C., Embryological Collection, Department of Zoology, University of Kansas; S.C., author's collection.

expansions of the primary ones. The secondary tubules are short conical projections rarely over one and one-half times as long as their greatest diameter. They arise from the sides of the distal expansion and almost always extend out at right angles to the axis of the primary tubule. Those primary tubules which arise from the sides of the hepatic pouches and are packed in among their fellows commonly assume a T-shaped form with the limbs of the T directed antero-posteriorly. The primary tubules which arise from the margins of the hepatic pouches become somewhat larger than those formed from the tubule ridges and give rise to from three to seven secondary tubules from their expanded distal chambers. These secondary branches may again subdivide into branches of the third order and upon these in turn there may occasionally be found nipple-like projections which represent the fourth order of tubules. In the large majority of cases, however, anastomosis takes place before tubules of the fourth or even the third order are formed. Figures 19 and 20 are of wax reconstructions of tubules from the lateral hepatic pouch of an embryo 13.3 mm. in length (S.C. No. 18). In figure 19 one sees the beginning of the outpouching of the distal expansion into secondary tubules. These structures are well marked projections in the older tubule shown in figure 20. Figures 22 and 23 are two views of a hepatic tubule from the dorsal margin of the left hepatic pouch of an embryo 15 mm. long (H.E.C. 227). The latter figure shows tubules of the fourth order. Figure 21 is a wax reconstruction of two young primary tubules from the ventral surface of the pars hepatica medialis of the same 15 mm. embryo. One of these shows the beginning of tubules of the second order.

When the tubule ridges first appear on the hepatic pouches they are due to the increased thickness of the epithelium in these places and not to an evagination of the pouch wall. In cross sections of the ridges (see fig. 28, a cross section of the lateral wall of the left hepatic pouch of an embryo of 8 mm. K.U.E.C. 542) it is noticeable that the nuclei which in other parts of the wall are arranged in two interlocking rows tend to be reduced

to a single row which lies near the external surface of the epithelium. There seems to be but a single layer of very high columnar cells at this point but the cell boundaries are not very clear. There is always a broad clear zone of cytoplasm towards the lumen of the pouch opposite the tubule ridge. Mitotic figures do not occur in the ridges but are frequent in the zones of epithelium between them. Following the formation of the ridge there appears a very shallow trench on the internal surface of the epithelium. The lumina of the individual tubules appear as the tubules themselves are differentiated through the breaking up of the ridges into rounded anlagen. One then finds in each tubule anlage a narrow slit-like cleft passing between the cell walls at right angles to the pouch cavity. This is shown in figure 29. Thus at first the tubule cells do not lie at right angles to the tubule lumen but nearly parallel to it. Later the cells assume an oblique position (see again fig. 29) and finally come to lie in the typical radiating position in relation to the lumen (fig. 30). As the tubules develop the cells become shorter and their nuclei change from elongately ovoid to nearly spherical bodies.

3. The anastomosis of the hepatic tubules

The liver is transformed from a gland of the branching to one of the reticular type by the anastomosis of its end pieces. The process begins in Acanthias in embryos from 12 to 15 mm. in length. Such embryos have from 70 to 85 pairs of somites and correspond roughly with Balfour's stages L and K and with Nos. 25–26 of the Normal plate series. The tubules arising from the dorsal portions of the lateral liver pouches precede in their differentiation those of other regions and it is generally among these dorsally placed tubules that anastomoses are first found. Later the tubules of the lower parts of the lateral pouches and finally those of the pars hepatica medialis enter upon the process. Variants from this general plan of procedure are not uncommon. Figure 2, of a sagittal section of the left hepatic pouch of an embryo 14 mm. long (S.C. 30) shows how general anastomoses are when once they begin in a given region.

No particular degree of differentiation seems necessary before a tubule takes part in an anastomosis, and when this process begins in any region both branched and simple tubules fuse indifferently. The commonest form of anastomosis is that established by the end to end fusion of tubules, but tubules may

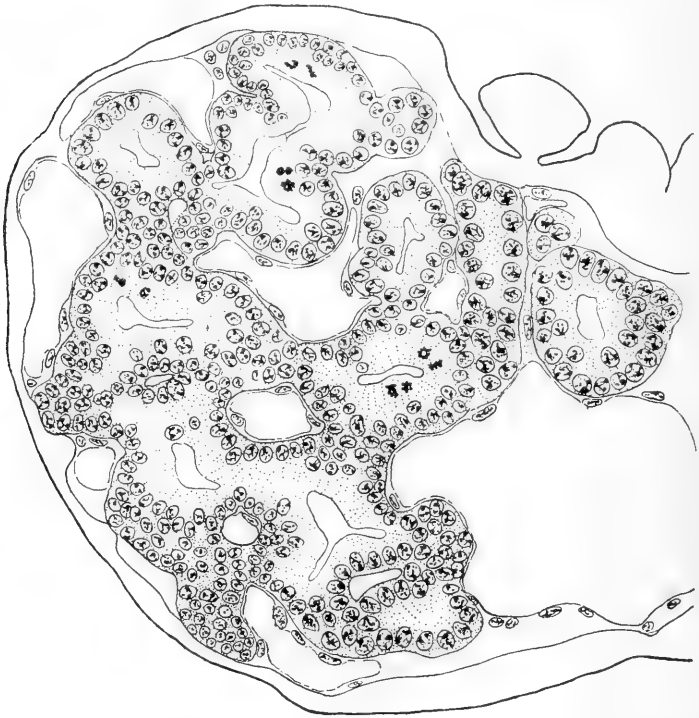


Fig. 2 Sagittal section of the anastomosing tubules of the left hepatic pouch of an Acanthias embryo 14 mm. long (S.C. 30.) × 100.

also join end to side or side to side. The last type is much commoner in later stages when more opportunities are offered for this form of contact. Anastomoses also seem to take place with equal frequency between tubules formerly quite separate or between minor branches of the same tubule when it is possible for such branches to come in contact.

Figure 24 is of a wax reconstruction of three anastomosing tubules from the left hepatic pouch of an embryo 15 mm. in length (H.E.C. 227). The pouch wall from which the tubules spring is cut away squarely around the base of each tubule. Tubules *A* and *B* are completely anastomosed end to end but there is still a distinct constriction at the plane where they have joined and the lumen within is considerably narrower than in the bodies of the tubules. Although not clearly shown in the figure both *A* and *B* are T-shaped and in each case the other tip of the tubule ends freely without anastomosis. Tubule *C* is a compound one and the subdivision which passes over to join with *B* is a branch of the third order. There is as yet no actual anastomosis between *C* and *B*, but the epithelial walls of the two are in direct contact.

The lack of any account of the histology of anastomosis seems to warrant a rather full description of the process here. The process may be rather arbitrarily divided into three steps or stages. In the first step the tubules come in actual contact; in the second there occurs the fusion of their walls and a rearrangement of the cells forming them, and in the third there is an establishment of a connecting channel between the two original lumina.

These stages of anastomosis can be observed in the liver of Acanthias embryos of any length from 13 to 45 mm. They are more easily followed, however, in young specimens. The figures which are used here to illustrate the process have been taken from embryos under 20 mm. in length. In each case the sections have been followed through and the tubules involved have been reconstructed to make sure that the picture presented was not due to an oblique plane of section or a misinterpretation of a segment of a single complicated tubule.

Early stages of contact of the anastomosing tubules are illustrated by figure 30 which is a transverse section of the left hepatic pouch of an embryo 13.3 mm. long (S.C. 18). Numerous tubules extend outward from the lateral wall of the hepatic pouch. Of these the larger number are still in the form of simple tubules which are expanded distally but a few have entered upon anasto-

mosis. The blood vessels pass among the tubules covering them in part with a thin film of endothelium which is interrupted at some places by the attachment of strands of mesenchyma and at others by the apposition of the distal surfaces of tubules with the insheathing layer of splanchnic mesothelium. Whether this endothelial covering forms an absolutely continuous partition without fenestra between the blood and the tubule epithelium cannot be definitely determined without injected specimens, but there seem to be places where the separation is not complete. Anastomosis is inaugurated by the contact of the involved tubules. At first a few mesenchymal or endothelial cells may separate the tubules but these are apparently pressed to either side so that the entodermal cells soon lie in actual contact. Often at first only four or five tubule cells actually meet, but shortly there is formed an area of contact which generally is not quite so large as the caliber of either tubule involved. Sometimes the cells of one tubule indent the wall of the consort but this is not the common rule. There are no basement membranes about the hepatic tubules, but the basal margins of the cells seem a little thickened so that for a time after contact the line between the cells of the two anastomosing tubules is still distinct. With the disappearance of this line the tubules may be considered as fairly fused.

The connection between the two fused tubules is often drawn out a little forming a short stalk or bridge between them. This will be termed here the connecting stalk. An indentation upon the external surface of this stalk indicates the line of fusion of the tubules. The connecting stalk, when present, is generally a little less in diameter than are either of the tubules and the boundaries of the cells forming it are not clear. One can follow the cells, however, by the position of their nuclei. Although at first sight the nuclei appear scattered without order in the stalk or at the point of anastomosis, a little study enables one to determine with some accuracy which nuclei are contributed by each tubule. The two rows of nuclei approach one another and may interlock but their radial arrangement in regard to the lumina of the two original tubules is not lost at first. They then pass to one side or the other of the connecting stalk, leaving a clear cytoplasmic

core in which cell boundaries are faintly distinguishable. In so doing the axes of the nuclei rotate through an angle of 90° so that instead of being parallel to the long axis of the connecting stalk as at first, they are now at right angles to it. Such rotation can be determined, of course, only in young tubules where the nuclei are oval and not circular in section. Figure 26, drawn from an embryo 19 mm. in length (S.C. 3), shows an early step in these changes. Figure 3 A is a graphic reconstruction of the tubules shown in cross section in figure 26. In this section the rows of nuclei belonging to the two tubules involved are distinguishable although the process of migration of the nuclei to the sides of the connecting piece is clearly under way. In figure 36 of a later stage from an embryo 14 mm. in length (S.C. 30), all the nuclei with the exception of one have passed to the sides of the connecting stalk.

In following the course of the nuclei in tubule anastomosis one is but tracing the movements of the cells in which they are contained, for it is hardly to be considered that the nuclei shift their axes within the cells, and moreover the few faint cell boundaries which may be made out show the same changes in position as do the nuclei. The hepatic cells of the connecting stalk have shifted through an arc of about 90° and when a lumen is established through the center of the connecting stalk it is bounded at least in greater part by the same cell surfaces which were presented to the lumina in the original tubules. In other words, while the tubule cells involved in anastomosis have shifted in position, their surfaces and their axes will bear the same relation to the new lumen which they did to the former one. Their long axes will be at right angles to the lumen while the inner and outer surfaces of the cell remain constant in both the original position in the simple tubule and the later position in the anastomotic segment. The polarity of the cell, in the sense of the term as used by Rabl ('88, '90), is not disturbed by anastomosis.

The lumen of the anastomosis is formed by clefts which extend out from the lumina of the formerly simple tubules. These clefts are at first small and irregular. They pass between the rather irregular borders of the radially arranged cells of the con-

necting stalk or plate, and, meeting, become confluent. It is not until some time after these clefts have joined that the lumen of the anastomosis acquires its full size and regularity. A late stage in the establishment of this connecting channel is shown in figure 27 from an embryo 19 mm. long (S.C. 3). Here the clefts are still separated by a single cell. Figure 3 B is a graphic reconstruction of the tubules involved in this anastomosis.

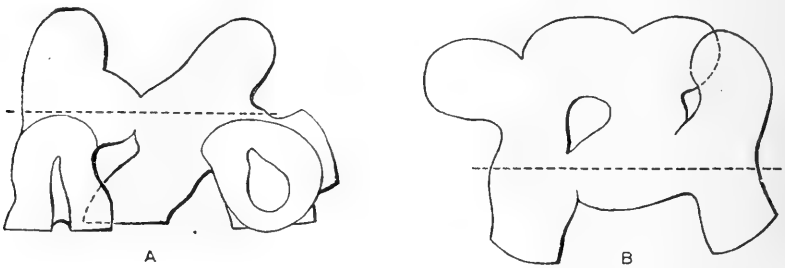


Fig. 3 Graphic reconstructions of anastomosing hepatic tubules in Acanthias. Detailed drawings of the sections indicated with dotted lines will be found in plate 2. *A*, tubules from an embryo 19 mm. long (S.C. 3) (see fig. 26). *B*, tubules from an embryo 19 mm. long (S.C. 2) (see fig. 27). × 100.

4. Later history of the hepatic cylinders

a. Growth of the hepatic network. Immediately after anastomosis has occurred the liver increases in size very rapidly. At first this growth is due almost entirely to the increase in number of the hepatic cylinders, but later the greater portion is brought about by the tremendous enlargement of the hepatic sinusoids. After anastomosis new cylinders are added to the existing network in three ways: by the formation of tubules from the remains of the hepatic pouches, by the formation of blind sprouts or buds from the sides of cylinders forming the network and by the production of new cylinders at the periphery of the network from the cylinders located there. These methods of addition to the hepatic network cease in the order given and the proportional amount contributed to the network by the several methods is in inverse order to that in which they are listed above.

The direct production of new hepatic cylinders from the hepatic pouches contributes but little to the bulk of the hepatic network and continues for only a short period after anastomoses are formed. As described in section 2, there are numerous tubules which arise at the bases of or between tubules which have previously sprung from the hepatic pouches. These younger tubules are at first small nipple shaped elevations arising singly and not from ridges. So long as the earlier tubules remain simple the later tubules follow them closely in their development, but when anastomoses become common among the older tubules and the sinusoidal circulation is well established, the younger tubules anastomose almost immediately after their formation either with one another or with cylinders of the already established network. In later stages hepatic tubules may sometimes arise as loops, the ends of which are attached to the wall of the hepatic pouch. By the time the embryo reaches a length of approximately 20 mm. (Normal plate Nos. 27–28, Balfour's stage N) the hepatic pouches are transformed into veritable hepatic ducts and thereafter no new hepatic tubules arise from them.

Hepatic cylinders, as has been seen, give rise to secondary buds while still in the form of single tubules, and this budding process continues long after anastomosis. The process can be most clearly demonstrated by the means of thick sections of which figure 41 from an embryo 24 mm. in length is an example.

[4] The method of preparation of the thick sections used in this study was as follows: The embryo was infiltrated with celloidin and cemented to a piece of infiltrated spleen or liver which in turn was fastened to a fiber block. The specimen was then cleared by Gilson's method, which makes the block almost transparent. The block was then placed in the microtome clamp and a strong beam of light from a condenser directed upon the object. A Greenough binocular microscope equipped with low power lenses was set up over the object. With this arrangement it was possible to follow in detail the process of section cutting. Sections were then carefully cut away until the exact region desired was reached. By focusing with the binocular it was possible to determine the approximate thickness of the section needed to just include the desired structures, and this section was then removed with a single cut. Sections made in this way are often superior to reconstructions for the study of the form of very small structures. They are best stained in a very dilute carmin solution and cleared for a long period in cedar oil, after which they can be observed with the binocular microscope, or better with the aid of an Abby binocular eyepiece.

In the area of approximately 1 mm. represented here there are five blind buds projecting from the cylinders into the sinusoids. This method of addition to the network continues for a considerable period. I have found no traces of new buds in the body of the liver after the great increase in size of the cylinders when the embryo reaches a length of about 40 mm. However, in the portions of the liver which are the last to be formed, i.e., the dorsal margins and the posterior tips of the lateral lobes, this method of cylinder formation continues until the embryo reaches a length of 50 to 60 mm.

While in the earlier stages of the development of the hepatic network the increase comes perhaps equally from peripheral and interstitial growth, in later stages the latter method is by far the more important. The hepatic network terminates peripherally in a large number of blind knobs which by their growth and division give rise to a large amount of hepatic tissue. The cells of these terminal knobs remain in a comparatively undiffentiated condition while those of the more central part of the network are undergoing rapid changes in structure. Figure 43 shows a small portion of the tip of the lateral lobe of an embryo 20 mm. long. The mesothelium covering the liver has been removed. Here the terminal knobs are seen projecting from the general network and are often attached to the mesothelial sheath by strands of mesenchyma. This specimen was prepared by cutting a thick celloidin section of the region desired by the method already described. The celloidin was then dissolved away and the mesothelial covering stripped from the fragment with fine forceps.

The peripheral addition to the hepatic network takes place at first over the entire surface of the liver. But like the interstitial method of addition it is later limited to the tips of the lateral lobes and to the dorsal margins of both the body and lateral lobes of the liver which at a comparatively late stage grow rapidly upward between the stomach and the lateral body walls. In embryos 60 mm. in length these areas are much restricted and they cannot be seen in an embryo 80 mm. long.

In the rapidly growing parts of the liver the cylinders often terminate peripherally in expanded end-bulbs or vesicles which contain a lumen many times the diameter of that of the typical cylinder. Such vesicles are found in embryos from 25 to 40 mm. in length but not in older specimens. Their walls consist of a low columnar or cuboidal epithelium. Figure 4 is a cross section of one of these structures from the lateral lobe of an embryo 36.6 mm. long (S.C. 10). The significance of these vesicles is unknown to me.

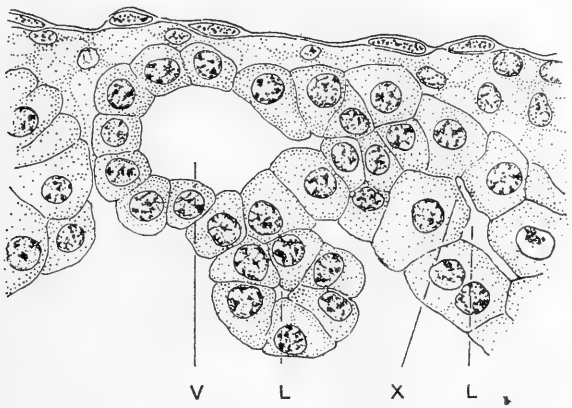


Fig. 4 Terminal vesicle of a hepatic cylinder from the lateral lobe of an Acanthias embryo 36.6 mm. long (S.C. 10). × 400. *L*, lumen of hepatic cylinder; *V*, terminal vesicle; *X*, side branch from central lumen of cylinder.

b. Changes in the size and structure of the hepatic cylinders. When the hepatic tubules enter upon anastomoses they are irregular in form and of variable caliber. The lumina of the tubules are large and irregular and are generally surrounded in cross section by 14 or 15 cells, if one may judge from the number of nuclei present, for cell boundaries are often indistinct at this time.

Table 1 shows some of the changes which take place in the course of the later development of the cylinders. The measurements and cell counts given in this table are in each case averages determined from twenty fair cross sections of cylinders of the

posterior part of the median lobe of the liver. The cross sections of cylinders were taken at random from this region, except that those lying in the rapidly growing peripheral zone were avoided in each case.

In the first specimen of the series the number of cells bounding the lumen in cross section averages 12.5. The diameter of the tubules has become more uniform and averages 58 micra. Examples of such tubules are illustrated in figure 31. At about this time the liver begins to increase greatly in size. This growth is due in part to the actual increase in number of hepatic cells, as

TABLE 1

Measurements of the hepatic cylinders in Acanthias embryos

LENGTH OF EMBRYO IN MM.	AVERAGE NUMBER OF CELLS IN CROSS SECTION OF TUBULE	AVERAGE DIAMETER OF TUBULES IN MICRA
15.0	12.5	58.0
20.6	10.0	50.0
25.0	8.0	37.6
28.0	7.0	35.6
33.0	6.6	37.0
37.0	6.0	38.5
47.0	5.2	40.0
50.0	4.6	46.0
60.0	5.3	39.0
80.0	5.0	43.0
95.0	3.7	54.0

is indicated by the presence of numerous mitotic figures in the hepatic cylinders, but a much greater part of the increment is due to the establishment of the huge hepatic sinusoids. With this increase in size of the sinusoids the tubules are distinctly reduced in size, their average diameter dropping from 58 micra in an embryo 15 mm. in length to 37.6 micra in one 25 mm. and 35.6, in one 28 mm. in length. This reduction in diameter may be due somewhat to the decrease in the size of the lumen, but is caused mainly by the actual decrease in the number of cells surrounding the lumen at any one plane. The process is a continuous one after anastomosis is established, but is more rapid

at first, dropping from an average of 12.5 in an embryo of 15 mm. to 7 in one of 28 mm. I think that this rapid and early reduction is due to the stretching of the tubules caused by the dilatation of the sinusoids among them. The tubules are attached to each other and to the mesothelial wall of the liver by strands of mesenchyma which may aid in the process by pulling upon the cylinders as the mesothelial wall is rapidly stretched in all directions. These mesenchymal strands can be seen in figure 43 of the liver of an embryo 20 mm. long.

After the embryo reaches a length of from 28 to 30 mm. the hepatic cylinders again begin to increase in size. They rise rapidly in diameter from an average of 35.6 micra in an embryo 28 mm. long to 46 micra in an embryo of 50 mm. and 54 micra in an embryo of 95 mm. This growth is still noticeable in an embryo of 220 mm. in which, however, the cells were too collapsed to admit of accurate measurement. While the diameter of the tubules increases, the size of the lumen and the number of cells surrounding it at any given plane as steadily decreases. Thus the average number of cells seen in cross section of a cylinder at 25 mm. is 8, at 50 mm. 4.6, and at 95 mm. 3.7. This indicates that the increase in size of the cylinder is due to the growth of the individual cells forming it and not to their multiplication, as is the early increase in size found in embryos from 13 to 16 mm. long.

Almost all this growth is due to the deposition of fat in the hepatic cells, the nuclei of which remain stationary or actually decrease in size. While the cells begin to increase in size even when the cylinders are becoming reduced in caliber, due to factors already mentioned, this growth is not sufficient to make up for the reduction until the embryo reaches a length of more than 30 mm.

The thick sections illustrated in figures 40, 41 and 42 show graphically some of the changes just described. Figure 40 is a section of a liver in which the process of anastomosis is well under way. It shows the large size and irregular caliber of both the cylinders and their lumina. Figure 41 is from a specimen in which the hepatic sinusoids have nearly reached their highest

development and in which the cylinders are reduced to slender tubes. Figure 42 is from a somewhat older specimen in which the cylinders have again begun to increase in diameter. The changes in the number of cells surrounding the lumen of the cylinder in cross section are shown in the four figures forming plate 3.

My remarks upon the changes in the finer structure of the hepatic cells can be regarded as little more than notes made in the course of the general study of the growth of the cylinders.

During the development of the hepatic tubules and cylinders, the nuclei of the cells forming them are modified in shape, size and structure. At the time when the tubule ridges first appear upon the hepatic pouches the hepatic nuclei are elongately oval in outline, their longer axes averaging from 14 to 15 micra and their lesser axes from one-third to one-half of this length (fig. 28).[5] With the definite outpouching of the hepatic tubules the nuclei become broader and shorter, but their volume remains practically unchanged (figs. 29, 30). In an embryo 10 mm. in length (S.C. 20) from which tubules have been described and figured in the preceding part of this paper, the hepatic nuclei average 8 micra in diameter and 11 micra in length. Anastomosis has apparently no effect upon either the size or structure of the nuclei. In embryos from 10 to 20 mm. long one can follow the change in shape of the majority of the nuclei from broadly oval to spherical bodies. In a 20.6 mm. embryo (H.E.C. 1494, fig. 32) the great majority of the nuclei are spherical and have a diameter of 10 micra. Thereafter they gradually decrease in size even as the cells grow in size through an increase in fat content. In an embryo 47.3 mm. long (S.C. 11) the average diameter of the nuclei is 7.5 micra and in one of 95 mm. (H.E.C. 1882) it is little if any less. As the fat accumulates in the liver cells the nuclei may again change in shape, being in many cases pressed against the margin of the cell and assuming an oval or crescentic outline suggesting the form of the nuclei found in true fat cells. The nuclei which remain spherical have an average diameter of 7 micra or a little less (in embryos 200–240 mm. long).

[5] All measurements given here were made from paraffin sections.

In early stages the nucleus is always in the basal portion of the cell. After anastomosis they are found more centrally located. In later stages they are found scattered, sometimes in the center of the cell surrounded with a film of protoplasm from which threads extend to the cell periphery; often close to the lumen wall of the cell and sometimes near some other point in its periphery.

Before the tubule anlagen appear the hepatic nuclei contain one, two or three large masses of chromatin which surround nucleoli and are generally applied closely to the nucleus wall. These chromatin masses are round or oval in shape and smooth in outline except for one or two small chromatin threads which may extend outward from each mass. The remaining space of the nucleus is filled with a clear nuclear sap through which run a few delicate and faintly staining fibrils. Such nuclei are characteristic of the young Acanthias embryo being found also in the cells of the mesenchyma, mesothelium, walls of the medullary canal and in the mesonephric tubules and duct. Those of the mesenchyma have been fully described by McGill ('10) in a study of the development of the striated muscle of the oesophagus in the dogfish. The figures in Neal's ('14) recent work illustrate the similar structure of the nuclei in the nervous system. Figure 28 shows the structure of a number of these nuclei stained with iron-hematoxylin. As the hepatic cells are differentiated the chromatin masses above mentioned become more irregular and there are given off from them a number of chromatin strands which eventually form a coarse network. The chromatin masses are reduced in size and may become detached from the nuclear wall. They also may be somewhat broken up and present a granular appearance. On the other hand, the chromatin threads which have originated from them may fuse forming secondary and generally smaller karyosomes which do not surround nucleoli. These changes are shown in figures 31 to 34, 38 and 39. The adult hepatic nucleus is rich in chromatin which is arranged in a coarse network containing several karyosomes some of which are probably the remains of the original ones and some of which are the result of secondary aggregation of chromatin granules from the chromatin threads.

Most of these karyosomes are applied to the nuclear wall. The hepatic cell retains the typical embryonic nuclear structure much longer than do the cells of the mesenchyma, mesothelium, nervous system or urogenital system. This typical embryonic structure is lost first in the cells forming the gall bladder and major hepatic ducts, next in the minor hepatic ducts which are formed from cylinders already well started upon a development towards typical hepatic parenchyma, and finally from the hepatic cells proper.

As has been remarked, fat droplets as indicated by vacuoles in the protoplasm of the hepatic cells appear, when the embryos obtain a length of about 25 mm. The use of special reagents would no doubt demonstrate the presence of fat prior to this stage. The droplets are found at first at the base of the cell, but later, in embryos 65 to 95 mm. long, droplets are found scattered through the entire cell body reducing the protoplasm to the network which has been described for the adult hepatic cell of selachians by Shore and Jones ('89) and Pilliet ('90).

In Acanthias embryos about 30 mm. long the gall bladder begins to press against the hepatic tissue which lies on either side and above it. This process is probably brought about by the great growth of the internal yolk-sac which lies below the gall bladder. This pressure of the gall bladder causes some degeneration of the hepatic tissue immediately surrounding it. Toldt and Zuckerkandl ('78) have described a similar process in the human embryo.

5. Development of the hepatic tubules in other forms of selachians

The development of the hepatic tubules in Torpedo, Raia and Mustelus differs somewhat from that of Acanthias. In these forms the lateral hepatic pouches do not reach the great development found in Acanthias and tubules are formed from these structures at a comparatively earlier stage. The omphalo-mesenteric veins are somewhat larger than in Acanthias and at the time when the individual hepatic tubules develop veinous channels are found both medial and lateral to the hepatic pouches.

The tubules which are formed from the dorsal parts of the pouches and of the pars hepatica medialis arise singly or in small clusters as do those in the same situation in Acanthias, but in the forms under discussion the dorsal or marginal tubules form a much greater part of the whole number produced than is the case in Acanthias. In Torpedo and in Mustelus the tubules from the ventral portion of the lateral pouches arise as in Acanthias from tubule ridges. These ridges are but slightly elevated and the corresponding grooves on the internal surfaces of the pouches are wide and shallow. The individual tubules which form from these ridges do not remain as mound-like structures arranged in rows but grow out almost at once and like the dorsal tubules appear as slender tubes extending outward between the veinous sprouts. In most cases the hepatic tubules which are first formed come in contact with the splanchnic mesothelium covering the liver.

The omphalo-mesenteric veins in these forms increase in size very rapidly and before tubule formation has progressed very far the anterior, ventral and lateral surfaces of the liver pouch are practically surrounded by a venous lake. All tubules which arise after this period project into this sinus and in their growth they push its endothelial wall before them. This process is illustrated in figure 44, which is of a thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The number of tubules arising directly from the pouch walls is small in these forms as compared with Acanthias for the surface area of the pouches is much reduced from the first. The large majority of the later tubules is formed by the branching of the earlier ones.

In Acanthias the hepatic tubules in the earlier period of their development are short, broad and pouch-like and the branches which arise from them are nipple-like projections. In the other forms studied both the primary and secondary tubules are slender elongated tubes which are early united in a complex branching and anastomosing network, the meshes of which are separated by sinusoids of large size. Figure 44 shows an anastomosis of a young hepatic tubule in Mustelus, and figure 45 of a similar

section of an embryo about 4 mm. longer of the same species shows the complete establishment of the hepatic network.

While the early tubules of Raia, Mustelus and Torpedo are smaller in cross-section than are those of Acanthias, the lumina of these tubules are considerably larger. The cells lining the lumina are cubical or low columnar in outline as compared with the high columnar type found in Acanthias. The nuclear structure is quite similar to that of the hepatic cells in Acanthias, the chromatin covered nucleoli described for that form being particularly prominent in Torpedo and Mustelus.

The later history of the cylinders is quite similar to that of Acanthias. The cylinders rapidly increase in diameter and the contained lumina become smaller. The increase in diameter of the cylinders is due, as in Acanthias, to the growth of the individual cells, and the number of cells about the lumen in any given section steadily decreases. The nuclear changes are similar to those found in Acanthias. In such specimens of Mustelus and Squatina as I have examined the fat contained in hepatic cells remains in discrete droplets instead of forming one large mass as is generally the case in Acanthias. The same is true to some extent of Torpedo (fig. 35).

Pilliet ('90) has described areas of comparatively undifferentiated cells in the adult selachian liver. These cells form the portions of the cylinders which lie about the hepatic-portal veins. They are of comparatively small size and contain centrally placed nuclei which stain deeply with alum-carmin. The fat content of the cells is less than of those cells located elsewhere, and particularly of those lying in the neighborhood of the hepatic veins. Pilliet regards these smaller cells as reserves or nests of young cells which, from their position near the nourishing vessels, contribute to the growth of the organ. Apparently he did not find them in all the specimens which he examined.

I have seen no evidence of a retardment of the differentiation of the cells near the hepatic-portal veins in Acanthias embryos, and if such occurs it must be at a late period in the development of the organ. There are numerous small cells in the immediate neighborhood of the hepatic-portal vessels, but they all form

portions of the terminal bile ducts, the development of which will be described in a later part of this paper. Such embryos of Torpedo as I have examined agree with Acanthias in lack of any definite nests or reserves of undifferentiated cells about the hepatic-portal veins. In well advanced Mustelus embryos, however, the hepatic cells which surround the larger branches of the hepatic-portal veins do remain somewhat smaller than those of the remainder of the liver and are not so completely charged with fat.

In summary it may be said that the chief differences between the two types of selachian liver, that represented by Acanthias and that by the several other forms mentioned, lies almost entirely in the earlier stages. These differences seem to be dependent on the difference in the size and arrangement of the omphalomesenteric veins at the time of the formation of the hepatic tubules.

DEVELOPMENT OF THE MINOR RAMI OF THE HEPATIC DUCTS

The formation of the minor rami of the hepatic ducts is closely associated with the history of the hepatic cylinders. In a preceding publication (Scammon '13) it was stated that all of the major and some of the minor rami of the hepatic ducts arise from the constricted bases of certain fairly definitely placed clusters of hepatic tubules. In following the history of these tubule clusters it was found that they become separated from the hepatic pouch from which they arise by a broad and rather indefinite peduncle which is at first hardly more than an extension of the pouch wall. Later this peduncle becomes constricted and elongated, forming a small branch from the pouch which by this time is transformed into a segment of the hepatic duct. This development begins before tubule anastomosis gets fairly under way and continues at the time when anastomoses are taking place. No new major rami arise as outpouchings after anastomosis is established and aside from the actual lengthening of already established rami, which is not great, all further growth of these structures takes place by the transformation of pre-existent hepatic tubules into ducts.

This illustrates well the two methods of hepatic duct formation. The first and more primitive type is that suggested by Minot ('93, p. 763) in which the duct is the result of a direct outpouching of the wall of the hepatic diverticulum. In the second and specialized type the duct is produced by the transformation of portions of the network of hepatic cylinders. Selachians clearly stand near the bottom of the scale in this phase of development. Here the ductus choledochus, the hepatic ducts and their major rami arise as outpouchings and only the minor rami and the most distal portions of the major ones are of trabecular origin. In ganoids, amphibians, reptiles and birds the ductus choledochus and the proximal part, at least, of the hepatic ducts are formed by outpouching and the remainder of the duct system from trabeculae. In the mammals apparently the ductus choledochus alone is the result of an outpouching, but further investigation may change this conception.

The differentiation of hepatic cylinders into bile ducts is very closely associated with their relations to the blood vessels. Bile ducts are only formed from cylinders which are in contact with the main trunks of the hepatic-portal veins or their larger and more definite branches. Still more striking is the fact that the side of the cylinder towards the vessels precedes in a very marked degree the differentiation of the opposite side and in fact in the smallest ducts the cells of the opposite side of the cylinder may never be transformed into duct epithelium at all but complete the ordinary development of true hepatic cells. Such a terminal duct from the liver of an embryo 95 mm. in length is shown in figure 37. On the other hand, of the many cylinders which closely surround the vascular trunk only a very small percentage is transformed into ducts. The development of the minor ducts is extremely small in proportion to the amount of hepatic parenchyma, smaller, I think, than in any other group of vertebrates. There is absolutely no indication of any system of intercalated ducts.

In the differentiation of a hepatic cylinder into a bile duct the former first approaches more nearly a perfect circle in cross

section and the lumen distinctly enlarges. The cells on the vascular side of the lumen are reduced in actual height but become more columnar in form because of the still greater reduction of the size of their bases. At the same time the nuclei which in hepatic cylinders are round or broadly oval in section and lie near the center of the cells become elongately oval in outline and tend to retreat to the bases of the cells. The cells are so diminished in size that the nuclei which increase little, if any, in bulk almost fill them. The nuclei lose their typical structure of a clear karyoplasm containing one or two large chromatin masses from which radiate chromatin threads and present instead a reticular chromatin network made up of evenly distributed granules of about the same size. The protoplasm becomes homogeneous and colors darkly with plasma stains. An example of such a developing duct at an early stage is shown in figure 39. Approximately one-third of the duct which abuts upon a blood vessel shows considerable progress in differentiation, while the cells of the segment opposite it are true hepatic cells. Between the two are zones of transitional cells. To the side of this duct is a smaller one in still an earlier stage of differentiation. The outline sketch shown in figure 5 illustrates the changes in shape of the hepatic cells at the time of duct formation.

The cells of the large hepatic ducts which are formed from the hepatic pouches and their evaginations show much the same steps in cytomorphosis as do those just described. The nuclei of the larger ducts, however, are oval at the start and so undergo no changes in form, but the change in chromatin arrangement is the same as in the minor ducts. In the gall bladder the same changes also take place but at a late stage (60–80 mm.) the nuclei again become circular in cross section and come to occupy the centers of the cells which are much elongated.

It is sometimes stated that the bile duct epithelium is formed of cells of a more primitive type than those of the cylinders or trabeculae. In the forms under discussion, however, the bile duct cells have departed farther from the embryonic type than the parencyhmal cells, if we may judge by their nuclear structure.

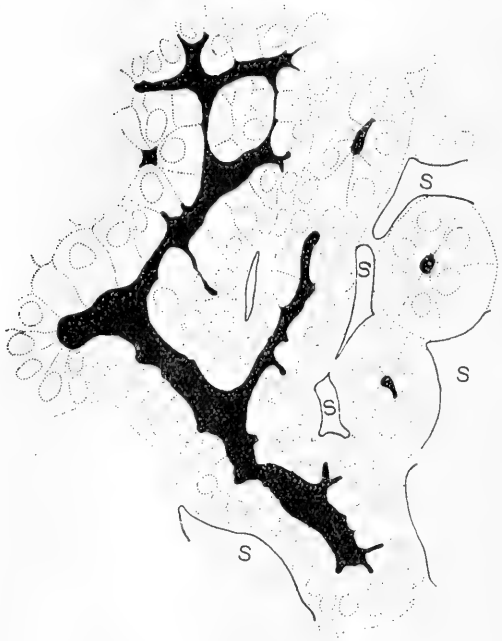


Fig. 5 Minor hepatic duct with connecting hepatic cylinders from the median lobe of the liver of an Acanthias embryo 37 mm. long (H.E.C. 353). × 400. Lumina of hepatic duct and cylinders solid black; hepatic cells in stippled outline; sinusoids, *S*, in unbroken outline.

DEVELOPMENT OF THE HEPATIC MESENCHYMA

It is well known that the mesenchymal tissue of the selachian liver is extremely scanty in the embryo although fairly abundant around the blood vessels in the adult. No particular study has been made of its origin, but Minot ('01) in his study of sinusoids, and more recently Debeyre ('09) in following the development of the hepatic cylinders, have noted the occasional delamination of mesenchymal cells from the mesothelium covering the liver and the presence of such elements along the walls of the hepatic sinuses. Debeyre also figures a cross section of a small portion of the periphery of the liver of an Acanthias embryo 30 mm. long, which shows small spurs of mesenchymal tissue extending inward from the mesothelium between the hepatic

cylinders. The origin of the hepatic mesenchyma can be followed with little difficulty in Acanthias. Here it appears that this material is probably derived completely from the mesothelium but at two distinct periods and from two different regions, and that in both cases this proliferation is associated with distinct irregularities of splanchnic mesothelium.

The first mesenchymal proliferation appears at a little later time than the formation of the stroma of that portion of the gut which lies posterior to the liver. In Acanthias embryos 5 mm. in length the liver pouch is but slightly differentiated and consists of two shallow diverticula which lie in the lateral walls of the archenteron and are fused anteriorly to form the median hepatic pouch. The archenteron of this region is clothed on either side by a layer of splanchnic mesoderm which is continuous above with the radix mesenterica and below with the splanchnic layer of the blastoderm. At the radix this layer is much thickened but it is reduced to a moderately thin layer over the sides of the archenteron. The irregular endothelial walls of the omphalomesenteric veins intervene between the ventral part of the archenteron and the mesothelium, but no mesenchyma is present. Irregular processes from the mesothelium, however, do extend inward and in places it appears as though cells were about to be delaminated. A short time later the hepatic pouch increases much in size and extends anteriorly far in front of the anterior intestinal portal. With this change the ventral parts of the investing layers of splanchnic mesothelium are brought in contact and eventually fuse, thus forming for some time a ventral mesentery. In connection with this process there appear two distinct sets of mesothelial irregularities. These consist of the mesothelial villi on the right side and of numerous irregular folds on the left.

The mesothelial villi found in connection with the covering of the selachian liver were first described by Choronshitzky ('00), although they were observed in other forms long before that time. Hochstetter ('00) has given their later history in Acanthias in connection with his study of the formation of the septum transversum. As Hochstetter has stated, these structures are

found only on the right side. They appear in embryos of 45 to 30 segments as a thickened plate of the mesothelium overlying the right omphalo-mesenteric vein (fig. 6 A). This plate is soon thrown into a series of pouch like irregularities, and the spaces thus formed are filled with a delicate network of protoplasmic processes from the mesothelial cells. The cores of the villi are thus at no time really empty, and are soon occupied by delaminated mesenchymal cells. From the bases of these villi mesenchymal cells are proliferated apparently both from the walls and the mesenchymal core, and soon come to form a small mass on the right side lying just below the omphalo-mesenteric vein. On the opposite side the irregularities are not villi but longitudinal folds. Unlike villi they do not arise from a thickened plate, the mesothelium at this point remaining as thin as elsewhere at the time of formation. Afterward the cells become more columnar and a mass of mesenchyma, larger but similar in other respects to that of the opposite side, is proliferated and underlies the left omphalo-mesenteric vein. Figure 7 shows the position and extent of these two mesenchymal proliferations in an embryo 7.5 mm. long (H.E.C. 1496). Soon after this stage the ventral mesentery breaks down and the ventral surface of the liver is free throughout its extent. With this process there is a delamination of mesenchymal cells along the median ventral line which unites the lateral ones already described, and there is thus formed a general ventral bed of mesenchyma which, as growth proceeds, forms a coating about the gall bladder and constitutes a large loose-meshed mass which extends forward from the gall bladder to the anterior mesothelial wall of the liver.

This constitutes the first and ventral contribution of mesenchyma to the liver from its mesothelial envelope. In its entire extent it produced the mesenchyma which surrounds the gall bladder, and later the vessels adjacent to it, the covering of the cystic duct, and to an undeterminable degree the sparse mesenchymal tissue of the lower part of the hepatic parenchyma. The irregularities of the mesothelium on the right side do not continue for any great period. By the time the embryo reaches

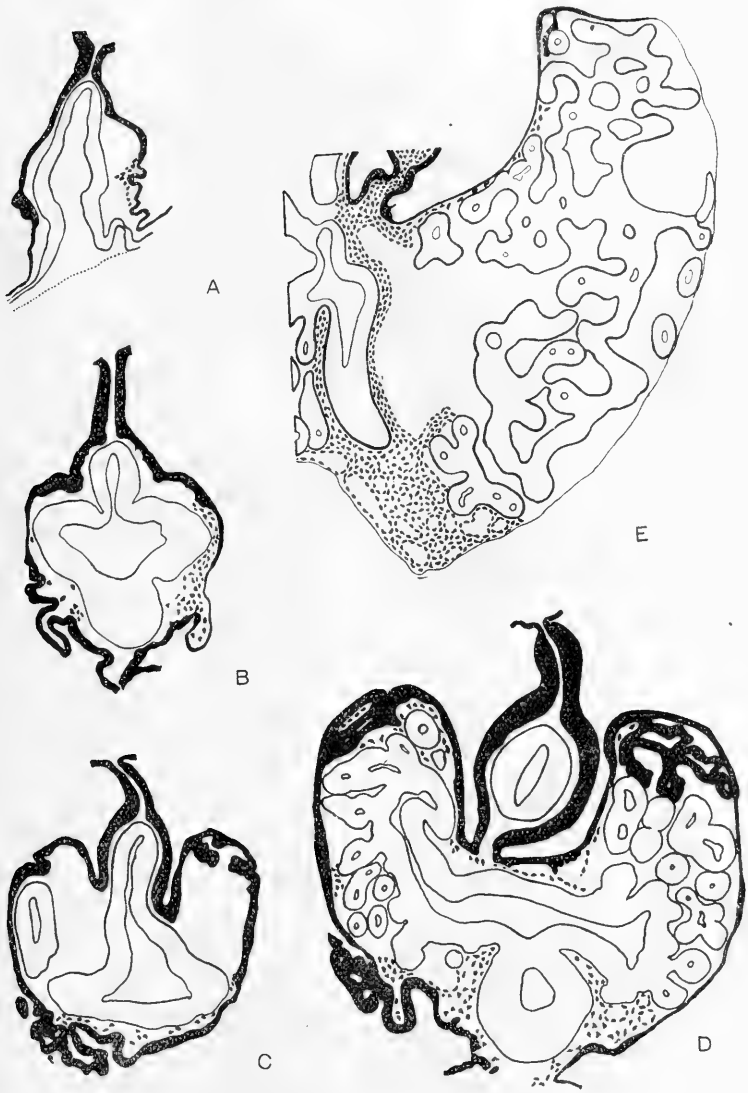


Fig. 6 A series of transverse sections of liver anlagen of Acanthias embryos of different ages. The mesothelium is represented in solid black, the mesenchyma in coarse stipple and the entodermal structures in outline; the blood-vessels are omitted. *A*, embryo 6.4 mm. long (S.C. 19); *B*, embryo 9 mm. long (H.E.C. 1495); *C*, embryo 10 mm. long (C.S. 20); *D*, embryo 15 mm. long (H.E.C. 1494); *E*, embryo 20.6 mm. long (H.E.C. 1494); all × 50.

a length of 15 mm. the mesothelium is smooth and much reduced in thickness. When the embryo reaches a length of 18 mm. the villi are reduced to small conical projections and are absent in embryos of 20 mm. Thereafter the mesothelium covering the ventral part of the liver on both sides becomes steadily reduced in thickness, and in embryos of 25 mm. and longer it is an exceedingly thin layer of squamous epithelium.

The second and dorsal mesenchymal proliferation begins at a later stage than does the ventral one. In an embryo 6.4 mm. in length (S.C. 19) (fig. 6 A) the dorsal portion of the mesothelium is seen to be slightly thinner than that of the ventral zone. As the lateral pouches of the liver are pushed upward, the omphalomesenteric veins come to lie mainly dorsal to these structures and the mesothelium over them is gradually thickened. This thickening is most noticeable at the lateral margins of the dorsal surface (fig 6 B). Soon after, in embryos of 10 to 12 mm., the mesothelium of this region becomes distinctly thickened in places and is invaginated forming tubules the walls of which are continuous with the splanchnic mesothelium and the lumina with the coelomic cavity (fig. 6 C and fig. 7). Often these tubules first appear as long trenches on the coelomic surface of the mesothelium. The sides of these trenches coalesce, thus roofing over the depression and forming tubules which open into the coelom at both ends. Other tubules grow in and end blindly. Examples of these early tubules are shown in figure 8. Although fairly regular at the start, these structures soon become irregular in caliber and form, and very often anastomose. Their lumina may be occluded in places thus forming mesothelial cysts which are generally connected by solid stalks with the covering mesothelium. At the time of their highest development the tubules form an anastomotic network which fills the dorsal fifth of the anterior part of the liver and they are then easily mistaken at first sight for true hepatic tubules (fig. 6 D). More careful study shows a number of differences between the two structures. Aside from the nuclear differences to be mentioned later, the mesothelial tubules are more irregular than the hepatic cylinders, their walls are thinner both actually and relatively as compared

to their lumina, the cells are more columnar and there is no definite external cuticula as there seems to be in the hepatic cells at this time. The cytoplasm of the cells is also more granular. The mesothelial tubules may extend downward and come

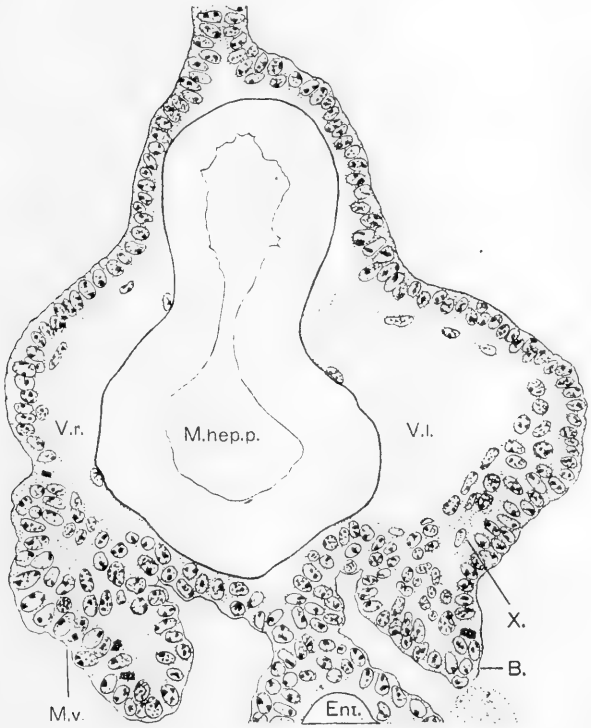
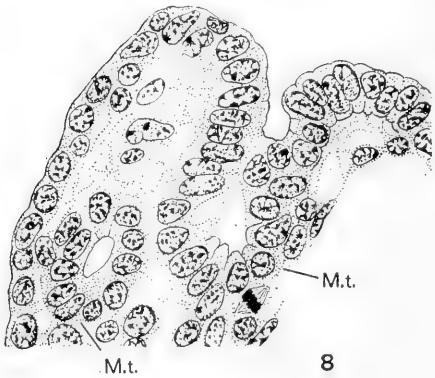


Fig. 7 Transverse section through the anterior part of the anlage of the liver of an Acanthias embryo 7.5 mm. long (H.E.C. 1496). × 400. *B*, tangential section of the mesothelial wall surrounding radicle of the left omphalo-mesenteric vein; *M.hep.p.*, median hepatic pouch; *M.v.*, mesothelial villus; *V.l.*, left omphalo mesenteric vein; *V.r.*, right omphalo-mesenteric vein; *X*, proliferated mesenchyma.

in contact with the hepatic cylinders, but generally they are replaced in this region by cords of mesenchymal cells. Figure 9 is a cross-section of a portion of the liver of an Acanthias embryo 15 mm. in length (H.E.C. 227) showing these tubules (*Mes.t.*) in the highest state of development.



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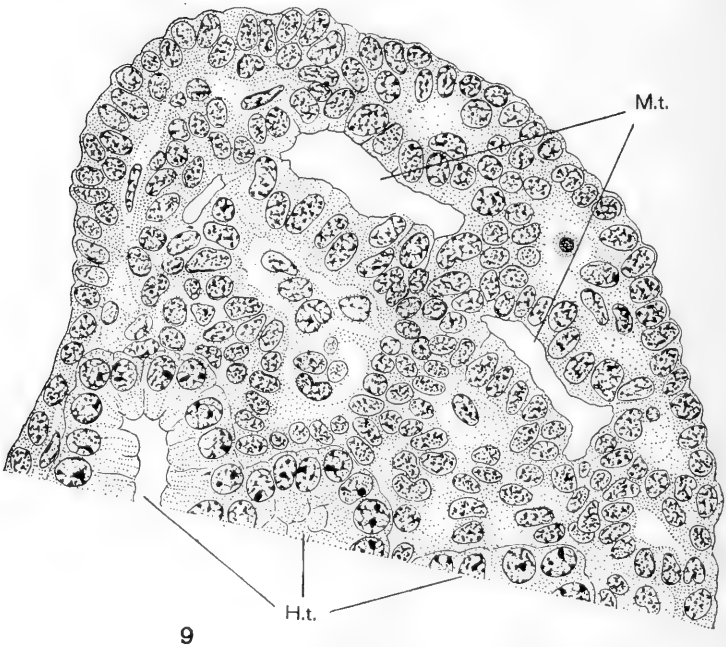

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Fig. 8 Transverse section of the mesothelial covering of the dorso-lateral margin of the liver of an Acanthias embryo 13.3 mm. long (S.C. 18). × 400. *M.t.*, mesothelial tubules.

Fig. 9 Transverse section of the dorsal portion of the liver of an Acanthias embryo 15 mm. long (H.E.C. 227). × 400. *H.t.*, hepatic tubules; *M.t.*, mesothelial tubules.

The mesothelial tubules do not remain long as such. The lumina are soon obliterated and the solid cords thus formed break up into mesenchymal strands. This process begins at the inner end of the cords and can be seen in figure 9. By the time the embryo reaches a length of 23 to 25 mm. the cords are entirely replaced by such mesenchymal strands which run in among the tubules which have now invaded this region (fig. 38). At 28 to 30 mm. even these strands have entirely disappeared and mesenchymal cells can be found only occasionally. The formation of mesenchyma by tubular ingrowths of mesothelium does not preclude the occasional delamination of single cells from the splanchnic mesoderm direct, but this delamination contributes but little to the total of the hepatic mesenchyma.

The nuclei of the splanchnic mesothelium in embryos 5 or 6 mm. in length, before mesenchymal delamination begins, are broadly oval in outline. They have the typical structure which is found in the nuclei of most of the tissues at this time. Each nucleus contains two or three large masses staining black with Heidenhain's hematoxylin. Each of these masses consists of a nucleolus surrounded by a thick layer of chromatin. The remainder of the nucleus is filled with a clear karyoplasm with a few faint strands of chromatin. These nuclei have been well described by McGill ('10) who studied the mesenchyma of the foregut region in the dogfish. As cells are delaminated from the mesothelium and as the mesothelial tubules are broken up into a mesenchymal syncytium, most of the nucleoli disappear and the chromatin is gradually scattered in a finely divided network. Similar changes take place in the nuclei of mesothelial cells but at a later period. A comparison of figures 7 and 38 will show the two extremes of this change. Mitotic figures are numerous in the mesothelium but scarce in the mesenchyma derived from it.

The relation of the mesothelial tubules to the sinusoids is of interest in the light of Bremer's ('14) recent work on the earliest blood vessels in man. Bremer found mesothelial cords from the extra-embryonic coelome connected with the angiocysts and solid cords of the vascular net in the body stalk, and he suggests that the elements of the vascular net arise from these ingrowths.

I have not found that the cavities in the mesothelial tubules or cysts of the selachian liver connect with the blood spaces. As the walls of these structures break up into mesenchymal strands it is impossible with ordinary section and staining methods to distinguish between them and the endothelium surrounding the adjoining sinusoids. This is particularly true if one tries to follow the mesenchymal strands which penetrate between the hepatic cylinders in later stages such as the one shown in figure 38. The mesenchymal and endothelial cells form free anastomoses. McGill ('10) apparently found the same condition in a much younger embryo, judging from figure 9 of her paper with which the section shown in my figure 7 is in agreement.

The reduction in the thickness of the mesothelial covering of the liver and the disappearance of the mesothelial villi is no doubt due in part to the great growth of the contents of the former, particularly of the hepatic cylinders and sinusoids. This increase takes place rather suddenly in embryos from 18 to 20 mm. in length and the reduction in the thickness of the mesothelium is to some extent coincident with it. It is noticeable, however, that in many cases when a hepatic cylinder comes in contact with the covering mesothelium the latter layer is distinctly reduced in thickness, its constituent cells change from a high columnar to a cuboidal form and the nuclei from oval to nearly spherical bodies. This change is probably not due to pressure for in places where two tubules come in contact with the mesothelium and are separated by a distance hardly equal to their own diameter the mesothelium becomes thin at the points of contact and remains thickened over the small intervening portion. Were the effect of tubule contact only a matter of pressure, one might expect some flattening of this intervening place as well. Also the mesothelium is not pushed outward at these points of contact but remains on a level with the surrounding surface.

One is tempted to suggest the homology of this dorsal mesenchyma-producing mass with the 'Vorleber' of Kölliker and His. But this similarity is only a superficial one as the mass just described does not appear until after the liver is well developed,

while in birds and mammals the 'Vorleber' is a precursor of that organ. Also the selachian mesenchymal mass lies in the dorsal and posterior part of the liver along its free upper surface, while the 'Vorleber' lies anteriorly and is broadly connected with the dorsal wall of the coelom. Ziegler ('88) showed long ago that the mesenchyma of the intestine in selachians arose from two longitudinal zones of proliferation from the splanchnic mesoderm. One of these zones lies above the archenteron and the other

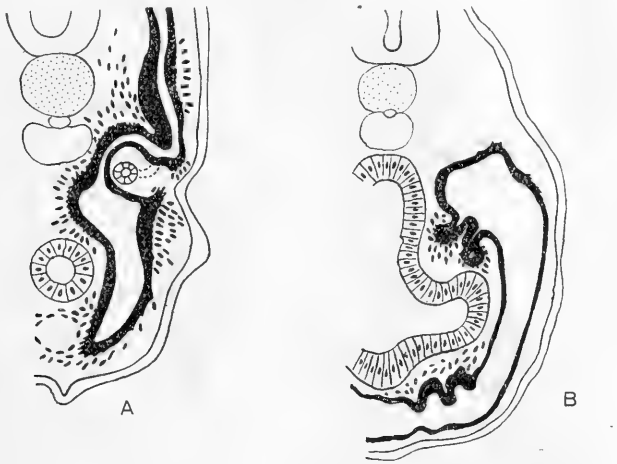

Fig. 10 A comparison of Ziegler's figure: *A*, of the mesenchymal tissue in the trunk region of a selachian embryo with a semi-diagrammatic figure; *B*, showing the origin of the mesenchyma in the hepatic region of an Acanthias embryo.

ventral to it. The method of development of the hepatic mesenchyma indicates that these dorsal and ventral zones of proliferation extend forward into the liver region as well and remain distinct for a considerable period even after the liver has separated from the gut above it. A comparison of a semi-diagrammatic figure of a cross-section through the hepatic region with Ziegler's diagram of a similar section through the middle of a trunk segment shows the essential agreement in the origin of the splanchnic mesenchyma in the two regions (fig. 10).

HISTORY OF THE HEPATIC SINUSOIDS

The sinusoids of the selachian liver are most striking objects and at one time form decidedly more than half of the entire bulk of the organ. Minot ('00) has given the only description of them which is at all complete as a part of his exposition of the nature of the sinusoidal circulation. Brief notes are also to be found in the papers of Holm ('97) and Debeyre ('09). The general development of the veins of the liver in these fishes has been studied by Rabl ('92), Hoffmann ('93) and Hochstetter ('93). The early development of the omphalo-mesenteric veins has been observed in detail by Mayer ('87) and by Ruckert ('88). Therefore I shall give only the briefest outline of the general history of the veins, and will begin with the conditions found in Acanthias embryos from 8 to 10 mm. in length. In such embryos there are paired omphalo-mesenteric veins extending forward to the sinus venosus on either side of the gut. The left vein is somewhat larger than the right. At first the right and left trunks are quite separate but soon they form two anastomoses, one posterior to the pancreas and the other, a little later, just posterior to the liver below the foregut. From the latter anastomosis the veins pass forward on either side of the foregut and medial to the lateral hepatic pouches until they reach the anterior end of the liver where they join and form the sinus venosus. It is important to note that in Acanthias in early stages only the medial surfaces of the lateral pouches are in contact with vascular channels. There are no vascular channels between the lateral surfaces of the hepatic pouches and the mesothelium covering them.

Figure 11 A shows a reconstruction of a somewhat later stage in which important changes have taken place. Now only the left omphalo-mesenteric vein passes through the groove between foregut and lateral hepatic pouch to join the sinus venosus. The right vessel ends blindly anteriorly and is connected with the left by the large anastomosis which lies behind the liver. In the meantime either a single vessel or several vascular sprigs have grown back on either side from the sinus venosus and passing

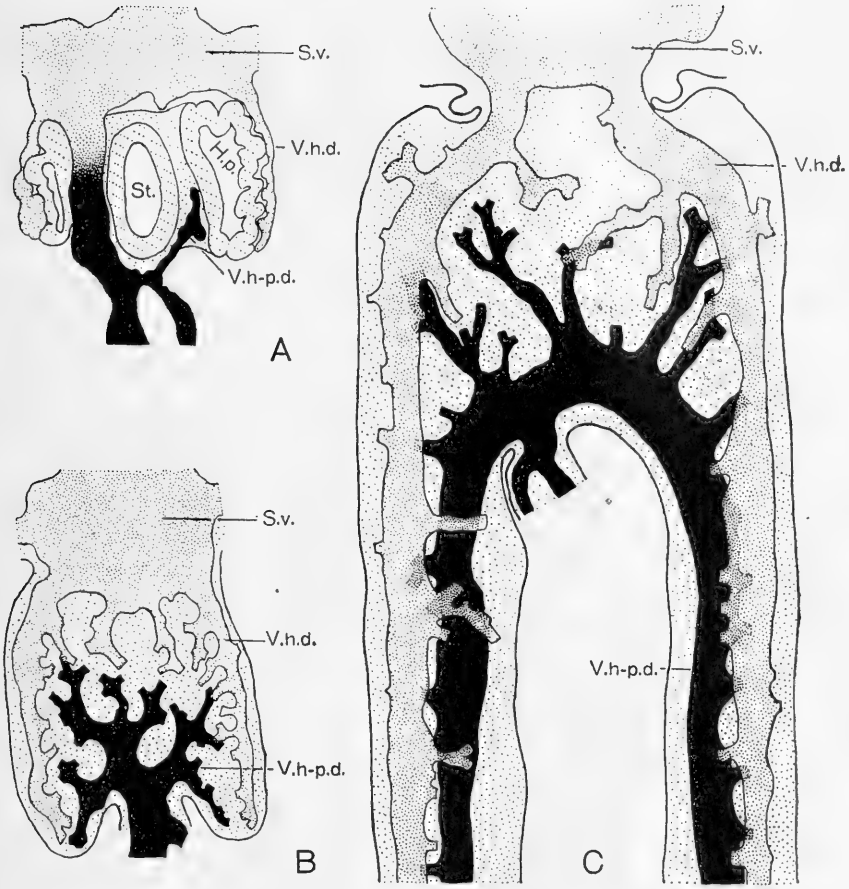

Fig. 11 Semi-diagrammatic graphic reconstructions (from frontal sections) of the liver veins, as seen from above, of three Acanthias embryos. The hepatic-portal veins are represented in solid black, the hepatic veins and sinus venosus in heavy stipple and the hepatic parenchyma in light stipple. In figure 12 A, the connection between the liver and the foregut and the upper parts of the lateral hepatic pouches are represented as cut away dorsally. *A*, embryo 15 mm. long (H.E.C. 230); *B*, embryo 20 mm. long (S.C. 31); *C*, embryo 41 mm. long (H.E.C. 371). *H.p.*, right lateral hepatic pouch; *St.*, stalk connecting liver pouch with foregut above; *S.v.*, sinus venosus; *V.h.d.*, right hepatic vein; *V.h.-p.d.*, right hepatic-portal vein.

along on the lateral surfaces of lateral hepatic pouches have formed a vascular plexus consisting of several large sinuses interrupted by small lacunae. In the drawing the omphalo-mesenteric veins, properly speaking, are represented in solid black and the sinus venosus and the veins developed from it in stipple. The posterior part of the omphalo-mesenteric veins may now be called 'hepatic-portal' veins, this being the term commonly used to designate them in the adult, while the veins which are efferent in their drainage will be designated by the term hepatic veins, which is the name applied to them in works on adult anatomy.

In the stage just described tubules have formed only on the lateral surfaces of the hepatic pouches. Soon thereafter tubule formation takes place with great rapidity along the margins of the pouches and from the pars hepatic medialis as well. The latter process results in breaking up the clear passageway of the blood along the left omphalo-mesenteric vein to the sinus venosus and in place of this one discrete passage there are formed a number of small irregular venous channels which pass from the posterior portions of the omphalo-mesenteric veins (hepatic-portal veins) in among the tubules and join the hepatic veins.

At this time there is a marked growth of the posterior lobes of the liver which brings about several changes in the position of the venous trunks. These may be seen by comparing figures 11 B and 11 C which are graphic reconstructions of embryos 18 mm. and 41 mm. long respectively. The branches of the early hepatic-portal vein which extend backward along the inner surface of the posterior lobes assume more and more importance, becoming in the later stage the posterior continuations of the main trunks of the vein. The former main trunks which extended forward are relatively reduced in size and appear as branches of these posterior vessels. At the same time the sinus venosus becomes more completely separated from the liver as the septum transversum is formed and the lateral hepatic efferent trunks converge towards the middle line. In so doing they take up the two larger venous radicles which formerly lay medial to them and represented the remains of the extreme anterior ends of the former omphalo-mesenteric veins. Thus these vessels, as may

be seen by comparing *B* and *C* of figure 11, become branches of the hepatic veins instead of direct tributaries of the sinus venosus. In older embryos an increase in the caliber of the hepatic veins posterior to their entrance into the sinus venosus indicates the position of the hepatic sinuses which become so prominent in some selachians and which have been discussed in some detail by Neuville ('01).

In selachians as compared with the higher groups of vertebrates the primitive liver veins are retained in the adult almost in their entirety. The main vessels are established early before the sinusoidal complex is well developed and these trunks remain almost complete, although they may vary in size with changes in the hepatic parenchyma. In figure 14, for example, the main trunks of the hepatic vein can be seen in each section although the size of these trunks varies greatly. The only primitive hepatic vessel which is really completely broken up into sinusoids by the hepatic cylinders is the small segment of the left omphalomesenteric vein which passes over the pars hepatica medialis to join the sinus venosus and even the anterior and posterior ends of this vessel remain as branches of the left hepatic and the hepatic-portal veins respectively. The growth in length of the hepatic and hepatic-portal veins takes place at the ends of the lateral lobes of the liver. Here there exist venous sinuses which are joined by both veins and which are interrupted by only occasional hepatic cylinders sheathed with endothelium. From these sinuses the posterior ends of the afferent (hepatic-portal) and efferent (hepatic) veins are differentiated by the growth of a septum of hepatic cylinders which at first form a loose meshwork and later become a compact wall through which only small capillary sinusoids pass from one vein to another. Some of the venous branches of the second order represent remains of original venous trunks as for example the two largest tributaries of the hepatic veins from the median lobe. The others are the representatives of the larger passageways which have pushed between the fairly constant tubule clusters which arise from the hepatic pouches. Each of the larger branches of the hepatic-portal vein in the median lobe of the liver accompanies for a short dis-

tance a secondary hepatic duct derived from one of these tubule clusters. As a rule the tubule ridges of the lateral surfaces of the hepatic pouches are completely formed and are beginning to break up into rows of individual anlagen before these blood vessels invade their vicinity. Different embryos, however, show some variation in this respect. The vessels pass backward between these rows of tubules and form a vascular plexus interrupted by a few small lacunae. After this process is quite well under way, the main vascular trunk of the left omphalo-mesenteric vein is interrupted by the growth of the pars hepatica medialis and as a result vascular sprouts extend out from the posterior part of this vessel and its fellow of the opposite side. These sprouts join with the plexuses of the hepatic veins and there is thus produced a set of sinusoidal vessels connecting the remains of the omphalo-mesenteric (hepatic-portal) venous system with the true hepatic veins. The extension of this network is gradual, and is not completely established until the tubules are well differentiated and anastomosis between the tubules begins to take place. The establishment of the complete sinuosidal circulation and of tubule anastomosis is practically simultaneous. In Acanthias, as in the pig according to Hilton ('03) and in reptiles (Hammer '93), there is no reason to believe that the blood vessels have anything to do with the formation of the earliest hepatic tubules. Certainly there is no breaking up of a solid mass of liver cells as described in the frog by Shore ('93) or any indentation of the wall of the lateral liver pouches as has been seen in Torpedo by Choronshitzky ('00). The vessels last of all penetrate among the tubules of the ventral surface of lateral hepatic pouches. Figure 12 is a frontal section through the tubules of this region of an embryo 15 mm. in length (H.E.C. 228). It will be seen that the tubules are separated only by scattered mesenchymal cells with the exception of a single vascular sprig.

Minot ('00) has remarked that in the liver, and in the pronephros and mesenephros as well, the sinusoids which at first may be small, increase in size until they reach a certain maximum and then decrease. To secure some idea of the extent of the changes which take place in this process, the area in cross-section

of the blood spaces of selected sections have been measured from a series of livers of selachian embryos. Eight sections were selected for measurement from each liver. To secure sections representing about the same relative planes in each specimen

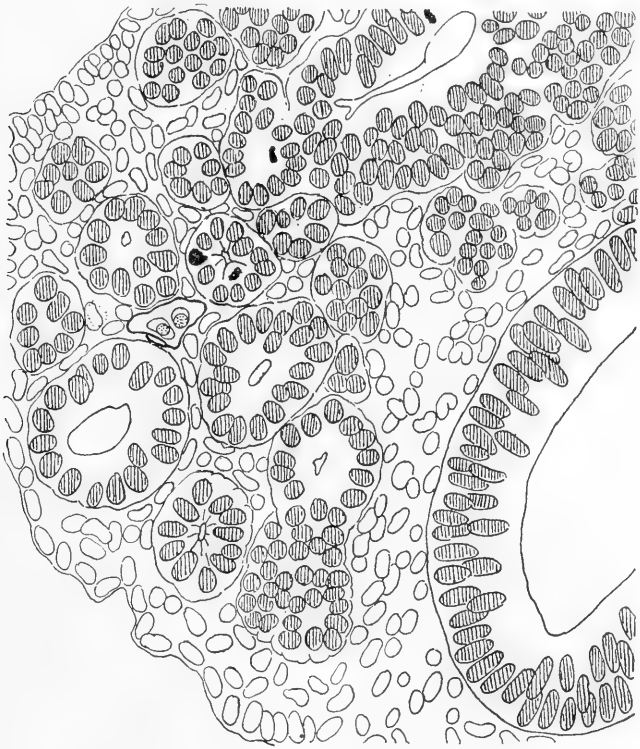

Fig. 12 Frontal section through the tubules arising from the ventral surface of the pars hepatica medialis of an Acanthias embryo 15 mm. long (H.E.C. 230). × 150. Hepatic cell nuclei are shaded with vertical lines, nuclei of mesenchymal cells drawn in outline. A single vascular twig is seen in cross section above the largest hepatic tubule.

the following method was employed. The liver was considered as divisible into three parts, as represented in figure 13. The first segment (*A* to *B*, fig. 13) consisted of that part of the liver anterior to the foremost part of the curved cystic duct. The second segment (*B* to *C*) extended from the cystic duct to the

plane where the anterior lobe of the liver joins with the two lateral and posterior ones. The two lateral lobes form the third segment (*C* to *D*). The first segment, *A* to *B*, was divided into three equal parts and the sections falling upon the planes separating these parts (*1* and *2*) were used for measurement. Two sections were selected in the same manner from the second segment *B* to *C*. The third segment *C* to *D* was divided into five equal parts and the four sections falling on the dividing planes

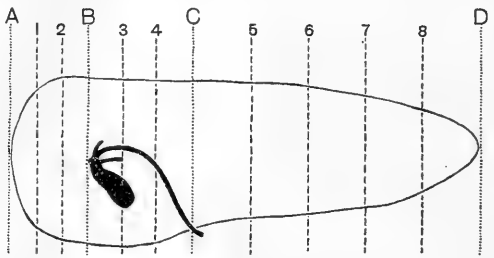

Fig. 13 Diagram of the liver of an Acanthias embryo showing method of selecting sections for measurements of the blood vessels. Bile ducts and gall bladder represented in black.

used for measurement. In this segment only the left lobe was measured in each case. The sections thus determined were drawn with the camera lucida or projection apparatus and the total area, the total area of the vascular bed in cross-section, and the area of the larger vascular trunks in cross-section were then determined by means of a planometer.[6] From this data the figures given in table 2 were calculated. Such a method can lay no claim to great accuracy. The possibility of error is quite large and the method of determining the position of the sections has some objections, for all parts of the liver do not grow at the

[6] It is impossible to determine the exact area of the large trunks in the earlier stages because of their great irregularity and their many connections with the adjoining sinusoids. In embryos 20 to 21 mm. long the area of the main venous trunks forms about 20 per cent of the entire cross section area of the vascular spaces of the liver. This drops to about 15 per cent in embryos of 25 to 28 mm. and increases thereafter with the relative reduction of the sinusoids. In the last member of the series in table 2, the main trunks formed about 30 per cent of the total cross section area of the blood vessels.

same rate and the development of the vascular supply of the organ is closely related to its growth. Such a study, however, does give us some rough approximations which may be of value.

A study of table 2 brings out clearly the three stages in the vascularization of the liver in Acanthias. The first stage found in an embryo between 12 and 20 mm. in length is one in which the tubules are growing with great rapidity and the sinusoids are only cleft like endothelium lined spaces (figs. 14 A and B). It is only in the lateral lobes of the liver where the hepatic and the hepatic-portal veins unite in large sinuses that the vascular percentage of the organ is high. During the latter part of this period tubule anastomoses are forming in large number. With anastomosis the vascular supply of the organ increases with the greatest rapidity and the tubules, taking on the form of slender cylinders, are separated by very large vascular spaces. This phase, which is inaugurated rather suddenly, continues while the embryo grows from a length of about 20 mm. to a length of

TABLE 2

Measurements of the hepatic vascular spaces in Acanthias embryos

LENGTH OF EMBRYO IN MM.	DESIGNATION	AREAS IN MM.	LEVELS OF SECTION							
			1	2	3	4	5	6	7	8
15.0	H.E.C. 227	T.a.[1]	0.238	0.321	0.314	0.314		0.029		0.019+
		V.a.[1]	0.035	0.044	0.020	0.021		0.005		0.006−
		V.%[1]	**18.3**	**13.8**	**6.3**	**6.7**		**26.3**		**30.0**
20.5	S.C. 5	T.a.	0.308	0.423	0.581	0.502	0.160	0.099	0.065	0.027
		V.a.	0.030	0.047	0.031	0.112	0.172	0.024	0.012	0.007
		V.%	**9.8**	**11.2**	**14.0**	**22.4**	**10.8**	**24.5**	**19.7**	**27.7**
20.6	H.E.C. 1494	T.a.	0.738	0.942	0.742	0.934	0.412	0.232	0.234	0.178
		V.a.	0.349	0.373	0.385	0.392	0.172	0.124	0.141	0.112
		V.%	**47.3**	**39.7**	**52.0**	**42.0**	**41.8**	**53.4**	**60.3**	**68.5**
28.0	S.C. 6	T.a.	1.120	1.440	1.210	1.190	0.357	0.324	0.303	0.204
		V.a.	0.698	0.616	0.591	0.522	0.205	0.167	0.173	0.157
		V.%	**62.4**	**42.8**	**48.9**	**43.9**	**57.5**	**51.5**	**57.0**	**77.0**
36.6	S.C. 29	T.a.	2.060	2.120	2.340	2.270	0.669	0.595	0.590	0.357
		V.a.	0.403	0.578	0.559	0.644	0.152	0.108	0.084	0.051
		V.%	**19.6**	**27.3**	**23.9**	**28.4**	**22.8**	**18.3**	**14.4**	**14.4**

[1] T.a., total area of cross section in square mm.; V.a., area of vascular spaces in square mm.; V.%, percentage of total area occupied by vascular spaces.

about 30 mm. During this period roughly one-half of the bulk of the liver is made up of vascular spaces and of this half much the greater part is in the form of sinusoids. Figure 14 C is a section through a plane corresponding to figure 14 B, and shows graphically the extent of the blood vessels at the time when they form the larger part of the liver. New tubules develop in great numbers during this time. Finally a period of reduction in the size of the sinusoids sets in. This reduction is brought about entirely by the increase in the size of tubules already formed. By this increase in the parenchyma the sinusoids are reduced to the 'capillary sinusoids' of Minot. This process is very noticeable in embryos between 35 and 45 mm. in length. It continues probably to the time of birth. Figure 14 E is of a cross section of the liver of an embryo 47.3 mm. long and shows the marked decrease in the size of the sinusoids at that time.

The increase in size of the sinusoids is both actual and relative. The decrease in the total area (in cross-section) of the sinusoids is at first only a relative one, but later, for a short period at least, it is actual as well as relative.

A point which table 2 does not bring out is that the size of the sinusoids is not determined by their position in relation to the larger vessels but is dependent upon the stage of development of the parenchyma with which they are interwoven. The sinusoids do not form a tapering system of vessels largest near the veins which receive them. This has been in a way pointed out in the section upon the growth of the hepatic cylinder network. In the lateral lobes for example, the growth of the tissue is at first entirely backward and during this period sinusoids of large size are generally distributed throughout the lobe (fig. 15 A). After the lobes have completed the greater part of their posterior growth there begins a great increase in the hepatic tissue along their dorsal margins and the lobes gradually push upward on either side of the intestine. With this change in the area of rapid growth there also occurs a change in the sinusoids which, as is shown in figure 15 B, continue to form practically half of the dorsal portion of the lobes while in the ventral portions they form less than 10 per cent of the total area in cross-section.

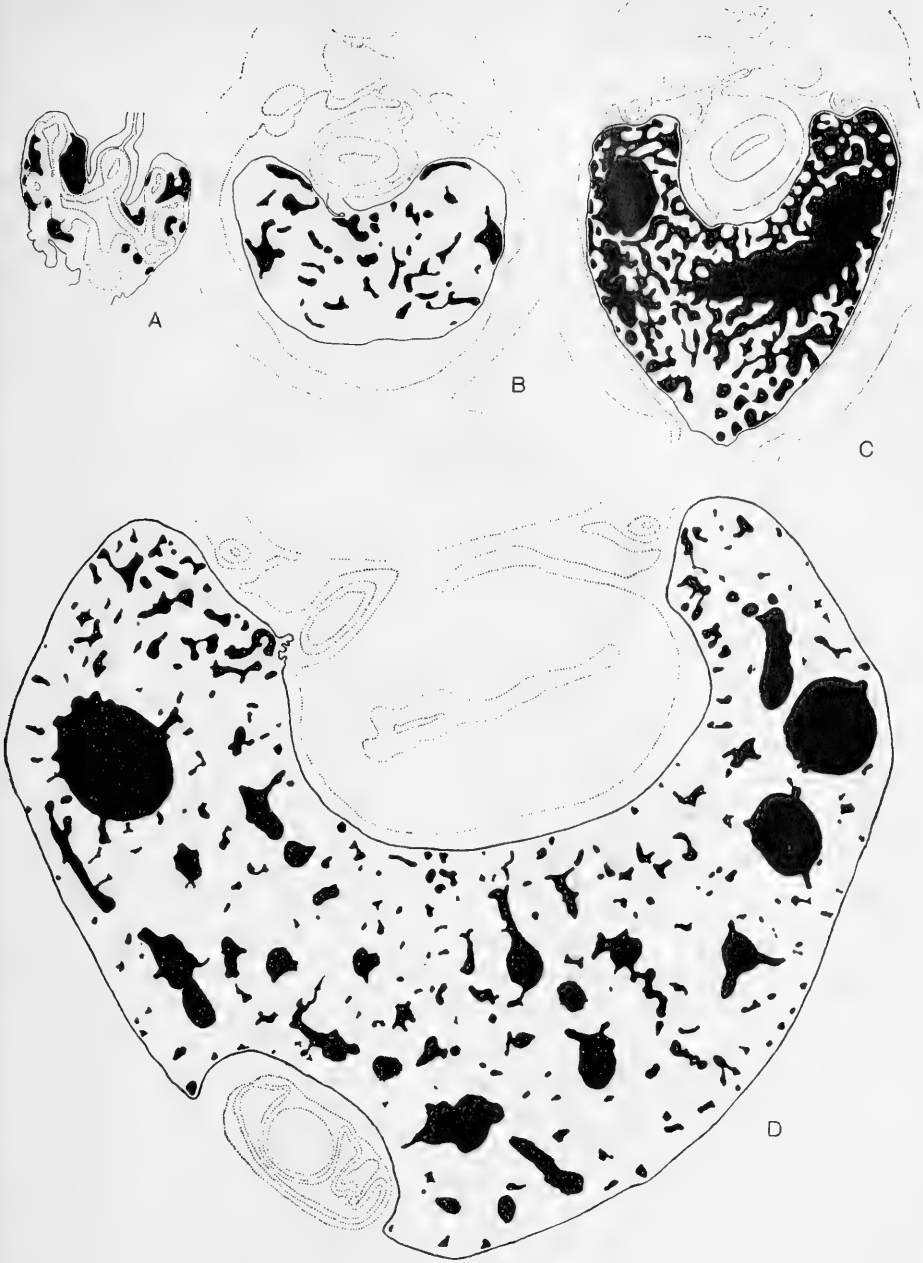

Fig. 14 A series of transverse sections of the livers of Acanthias embryos of different ages showing the development of the sinusoids which are represented in solid black. All sections, with the exception of *A*, are taken at a plane equally distant from the anterior end of the liver and the anterior end of the gall bladder; all × 35. *A*, embryo 13.3 mm. long (S.C. 18); *B*, embryo 20.5 mm. long (S.C. 5); *C*, embryo 28 mm. long (S.C. 6); *D*, embryo 47.3 mm. long (S.C. 11).

Similar changes are seen in earlier stages in the main median lobe of the liver and in the process of the cystic lobe which extends into the yolk stalk coelome.

In Mustelus and Torpedo the early development of the hepatic sinusoids is quite different from that seen in Acanthias. In these forms the omphalo-mesenteric veins become much enlarged at an early stage and lie both dorso-medial and ventro-lateral to the hepatic pouches instead of only medial to them in Acanthias. As a result the hepatic tubules are in contact with the walls of large vascular chambers from the time of their first appearance and as they grow they can only do so by pushing the endothelial walls of the vessels before them. The tubules, as has been remarked, are slender and elongated and unite by anastomoses soon after their formation. Thus there is no stage in these forms corresponding to the early one in Acanthias where the sinusoids are small cleft-like spaces. The proportion of the volume of the liver formed by sinusoids is very high at first but is steadily reduced as development proceeds. Figure 16 of two transverse sections of Mustelus embryos 16.7 mm. and 22.5 mm. in length respectively illustrates these points, as do also the thick frontal sections shown in figures 44 and 45. The larger vascular trunks of the liver in Mustelus and Torpedo are mapped out by the growth of the hepatic cylinder network between them. As in Acanthias the main liver veins are marked out early in the development of the organ, and are not formed by the fusion of smaller vascular spaces.

In both the forms under discussion and in Acanthias the hepatic sinusoids are formed by the intercresence of the hepatic cylinders and the omphalo-mesenteric veins. In Mustelus and Torpedo this intercresence is due to the invasion of the space occupied by the vein. In Acanthias, *in early stages*, the intercresence is due to the penetration of the venous sprouts about the tubules which are already established. In later stages in Acanthias the cylinders increase by growing into venous spaces and the end result in either case is the same. Thus both the methods of sinusoid development postulated by Minot ('00) and by Lewis ('04) are found in these forms.

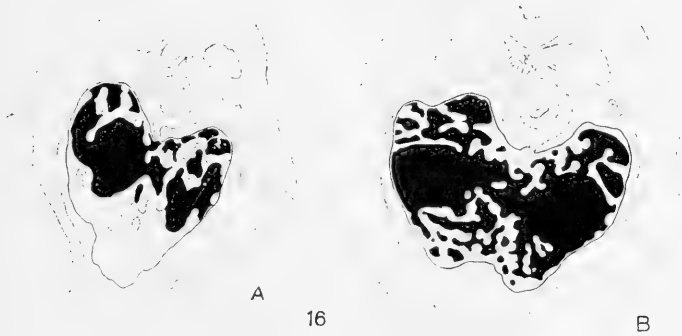

Fig. 15 Two transverse sections through the middle of the left lateral lobe of Acanthias embryos; both × 75. *A*, embryo 28 mm. long (S.C. 6); *B*, embryo 47.3 mm. long (S.C. 11); sinusoids represented in black.

Fig. 16 Transverse sections through the middle of the median lobe of two embryos of Mustelus canis. *A*, embryo 16.7 mm. long (S.C. 32); *B*, embryo 22.5 mm. long (S.C. 33); both × 35; sinusoids represented in black.

In Acanthias the establishment of the sinusoidal circulation and the process of anastomosis of the hepatic tubules occur almost simultaneously. In Mustelus and Torpedo the hepatic tubules anastomose immediately after their intercresence with the omphalo-mesenteric veins. At first sight there would seem to be some relation between the sinusoidal type of circulation and the anastomosis of glandular end-pieces. This seems to be supported by the evidence that the pancreas of ganoids has free anastomoses with a sinusoidal circulation and these conditions are also found in the paraphysis of Necturus (Warren '05) and in the islands of Langerhans in Mammalia (deWitt '06). Sinusoids also occur in other ductless glands which anastomose. On the other hand I have found no report of anastomoses between the tubules of the mesonephros, and a free sinousoidal circulation exists in this organ. The tubules of the embryonic mammalian pancreas are known to anastomose freely, and there is no record of a sinusoidal circulation in this organ. Similarly Braus ('00) finds a free anastomosis of the end pieces of the bulbo-urethral gland which has the capillary type of circulation and Bremer ('11) has found the same conditions in the testis.[7] It is evident that no general rule can be laid down regarding this relation, at least with our present knowledge of the subject.

SUMMARY

A. Development of the hepatic parenchyma

1. Hepatic tubules are first represented by longitudinal ridges formed on the external surfaces of the pars hepatica lateralis and medialis, and by slight irregularities of the margins of the pouches forming the pars hepatica lateralis.

2. The hepatic ridges are converted into irregular rows of tubule anlagen by transverse constructions.

3. The individual tubule anlagen thus formed grow outward and are differentiated into expanded terminal chambers and

[7] Occasional anastomoses are found in a number of glands such as the gastric and uterine glands. It is to be expected that such occasional connections will be found in almost any gland which is studied carefully with the aid of reconstructions.

constricted proximal necks. Secondary tubules of the second, third or fourth order may be produced from the terminal expanded chamber before tubule anastomoses are formed.

4. In Acanthias the hepatic tubules may differentiate in regions into which blood bessels have not penetrated.

5. Tubule anastomosis and the complete establishment of sinusoidal circulation are practically simultaneous.

6. Anastomoses of tubules may take place end to end, side to end, or side to side. The frequency of the several types is in the order stated.

7. The process of anastomosis involves the stages of (a) the contact and fusion of the tubules involved, (b) the rearrangement of cells in the area of confluence, and (c) the establishment of a new connecting lumen.

8. While the cell position is shifted in anastomosis, the cell axis remains unchanged.

9. After anastomosis the increase in the number of hepatic cylinders takes place in three ways, viz: (a) the formation of hepatic cylinders directly from the pouch wall; (b) the interstitial development of cylinders from blind sprouts from the network; (c) the peripheral growth of the terminal hepatic cylinders.

10. These methods of increase cease in the order named and contribute to the network inversely to the order given above.

11. Increase in the number of cylinders of the hepatic network first ceases in the body of the median lobe and last in the tips of the lateral lobes and along the dorsal margins of the lateral lobes and connecting portion.

12. The number of cells surrounding the lumen of the hepatic cylinder in cross-section drops from an average of 12 + in the tubule at the time of anastomosis (14 to 15 mm.) to 3 ± in an embryo 95 mm. long.

13. The diameter of the hepatic cylinders increases up to and immediately after the time of anastomosis. With the enormous increase in the size of the sinusoids following a short time after anastomosis, the diameter for a time decreases. Thereafter the diameter of the cylinders undergoes a steady increase at least to the time of birth or hatching.

14. The early increase in size of the cylinders is due to a multiplication of cells. The later increase is due to a growth in the size of the individual cells.

15 The hepatic nuclei which are originally large and elongately oval, become somewhat reduced in size and spherical in shape at the time when the tubules are formed from the tubule ridges. Thereafter they undergo a slow shrinkage in size up to the time of birth. In later stages they may again become oval or gibbus in outline, due to the pressure of the intracellular fat. The nuclei are at first basal in position and later become either central or peripheral.

16. Hepatic nuclei first have the common embryonic type of structure which they retain longer than do the nuclei of most tissue. The embryonic type of structure is lost first in the nuclei of the gall bladder and major hepatic ducts, next in the minor hepatic ducts, and finally in the hepatic cylinders.

17. Fat appears in the cylinders at an early stage and eventually fills almost the entire cell as in the typical fat cell.

18. Selachians in which the omphalo-mesenteric veins are early developed to large proportions have slender hepatic tubules which indent the walls of the vessels and anastomose at a very early stage.

B. Development of the minor ducts

1. Two forms of bile duct formation exist in the selachian liver, that of evagination of the liver pouch, and that of transformation of pre-existing cylinders into bile ducts. The first and most primitive is more active in the selachians than in higher vertebrates and forms the major ducts and the proximal parts of the minor ones. The distal parts of the minor ducts are formed by cylinder transformation.

2. Ducts are only differentiated from cylinders which lie in contact with branches of the omphalo-mesenteric veins. The side of the cylinder lying towards the vein is always the first to differentiate and in the smaller ducts is the only part to be differentiated from the true hepatic cells.

3. The cells of the bile duct epithelium are structurally farther removed from the primitive hepatic cell type than are the cells of the hepatic cylinders.

C. The hepatic mesenchyma

1. The hepatic mesenchyma is derived from the splanchnic mesothelium from two zones and at two periods. In both cases the mesenchymal proliferation is associated with marked irregularities of the mesothelium.

2. The first mesenchymal proliferation is ventral and is associated with the formation of mesothelial villi on the right side and irregular mesothelial growths about the omphalo-mesenteric vein on the left side. This proliferation forms the mesenchymal tissue about the gall bladder, cystic duct, main trunk of the omphalo-mesenteric vein, and to an indeterminable degree the interstitial mesenchymal tissue of the ventral part of the liver.

3. The second and dorsal proliferation is associated with the formation of mesothelial funnels and anastomosing mesothelial tubules which at one time occupy the dorsal fifth of the liver. These mesothelial invaginations break down into mesenchymal cords which in turn form the interstitial mesenchymal tissue of the dorsal part of the liver.

4. These two zones of mesenchymal proliferation correspond with the two zones of splanchnic mesenchymal proliferation of the trunk region of selachian embryos as described by H. E. Ziegler.

5. The mesothelial covering of the liver is at first made of columnar cells which later become squamous and thereafter apparently give rise to little or no mesenchymal tissue. The last areas from which the primitive form of mesothelium disappears are the tips of the lateral lobes and the dorsal margins of the lateral and median lobes.

6. Generally the splanchnic mesothelium becomes reduced at once from a columnar to a squamous cell layer upon contact with a hepatic tubule or cylinder.

7. The primitive embryonic type of nuclear structure is lost in the mesenchymal cells as they are proliferated from the mesothelium. No sharp line of demarcation could be drawn between endothelial and mesenchymal cells.

D. Hepatic sinusoids

1. Hepatic sinusoids arise in selachians by intercresence of the hepatic cylinders with the omphalo-mesenteric vein. This intercresence may be brought about either (a) by the growth of the rami of the omphalo-mesenteric vein about the cylinders as in Acanthias, or (b) by the invagination of the vessel wall by growing cylinders as in Mustelus and Torpedo.

2. In the first type there may be recognized three stages of sinusoid development: (a) The first in which the sinusoids are sparse and differ from capillaries only in their terminations at either side in veins; (b) The second stage in which the sinusoids are greatly enlarged constituting approximately 50 per cent of the liver; (c) The final stage in which the sinusoids take the form of the capillary sinusoids of Minot. In the second type of intercresence only the last two stages are present.

3. The reduction of the sinusoids from stage (b) to stage (c) is due to the growth in size of the hepatic cylinders and not due to their increase in number, for the most active increase in number of cylinders takes place prior to or early in stage (b).

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PLATE 1

EXPLANATION OF FIGURES

17 Reconstruction of the hepatic anlage of an Acanthias embryo 7.5 mm. long (S.C. 14) showing longitudinal tubule ridges upon the wall of the lateral hepatic pouch.

18 Reconstruction of the hepatic anlage of an Acanthias embryo 10 mm. long (S.C. 20) showing the formation of individual tubule anlagen from the tubule ridges.

19 Reconstruction of a hepatic tubule of an Acanthias embryo 13.3 mm. long (S.C. 18) showing division into dorsal chamber and proximal neck and the secondary tubules arising from the former.

20 Reconstruction of a more highly developed tubule; from the same specimen as figure 19.

21 Reconstruction of two simple tubules from the lateral hepatic pouch of an Acanthias embryo 15 mm. long (H.E.C. 227).

22 and 23 Two views of a reconstruction of a highly developed hepatic tubule just prior to anastomosis; some of the secondary tubules are in contact; from the same specimen as figure 21.

24 Reconstruction of three anastomosing tubules; from the same specimen as figure 21; tubules *A* and *B* are completely fused; tubules *C* and *A* are in an early stages of the process.

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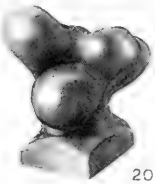

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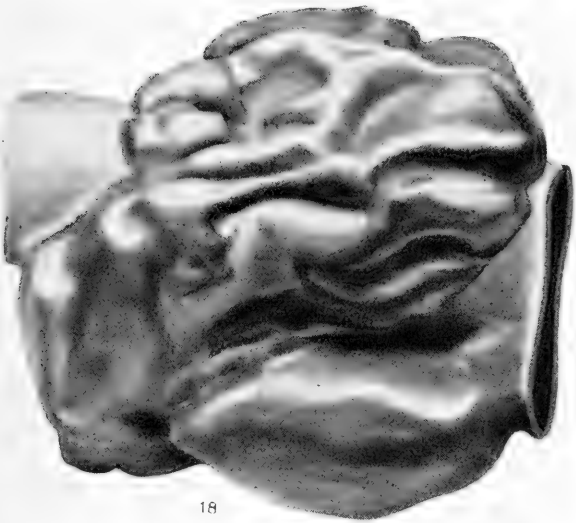

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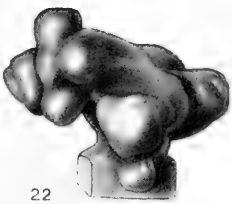

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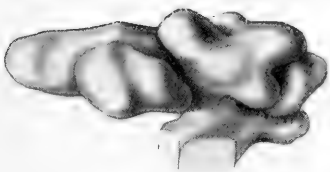

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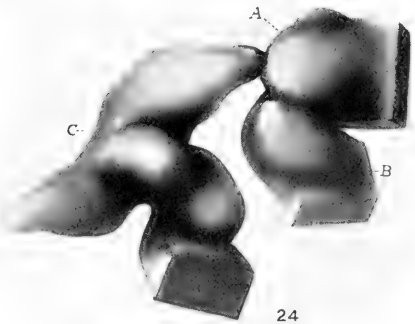

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PLATE 2

EXPLANATION OF FIGURES

25 to 27 Sections of hepatic tubules of Acanthias illustrating the process of anastomosis; (fig. 36 represents a step between figs. 26 and 27).

25 Early contact stage in anastomosis; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.

26 Fusion of tubules; from an embryo 19 mm. long (S.C. 3); alum hematoxylin. × 400.

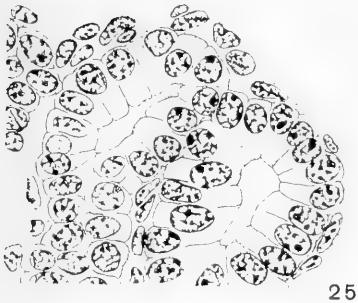
27 Establishment of connecting lumen between two anastomosed tubules. From an embryo 19 mm. long (S.C. 2); alum hematoxylin. × 400. Graphic reconstructions of these tubules, shown in cross section in figures 26 and 27, are illustrated in figure 2, page 224.

28 to 30 Sections of the external walls of lateral hepatic pouches of Acanthias embryos illustrating the formation of the hepatic tubules.

28 Formation of tubule ridges; from an embryo 8 mm. long (K.U.E.C. 545); iron hematoxylin. × 600.

29 Early outpouching of individual tubules. From an embryo 10 mm. long (S.C. 20); alum hematoxylin. × 400.

30 Completely formed tubules at the time when anastomoses first appear; from an embryo 13.3 mm. long (S.C. 18); alum hematoxylin. × 400.



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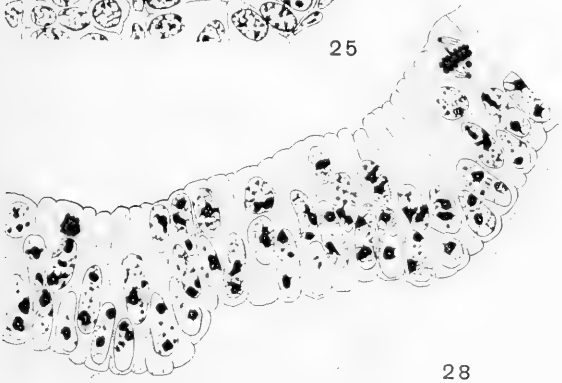

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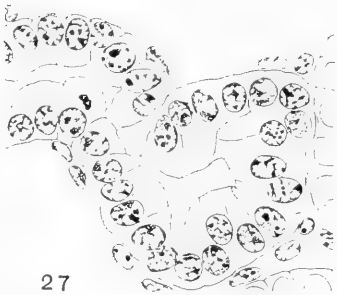

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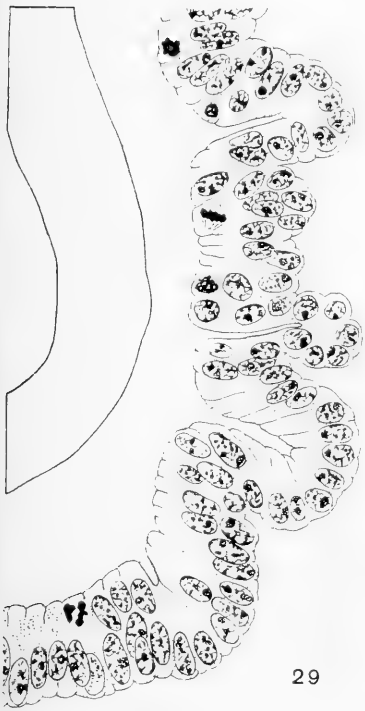

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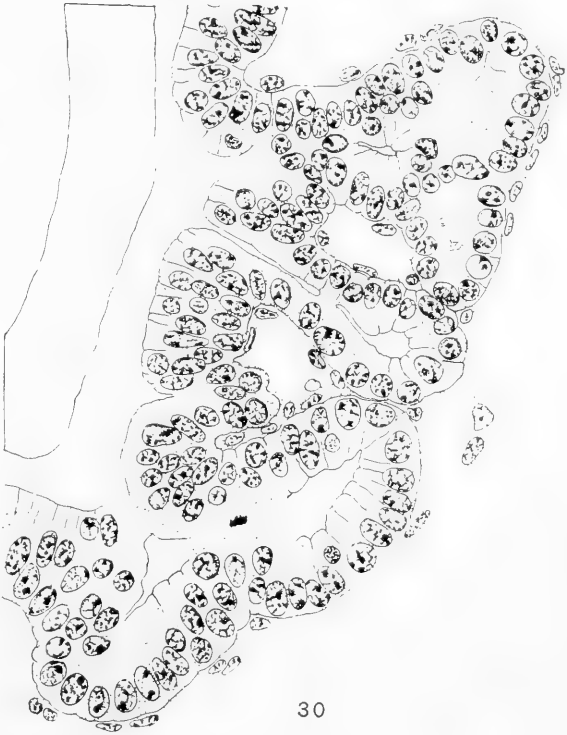

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PLATE 3

EXPLANATION OF FIGURES

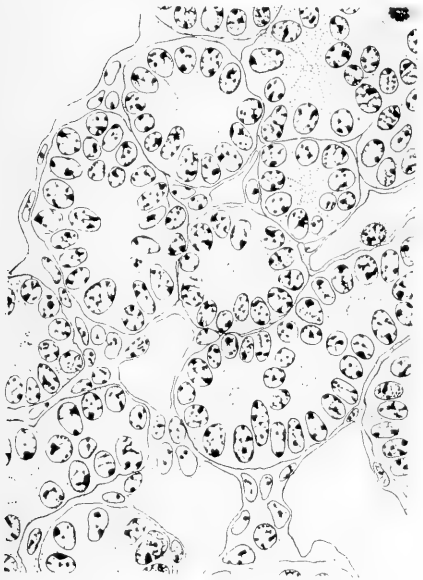
31 to 34 Four sections of livers of Acanthias embryos of different ages, illustrating the changes in the hepatic cylinders; all × 400.

31 Section of the liver of an embryo 16 mm. long (K.U.E.C. 547); iron hematoxylin.

32 Section of the liver of an embryo 20.6 mm. long (H.E.C. 1494); iron hematoxylin.

33 Section of the liver of an embryo 32 mm. long (H.E.C. 1652); iron hematoxylin.

34 Section of the liver of an embryo (of Squalus sucklii) 95 mm. long (H.E.C. 1882); alum cochineal.



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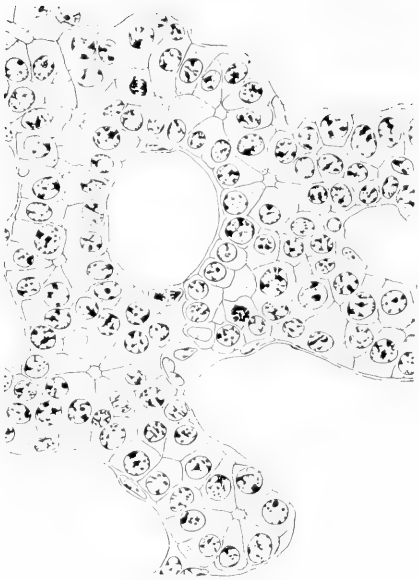

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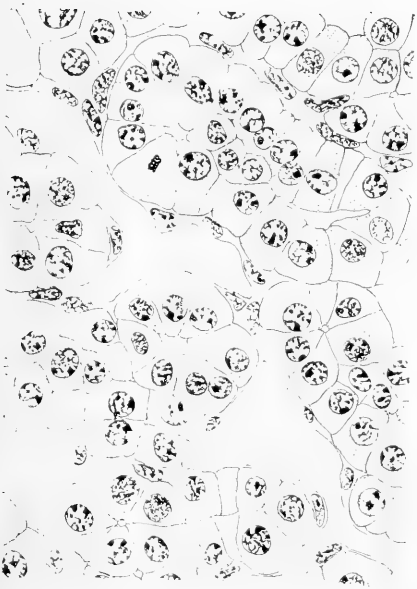

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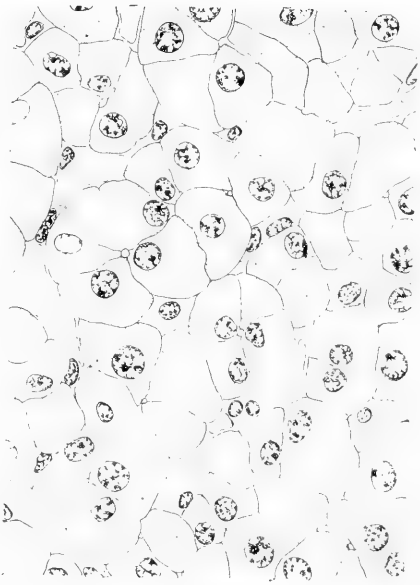

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PLATE 4

EXPLANATION OF FIGURES

35 Transverse section of the margin of the liver of an embryo of Torpedo ocellata 29 mm. long; alum hematoxylin. × 350.

36 Section of an anastomosis between two hepatic tubules of an Acanthias embryo 14 mm. long (S.C. 30); alum hematoxylin. × 500. (This section shows a stage intermediate to those shown in figures 26 and 27).

37 Transverse section of a terminal bile duct of an embryo 95 mm. long (H.E. C. 1882); alum cochineal. × 400.

38 Hepatic tubules and mesenchymal ingrowths from a transverse section of the dorsal margin of the liver of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400. *M*, mesenchymal ingrowths. *H.t.*, hepatic tubules.

39 Transverse section of a developing bile duct of an Acanthias embryo 24.7 mm. long (H.E.C. 1492); iron hematoxylin. × 400.

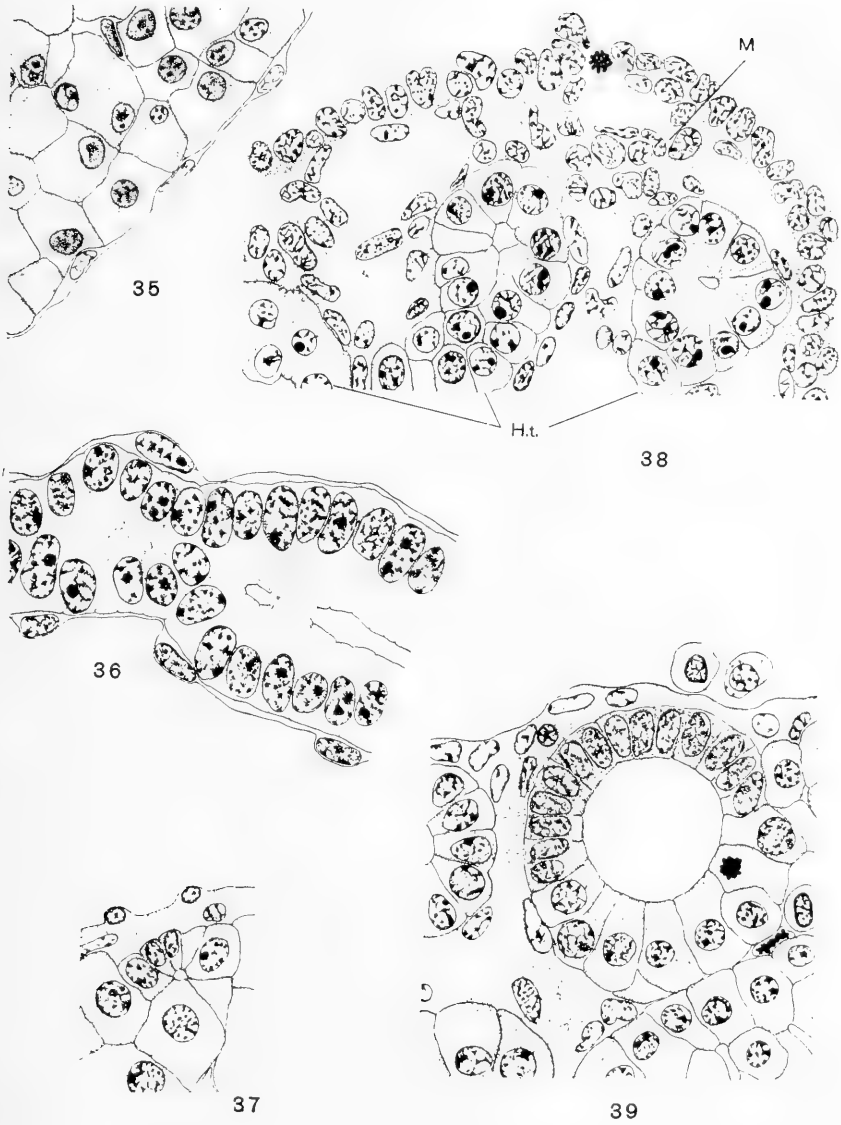

35
M
H.t.
38
36
37
39

PLATE 5

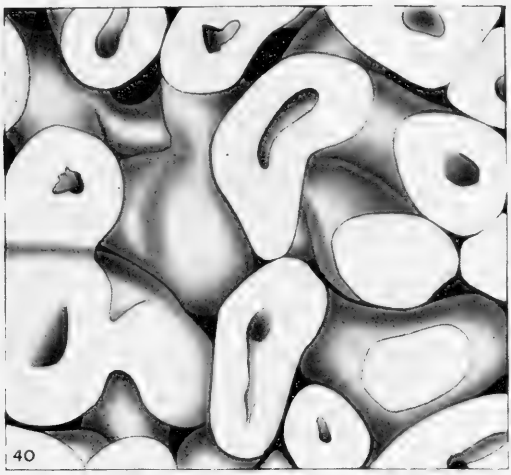
EXPLANATION OF FIGURES

40 to 42 Portions of thick sections of livers of Acanthias embryos of different ages. The method of preparation is described in footnote 4, page 225. All approximately × 200.

40 Thick section of the liver of an embryo approximately 15 mm. long.

41 Thick section of the liver of an embryo approximately 21 mm. long.

42 Thick section of the liver of an embryo approximately 30 mm. long.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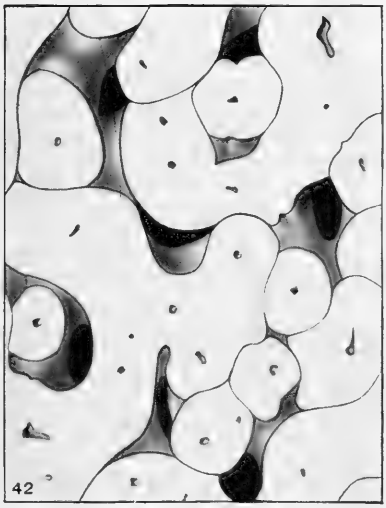
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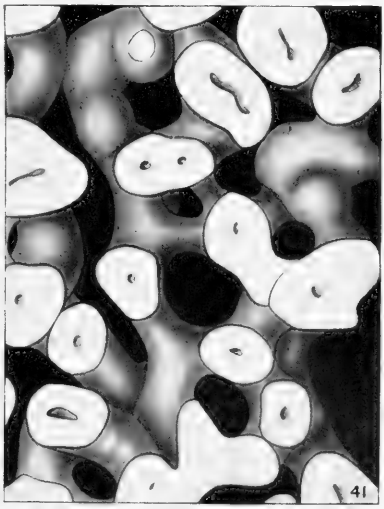
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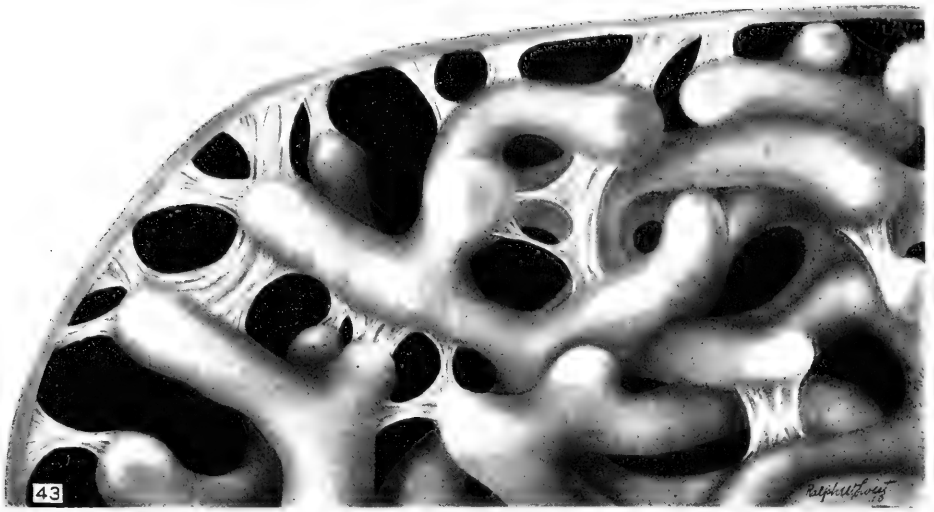
PLATE 6

EXPLANATION OF FIGURES

43 Preparation of the periphery of the posterior lobe of an Acanthias embryo approximately 20 mm. long showing the terminal buds of the hepatic network and the attachment of the network to the mesothelial covering by means of mesenchymal strands.

44 Thick frontal section of the liver of an embryo of Mustelus canis approximately 12 mm. long. The large hepatic duct and the hepatic tubules lie in a vascular sinus which is represented in black. The vertical band seen on the right hand side is a section of the mesothelial covering of the liver.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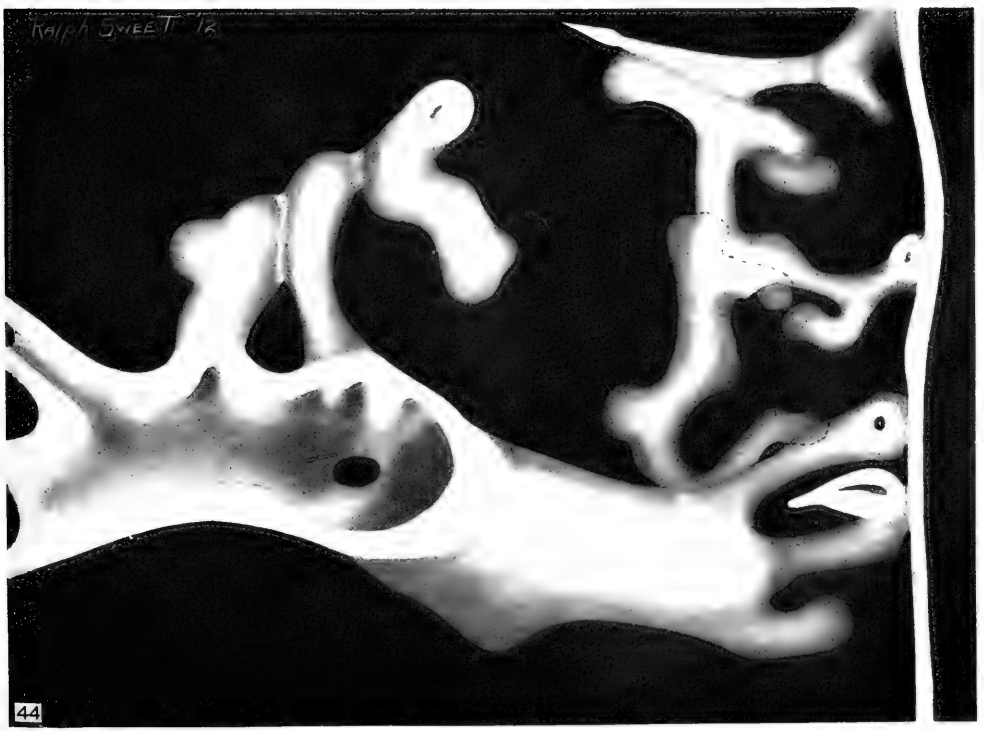
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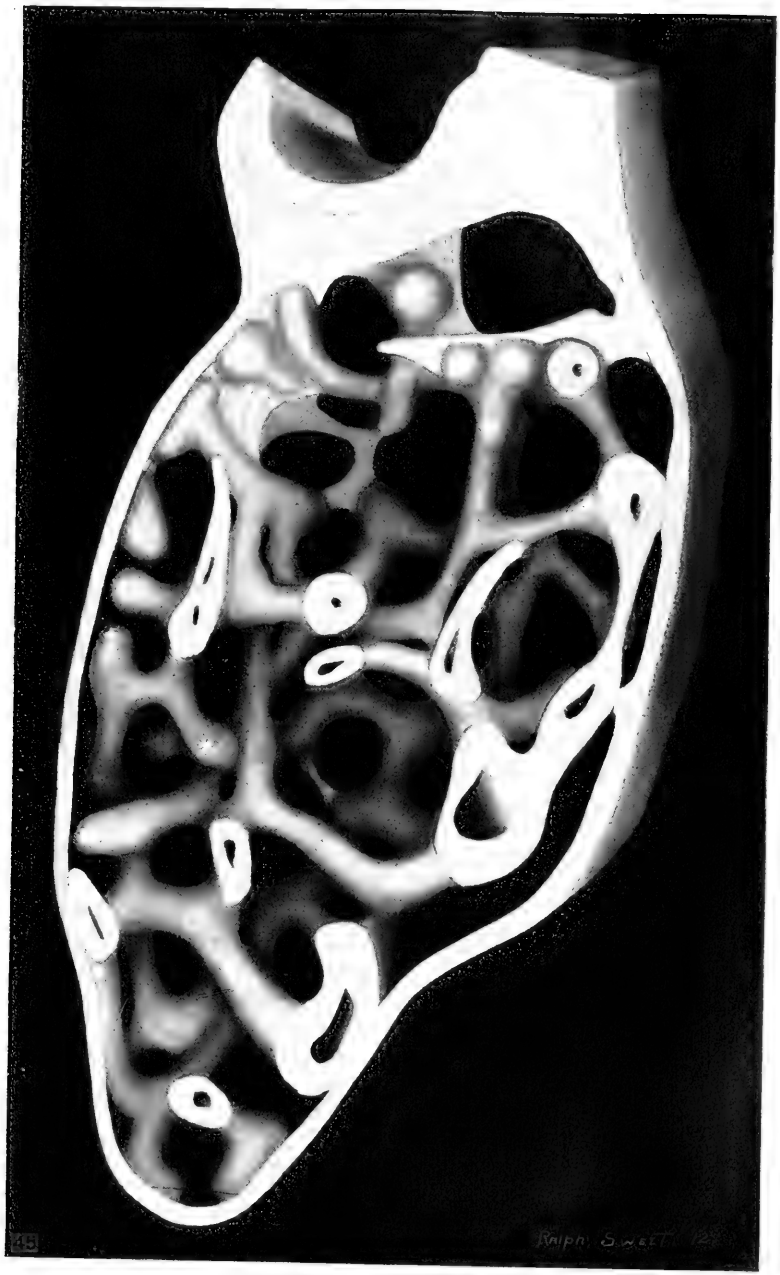
PLATE 7

EXPLANATION OF FIGURE

45 Thick frontal section of the liver of an embryo of Mustelus canis approximately 16 mm. long.



THE DEVELOPMENT OF THE THYMUS IN THE PIG

I. MORPHOGENESIS

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TWELVE FIGURES—TWO PLATES

HISTORICAL

It has been definitely established by many investigators that the thymus of all mammals is of epithelial origin. More recent investigations have shown, however, that the epithelial anlage of the thymus is not derived from the same germ layer in all mammals. Investigators are agreed on the point that the thymus of mammals, when it is of a purely entodermal origin, is a derivative of the ventrally directed epithelial diverticulum of the third pharyngeal pouch. It has also been quite definitely settled that in some mammals (mouse, Roud '00) the thymus is entirely of ectodermal origin. The mixed (ectodermal-entodermal) origin of the thymus in some mammals has not yet been generally accepted. In pig embryos it is the close topographical relation that exists between the cervical vesicle and the third pharyngeal pouch that makes a mixed origin of the thymus possible.

Among some of the workers on the early development of the thymus of the pig may be mentioned Fischelis, Kastschenko, Zotterman, Born, Bell, and Fox, the first three of whom attribute to the thymus an ectodermal-entodermal origin. Fischelis ('85) derived the thymus from the third pharyngeal pouch and the third branchial groove. According to this investigation these two fuse, and from their point of fusion each contributes about one-half to a ventrally directed downgrowth, the anlage of the thymus. This conclusion is erroneous, for that portion of the

thymus which is derived from the third pharyngeal pouch is a comparatively long mid-ventrally directed epithelial tube before the cervical sinus is fused to it. He also makes no mention of the XII cranial nerve which plays an important part in modifying the topographical relations of the anterior portion of the thymus to surrounding structures. Basing his conclusions on inaccurate observations, his views in regard to a mixed origin of the thymus have now only an historical value.

The first detailed study of the early development of the thymus in the pig was made by Kastschenko ('87). He describes the mesial portion of the sinus cervicalis, which he calls the 'vesicula thymica,' as fusing with the anterior end of the epithelial anlage of the thymus, which is derived from the third pharyngeal pouch. In the shiftings of some of the structures in the neck, that occur in young embryos during growth, the lateral portion of the cervical vesicle is separated mechanically from its mesial portion by the hypoglossal nerve. The free lateral portion of the cervical vesicle gives rise to the 'thymus superficialis' which is necessarily of ectodermal origin. He claims that the superficial thymus is not a constant structure, for, in a 30 mm. embryo that he examined, it was not present. The anterior end of the thymus to which the mesial portion of sinus cervicalis has fused, plus the parathyroid, that lies close to it, he designates the thymus head; while the large remaining portion of the thymus which is of a purely entodermal origin, plus the thymus head, he calls the 'thymus profunda.' The largest embryo examined by him was 82 mm. in length.

Zotterman ('11) also made a detailed study of the morphogenesis of the thymus of the pig. Her conclusions are in accord with those of Kastschenko with the exception that in about one-half of the specimens examined the superficial thymus was connected with the thymus head by a cord of cells that looped over the hypoglossal nerve. The superficial thymus was found in all the specimens examined and in the largest (105 mm.) investigated all the features common to the thymus (cortex, medulla, Hassall's corpuscles, etc.) were present.

Fox ('08) agrees with Kastschenko that the superficial thymus

of the pig arises by a constriction of the fundus praecervicalis, but claims that in embryos up to 35 mm. in length, the oldest stage he examined, its histological structure does not resemble that of the thymus more than any other branching epithelial mass.

Born ('83) derived the thymus anlage in pig embryos from the third pharyngeal pouch, while Bell ('06) also is inclined to believe that the ectoderm takes no part in the formation of the thymus.

MATERIAL AND METHODS

For investigation of the early stages of the morphogenesis of the thymus, the excellent collection of pig embryo series, 3 to 42 mm. in length, in the Department of Histology and Embryology of Cornell University, proved very helpful. In addition to these, five embryos ranging from 9 to 21.5 mm. in length, and the neck and upper thoracic region of eight embryos ranging from 32 to 95 mm. in length, were sectioned transversely. These sections, 10 microns in thickness, were stained with hematoxylin and eosin. From series of this group reconstructions of the pharyngeal region were made. Many dissections exposing the thymus were made of the neck and upper thoracic region of embryos from 100 to 280 mm. in length (full term). The thymus of a pig one day old was also examined.

MORPHOGENESIS

In the investigation on the morphogenesis of the thymus special attention was constantly directed toward the development of the superficial thymus because its existence is not yet generally accepted and, since the latest developmental stage in which it was investigated by Zotterman was only 105 mm. in length, its fate is not definitely known.

An 11 mm. embryo was the developmental stage chosen as the starting point for the study of the morphogenesis of the thymus. At this stage the ectodermal and entodermal parts of the branchial grooves and pharyngeal pouches can still be

distinguished from each other without difficulty. The sinus cervicalis, formed by the rapid growth in a caudal direction of the mandibular and hyoid arches and the more retarded growth of the branchial arches proper, is already well mapped out. As this stage shows well accepted relations and developmental steps it needs no further description.

Embryo of 14.5 mm. (figures 1 and 2). This is the youngest developmental stage from which a reconstruction of one side of the neck was made. Since the determination of the real origin of the thymus was one of the crucial points in this investigation only that part of the neck containing the anterior portion of the thymus anlage and the vesicula cervicalis was modelled.

The posterior edge of the hyoid arch has grown over the opening of the sinus cervicalis, shutting it off from the exterior. The cavity thus formed is the vesicula cervicalis (*V.c.*) or the 'vesicula thymica' of Kastschenko.[1] The vesicula cervicalis, now widely separated from the ectoderm (*Ect.*), is still connected with it by a heavy cord of cells, the ductus cervicalis (*D.c.*). Only in places through its entire extent are traces of a lumen left. To the outer end of the vesicula cervicalis is attached a cord of cells that runs in an antero-ventral and mesial direction and connects with the second pharyngeal pouch. This is the ductus branchialis (*D.b.*). The anterior one-fifth of this structure possesses a lumen which is continuous with that of the second pouch. At this stage it is impossible to determine the extent of the part that is of entodermal origin and the extent that is of ectodermal origin. The boundary line between the two has disappeared through the obliteration of the lumen. Fox ('08) was unable to find the ductus branchialis in pig embryos but demonstrated a long diverticulum—'filiform process'—arising from the ventro-lateral angle of the second pouch and connected with the ecto-

[1] "According to H. Rabl ('09) the term 'vesicula cervicalis' is to be applied to the entire complex, including the two ductus branchiales; Hammar uses the term 'vesicula praecervicalis' only for the vesicular portion that is associated with the third pharyngeal pouch, this portion being approximately identical with the 'fundus praecervicalis' (cervicalis) of His and H. Rabl, as well as with the 'vesicula thymica' of Kastschenko and the 'sinus vesicle' of Zuckerhandl." (Quoted from Keibel and Mall's Human embryology, vol. 2, p. 456).

derm. This process was not present in any of the embryos I examined.

The vesicula cervicalis (fig. 2, *V.c.*) lies lateral to the third pharyngeal pouch, between the cephalo-dorsal portion of the parathyroid (*Pt. 3*) and the caudo-ventral part of the ganglion nodosum (*G.n.*) and a short distance anterior to the hypoglossal nerve (*N.XII*). Its general shape is fusiform, with its long axis almost perpendicular to the surface ectoderm. The middle third is solid, but each extremity contains a cavity. The expansion of the central portion is due to a proliferation of the cells of its anterior wall which presses tightly against the dorsal surface of the parathyroid. The pressure against the parathyroid has apparently caused the obliteration of the cavity of the vesicula cervicalis in its central portion. Its inner third lies closely along the ventral side of the ganglion nodosum into which its curved end projects and with which it apparently is fused.

The parathyroid (*Pt. 3*) is now a massive structure lying lateral to the third pharyngeal pouch (*S.b.3*), anterior to the hypoglossal nerve with which it is in contact, ventral to the ganglion nodosum and dorsal to the carotid artery (*A.c.*). Its general shape is that of a hemisphere with its flat side turned toward the vagus nerve and the vesicula cervicalis. A depression in both the vesicula cervicalis and the parathyroid mark the points of most intimate contact between the two structures.

The entodermal anlage of the thymus (*T.e.*) is now, throughout its greatest extent, a solid cord of cells, and still attached to the third pharyngeal pouch. Its anterior end lies closely against the parathyroid and the hypoglossal nerve. From its point of origin it extends caudally, and, with the exception of about one-fourth of the posterior portion, lies ventro-laterally to the carotid artery and the vagus nerve. The caudal portion makes a rather sharp turn in a ventro-mesial direction and occupies the upper part of the pericardial region. The diameter of the caudal part is considerably greater than that of the remaining portion. This is due to the presence of a large lumen and thick walls in this region. In the central and anterior portions only a few slight traces of a lumen persist. The vesicula cervicalis and the an-

terior portion of the entodermal thymus do not come in contact with each other in this developmental stage.

Embryo of 17.5 mm. (figures 3 and 4). During the interval between this and the previous stage shiftings in the pharyngeal region have taken place that have changed the relation of some of the parts to each other. The ductus cervicalis (*D.c.*), now a solid cord of cells, is still connected with the ectoderm. The ductus branchialis on the left side, which was not modelled, has lost its connection with the outer end of the vesicula cervicalis. No traces of it in this region can be seen. It is, however, still connected with the second branchial pouch from which it extends for a short distance toward the point of its former attachment.[2] On the right side it is still a continuous solid cord of cells extending from the second pouch to the vesicula cervicalis. In a 21 mm. pig embryo Zotterman ('11) had demonstrated the ductus branchialis as a continuous cord of cells while the ductus cervicalis as being broken. In the embryos which I examined the ductus branchialis was always the first to become discontinuous.

The vesicula cervicalis (*V.c.*) no longer lies perpendicular to the ectoderm. The vesicula cervicalis medialis (*V.c.m.*)[3] extends from its point of attachment to the ganglion nodosum in an antero-lateral direction to the hypoglossal nerve (*N.XII*) around which it forms an acute angle. From the nerve the vesicula cervicalis lateralis (*v.c.l.*) extends for a short distance in a caudo-lateral direction. This is the same general direction taken by the ductus cervicalis which is connected to the vesicula cervicalis lateralis and the ectoderm. The vesicula cervicalis medialis is tightly wedged in between the vagus on its dorsal side and the parathyroid gland and a small portion of the thymus on its

[2] A reconstruction of this remnant was deemed unnecessary since it takes no part in the formation of the thymus and would have needlessly increased the size of the model.

[3] From the reconstruction as represented in figures 3 and 4 it will be seen that the vesicula cervicalis now loops over the hypoglossal nerve. For the sake of simplicity as well as for clearness, that portion of the vesicula cervicalis lying between the nerve and the pharynx will be termed the 'vesicula cervicalis medialis' while the part lying between the nerve and the surface ectoderm will be termed the 'vesicula cervicalis lateralis.'

ventral aspect. Its caudal portion is greatly flattened but as it approaches the nerve it gradually assumes a cylindrical form which also is the shape of the vesicula cervicalis lateralis. A part of its flattened caudal portion dips into the ganglion nodosum while a portion lies in close contact with the thymus. Fusion between the thymus and the vesicle has apparently not yet taken place, for the boundary of both can still be clearly determined. The lumen of the vesicula cervicalis is for the most part obliterated. Only slight traces here and there in its course persist. It is largest in the portion that dips into the ganglion nodosum. Here the lumen is large and the wall of this portion of the vesicle is no thicker than that of earlier stages. Apparently no cell proliferation takes place in this region. The surface of the entire vesicula cervicalis is more or less irregular. An idea of its shape can best be obtained by referring to figure 4 in which the hypoglossal nerve and a part of the ganglion nodosum were removed, thus almost entirely exposing it.

The parathyroid (*Pt. 3*) is an elongated and very irregular mass of cells that is tightly packed in between the cervical vesicle and vagus nerve on its dorsal aspect, and the carotid artery on its mesial surface. Its caudo-mesial and caudo-lateral portions are in contact with the thymus while its anterior portion is on a level with the arch of the vesicula cervicalis over the hypoglossal nerve.

The thymus (*T.e.*) is considerably longer than in the preceding stage. Its cephalic and caudal ends have about the same relative position to the other structures as in the 14.5 mm. embryo. Its greater length at this stage is due to growth which has kept pace with the growth of the pharynx. It is still connected with the third pharyngeal pouch by a greatly attenuated cord of cells. Its anterior portion (figs. 3–4) is fused to the caudal aspect of the parathyroid from which it extends caudally. As in the preceding stage, the caudal portion makes a sharp turn in a ventromesial direction and lies over the upper portion of the pericardium. The caudal portion of the right thymus extends across the mesial plane while the same region of the left thymus lies to the left of the mesial plane and extends farther caudally than

the right one. The anterior and central portions are cylindrical in outline, having an almost uniform diameter. In the left thymus the lumen of the anterior and central regions has entirely disappeared, while in the right thymus only a trace of it persists in the central portion. The caudal portion in the pericardial region is greatly enlarged. The lumen in this region is broken but in places is quite large in diameter. The walls are very thick and irregular, no longer retaining their cylindrical shape.

The anterior portion of the thymus also extends for a short distance along the ventro-lateral aspect of the parathyroid. It thus has two prongs between which lies the epithelial body. This condition is not present on the right side and was not observed in other specimens of about the same developmental stage. In stages earlier than this the parathyroids are anterior to the hypoglossal nerve. The anterior portion of the thymus is in close contact with both, as shown in figure 2. In the shiftings that occurred during the interval between this and the previous stage it appears that a portion of the thymus was carried along by the nerve and strung along the parathyroid thus bringing about the split condition of its anterior end.

Embryo of 21.5 mm. (figure 5). In this stage the vesicula cervicalis has lost its connection with the ectoderm. The ductus cervicalis has entirely disappeared. The vesicula cervicalis lateralis (*T.s.–V.c.l.*) lies lateral to the hypoglossal nerve (*N.XII*). It is a large fusiform shaped mass of cells containing in its anterior portion a narrow tortuous lumen. This structure is of a purely ectodermal origin and represents the 'thymus superficialis' of Kastschenko. It is connected to the vesicula cervicalis medialis by the pars intermedia or connecting band (*P.i.*) that loops over the hypoglossal nerve. This band was not observed by Kastschenko, hence he held that the superficial thymus remained free from the remaining portion of the thymus. The vesicula cervicalis medialis, also greatly expanded, has lost its connection with the ganglion nodosum, possesses no lumen, and lies along the antero-lateral side of the massive parathyroid (*Pt. 3*) where it is fused with the anterior portion of the thymus.

The anterior portion of the thymus (*T.e.*) has lost its connection with the pharynx and lies on the dorso-lateral side of the parathyroid and is fused with the vesicula cervicalis medialis. In the region of the fused portion it contains a cavity of considerable size while the remaining portion along the epithelial body is without a lumen. From the epithelial body the thymus extends in a caudal and a slightly medial and ventral direction as a solid cord of cells. Just anterior to its entrance into the thoracic cavity it is slightly enlarged. The extreme caudal portion which lies within the thoracic cavity turns abruptly in a ventral direction, is greatly flattened, and in contact with the pericardium. The thoracic segments of the right and left thymus at this stage lie closely together but are not fused. Bell, however, in a 20 mm. embryo, describes them as being fused.

The hypoglossal nerve now forms an acute angle with that portion of the vagus lying immediately posterior to it. In the two preceding stages that were modelled, the corresponding angle formed by these two nerves was obtuse instead of acute. This change in the form of angle between the earlier and later stages apparently is due to shiftings—a consideration of which is to follow—that take place in the neck during the growth of young embryos by which a stress appears to be exerted on the hypoglossal nerve by the cervical vesicle.

The thymus at this early stage (21.5 mm.) can be divided into seven regions, most of which in the later stages become very pronounced. They are: (1) The 'superficial thymus' which represents the vesicula cervicalis lateralis and is of a purely ectodermal origin; (2) the 'thymus head' which represents the structure formed by the fusion of the vesicula cervicalis medialis and the anterior portion of the entodermal anlage of the thymus; (3) the 'connecting band' which loops over the hypoglossal nerve and connects the superficial thymus with the thymus head and is of a purely ectodermal origin; (4) the 'mid-cervical segment' which is an enlargement of the thymus between the intermediary and cervico-thoracic cords; (5) the 'intermediary cord' which connects the thymus head with the mid-cervical segment; (6)

the 'thoracic segment' which lies in the anterior portion of the thorax and is spread over a portion of the pericardium; and (7) the 'cervico-thoracic cord' which unites the mid-cervical segment to the thoracic segment. This system of nomenclature, for which we are indebted to Kastschenko, Zotterman, and Bell, will be used in the discussion of all the later developmental stages.

The different regions of the thymus described in the 21.5 mm. embryo were examined microscopically in the following five stages. These will be briefly described in order to present a more complete developmental history up to a 95 mm. embryo, which was the oldest stage in which a part of the pharyngeal region was reconstructed.

Embryo of 26 mm. The thymus as a whole is considerably larger than in the preceding stage. The surface of the superficial thymus, the thymus head, and the thoracic segment has become very irregular due to outgrowths of epithelial buds from the main stem. This budding represents the beginning of lobulation and had already started in the preceding stage. Lobulation of the mid-cervical segment has just begun. The superficial thymus extends only slightly farther caudally from the hypoglossal nerve than in the preceding stage. The connecting band on the right side has disappeared but the superficial thymus has retained its usual topographical relation to the thymus head. From the parathyroid body the general direction of the thymus is in a caudo-mesia land ventral direction. The intermediary cord on the right side is only a very slender cord of cells while that of the left side has a considerably greater diameter. The cervico-thoracic cords are short and have a uniform diameter of small dimension. The thoracic segments lie in contact with the anterior and ventral portion of the pericardium. The two segments lie close together and have fused in some places along their median sides.

Embryo of 32 mm. The connecting band on the left side is broken. No traces of it can be seen in connection with the thymus head but a remnant of it is still attached to the super-

ficial thymus and extends as a greatly attenuated cord of cells toward the hypoglossal nerve. The general features of the entire thymus at this stage so closely resemble those of the 26 mm. embryo that a detailed description is unnecessary. The only difference of importance is the greater size of the organ as a whole and of the epithelial buds from the main stem.

Embryo of 40 mm. The connecting band is continuous around the hypoglossal nerve on both sides. The parathyroids are slightly elongated and lie along the dorso-mesial side of the central third of the thymus head. Many of the primary epithelial buds of the enlarged segments (the superficial thymus, thymus heads, mid-cervical and thoracic segments) have sent out processes, thus marking the beginning of secondary lobulation. The intermediary cords are greatly attenuated and show no signs of budding. The transition from the thymus head to the intermediary cords and from the latter to the mid-cervical segment is very abrupt. The cervico-thoracic cords are short and lie near each other a short distance ventral to the trachea. The thoracic segments of both the right and left thymus are now fused along the greater part of their median plane. They are a little larger than those in the previous stage and have the same general position over the anterior and ventral portion of the pericardium.

Embryo of 52 mm. The connecting band on each side loops over the hypoglossal nerve and connects the thymus head with the superficial thymus. The one on the left side is a comparatively large and irregularly modelled cord of differentiated thymic tissue while the one on the right side is a slender and greatly attenuated cord of epithelial cells. The intermediary cords are still greatly attenuated cords of epithelial cells but are now studded here and there with small epithelial buds. The left cervico-thoracic cord is still slender with a nearly uniform diameter while the right one is much larger and has undergone lobulation. Both are now of differentiated thymic structure. The enlarged segments of the thymus are appreciably larger than those in the 40 mm. embryo. They have undergone extended

secondary lobulation the lobes of which on account of their large size lie in general more closely together than those in the previous stage. The parathyroid lies partly imbedded in the dorso-mesial aspect of the thymus head slightly anterior to its central portion. The superficial thymus lies closely along the antero-lateral aspect of the thymus head but is not fused to it.

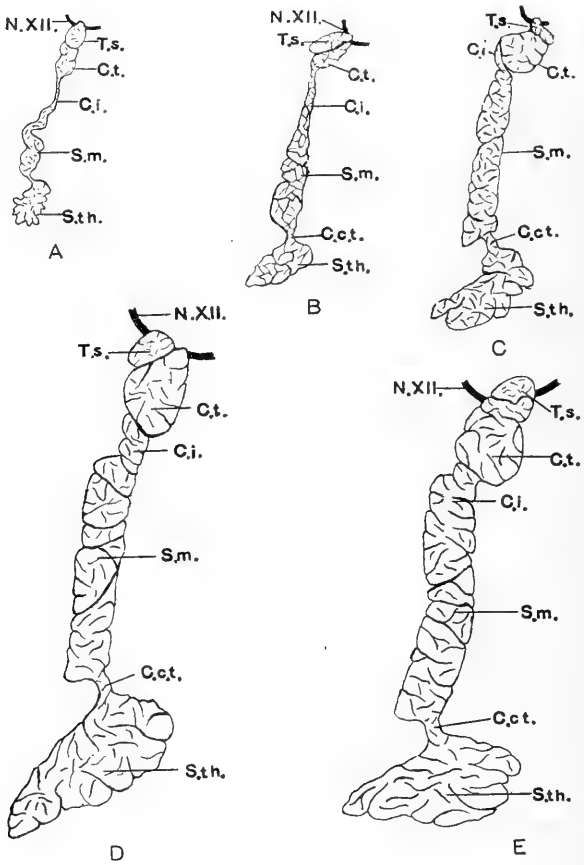
Embryo of 63 mm. The connecting band on both the right and left sides loops over the hypoglossal nerve. They are comparatively large and have an irregular surface similar to the left connecting band in the preceding stage. Aside from their greater size the large segments of the thymus in this stage present no striking morphological changes from those of the 52 mm. embryo.

Embryo of 95 mm. (*figures 6 and 7*). This is the oldest stage in which the anterior portion of the thymus was modelled. The left thymus was chosen for reconstruction although the right one would have done equally well. Figure 6 represents a lateral aspect of the thymus head (*C.t.*) and the superficial thymus (*T.s.*) The thymus head lies alongside the common carotid artery (*A.c.*), its anterior end lying near the bifurcation into the external and internal carotid arteries. The parathyroid (*Pt. 3*) lies about midway between the two ends of the thymus head along its dorsal border and is closely attached to it. The superficial thymus (*T.s.*) lies along the anterior half of the lateral aspect of the thymus head. Its dorsal and ventral borders are almost parallel to each other. The caudal border is rounding while its anterior part tapers irregularly into the slender connecting band (*P.i.*) which loops over the hypoglossal nerve (*N.XII*) and is connected with the thymus head. Figure 7 represents a ventral aspect of the same structures as seen in figure 6. The superficial thymus (*T.s.*) and the thymus head (*C.t.*) are flattened laterally. The anterior portion of the thymus head lies in contact with the dorsal border of the hypoglossal nerve. The superficial thymus lies closely against the thymus head but is not fused with it. Its posterior border on the right side gradually tapers down to a thin edge in contrast to the blunt posterior border of the left superficial thymus. The diameter of the inter-

mediary cords is considerably greater than in the preceding stages. Lobules are now present along their entire extent. They are a little shorter in this stage than in the 63 mm. embryo while the mid-cervical segment is somewhat longer. The cervico-thoracic cords are short and lie closely together. The thoracic segments are thin and flat and spread out over the pericardium to the left of the median line. They extend only a short distance to the right beyond the median line.

Embryos of 105, 140, 170 and 280 mm., and pig 1 day post partem (text figures A, B, C, D and E, respectively). In these stages the structures covering the thymus were removed and the entire organ on the left side, undisturbed, was exposed to view. Diagrammatic drawings, representing accurately the outline of the lateral aspect of the different regions of the thymus, were made. In all cases specimens were selected in which the connecting band on both sides looped over the hypoglossal nerve and connected the superficial thymus with the thymus head.

By referring to the figures cited above it will be seen that the comparative size of the superficial thymus (*T.s.*) and the thymus head (*C.t.*) vary somewhat in different developmental stages. In general, the proportional size of the former to the latter is greater in earlier than in later developmental stages. From numerous dissections that were made it was found that the comparative sizes of the two structures vary considerably in embryos of about the same developmental stage or even in those of the same litter. Figure *A* represents about the average comparative size of the superficial thymus and the thymus head in embryos of about 105 mm. in length, while the size of the superficial thymus of an 140 mm. embryo as represented in figure *B* is considerably larger than it ordinarily occurs in corresponding developmental stages. Variations in size of the superficial thymi in the same embryo also occur; e.g., the right one in a 170 mm. embryo was an oblong flap that covered the anterior one-fourth of the lateral surface of the thymus head, while the left one is much smaller as represented in figure *C*. In all the embryos examined the superficial thymus was always closely associated with the thymus head but never fused with it.



Text figs. A, B, C, D Outline drawings of the exposed left thymus of embryos respectively 105, 140, 170 and 280 mm. (full term) in length; natural size. *C.ct.*, cervico-thoracic cord; *C.i.*, intermediary cord; *C.t.*, caput thymus = thymus head; *N.XII*, hypoglossal nerve; *S.m.*, mid-cervical segment; *S.th.*, thoracic segment; *T.s.*, thymus superficialis.

Text fig. E Outline drawing of the exposed left thymus of a 'runty' pig, one day old and only 240 mm. in length; natural size. The thymus in this specimen was a few millimeters shorter than that in the full-term embryo; this is perhaps due to the fact that the specimen was a 'runt'. *C.ct.*, cervico-thoracic cord; *C.i.*, intermediary cord; *C.t.*, caput thymus = thymus head; *N.XII*, hypoglossal nerve; *S.m.*, mid-cervical segment; *S.th.*, thoracic segment; *T.s.*, thymus superficialis.

From the thymus head the intermediary cord (*C.i.*) and the mid-cervical segment (*S.m.*) extend in a meso-ventral direction to the anterior aperture of the thorax ventral to the trachea. In comparatively early stages they are more or less tortuous as represented in figure *A*, while in later stages their course is nearly straight. The mid-cervical segment in early stages is short and lies immediately anterior to the thorax while the intermediary cord is comparatively long as represented in figure *A*. As development proceeds the mid-cervical segment gradually becomes longer while the intermediary cord becomes shorter as represented in the figures.

The cervico-thoracic segment (*C.ct.*) in all stages is short and of a comparatively small diameter. It lies in the extreme ventral portion of the anterior aperture of the thorax. In early stages the cords of the right and left thymus lie closely together and in later stages they fuse with each other.

The thoracic segment (*S.th.*) in later developmental stages is composed of the thoracic portions of both the right and left thymus which have fused in this region. In embryos about 105 mm. in length, and later stages, it is spread over the antero-ventral surface of the left side of the pericardium. The swinging of the right segment toward the left side is already noticeable in a 42 mm. embryo. This segment is thickest along the median line (3.5 to 4 mm. in full term embryos) and gradually tapers down to a thin irregular edge.

The connecting band was present on both sides in the majority of specimens examined. It may, however, be absent either on one or on both sides. Its rupture is apparently due to the growth in length of the thymus not keeping pace with the growth in length of the neck. The expanded caudal portion of the thymus being firmly anchored in the anterior portion of the thoracic cavity, on account of the unequal rate of growth between the neck and the thymus, will exert a pull on that portion of the organ in the neck and thus greatly attenuate or tear the connecting band. Also there is thus a stress exerted on the hypoglossal nerve which apparently tends slightly to change its direction, as stated in the description of the thymus in 21.5 mm. embryo (p. 325).

A microscopical examination was made of the superficial thymus in various developmental stages, including that of two full-term embryos. It was found that the histogenetic processes of this segment kept pace with those in the segments of the thymus which have a purely entodermal origin.

CONCLUSIONS

The thymus of the pig has an ectodermal-entodermal origin. The respective origin of each segment is as follows:

1. The superficial thymus, which is a derivative of the cervical vesicle, has a purely ectodermal origin. It is a constant structure and, therefore, forms an integral part of the organ.
2. The connecting band is also a derivative of the cervical vesicle and has, therefore, a purely ectodermal origin. In the majority of embryos it persists to birth but may be absent either on one or on both sides.
3. The thymus head, in which is lodged the parathyroid III, is formed by a fusion of a portion of the cervical vesicle to the anterior end of the epithelial diverticulum derived from the third pharyngeal pouch. It has, therefore, an ectodermal-entodermal origin.
4. The intermediary and cervico-thoracic cords, and the mid-cervical and thoracic segments are derived wholly from the epithelial diverticulum of the third pharyngeal pouch and have, therefore, a purely entodermal origin.

I wish to thank Dr. B. F. Kingsbury for the aid given me in this work. I am also indebted to Dr. David Marine, of the Western Reserve University, for sending me many formalin-preserved embryos of various sizes from which most of the drawings of the exposed thymus were made.

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ABBREVIATIONS

A.c., carotid artery
C.t., caput thymus = thymus head
D.b., ductus branchialis
D.c., ductus cervicalis
Ect., ectoderm
G.n., ganglion nodosum
G.s.c., superior cervical ganglion
N.X., vagus nerve
N.XII., hypoglossal nerve
P., pharynx
P.i., connecting band
Pt.3, parathyroid derived from third pharyngeal pouch
S.b. 2, sacculus branchialis II = second pharyngeal pouch
S.b.3, sacculus branchialis III = third pharyngeal pouch
S.b.4, sacculus branchialis IV = fourth pharyngeal pouch
T.e., entodermal thymus
T.s., thymus superficialis
V.c., vesicula cervicalis
V.c.l., vesicula cervicalis lateralis
V.c.m., vesicula cervicalis medialis

PLATE 1

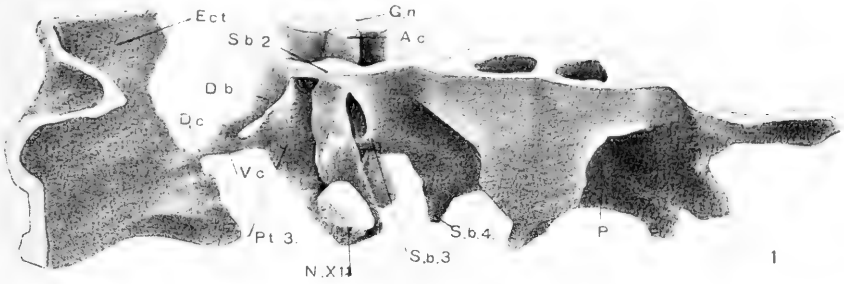
EXPLANATION OF FIGURES

Figures 1 to 7 were drawn by Miss Cora Whitman, from wax models which were made by the author. The text figures *A* to *E* were drawn by the author.

1 Drawing of a reconstruction of the pharynx and derivatives of the second and third pharyngeal pouches of the right side, including portions of the structures closely associated with the pharyngeal derivatives and a portion of the ectoderm. Ventral aspect; pig embryo 14.5 mm. in length. Model × 92, reduced one-half.

2 Drawing of a reconstruction of a portion of the pharynx, the third pharyngeal pouch, anterior portion of the thymus anlage, parathyroid 3, cervical vesicle, and associates of the above named structures. Caudo-ventral aspect. The model represented in this figure was made from the same side of the same embryo (14.5 mm.) from which the model represented in figure 1 was made. Model × 245, reduced one-half.

3 Drawing of a reconstruction of the same structures as enumerated under figure 2. This model was made to show specially the relation of the cervical vesicle to the thymus anlage and the hypoglossal nerve after the shifting of the structures in the neck have become quite noticeable. Ventral aspect, left side; pig embryo 17.5 mm. in length. Model × 182, reduced one-half.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A c
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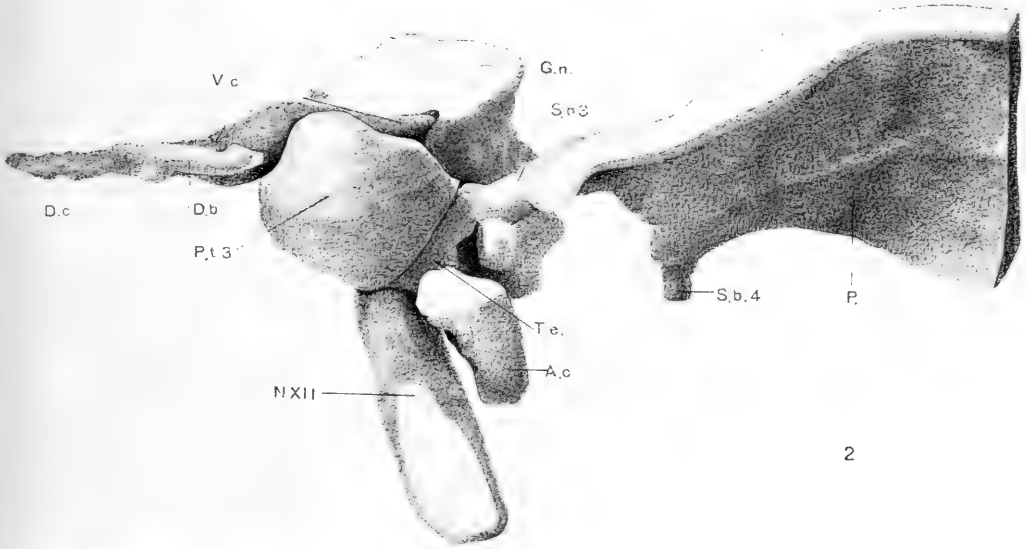
D c
V c
Pt 3.
S.b.4
P
N.XII
S.b.3

1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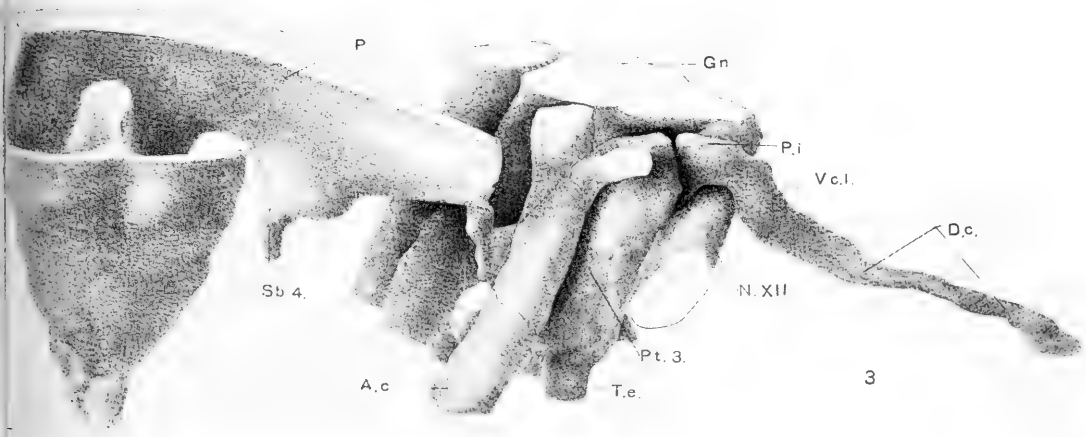
N XII

2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D.c.
Sb 4.
N. XII
Pt. 3.
A.c
T.e.

3

PLATE 2

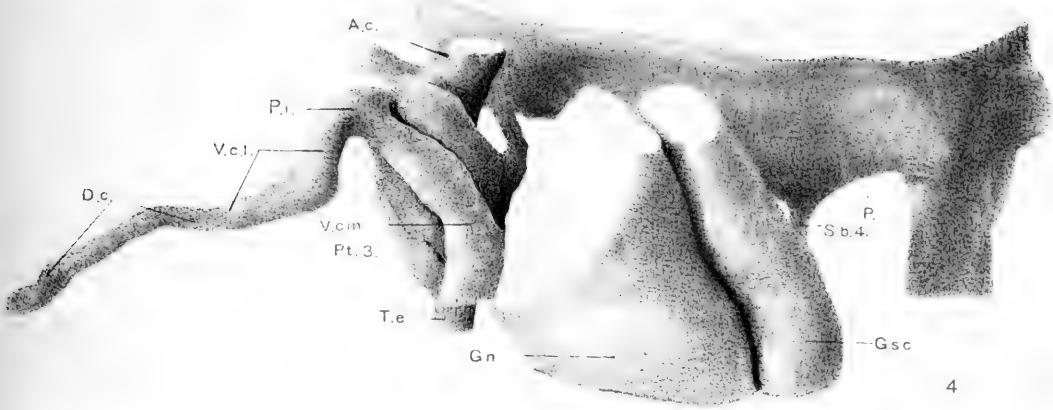
EXPLANATION OF FIGURES

4 Drawing of the same model as represented in figure 3, with the hypoglossal nerve and a portion of the ganglion nodosum removed to expose the cervical vesicle and more clearly to show its relation to the thymus anlage. Dorso-lateral aspect; reduced one-half.

5 Drawing of a reconstruction showing the relation of the vesicula cervicalis lateralis (*T.s.*) to the thymus head (*C.t.*) and their topographical relation to neighboring structures. In this stage the thymus and the cervical vesicle, which have fused, have lost their connection respectively with the entoderm and ectoderm. Right side, lateral aspect; pig 21.5 mm. in length. Model × 182, reduced one-half.

6 Drawing of a reconstruction showing the topographical relation of the superficial thymus to the thymus head. These two structures, in the specimen from which the reconstruction of the thymus was made, are connected with each other by the connecting band (*P.i.*) which loops over the hypoglossal nerve. Left thymus, lateral aspect. × 30, reduced one-half.

7 Drawing of model represented in figure 6; ventral aspect.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T.e
Gn
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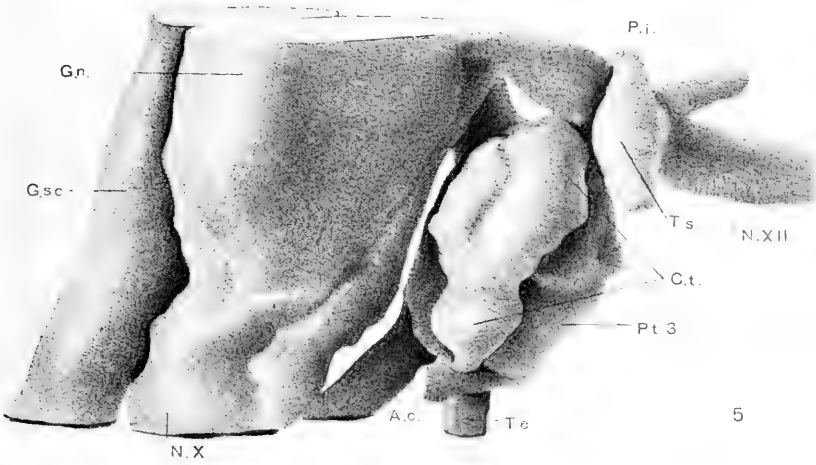
P.
S.b.4.

4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T.e
N.X

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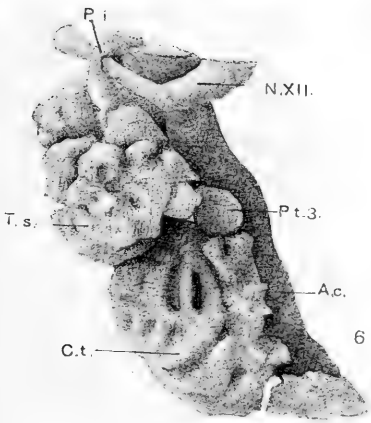
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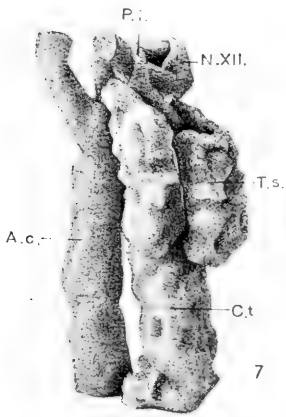
6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C.t

7

MITOCHONDRIA (AND OTHER CYTOPLASMIC STRUCTURES) IN TISSUE CULTURES

MARGARET REED LEWIS AND WARREN HARMON LEWIS[1]

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TWENTY-SIX FIGURES

CONTENTS

[1] We are indebted to the Marine Biological Laboratory for the use of a room during the summer of 1914.

INTRODUCTION

Tissue cultures afford a new and somewhat different method from that usually employed for the study of many cell structures. It enables one to compare the living with the fixed material. In fact, one can study the same cell while living, during the process of fixation, and later as a stained permanent preparation. It also enables one to follow the changes which take place in the living cell from minute to minute. Above all, tissue cultures afford a method by which we can experiment on the cells and mitochondria. And through such methods only do we believe a correct interpretation of the significance of mitochondria is to be found.

In spite of the new and different environment of the tissue, i.e., its isolation from the rest of the embryo; the substitution of a simple Locke's solution for normal plasma; the contact with the cover-slip; and the absence of a circulation, which continually renews the food-supply and removes the waste, the cells of the tissue cultures are apparently quite normal during the first two or three days and exhibit no noticeable changes except the characteristic configuration of the growth. How greatly the new environment disturbs the normal metabolic processes of the cell is impossible to surmise. The cells are in such a thin layer that each cell is probably as well bathed by the Locke's solution as in the embryo it would have been bathed by plasma or lymph.

In the older cultures the cells lose their normal appearance and show signs of degeneration. Migration, growth and mitosis cease, the cells become smaller and show both cytoplasmic and nuclear changes. This may be due to the fact that the medium lacks both the inorganic and organic substances necessary for the prolonged continuance of life, but when we consider that the same degeneration takes place when tissues are explanted into a plasma medium it seems more probable that the degeneration is due to an excess of waste products accumulated around the cell.

It is during the first two or three days then that we may compare the cells and their structures with those found in the embryo. The mitochondria have been studied during this early period when their appearance and behavior can be considered normal. The close resemblance of the mitochondria found during this early period to those found in the chick by other observers (Benda, Meves, Duesberg, Dubrueil, Cowdry, etc.) shows that they at least are not noticeably altered in the culture. We are justified, we believe in assuming that our findings concerning mitochondria apply as well to the normal cells within the embryo as they do to the cells of the tissue culture.

TECHNIC

The ordinary technic for the cultivation of tissues in Locke's solution as described by Lewis and Lewis ('11, '12) was used. We found great variations in the amount, duration and character of growth in different solutions. This was apparently not due to the slight variation that occurs in the weighing out of the salts or sugar, which enter into the composition of the solution, since these can be varied considerably and good growth obtained. The trouble lies either in the distilled water, a contaminated container for the solution, poor chick material, or some manipulation during the process of explantation, which we vary unknowingly. In repeating this work one should make several trials until a solution favorable for growth is obtained. When a favorable solution is once obtained it can be kept for months, provided the dextrose is not added to the stock solution.

Chick embryos were taken out of the egg under aseptic conditions and put into 10 or 20 cc. of sterile Locke's solution (NaCl 0.9 per cent, $CaCl_2$ 0.025 per cent, KCl 0.042 per cent, $NaHCO_3$ 0.02 per cent, dextrose 0.25 per cent at 39°C. A piece a few millimeters in diameter of the desired tissue was then cut out and placed in another dish which contained 10 or 20 cc. of sterile Locke's solution at 39°C. This small piece was then cut up into numerous very small pieces. These were drawn up into

a fine pipette, usually one at a time, with some of the solution and placed each on a sterile cover-slip which was inverted onto a vaseline (melting point, 46 ± °C.) ring on a hollow ground slide. All instruments and cover-slips were sterilized by passing them through the flame, and aseptic precautions were observed throughout.

Great care should be taken to insure absolute cleanliness of the cover-slips. The migrating and dividing cells, as we have already stated, adhere to the cover-slip and utilize it as a means of support, and the presence of grease seems to prevent them from getting a foothold. The small drop should spread out evenly and thinly over the center of the cover-slip so that the surface tension keeps the explanted piece in contact with the cover-slip. The stereotropic cells can thus easily creep out from the piece to the cover-slip on which they migrate towards the periphery of the drop. In cases where the drop is too deep and the small explanted piece falls away from the cover-slip the convex surface of the drop may act as a support for growth.

Growth began within ten to twenty hours and reached a maximum in extent and showed the greatest number of mitotic figures on the second or third day. The cultures were incubated at 39°C. to 40°C. in an electric incubator (with a glass in the door). The presence of the electric light which was placed in the same chamber with the cultures for the purpose of maintaining the temperature of the incubator did not seem to affect the growth. Cultures apparently grow as well in the light as in the dark.

Around the piece of explanted tissue the new growth forms a more or less radiating reticulum, a syncytium, or a membrane-like sheet of cells with varying numbers of isolated cells. The growth may be several cells in thickness near the old piece, but toward the periphery there is usually only a single layer of flattened cells which are often scarcely 2 μ in thickness (fig. 1). The entire contents of these peripheral cells can be observed with very little change in focus. The growth is so closely attached to the cover-slip that in many cases the explanted piece can be torn away without injury to the new growth.

Fig. 1 Photograph of part of a 2-day culture of intestine from an 8-day chick. The black mass is the explanted piece, which is surrounded by the new growth of connective tissue and smooth muscle; there are 13 mitotic figures in this part of the culture; osmic vapor and iron hematoxylin.

Although several different kinds of cells have been identified in the living cultures (Lewis and Lewis) as, for instance, the mesenchyme and connective tissue cells, the heart and smooth muscle syncytium, the endodermal membrane, the yolk membrane, the nerve cell, the kidney tubule cell, etc., nevertheless, the general cytoplasmic structure of the living cell, regardless of the kind of cell, is practically the same except in cases where the cells contain secretory granules. The cytoplasm appears as a homogenous substance within which are several types of granules, i.e., refractive fat globules, various shaped mitochon-

dria and other granules. The nucleus appears as a finely granular body surrounded by a definite nuclear wall with one or more nucleoli. The nucleolus is never a round compact body, but instead is a coarsely granular ragged body, often large in proportion to the size of the nucleus. The nucleolus can readily be seen with the low power even when the outline of the nucleus cannot be distinguished. At one side of the nucleus there is usually present the central body (idiozome).

When permanent preparations were desired the cover-slip was removed from the vaseline ring and the entire culture fixed to the cover-slip by means of osmic acid vapor. After fixation the explanted piece was often torn off from the cover-slip, leaving the new growth, in order to facilitate certain staining processes. Since the growth is very thin it was unnecessary to cut sections. The cover-slip with the fixed growth was treated as one would sections on a slide.

FIXATION

The entire process of fixation can be watched and studied upon any cell, as, for example, one that has been under observation for some time. While the preparation is observed under the microscope some of the fixing solution can be introduced into the cavity of the slide through an opening made in the vaseline ring, which seals the coverslip to the slide. The specimen can be fixed with either vapor or fluid. If a vapor is used, as of osmic acid, a small drop of a 2 per cent solution of osmic acid is introduced on the bottom of the cavity so that it does not come in contact with the hanging drop. If a solution is used, enough is introduced to fill the entire cavity and mix with the hanging drop.

The vapor from a freshly made 2 per cent osmic acid solution gave the best results, and when used with care resulted in a fixed cell, which more closely resembled the living cell than any other method we have used. The osmic acid vapour seems to cause a precipitation of the cell structures in the form of minute granules. Even after such fixed specimens are stained by means of iron hematoxylin the general character of the cyto-

plasm and nucleus are not noticeably altered except for the staining.

B. F. Kingsbury ('12) states that according to Rawitz ('07), Kollarewsky ('87) and Eisen ('00) osmic acid does not preserve nuclear details. So far as can be seen from our material the living cell exhibits few nuclear details and even osmic acid vapor differentiates more clearly the nuclear structures than they can be distinguished in the living cell.

The mitochondria are so well fixed by osmic acid vapor or by a fixing solution which contains osmic acid that it has been suggested that the mitochondria may be artifacts due to osmic fixation. Vapor from strong formalin which has been carefully neutralized (Mann '02 and also Bensley '11 recommend that formalin be freed from acid by careful neutralization and redistillation) gave good results in regard to fixation not only of the mitochondria but of all cellular structures. Unfortunately, the mitochondria did not stain well after formalin fixation. Iodine vapor from a crystal of iodine often afforded good results in regard to the spindle fibers and also the mitochondria, especially so when followed by Bensley's anilin fuchsin, methylene green stain, but iodine was an uncertain fixative. Osmic acid solutions do not give as uniformly good results as the vapor.

Any fixing solution which contained acid (acetic, hydrochloric, sulphuric, etc.) proved useless as a fixative for tissue cultures. The vapor from such acids coagulated the entire cell before the fluid touched the preparations. The mitochondria rapidly changed into small granular rings, which later were completely lost in the coagulated network of cytoplasm. The nucleus lost its homogenous finely granular structure and a coarse network appeared. The nucleolus became a small round body. This resembles closely the usual textbook figure of a cell, which by long association one has come to believe represents a cell but which actually resembles the living cell not at all.

When a living cell (fig. 2 a) was exposed to the action of vapor from 2 per cent glacial acetic this coagulation effect was soon apparent, as shown in figure 2 b. The cytoplasmic and nuclear networks rapidly appeared while the mitochondria, which

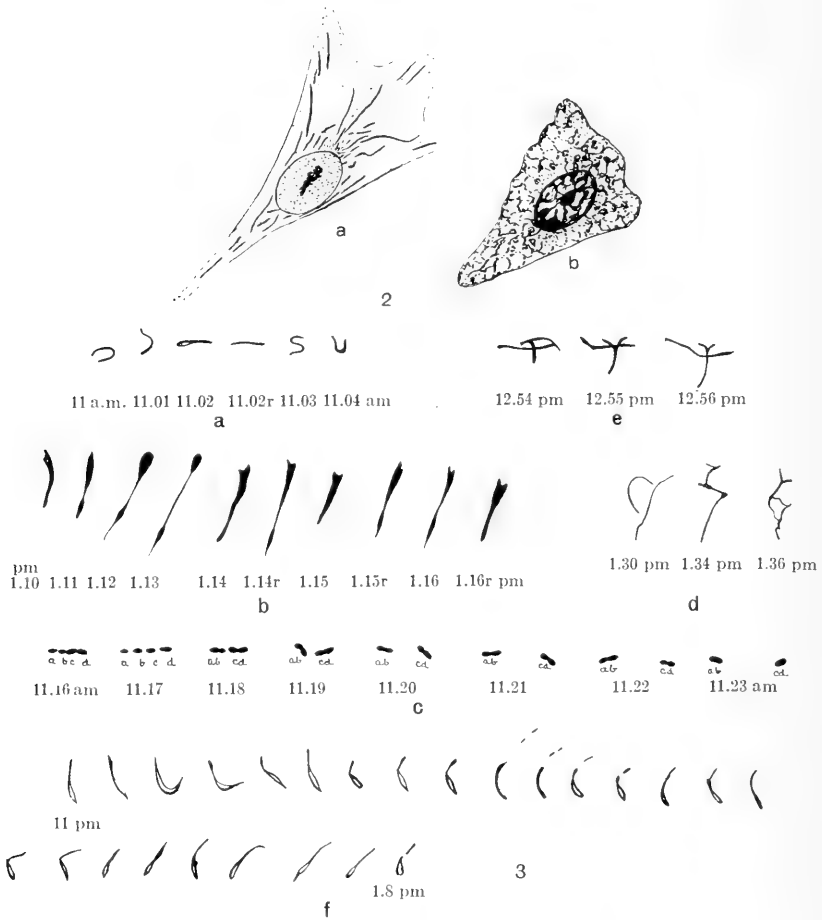

Fig. 2 *A*, sketch of living cell with nucleus and mitochondria, the cytoplasm should be homogenous. *b*, the same cell after exposure to the vapor of 2 per cent acetic acid; both cytoplasm and nucleus show reticular structure due to coagulation; the mitochondria are destroyed except for a few granular remains.

Fig. 3 Changes in shape of mitochondria as observed in living cells; *a*, changes by bending during a period of 4 minutes; *b*, by shortening and elongating and shifting along the mitochondrium of the mitochondrial substance, time 6 minutes; *c*, by fusion of granules; in the course of 7 minutes the four granules *a*, *b*, *c*, *d* became fused into two granules *ab* and *cd*; *a*, *b*, *c* from a 2-day culture of mesenchyme from a $4\frac{1}{2}$-day chick; *d*, changes in shape and fusion of mitochondria to form network in the course of 6 minutes; *e*, changes in shape and fusion, 2 minutes; *f*, changes in form of a single mitochondrium during a period of 7 minutes; *d*, *e* and *f* from a 3-day culture of mesenchyme from a 4-day chick.

were clearly seen in the living cell as long rods and threads rapidly disappeared. Brunn ('84) found that the Eberth bodies, which have since been shown to be mitochondria, are dissolved by acetic acid. Duesberg ('11) states the fact that they are destroyed by acetic acid to be one of the criteria for mitochondria. Prolonged fixation in osmic acid after the osmic vapor has already fixed the preparation did not cause any distortion of the nucleus or the cytoplasmic structures. The mitochondria became somewhat blackened and the fat globules were first yellow-brown and later became a dark brown. Even after a month no change appeared in the cytoplasm which in any way indicated the presence of a canilicular apparatus as was found in certain other cells by Kopsch ('02), Sjovall ('06), and Cowdry ('12).

A careful study of the living cell together with a study of the effect of various fixatives shows that while the mitochondria are only successfully fixed by osmic acid, they are by no means artifacts due to osmic acid fixation.

MITOCHONDRIA

The mitochondria are always present in the living cells of the tissue cultures and after some experience can be easily recognized. They are never as conspicuous, however, as the fat globules or the nucleus. The mitochondria are slightly refractive bodies which vary greatly in shape, size, and position. In the living cell these bodies are never quiet, but are continually changing in shape, size, and position. Often as many as fifteen or twenty shapes may be exhibited by a single mitochondrium within as many minutes (fig. 3). This extreme plasticity of the mitochondria is a very important characteristic and was shown in every preparation examined. It is certainly a feature which must be reckoned with in any attempt to classify or to analyze their behavior from fixed material.

The chaotic condition of the literature in respect to the terminology and criteria for mitochondria and other cytoplasmic bodies renders it difficult often to correlate our findings with

those of other observers. It is important then that we should as far as possible submit the bodies herein under consideration to already established criteria for mitochondria.

While these bodies fulfil Benda's ('99) original criterion for mitochondria in embryonic cells in that they stain blue with alizarine, we have made no effort to fulfil Montgomery's ('11) criterion that they must show an unbroken cycle from egg to somatic cell to anlage sex cell and back to the fertilized egg. They do, however, correspond with Duesberg's ('11) criterion for mitochondria in the adult cell, in that they are seen in the fresh preparation, dissolved by acetic acid, preserved by osmic acid, and stain by the same dyes as the mitochondria in embryonic cells, that is, green with Janus green in the fresh preparations (Michaelis '99, Laguesse '99, Bensley '11, Cowdry '12); stain blue with Benda's stain (Benda '03, Meves, '08, Duesberg '09); red with Bensley's anilin fuchsin, methylen green stain (Bensley '11, Cowdry '12), and black with Heidenhain's iron hematoxylin.

Janus green caused the death of the growth after a few hours, and frequently the mitochondria separated into granules (fig. 21). For this reason Janus green was used only to identify various granules as mitochondria but never for any observations upon the changes in shape, size or quantity of mitochondria.

These bodies have been given various names—mitochondria and chondriomiten by Benda; chondrioconten, chondriosomen, chondrion and plastosomen by Meves; plasmafaden, plasmakören by Retzius; paramiton or miton by Flemming; microsomen by Van Beneden; granules and filament by Altman, etc.

Position of mitochondria

Great variation occurs in the arrangement of the mitochondria even in the same kind of cells in the same preparation, not only in the living but also in the fixed preparations. It is not uncommon for the mitochondria to be more or less evenly scattered throughout the cytoplasm and the various processes of the cell. They have been observed even in the extremely

slender processes that are scarcely larger in diameter than a mitochondrium. This rather uniformly scattered arrangement usually occurs during mitosis and here likewise the cell processes may contain mitochondria. The spindle area is usually free from mitochondria (figs. 4, 15, 16, 17). Infrequently in the late anaphase the mitochondria may collect along the plane of division. In elongated cells the mitochondria are usually arranged at either end of the nucleus with their long axis more or less parallel to the long axis of the cell. However, in many of the cells the mitochondria are more numerous about the nucleus or about the central body than towards the periphery of the cell, where they may be scattered or entirely absent (figs. 4, 5, 12, 17). The central body is an extremely finely granular body at one side of the nucleus and has been so-called by us because the mitochondria frequently radiate around this body and because it is a non-committal term. Usually the idiozome (or nebenkern, for discussion of correct terminology see Wilson '11) can be seen within this body and occasionally the centrasome can be made out within the idiozome. The central body is more clearly seen in the living cell than in the fixed cell, but in some cells this body cannot be distinguished and the mitochondria appear more or less radially arranged around the nucleus. At times the mitochondria may be confined to one side only of the nucleus, usually the side on which lies the central body. This radial arrangement about the central body has been described by Eberth (66), Vejdovsky ('07), Meves ('09), Veratti ('09). In some preparations this arrangement is so marked that one cannot but wonder if there is not some definite relationship between the two, and it is not difficult to understand why Vejdovsky ('07) believed that the mitochondria were products of the activity of the centrasome.

The mitochondria, however, are continually altering their position, not only in relation to the nucleus and central body but also in relation to each other. They seem to be continually emerging from the mass near the nucleus or near the central body and to migrate out towards the periphery. Also those towards the periphery often return to the central mass. There

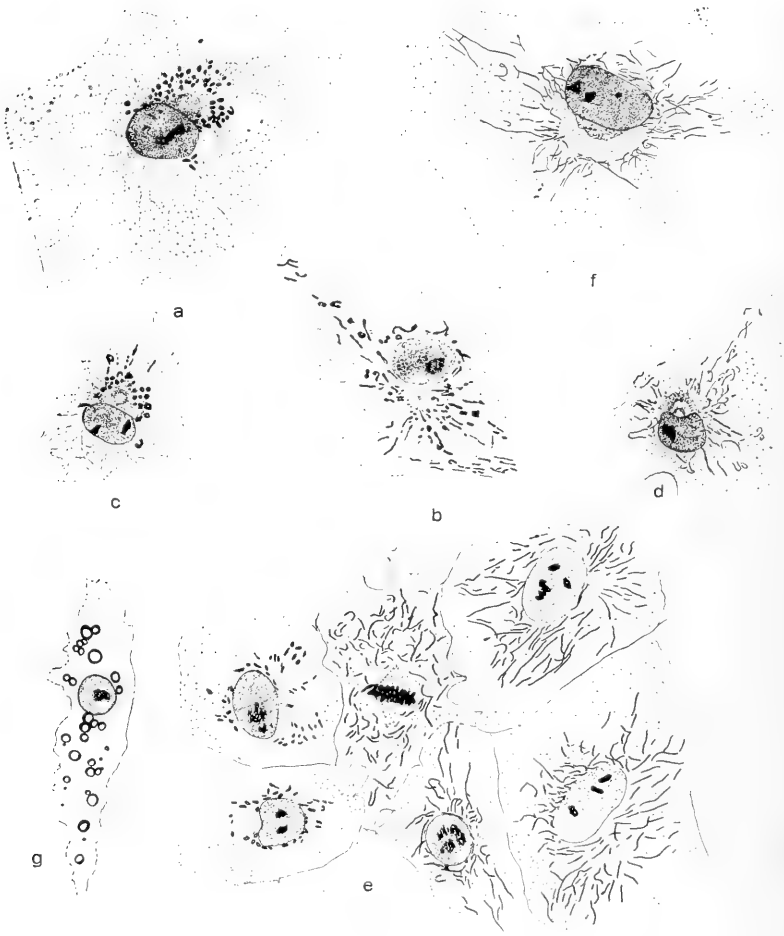

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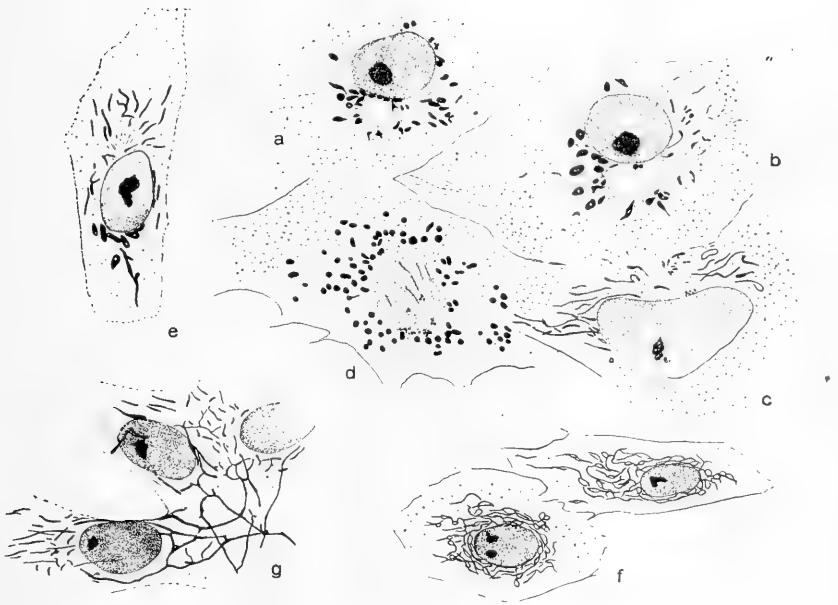
Fig. 4 *A*, *b*, *c*, *d*, *e*, cells from a 2-day culture of heart from a 5-day chick. *a*, cell with 69 mitochondria of granular type, somewhat radially arranged about the central body. *b*, cell with 125 mitochondria of very different shapes and sizes, about the central body and scattered through the cytoplasm. *c*, cell with 37 mitochondria about the central body, mostly granular in type; × 1080 diam. *d*, cell with about 90 mitochondria about nucleus and central body, mostly rod- and thread-shaped; × 540 diam. *e*, group of six adjoining cells; as in *a* the cells with granular and short, rod-shaped mitochondria show the latter arranged about the nucleus and central body; one cell contains 40 and the other 54 mitochondria of the short and long rod- and thread-shaped types, which are arranged more



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about the nucleus and central body; the dividing cell has 118 mitochondria which are scattered more evenly through the cytoplasm than in the other cells; × 790 diam.; osmic acid vapor and iron hematoxylin; *f*, cell from a 2-day culture of heart from a 6-day chick cell with 152 mitochondria, mostly rod- and thread-shaped, arranged about nucleus and central body; × 790 diam. *g*, cell from a 2-day culture of heart from a 4-day chick. Only two cells in entire culture show these ring-formed mitochondria and in these two the cytoplasm was abnormal, perhaps dead. Bensley stain; × 790 diam.

Fig. 5 *A*, *b*, *c*, *d*, four adjoining cells from a 2-day culture of heart from a 5-day chick; × 790 diam. The resting cells, *a*, *b* and *c*, have 47, 51 and 48 mitochondria respectively, while cell *d* in early prophase has 89. These cells exhibit great variety in the shapes of the mitochondria; small and large granules, spindles, short rods, long rods and threads are present; the mitochondria are somewhat more scattered than the cytoplasm in cell *d*. *e*, cell with 38 mitochondria which vary greatly in size and shape; from the same specimen; × 540; osmic acid vapor and iron hematoxylin. *f*, *g*, mesenchyme cells from 2-day cultures of intestine from a 7-day chick embryo; *f*, many of the mitochondria are united in networks; × 540 diam.; *g*, some of the mitochondria branch and anastomose to form a complicated network which appears to extend from one nuclear area to another, where the cells form a syncytium; × 790 diam.; only a portion of the cells and network is shown; osmic acid vapor and iron hematoxylin.

is probably no relation between the movement of the cell and the movement of the mitochondria, for the cell processes change their position so slowly that there is often no noticeable change for several hours, while the mitochondria change position rapidly and continually.

Occasionally in a syncytium of cells a mitochondrium may pass over the cytoplasmic bridge from one cell to another (fig. 5 g). In some cases a mitochondrial thread may pass over the bridge into another cell and later return.

What is it that governs the arrangement of the mitochondria? Is it the shape of the cell, the influence of the central body or of the nucleus, the internal structure of the cytoplasm, or do the metabolic activities of the cell govern the size, shape and arrangement of the mitochondria?

Shape of mitochondria

The mitochondria exhibit extraordinary diversity of form often in the same preparation, even in adjoining cells of the same type (figs. 4, 5). Not infrequently a single cell may contain mitochondria of diverse shapes (fig. 5 e). These different mitochondrial shapes may be more or less localized in different parts of the cytoplasm (fig. 5 e) or may be more or less mixed together (fig. 4 b). The extraordinary diversity in form of the mitochondria shown by cells of the same type lying side-by-side in the same preparation is sometimes very striking. Such differences occur in the young growing cells after division, in older resting cells and even during the various stages of mitosis. Again, we may find in the same preparation groups of cells in one part of the growth, that have very similarly shaped mitochondria, while in another part practically all of the cells may have quite differently shaped mitochondria. In such preparations all gradations in shape and size, from minute granules to larger and larger ones, or from rods to threads of various lengths, or threads and networks, etc., can be seen in adjoining cells of the same type or even in the same cell. Just as the fixed preparations show such gradations, we find that all sorts

of transformations from one shape into another can be watched in the living cell.

Mitochondria of various shapes have been described by other observers, and so definite did some of the shapes appear to be that they were given various names, which today are without much significance. Nevertheless, it is convenient to classify mitochondria as follows (fig. 10).

Small granules	Threads
Dumb-bell-shaped granules	Loops
Spindle-shaped granules	Rings
Large granules	Network
Rods	

The degenerate mitochondria also show more or less definite shapes (fig. 13).

Mitochondria continually change shape as by bending in various directions (fig. 3 a), or by shortening and thickening or elongating and thinning (fig. 3 b); at times this thickening and thinning seems almost like a pulsation along the length of the mitochondrium. These various shapes of mitochondria are not fixed or constant in any cell. Rods or threads may change into granules; threads may fuse or branch into networks (fig. 3 d, 6, 7); or granules may fuse to form larger granules (fig. 3 c). Degenerating mitochondria may separate into granules and vesicles (fig. 13).

Ring-shaped mitochondria are seldom found in these preparations. Occasionally a living cell may contain one or two large or small ring-shaped mitochondria which rapidly change into threads, rods or granules. A few fixed and stained preparations show one or two cells at the periphery of a large growth which contain ring-shaped mitochondria exclusively (fig. 4 g). Kingsbury ('11) has suggested the possibility that mitochondria which contain a large amount of lipoid are reduced by osmic acid only at the surface, and the central part later dissolves out, which produces the appearance of rings. These ring-shaped mitochondria can hardly produce fat or lipoid droplets (Dubreuil '11, '13) since they are seldom present in cells in which fat is being formed.

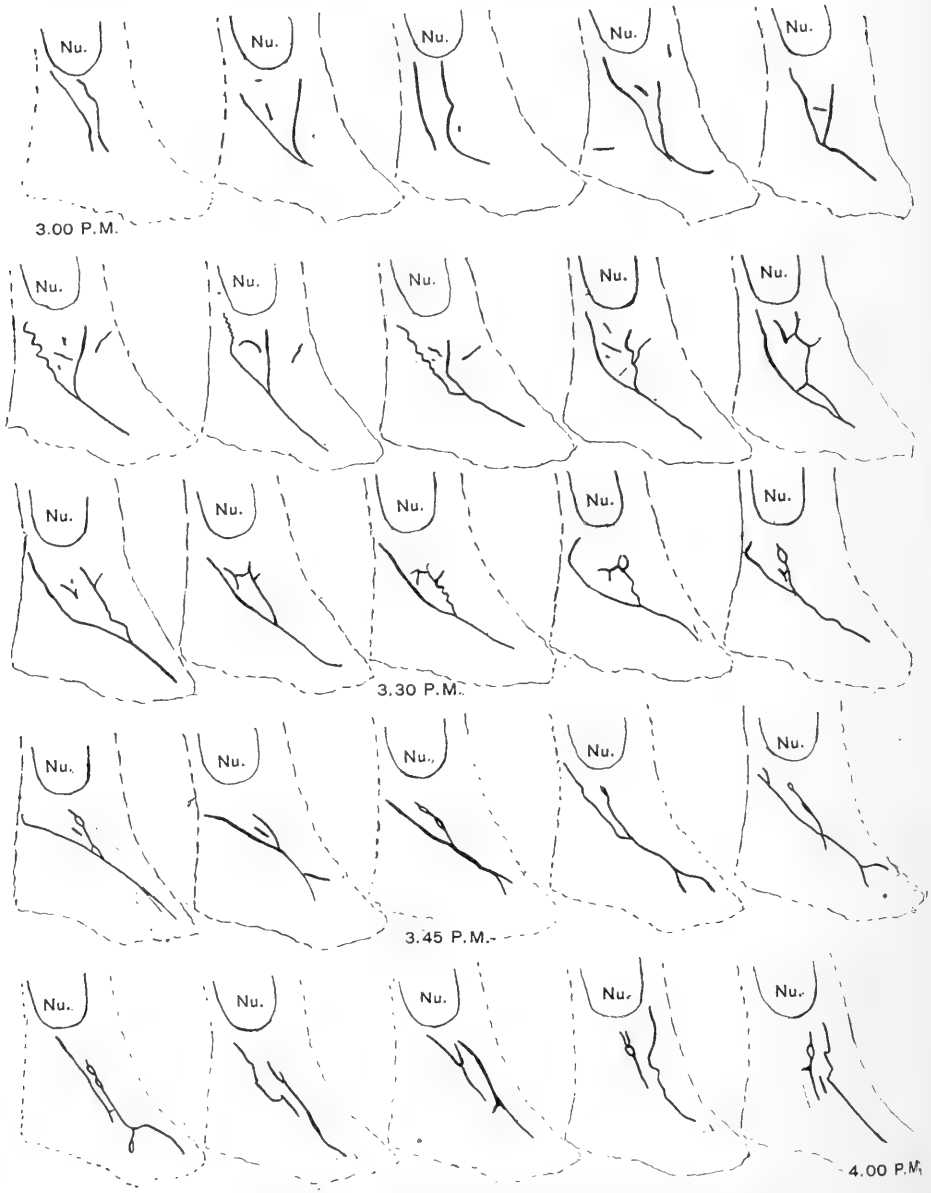


Fig. 6 From a living culture about 24 hours old, of a piece of heart from an 6-day chick, showing branching, fusion and splitting of two or three mitochondria during a period of about 1 hour.

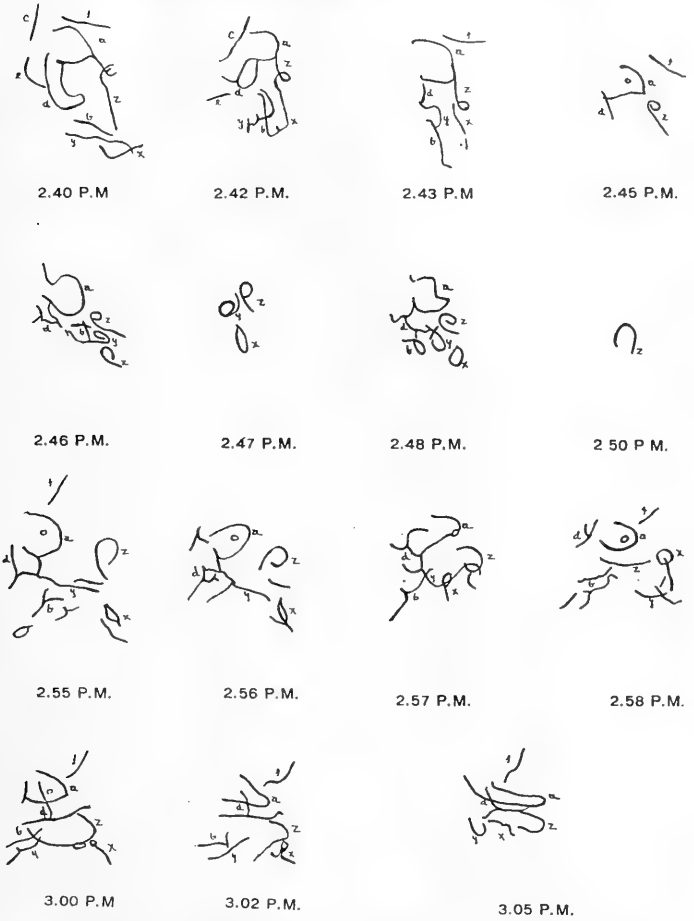


Fig. 7 Changes in shape and anastomoses of a few mitochondria during a period of 25 minutes in a living mesenchyme cell. The changes were so rapid that it was not always possible to draw each mitochondrium; they can be followed by the lettering; 24-hour old culture from a 6-day chick embryo.

The mitochondria are frequently arranged in the form of a network (fig. 5 f, g) which may involve many of the mitochondria or only a few of them. A study of the fixed preparations and especially a study of the living cells shows conclusively that Mislavsky ('11) is correct in his contention that the mitochondria do fuse and branch to form networks. We have observed all

stages of the formation of network in the living cell. These networks continually change shape (figs. 6, 7). New branches appear, old ones change shape or position or break away, and at times the entire network may break down into loops, threads and granules without any apparent change on the part of the cell. From our observations it appears that the network is

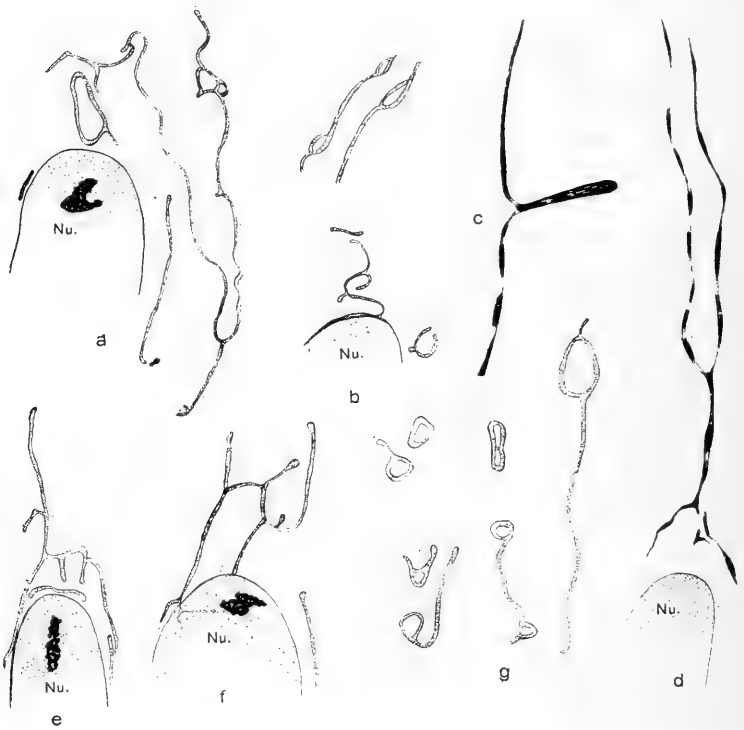


Fig. 8 Mitochondria from a 3-day culture of intestine from a 7-day chick; osmic vapor and iron hematoxylin; × 2250 diam.; various forms of mitochondria, which come from the breaking down of a network, into loops, rings, threads, etc.

very unstable and rapidly breaks down into granules, loops and threads. Figure 8 shows such loops and rings in a fixed specimen.

There has been some discussion as to which shape of mitochondria is the more primitive. Meves ('08) claimed that in the twenty-hour chick embryo the mitochondria are present

only as very thin threads, but that at forty-eight hours the threads are thicker and also some granules are present. These mitochondria have heavy stained edges with a clear mark substance.

Duesberg ('08) finds the same for the chick, but in the rabbit he describes the mitochondria in the early fertilized egg as small granules, which increase in volume and become large granules at the end of the third day. The large granules have a clear central part and dark outer edge. They flow together and build rods and threads. Rubaschkin ('11) finds only granules in the early guinea-pig development. He claims that the granular form of mitochondria is the most primitive and indifferent form.

So far as could be observed, there is no special difference in the shape of the mitochondria present in the cells of the growth from a piece of a three-day chick embryo from that present in the growth from a piece of ten-day chick embryo. Only those cells show exclusively the small granules, which contain many fat globules or vacuoles. We have observed the cells of a 51 hours growth which contained only the granular type of mitochondria to contain at 70 hours mostly thread types (fig. 9 a–f). The threads were formed by the stretching out of the granules rather than by fusion of granules although such fusion of granules does take place. When a preparation is studied from day to day it is clear that the shape of the mitochondria changes and that no one shape is constant for any one age.

Brown ('13) finds that in the male germ cells of Notonerta the mitochondrial fibers and threads arise in part at least from spherical-shaped mitochondria.

Schaxel ('11) claims that the shape of the mitochondria varies with the method of fixing and staining inasmuch as by the Benda treatment the rod-like forms predominate while after the Altman treatment the granular type predominates. While there have been few observations made as to the effect of various technical methods upon the shape of the mitochondria they appear to be such malleable structures that it is quite probable that their shape could be altered by different methods.

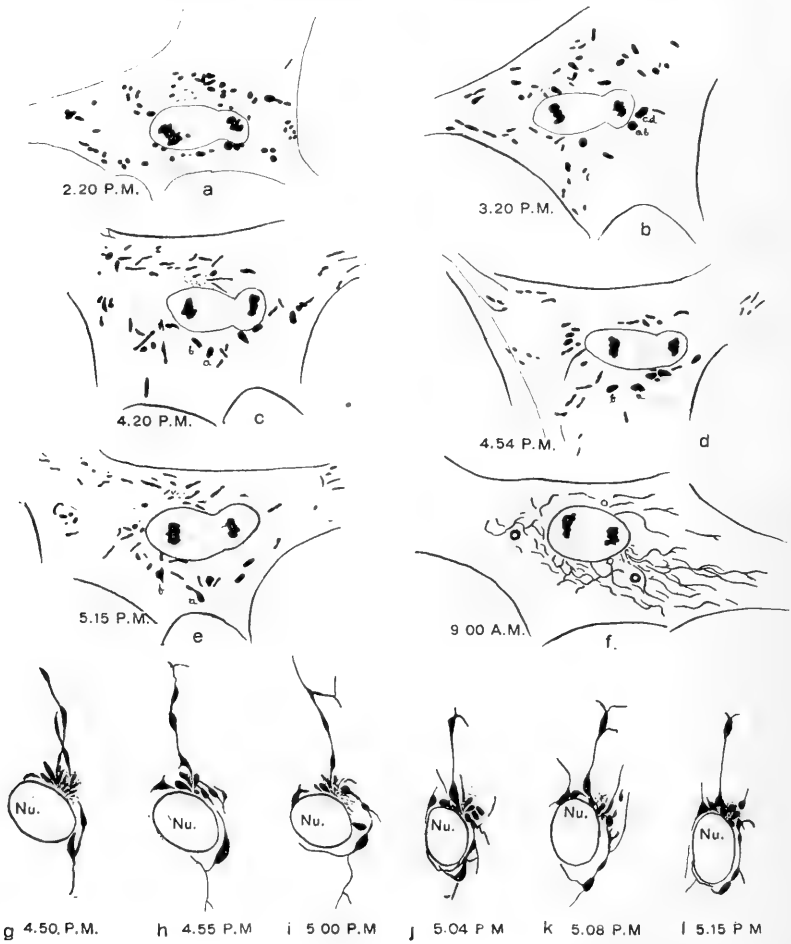


Fig. 9 Mitochondria in living cell from a culture of heart from a $4\frac{1}{2}$-day chick. *a*, camera drawing at 2.20 P.M., when culture was 51 hours old; *b*, same cell at 3.20 P.M.; *c*, at 4.20 P.M.; *d*, at 4.55 P.M.; *e*, at 5.15 P.M.; at this time all the cells had a similar type of mitochondria; *f*, camera drawing at 9.20 A.M. of next day, culture 70 hours old. All the cells in the culture had the same type of mitochondria as in *e*. At 4 P.M. most of the cells began to show signs of degeneration and a fresh drop of Locke's solution was put on preparation; the thread-like mitochondria, as seen in *f*, began to fuse into large spindle-shaped masses near the nucleus and central body, as in *g*, where all the mitochondria now present at 4.50 P.M. are shown, and the changes which they underwent during the next 25 minutes are in this particular cell shown in *h*, *i*, *j*, *k* and *l*.

Size of mitochondria

The mitochondria vary so greatly in size (fig. 10) that were it not for prolonged study of them and the use of a specific vital stain such as Janus green it would be difficult to believe that they all belong in the same class of granules. Even in a single cell great variation occurs from very minute granules which are scarcely visible to relatively large masses (figs. 4, 5, 9).

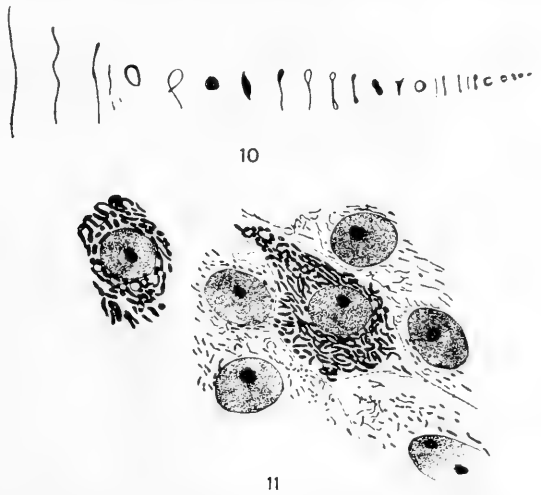


Fig. 10 Camera lucida drawings of mitochondria of various sizes and shapes from different cells and specimens; osmic acid vapor and iron hematoxylin; × 790 diam.

Fig. 11 Endodermal cells from 3-day culture of allantois from a 7-day chick; Bensley's aniline fuschsin methylene green stain; × 790 diam.

Occasionally a cell is seen in which all the mitochondria appear to be swollen up and much larger than those in the surrounding cells (fig. 11). To what this is due is not known.

A mitochondrium under observation frequently seems to change in size as well as shape, but so far no micrometer measurements have been made to determine this point. Definite increase in size has frequently been seen, due to fusion of two granules to form a larger granule or to fusion of rods into threads; and occasionally all the mitochondria in the cell may become col-

lected in several very large granules (fig. 9). In certain pathological conditions Barratt ('13) has found that the mitochondria become abnormally large and stain clearly.

Number of mitochondria

The number of mitochondria varies greatly in cells of the same kind in the same preparation (fig. 12) and in different preparations (figs. 4, 5, 12). Numerous counts of the mitochondria in the same kind of cells in the same preparation show that there is no one number of mitochondria peculiar to any one kind of cell or to any one stage in the development of the cell. The number of the mitochondria appear to decrease and to increase under various conditions. This may result from fusion or division of the mitochondria without much change in the quantity of mitochondrial substance; or this may be accompanied by a corresponding increase or decrease in the amount of mitochondrial substance, independent of any fusion or division of the already existing mitochondria. This would indicate that some of the mitochondria may at times entirely disappear and that possibly new ones may arise *de novo* in the cytoplasm. Sometimes most of the cells in a growth undergo such changes. When observed on one day they may have rather few mitochondria, while on the following day most of the cells may contain a marked increase in the number of mitochondria, or the opposite phenomenon may take place. This may or may not be accompanied by a corresponding change in the quantity of mitochondrial substance. Prolonged action of heat causes a decrease in the size and number of the mitochondria, and it is hoped that further experimental work will determine what conditions cause such changes in the ordinary cultures.

Quantity of mitochondria

By the quantity of mitochondria we mean the total mass of the mitochondrial substance within a cell. This can only be roughly estimated, as some cells with many very small mitochondria have a smaller quantity of mitochondrial substance than others with fewer but larger mitochondria. However, in

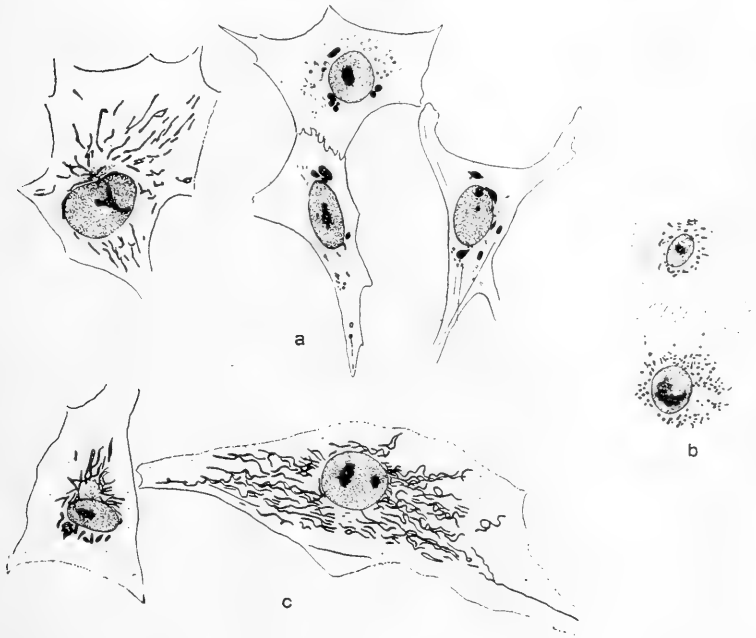


Fig. 12 A, mesenchyme cells from a 2-day culture of intestine from a 5-day chick, showing marked differences in shape, size and number of mitochondria. The four cells have 74, 8, 27 and 6 mitochondria; Bensley stain; × 790 diam.; *b*, two adjoining cells from a 2-day culture of heart from a 7-day chick; granular type of mitochondria, one cell has 38 and the other 111 mitochondria. Osmic acid and iron hematoxylin; × 540 diam.; *c*, two endodermal cells from a 2-day culture of allantois from a 4-day chick; the larger cell contains about 128 and the other 27 mitochondria; Bensley stain; × 790 diam.

many cases of adjoining cells (figs. 11, 12 b) or of the same kind of cells in different parts of the preparation (fig. 12 c) there can be no doubt that the quantity of mitochondria is markedly increased or very much decreased. This increase in the quantity of mitochondria is most marked in a few scattered cells in the growth from a piece of allantois (fig. 11).

Cells with few mitochondria do not necessarily have larger mitochondria and there seems to be no definite relation between size and number or number and quantity. The quantity in the cell differs so widely that it has so far been impossible to connect the quantity of mitochondria with any one factor. Possibly it is dependent upon the metabolism of the individual cell.

This is also true of cells undergoing division, for there seems to be no amount of mitochondria characteristic of any one phase of division. Also the variation in the quantity of mitochondria present in any one phase of division is considerable, as can be seen (figs. 14, 15, 16). Daughter cells usually have a smaller quantity of mitochondria than the metaphase cell or than the resting cell (fig. 17).

As to the question whether the amount of mitochondria increases during mitosis it is impossible to state. So far, we have only one definite observation that this is true. In this case the living culture was subjected to a temperature of 46°C. for two hours, during which period the mitochondria decreased decidedly in number and size. Two cells which were under observation suddenly began to pass into prophase and during this process the number of mitochondria in these two cells increased until they contained more than they had before the experiment was begun. Although several subsequent experiments with increased heat caused a decrease in the quantity of mitochondria no cell division was observed.

No agent but heat has so far been observed which caused a change in the amount of the mitochondria without injury to the cell. However, it is evident that certain metabolic conditions must cause a change in the quantity of mitochondria.

Relation between position, size, number and quantity of mitochondria

No definite relation between the position, size, number and quantity of the mitochondria has been observed in the cells of the tissue cultures, still there is a more or less marked manner in which the mitochondria occur in the cells. Frequently the long threads or short rods are plentiful and scattered throughout the cytoplasm with or without a definite central body. When the mitochondria are in the form of large granules and thick rods they are fewer in number and are arranged more or less radially around a central body. When only a very few mitochondrial granules are present they are usually of the large granule type.

All phases in the development of the cell, i.e., daughter cell, growing cell, resting cell and dividing cell can be found with any one of the above combinations of the mitochondria. However, it must not be forgotten that many cells contain part of one kind of mitochondria and part of another and that any one shape of mitochondria may turn into another at any time during observation, and that no one shape of mitochondria remains as such for a very long interval of time, but changes into another.

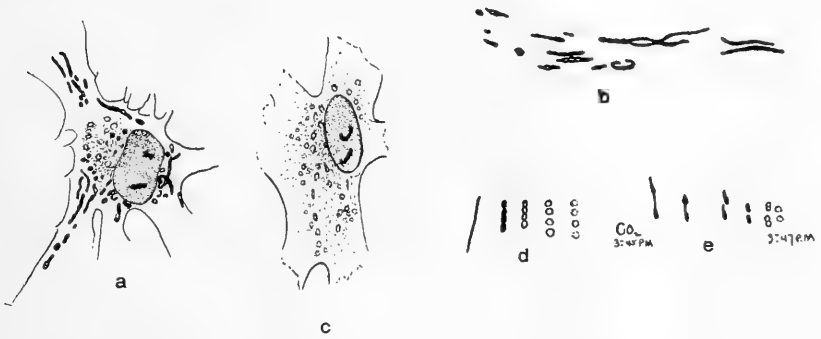


Fig. 13 A, cell from a 2-day culture of heart from a 7-day chick; practically all the mitochondria are degenerated, those in the region of the central body show most advanced stage of granular rings; × 790 diam.; *b*, degenerating mitochondria from mesenchyme cell of a 2-day culture of intestine from a 4-day chick; × 920 diam.; *c*, cell from a 2-day culture of heart of a 5-day chick; all the mitochondria have degenerated into granular rings; osmic acid vapor and iron hematoxylin; × 790 diam.; *d*, process of degeneration in a single mitochondrium produced by the action of acetic acid vapor on a living cell; *e*, effect of CO_2 on another mitochondrium in 2 minutes.

Degenerate mitochondria

Degenerate mitochondria of various shapes are occasionally found in these preparations (fig. 13). A study of the cells of the older growths shows that all the mitochondria do not necessarily degenerate at the same time. Some cells are found which contain many normal mitochondria, some partly degenerate, and others entirely degenerate.

This degeneration appears first in the mitochondria around the central body and later in those scattered at the periphery (fig. 13 a).

The process of degeneration of the mitochondria can be most successfully observed when produced by some outside agency such as carbonic acid gas or vapor from a weak acid solution (fig. 13 d, e). When the death of the cell is produced experimentally the mitochondria become first a series of granules which soon become slightly vesicular although at this stage they still stain in the characteristic manner. Then these vesicles separate and rapidly become small, finely granular rings or shadows. These no longer stain like mitochondria but more like the cytoplasm, i.e., brownish green with Bensley's anilin fuchsin, methylen green or pale gray with Heidenhain's iron hemotoxylin, and in the living cell the Janus green does not stain them green. It is apparent that some change has taken place which has completely changed not only the morphology but also the composition of the mitochondria.

These degenerate mitochondria correspond in many ways to the "grains du segregation" described by Dubreuil in the lymph cells, but are unquestionably degenerate mitochondria, and they can be produced in any cell of these growths by means of various agents such as carbonic acid gas, chloretone, acid vapor, hydrogen peroxide and potassium permanganate.

Meves ('10) and Duesberg ('10) simultaneously found that poorly fixed mitochondria show granulation and small bladder forms. Other observers have found that granulation is due to delay in fixation after death or to disease, as Mayer and Rathery ('07) experimental polyuria; Takaki ('07) polyuria or prolonged fast; Policard ('10) experimental poluria and after injection of phlorizin; Policard and Garnier ('07), Cesa Bianchi ('10), Heidenhain ('11) also obtained similar results.

Beckton ('10) claims that in a certain tumor no mitochondria were present in the tumor cells. In view of the observations of Beckwith ('14) it may be possible that certain cells can exist without mitochondria, but it seems more probable that the apparent lack of mitochondria in the tumor cell described by

Beckton may have been caused by delay in fixing the material so that the mitochondria became degenerate, or the mitochondria may have been present only as degenerate structures which did not stain.

Mitochondria in mitosis

Naturally, the question at once arises: What is the rôle of the mitochondria during division of the cell? Many observers believe that the mitochondria form a palisade about the spindle during late anaphase and then divide and one-half of each mito-

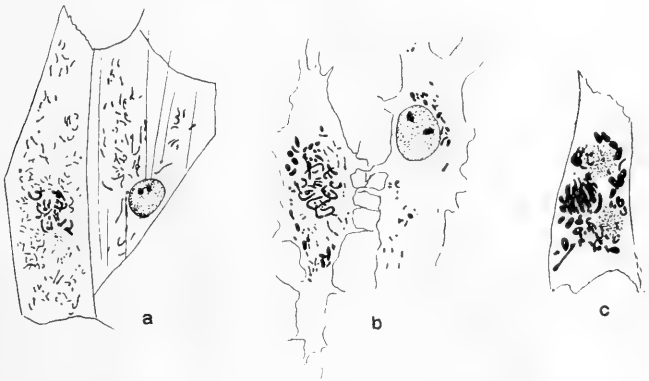


Fig. 14 Arrangement of mitochondria in the prophase stage; *a*, from a 3-day culture of intestine from an 8-day chick embryo; × 1080 diam.; *b*, from a 3-day culture of intestine from a 7-day chick; × 790 diam.; *c*, from a 2-day culture of heart from a 5-day chick embryo; × 540 diam.

chondrium passes to each daughter cell (Benda, Duesberg, Meves, etc.). Meves ('13) in his work on ascaris egg goes so far as to state that not only are the mitochondria present in the egg and spermatozoon, but also that the male mitochondria are carried into the egg by the spermatozoon and so each egg receives not only female but also male mitochondria and the granules resulting from the fusion of the male and female mitochondria are distributed to each cell of the embryo. In view of the behavior of the mitochondria Meves suggests that they may play a part in inheritance.

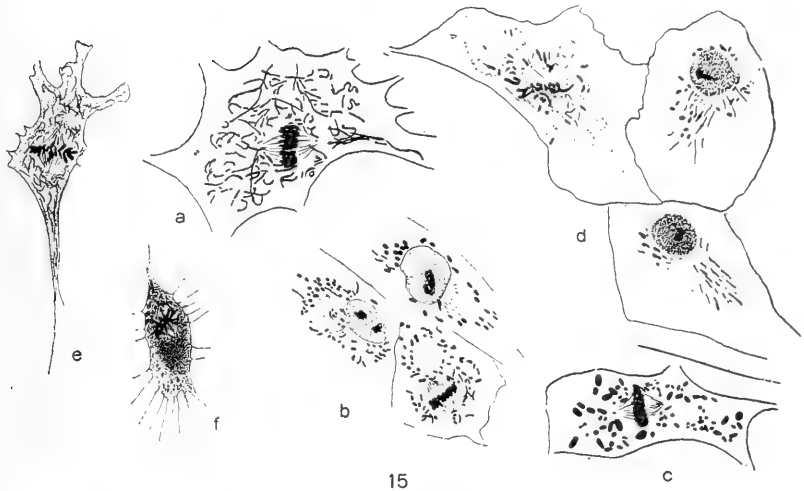
A study of the fixed specimens seems to show that the mitochondria retain somewhat their original character and shape during mitosis (figs. 14–17). They are, however, almost always shorter and more scattered through the cytoplasm than in the surrounding cells (figs. 4, 5, 14 a, 15 b, d, 17, 18). There are usually as many and often more mitochondria in the early stages of the dividing cell than in the neighboring cells (figs. 4, 5, 14 a, b, 15 b).

There is no indication in the fixed specimens of any arrangement of the mitochondria about the spindle in such a manner that they would undergo division into two parts in the plane of cleavage of the dividing cell. On the other hand, all of our specimens seem to show that the mitochondria tend to become more evenly scattered through the cytoplasm during division, and those that happen to be on either side of the cleavage plane are carried into the respective daughter cells.

Since each daughter cell contains only about one-half the number of mitochondria found in the mother cell at the time of division we must assume that there is an increase sometime during the life of the cell between one division and the next, otherwise the number would rapidly decrease during each successive division. Now the question is: When does this increase take place? Is it during the so-called resting period, or during mitosis? In some of the fixed preparations where mitotic figures, daughter cells and young growing cells are numerous, it is possible to arrange cells in a series according to the stage of recon-

Fig. 15 Arrangement of mitochondria during metaphase; *a*, *b*, *c*, *f*, cells from 2-day cultures of heart from 5-day chick embryos; *d*, *e*, cells from a 3-day culture of intestine from an 8-day chick embryo; × 540 diam.

Fig. 16 Arrangement of mitochondria during anaphase and telephase and young daughter cells, *a*, *b*, *c*, from a 3-day culture of intestine from an 8-day chick; × 540 diam. Cell *a*, anaphase has 156 mitochondria, the two daughter cells, *b*, have 122 and 125 each, while the older daughter cell, *c*, has 151 mitochondria; the neighboring adult cells in this region have been 70 to 160 mitochondria; *d*, 3-day culture of intestine from a 7-day chick; the two daughter cells with the smaller dark nuclei have 92 and 49 mitochondria, while the adjoining resting cells have only 77 and 48 mitochondria each; × 790; *e*, daughter cell from a 2-day culture of heart from a 5-day chick, with very different type of mitochondria; × 540 diam.; osmic acid vapor and iron hematoxylin.



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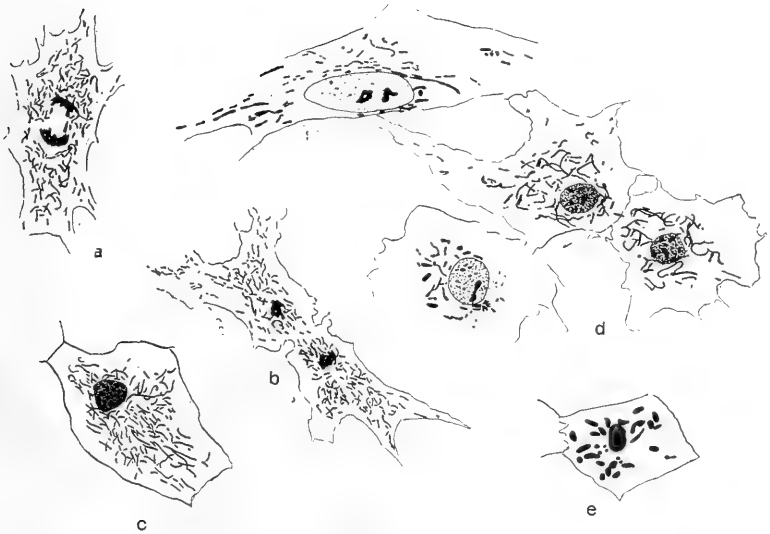

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struction of the nucleus, as indicated in figures 17 and 18. The younger nuclei are smaller, darker and more compact and the cells are smaller. The older cells are larger and contain larger nuclei which are less and less deeply stained. In such a series (fig. 17) the number of mitochondria increases from about 40 to 150. On the other hand, the old resting cell (k) with a very pale nucleus has only 32. In one series (fig. 18 i, j, k, l) the number increases from 24 to 140. In figure 18 one of the two daughter cells (c) has 37, the dividing cell (a) has 140, while the two neighboring resting cells (e) and (f) have 39 and 47. Again, in figure 18, the young daughter cells (d, d) have 37 each while older neighboring cells (g, h) have 56 and 58 each. On the other hand, another dividing cell (b) near this same group has but 60 mitochondria.

From such observations one might conclude that there is a gradual increase in the number and in the size of the mitochondria during the growth period of the daughter cells. The greatest increase both in number and size seems to occur then during the so-called 'resting' period which is in reality a period of growth both for the mitochondria and for the nucleus. On the other hand, while we are unable to determine definitely whether the number of mitochondria actually increases during the early stage of mitosis there are frequently indications that such cells have more mitochondria than mature cells Cell d (fig. 5) early prophase has 89 while the resting cells a, b, c have 47, 51 and 48 mitochondria each. In figure 4 e the dividing cell has 118 while the three neighboring cells have 102, 126 and 62 mitochondria each. The two cells with the larger nuclei are probably older resting cells and each has about the same number of mitochondria as in the dividing cell.

Numerous other specimens seem to show that the dividing cells often have more mitochondria than any of the fullgrown resting cells in the immediate neighborhood. Sometimes this is so marked that there is every indication that the number of the mitochondria in some instances may increase considerably during mitosis. It seems probable thereforee that mitochondria increase in number both during the resting period and during

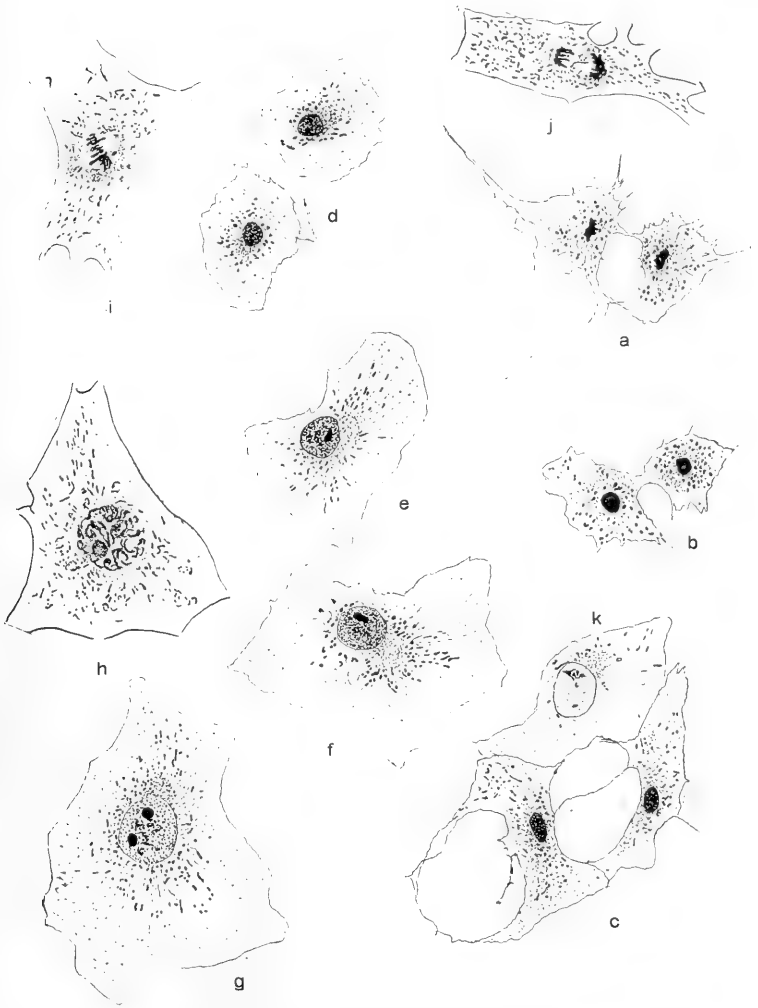

Fig. 17 Cells from a 2-day culture of heart from a 5-day chick embryo; × 540 diam.; *a*, very young daughter cells with 42 mitochondria in each cell; *b*, slightly older daughter cells with 42 and 35 mitochondria; *c*, older daughter cells with 48 and 75 mitochondria; *d*, older daughter cells with 43 and 38 mitochondria; *e*, older cells with 66 mitochondria; *f*, older cell with 96; *g*, still older cell with 156; *h*, prophase with 197 (?); *i*, metaphase with 117; *j*, anaphase with 174; *k*, old resting cell with only 32 mitochondria.

mitosis; perhaps in some more during the resting period; in others more during mitosis and in still others during both periods or only during one. It is very unlikely that one can arrive at a satisfactory solution of such a problem from fixed material, since the bodies we are dealing with are subject to such great changes in number and size during life. The number of mitochondria is not of much value as an indicator of the total quantity of mitochondrial substance.

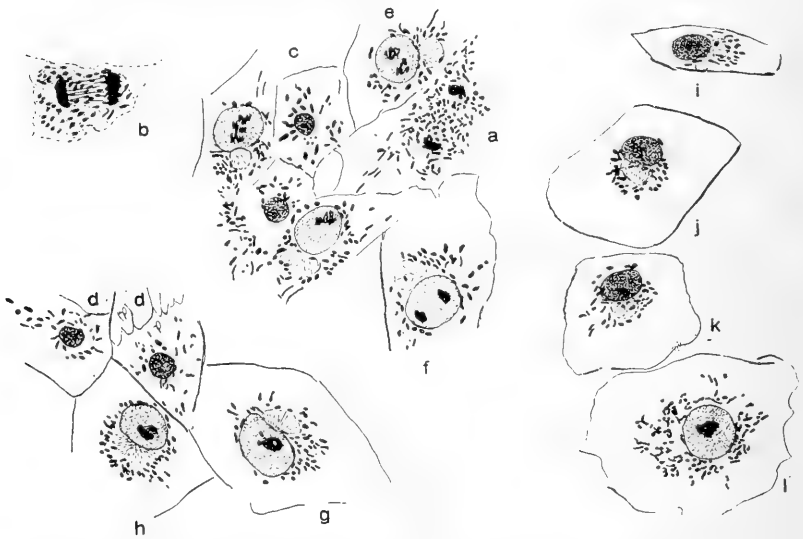

Fig. 18 Cells from a 2-day culture of heart from a 5-day chick; × 540 diam. Cell *a* in anaphase has about 140 mitochondria, while *b* has only about 60, the young daughter cell *c* has 37, the two daughter cells *d*, *d* have 36 each; the older cells *e*, *f*, *g* and *h* have 39, 47, 58 and 56 mitochondria respectively. In the series *i*, *j*, *k*, *l*, the young cell *i* has only 24, the older cells *j* and *k* have 41 and 42, while the mature cell *l* has 140 mitochondria.

We have indicated that the daughter cells not only have about one-half the number of mitochrondria found in the mature cells, but that the mitochondria are sometimes smaller.

Does the increase in number during the growth period come about through division of preëxisting mitochondria (a process which frequently takes place) or do mitochondria arise *de novo?* So far as our observations go, either or both processes may occur.

The only certain method to determine just how and when the mitochondria increase is to follow several living cells through complete cycles. Unfortunately, the cells of tissue culture often round up during late metaphase and anaphase (fig. 15 e, f) so that it is impossible, except in a few cases, to follow the individual mitochondrium throughout cell division.

The process of mitosis is an exceedingly slow one compared with that described in other tissues: Prophase 10 to 20 minutes, metaphase and anaphase, 1 to 2 hours; while the period from anaphase including telophase to the daughter cells is an exceedingly short one, never more than five minutes from the time the chromosomes are arranged at the opposite poles of the spindle until the cytoplasm is divided, except for slender processes, and such stages are correspondingly few in number in the permanent preparations.

We have not been able to follow the number of mitochondria through a complete cycle of the cell in the living cultures. We have, however, been able to watch the behavior of the mitochondria during mitosis in a few living cells. Usually the mitochondria are scattered throughout the cytoplasm and remain so during cell division. About one-half of the mitochondria pass to each daughter cell, namely, those which happen to be on one side or the other of the cleavage plane. In two or three cells during late anaphase most of the mitochondria became arranged in rather of a broad zone around the spindle in the area through which the division plane later formed and one-half of the number of mitochondria passed into each daughter cell. There was no indication of any division of the mitochondrial granules; in fact, in one cell it was clearly observed that several thread-shaped mitochondria passed over entire into one of the daughter cells. A division of the mitochondria such as observed by Meves ('08) and Duesberg ('10) was never observed. We find as did Buchner ('09, '10, '11) that this characteristic arrangement of the mitochondria during division of the cell is by no means a constant occurrence.

We have already stated that we are uncertain whether there is an actual or only an apparent increase in the amount of

mitochondria during mitosis. So far we have only one direct experimental observation to offer, and in this particular case there was an actual increase in the number and possibly also in the quantity. In this experiment the temperature had been raised from 39 to 46°C. and was retained at 46°C. for two hours. There resulted a decided decrease in the amount of mitochondria within all the cells. Two cells began to divide. The nuclear wall disappeared, the nucleoli faded and the chromosomes appeared. These cells, which a few minutes before had contained only a very few mitochondria, now became full of short dumbbell-shaped rods, while the resting cells did not undergo any change. So far as could be seen by most careful observation, this increase in quantity of mitochondria was not due to the division of the existing granules.

Mitochondria in different kinds of cells

Regardless of the fact that the mitochondria constantly change in shape, size and quantity in any one cell, there is a characteristic appearance of the mitochondria in certain kinds of cells, as, for instance, the short, rod and dumbbell shapes are most frequently found in the cells of the endodermal membrane; the long threads, rods and sometimes loops are found more frequently in the connective tissue; the small granules and short rods are frequent in nerve fibers and cells, and are often much smaller than those of the connective tissue cells over which the nerve fiber passes; a striated arrangement together with scattered granules is characteristic of the fibroblasts; and the large granules are more frequently seen in the heart and smooth muscle syncitium than in any other kind of tissue. At times the growth from the explanted intestine or heart contains only cells with thread- and rod-shaped mitochondria. Again, a large proportion of such cells contain only large granules. These granules are frequently so large that they are clearly seen with the low power. They are collected about the central body and appear to be more refractive than other types of mitochondria. Occasionally these large granules fuse. That they are not a

degenerate form of mitochondria is shown by the fact that such cells frequently divide. In case of mitosis the mitochondria spread around the nucleus, and the large granules become short rods or dumbbell-shaped rods.

While these certain characteristic appearances of the mitochondria are found as a rule in the different kinds of cells, nevertheless the shape, position, size and quantity vary so much that it is not always possible to distinguish the kind of cell by the appearance of the mitochondria.

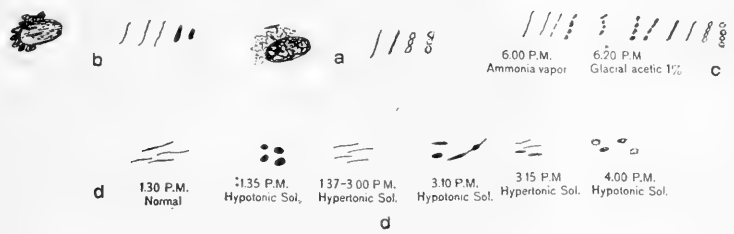

Fig. 19 *A*, effect of 2 per cent glacial acetic acid vapor on the nucleus and adjoining mitochondria and upon a single thread-like mitochondria; *b*, effect of strong ammonia water vapor on another nucleus with adjoining mitochondria and on a single thread-like mitochondrium, the reaction in both cases was almost instantaneous; *c*, effect of ammonia vapor on a single mitochondrium followed after 20 minutes by the vapor of 1 per cent glacial acetic acid; *d*, effect of hypo- and hypertonic solutions on 4 mitochondria.

EXPERIMENTAL WORK

Mitochondria in the living cell react rapidly and definitely to certain stimuli and in many cases they react more rapidly than either the cell as a whole or any other structure of the cell. This reaction, to be sure, often resembles a disintegration of the mitochondria and results in the rapid formation of varicose mitochondria and then the separation of the varicose mitochondria into a number of small, finely granular rings.

Reaction to acids

When the culture is subjected to the action of carbonic acid gas (fig. 13 e) or the vapor of acetic, sulphuric, hydrochloric, chromic and other acids (fig. 19 a, 13 d) the mitochondrial threads rapidly assume a varicose condition and soon separate into a

number of small granular rings of uniform size. Hydrogen peroxide, potassium permanganate and chlorotone, each produce a similar result.

Reaction to alkalies

Alkalies, ammonia gas and sodium hydroxide, on the other hand, cause the mitochondria to swell without any sign of varicosity. The nucleus also becomes larger and more transparent (fig. 19 b).

If the ammonia vapor is followed by vapor from acetic acid the acid will cause the mitochondria and also the nucleus to return to the normal condition. We have not succeeded in stopping the action of the acid at this point, however, and the mitochondria become degenerate rings (fig. 19 c).

Reaction to xylol, chloroform, ether

Xylol, chloroform, and ether simply remove the mitochondrial material, or possibly dissolve the mitochondria and leave shadow forms or slight traces of degenerate mitochondria.

Reaction to hyper and hypotonic solutions

Changes in osmotic pressure affect the mitochondria often before any change is seen in the cytoplasm. Hypertonic solutions shrink the mitochondria while hypotonic solutions cause them to become swollen. The effect of a hypertonic solution can be removed by a decrease in the osmotic pressure of the solution, and, vice versa, that of a hypotonic by an increase in the osmotic pressure (fig. 19 d).

Reaction to heat

Heat gives interesting results. With an increase in the temperature of the warm stage on which the preparation is studied from 40 to 48°C., the mitochondria become round granules within fifteen or twenty minutes, regardless of their previous shape (fig. 20). The size of these round granules is determined by

the size of the mitochondrial thread or rod before the heat began to act. When the heat is applied the mitochondria do not divide into a number of granules, as is sometimes the case when Janus green is used, but each one rounds up as a whole and forms one round granule for each mitochondrium. With rapid cooling of

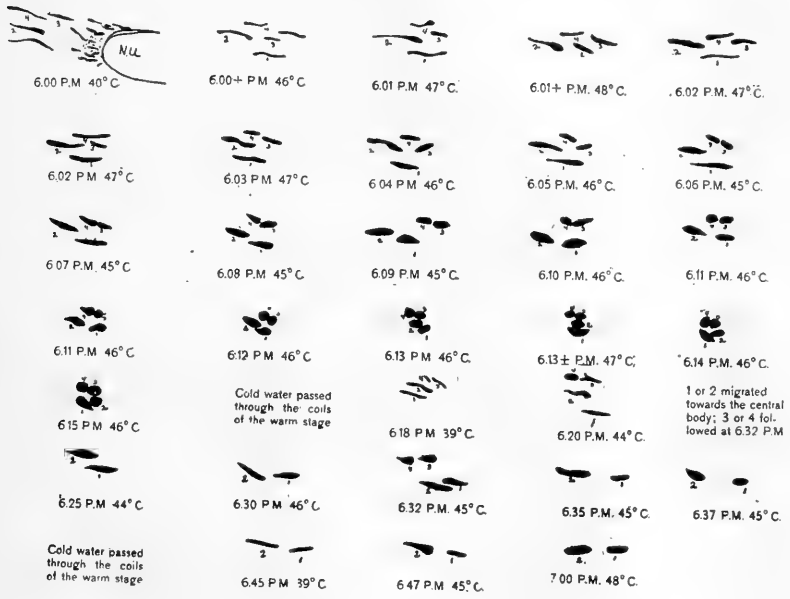

Fig. 20 Part of living cell, drawn with camera lucida, showing position of 4 mitochondria which were drawn at intervals, while the temperature was first increased and kept at 46 to 48°C. for 15 minutes, then cooled to 39° for 3 minutes and again increased to 44 to 46° for 20 minutes; again cooled to 39° for 8 minutes, and finally increased to 48° again.

the preparation, by passing cold water through the coils of the warm stage, the mitochondria return to their normal shape. Prolonged heat, such as 46°C. for over an hour, has in a few instances reduced the number and also the size of the mitochondria in a given cell.

VITAL DYES

Janus green

Janus green (di-ethyl saffranin azo di-methyl aniline) has been considered a more or less specific stain for mitochondria in the living cell, according to Laguesse ('99), Michaelis ('99), Bensley ('11), Cowdry ('12–'14). Unfortunately, in our preparations, while the dye stained the mitochondria a brilliant blue-green, it was also toxic to the cells, and even the weakest solution (1–200,000) which definitely stained the mitochondria caused the death of the cells within a few hours. Not only did the dye prevent further growth, but in most instances it also caused various amounts of distortion of the mitochondria. In a few

Fig. 21 Changes exhibited by 4 different cells after application of Janus green; in *a* and *b* the mitochondria were all long threads before the Janus green was applied and had begun to split up before the drawings could be made.

cases the mitochondria moved, changed shape and appeared quite normal, although distinctly stained, but usually the mitochondrial threads or rods separated into granules (fig. 21), shortly after the stain was applied. This is an indication of a slight degree of degeneration on the part of the mitochondria (see experimental work) and possibly the cell is already injured, although not so greatly as to interfere immediately with the activities of the cell, as in many cases the cell continued to move after the stain had been applied and in one observation on a heart muscle cell in which the mitochondria granules were deeply stained with a (1–100,000) Janus green solution the cell continued to beat for one hour and forty minutes. At the end of this time the stain had faded out and the cell ceased to beat.

The dye was dissolved in the Locke's solution, which was used for that particular explantation, and after a drop of the solution containing the dye warmed to 39°C. had been dropped

on the growth it was drawn off and the tissue again bathed in a fresh drop of the warm solution free from dye. The mitochondria take up the dye within a few minutes and remain stained from thirty minutes to two hours. So far as we have observed, the intensity with which the mitochondria stain does not depend upon the strength of the solution. A very weak solution (1–100,000) gives as intensely stained mitochondria as does a strong solution (1–5000). A weak solution, such as 1–100,000 Janus green, stains only the mitochondria a blue-green, while the cytoplasm, nucleus, and nucleolus remain clear. A strong solution (1–5000), however, stains the cytoplasm a pale green, the mitochondria a darker green, the nucleolus green, and the nucleus a more or less violet-green.

Nile blue B extra and brilliant cresyl blue 2 b

Aside from Janus green, no dye used in these observations stained the mitochondria in the living cell. Both nile blue A concentrated or B extra and brilliant cresyl blue 2 B, however, did stain the mitochondria after the death of the cell, especially after fixation either with neutralized formalin vapor or osmic acid vapor. This is interesting in connection with the work of Lorrain Smith ('08) on differential stains for fats. He states as follows:

> It was observed that watery solutions of nile blue sulphate (A), a colour stuff of the oxazine series, stains the fat globules contained in tissue cells in various colours. In the majority of cases the fat globules are stained a brilliant red; occasionally globules are present which take a blue stain, and not infrequently the colour is due to a mixture of blue and red. . . . . We may express the reaction in the following way: The fatty acid combines with the oxazine base to form a blue soap, whereas both neutral fat and fatty acid merely dissolve the relatively weak oxazone base (red).

He remarks in relation to tissues fixed with formalin that the globules stain readily either red or blue according to their composition:

> When a globule contains a small amount of fatty acid and a large amount of oxazone base is present in the solution of the dye, the globule becomes predominately red, whereas if the stain is relatively weak in oxazone the blue colour of the oxazine staining is more apparent.

We found that not only do nile blue (A concentrated and B extra) and brilliant cresyl blue (2 b) show the above changes of color found by Smith ('08) with fats but also that each dye changes from blue to pink in the presence of certain other substances as shown in table 1.

Both brilliant cresyl blue 2 b and nile blue B extra are toxic to the cell, and a preparation never lived more than an hour after even the weakest (1–200,000) solution of the nile blue B extra. Brilliant cresyl blue 2 b is less toxic than nile blue and each of these stains is in a way antiseptic, for no infection took place after the stain was used although the dye was not sterilized. The color reactions with these stains on the living and on the dead cells are shown in table 2.

TABLE 1

		NILE BLUE B EXTRA	BRILLIANT CRESYL BLUE 2B
Sodium carbonate	(in solution)	blue	blue
Sodium carbonate	(dry)	red	bluish violet
Lithium carbonate	(in solution)	pink	violet
Potassium hydroxide		pink	pink
Sodium hydroxide		pink	pink
Ammonia water		precipitate red	dirty brown solution

TABLE 2

	CYTOPLASM	NUCLEUS	NUCLEOLUS	FAT DROPLETS	VACUOLES (FIG. 22)	OTHER GRANULES	MITOCHONDRIA
			Living cell				
Nile blue B extra	clear	clear	clear	refractive	pink	blue	clear
Brilliant cresyl blue 2b	clear	clear	pale blue	refractive	pink	purple	clear
			Dead or fixed cell				
Nile blue B extra	pale blue	blue	blue-violet	blue	clear	blue	blue
Brilliant cresyl blue 2b	pale violet	blue-violet	blue	blue	clear	purple	gray-violet

The difference in the results obtained when these dyes are used upon dead cells and when used upon living cells shows clearly that the chemical conditions which exist in the living cell are quite different from those in the dead cell. What happens in the living cell to prevent the mitochondria and fat globules from taking on the pink or blue color which is assumed immediately upon the death of the cell? Was the dye itself oxidized and why did the vacuoles and certain other granules stain? The vacuole certainly does not take the pink color due to the presence of fat of any kind, for death of the cell would hardly remove the fat but would only change it possibly from neutral to acid fat and the vacuole should then change from pink to blue color instead of fading out entirely. If on the other hand the pink color is due to the alkaline nature of the vacuoles, why then does it not either remain pink or else become blue? Why does the nucleus remain unstained until death of the cell begins and then the nucleolus first take on the stain and later the nucleus? Is the pale blue color of the nucleus after brilliant cresyl blue 2 b in the living cell a delicate indicator that the cell is injured by the dye? These are but a few of the questions suggested by the different action of these dyes upon the dead and the living cell and which must be left for the physiological chemist to solve.

This change is most readily seen when a cell has first been stained while it is living and then fixed under the microscope. As the preparation dies the pink vacuoles fade out and the nucleolus, the nucleus, cytoplasm, fat globules and mitochondria stain. This is not due to the direct action of the fixative upon the stain itself since a fixed preparation which has been well washed with Locke's solution gives the same results with these dyes.

Iodine

It might be mentioned in this connection that while the vapor from a crystal of iodine did fix the mitochondria as reddish brown threads, rods and granules, there was no evidence of any port wine colored granules of glycogen attached to any

mitochondrium nor within the loop or ring shaped mitochondria. A few glycogen granules were occasionally present, however, as could be distinguished by the color reaction. The fat globules stain first a pale port wine color which later becomes blackened. If Guilliermond's ('13) conclusion that the loop-shaped mitochondria give off glycogen granules is correct, one would certainly expect to find that iodine used in connection with unfixed material would show this at least during the final stage in the formation of the glycogen when the granule lies free but still in the neighborhood of the mitochondrium from which it came.

CERTAIN OTHER CELL STRUCTURES AND THEIR RELATION TO THE MITOCHONDRIA

Granules

Certain other granules were present in most of the cells of these growths, but so far, these granules have not been carefully studied. They can be differentiated from mitochondria of similar shape by the greater rapidity with which the granules move through the cytoplasm. Certain of the vital dyes which color these granules leave the mitochondria unstained. Neutral red usually stains one or several granules near the central body. Nile blue B extra and brilliant cresyl blue 2 b also stain certain granules near the central body. In cells which contain the body we have termed vacuole (see below) one or more of these granules are present within the vacuoles and are stained blue within a pink vacuole (nile blue B extra) or purple within a pink vacuole (brilliant cresyl blue 2 b). These granules are few in number in the normal cell but plentiful in cells which contain many vacuoles. Other vital dyes stain certain granules within the cell, but so far as our observations go they are the same as the neutral red granules or else as the nile blue, and brilliant cresyl blue granules.

At times the granules within the vacuole take the Janus green color as a very pale green, but no other relation between these granules and the mitochondria has been found.

Vacuoles

There are two distinct types of degeneration of the cells of the tissue cultures. The cell either suspends activities, rounds up and dies, or else the cell continues its usual activities but the cytoplasm becomes filled up with vacuoles and the mitochondria become small granules (fig. 22). In a healthy cell a vacuole is often seen to come and go in the cytoplasm, but when several vacuoles remain in the cytoplasm degeneration has begun and the cell never again resumes its normal appearance, but continues to accumulate vacuoles until most of the cytoplasm is used up and only a network which contains scattered granules remains.

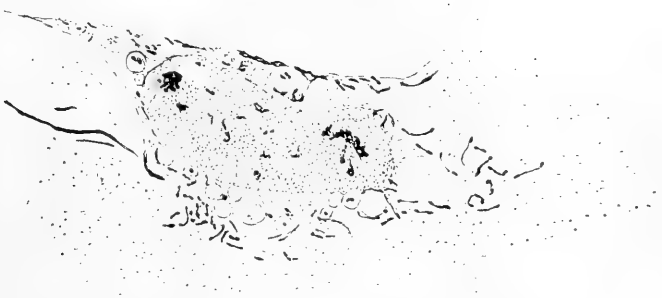

Fig. 22 Cell from a 3-day culture of intestine from a 7-day chick embryo; the cell has a number of vacuoles near the nucleus, most of the vacuoles contain one or more granules; × 1580 diam.

In the fixed and stained preparations the vacuole appears either as a clear space often difficult to differentiate from the fat globule space, or it appears as a clear space within which is a faintly stained granular substance (gray with Heidenhain's iron hematoxylin or red brown with Bensely's anilin fuchsin, methylen green stain).

In the living cell these vacuoles are distinctly different from the fat droplets. They appear to be fluid spaces not at all refractive, in fact, they resemble a hole in the cytoplasm. Small dancing granules which vary in number from one to many may be suspended in the fluid of the vacuole or closely attached to

the side. These granules usually stain a pale green with Janus green stain.

Nile blue B extra and brilliant cresyl blue 2 b each act as a differential stain for these bodies. The vacuole stains pink and the granules blue (nile blue B extra) or purple (brilliant cresyl blue 2 b, fig. 23).

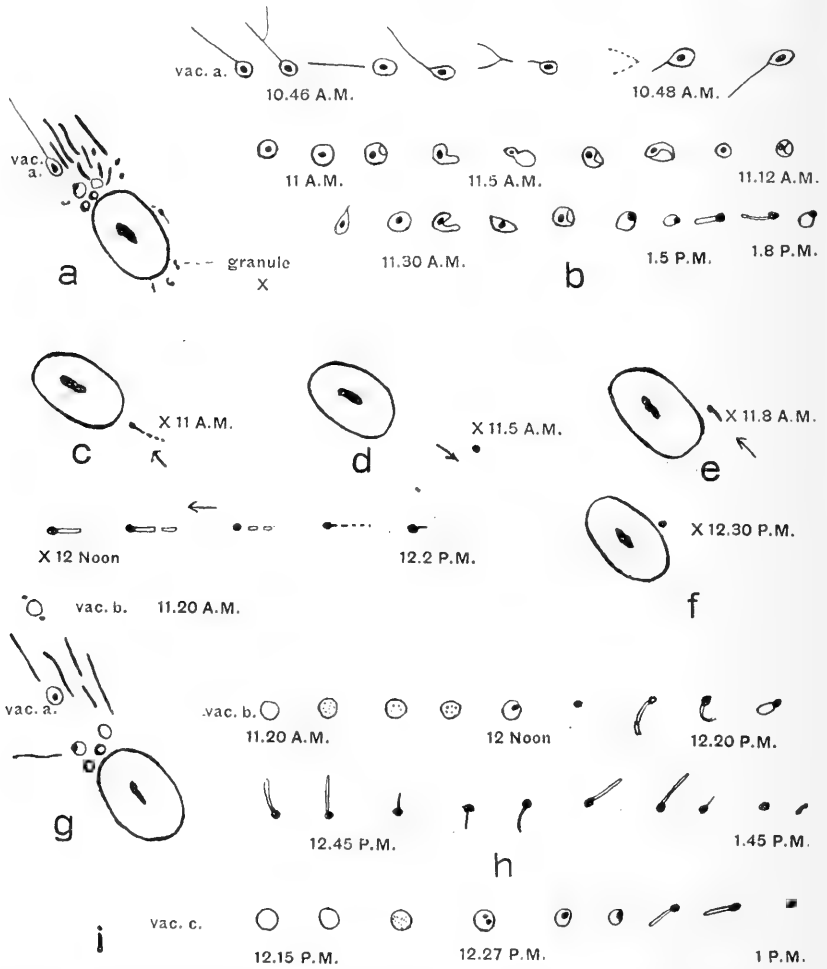

Fig. 23 Observations on the behavior of vacuoles in the living cell with brilliant cresyl blue 2 B (see text).

Brilliant cresyl blue 2 b, which is much less toxic than nile blue B extra, shows a most interesting behavior on the part of these vacuoles and granules. A vacuole may appear in the cytoplasm as a clear unstained space which at first contains no granules, but within five to ten minutes the granules appear as dancing bodies as though they were the result of some condensation and precipitation within the vacuole. This process continues and the vacuole takes on first a pale violet color, but later a bright pink, and the granules condense into one or two purple granules. Then the vacuole exhibits various movements such as sending out long pink streamers or threads or becoming U-shaped. Such a vacuole may decrease in size until only the purple granule can be seen. When a cell which contains many vacuoles is stained, all gradations between the pale non-granular vacuole to the single purple granule can be seen.

One of the many observations made upon healthy cells in which vacuoles appear is given in full below (fig. 23).

10.45 A.M. 1 gtt. (1–100,000) brilliant cresyl blue 2b in Locke's solution + 0.25 per cent dextrose was placed on the preparation.

10.50 A.M. The cytoplasm remains clear, several purple granules appear. The mitochondria are unstained, slightly refractive bodies, the fat globules are unstained and highly refractive, the nucleus is unstained, but the nucleolus is a pale blue (fig. 23 a). There is present one vacuole in the cell, which is stained a brilliant pink and contains a large purple granule. The vacuole sends off a long pink streamer quite as long as the thread-like mitochondria but not so thick (fig. 23, vac. a.). The vacuole manifests great activity. The streamer at times becomes detached from the vacuole and fades out. Again it appears to be drawn into the vacuole or sent out from the vacuole. Fig. 23 b.

11.00 A.M. The streamer no longer appears, and the vacuole itself begins to change shape (fig. 23 b) and continued until 11.30 when the vacuole began to grow smaller and a deeper pink. It then remained more or less quiet but grew much smaller in size.

1.05 P.M. The vacuole again sent off a pink streamer which lasted only two or three minutes, after which the small vacuole with one large granule remained quiet.

11.00 A.M. A small granule (x) at the other side of the nucleus moves rapidly to and fro between the nucleus and the periphery several times (fig. 23 c, d, e, f). A streamer of pink follows the purple granule until at 12 noon while the granule moved rapidly towards the nucleus the streamer broke off two pink granules which instantly faded out.

12.30 A.M. The granule shows no sign of pink vacuole or streamer and remains quiet near the nucleus.

11.20 A.M. A clear unstained vacuole (vac.b.) appeared in the cytoplasm between two of the purple granules (fig. 23 g) and behaved as follows, (fig. 23 h).

11.30 A.M. It became a pale violet vacuole with a few dancing unstained granules.

11.45 A.M. It was a violet vacuole with purple granules.

11.50 A.M. Violet vacuole became pink with purple granules.

12.00 NOON. The vacuole condensed into a small bright pink vacuole with only one purple granule.

12.13 P.M. The vacuole entirely disappeared and only the purple granule remained.

12.20 P.M. Purple granule sent out a pink streamer.

12.45 P.M. The pink streamer osculates and is rapidly sent out and drawn in again.

1.45 P.M. The granule became quiet and the streamer disappeared. The purple granules later moved as a rod from the periphery of the cell in towards the nucleus and back several times. It passed over and under the mitochondria without hindrance. Other purple granules in the cell moved rapidly without streamers, some as double granules, others as rods or as single round granules.

12.10 P.M. A pale space appeared in an adjoining cell (fig. 23 i).

12.15 P.M. This space became pale violet.

12.20 P.M. Violet color changed to pink.

12.25 P.M. Granules appeared in the vacuole.

12.27 P.M. Granules became deep purple granules.

The mitochondria and the fat globules remain unstained in all the cells of the growth. In some other cells of the growth many pink vacuoles are present and also many purple granules. In such cells the mitochondria are mostly small granules and only a few rod- or thread-like ones remain. In the cell under observation the mitochondria did not change type although they were continually changing shape.

There was no direct connection between the mitochondria and the formation of the vacuoles in the above observation, and yet in many cells there is often a coincident change in the shape of the mitochondria until in cells which contain many vacuoles within the cytoplasm the mitochondria are no longer in the shape of rods and threads but then appear as small granules.

As stated above, the fixed and stained preparations (fig. 24 a) do seem to show all stages in the formation of the vacuoles from the mitochondria just as Dubreuil and Guilliermond have shown the formation of bodies from the mitochondria. However, the fact that the vacuoles have been observed to arise

independently of the mitochondria, although there is a coincident change in the shape of the mitochondria makes one exceedingly wary of accepting any evidence from the fixed and stained preparations in this regard without corroboration from observations upon the living cell.

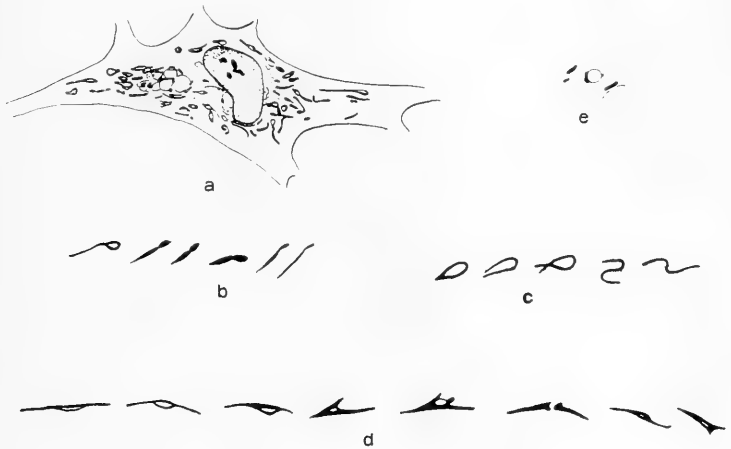

Fig. 24 One-day culture of heart from a 5-day chick embryo; *a*, cell with various shaped mitochondria similar to those figured by Dubreuil and Guilliermond, which led them to conclude that mitochondria formed fat and other bodies. Observations on this cell while living gave no evidence for the formation of such bodies from the mitochondria; *b* and *c* show successive forms of two mitochondria from the above cell and *d* also shows changes exhibited by a single mitochondrium that Guilliermond might have interpreted as showing the formation of a droplet; *e*, the rod-shaped mitochondrium which is applied closely to the vacuole was observed, while the cell was living, to migrate from some little distance to the vacuole; it had no connection with the formation of the vacuole. If the specimen had been fixed to show the condition, as in *e*, one might have concluded that the mitochondrium had something to do with the formation of the vacuole or droplet.

Certainly the mitochondria are intimately connected with any change in the cytoplasm, often as in the case of heat without manifestation of change by other bodies in the cytoplasm, and it is probable that any change which takes place in the cytoplasm such as would cause the formation of vacuoles or other bodies would also have an influence upon the mitochondria of that cell.

Fat globules

The connection between the mitochondria and the formation of fat is a very complex and much discussed subject. It has undoubtedly been shown that the mitochondria are bodies which contain lipoid (Fauré-Fremiet '09; Regaud and Mawas '09; Fauré-Fremiet, Mayer, Schaeffer, '10; Regaud '10; Mawas '10; Mayer, Rathery, Schaeffer, '10; Duesberg '11; Dubreuil '13; Cowdry '14). Our experimental work shows that the mitochondria act in many ways like bodies which contain lipoid. They are soluble in xylol, chloroform or ether, are slightly blackened by means of osmic acid, and in fixed preparations are stained blue by means of nile blue B extra and yellow by means of Sudan III. It seems probable that the bodies which contain lipoid should form the fat globules, and many observers have tried to establish this (Metzner '90, Zoja '91, Loyez '09, Russo '09, Dubreuil '13). Others have claimed that the mitochondria are indirectly connected with the formation of fat (Bluntschli '04, Van der Stricht '05, Van Durme '07, Lams and Doorme '08, Schoonjams '09).

The masterly papers of Dubreuil ('11, '13) appear to show clearly and concisely each step in the formation of fat droplets from the mitochondria, and without doubt from the fixed material which Dubreuil had at hand it seemed to be the logical conclusion that the fat is formed from the mitochondria. Guilliermond ('13) in a set of observations equally clear uses many figures similar to those of Dubreuil, but reaches the conclusion that the mitochondria form the glycogen granules of certain cells. It is certainly evident from our observations that no definite conclusions can be drawn from the morphology of the mitochondria present in any one cell at any one time. Various chemical tests and continued observation of a given mitochondrium are necessary to establish any morphological conclusion.

In our fixed preparations (fig. 24 a) all the figures shown by Dubreuil as evidence that the mitochondria form the fat can be found, i.e., threads, loops, rings and fat droplets, but the study of any one such mitochondrium in the living cell has

never shown that fat droplets arise from mitochondria (fig. 24 b, c, d). A thread may form a loop, but the loop changes back again into a thread instead of continuing into a ring. Various rings studied have never changed into globules during observation but have become rods or threads or granules. Such appearances as figure 24 e, were caused by the migration of a mitochondrium to the edge of a vacuole and not as both Dubreuil and Guilliermond might conclude, that the mitochondrium formed the vacuole. Certain granules or thick rods seen in the living cell have the appearance of hollow bodies in the permanent preparations and correspond to some of Dubreuil's figures. This appearance may be due to fixation as Kingsbury ('11) suggests, i.e., that the osmic acid reduced more at the surface and later the more soluble interior is dissolved out. Both Meves ('08) and Duesberg ('11) describe the clear inner part of the mitochondrium, to quote Duesberg, the mitochondria were first present in the early rabbit embryo as small granules but these increase in volume and become large granules at the end of the third day. They have a clear central part with a dark outer edge. Such appearance was seldom seen in the living cell and it is possible that these as well as certain figures of Dubreuil and Guilliermond were formed by the method of fixation. Cells which contain both loop and ring shaped mitochondria frequently show no sign of fat formation, while other cells which are accumulating fat show no mitochondria of the shape which Dubreuil leads us to suppose form the fat droplets.

There are three distinct types of fat in these tissue culture growths. First, that in the cells which grow out from tissues that at the time of explantation of the piece of tissue, contained fat droplets as the yolk membrane or the migrating fat cells. There seems to be a predetermined ability on the part of these cells to form fat, as is clearly shown where the growth from the yolk membrane adjoins that from the connective tissue (fig. 25). Each new yolk membrane cell contains fat droplets similar to those of the explanted piece of the yolk membrane. In these cells the mitochondria are usually in the form of small granules and the fat droplet is surrounded by granules which

stain like mitochondria. In the migrating fat cells which contain few fat globules some of the mitochondria may be in the form of threads or short rods but there is a coincident change in the shape of the mitochondria with the accumulation of fat droplets so that a cell which is crowded full of fat droplets contains

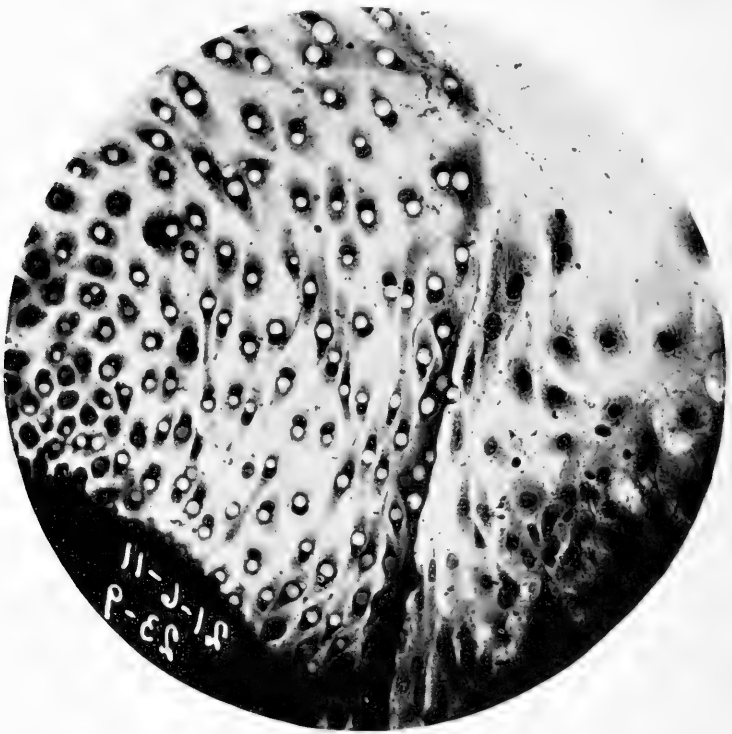

Fig. 25 Photograph of part of a 2-day culture of intestine from a 6-day chick. The explanted piece of intestine is from the region where the yolk-sac is attached, and the cells on the left of the culture are similar to those from cultures of the yolk-sac; each endodermal cell has one or two large fat globules; on the right are mesenchyme cells free or almost free from fat.

only small granule shaped mitochondria. The fat droplets are outlined by a row of granules which stain like mitochondria (fig. 26 b).

The second type of fat is one or two small round refractive granules found in almost all the cells of the growths. These fat globules have not been observed to increase markedly in

size or to change their shape. During mitosis they remain stationary and all may pass over to one daughter cell or part to one and part to the other daughter cell. No relation between these fat globules and the mitochondria was observed. Cells which contain one or no fat globules often contain loop or ring shaped mitochondria, but prolonged observation of these has not shown any increase in the amount of fat. The third type of fat is that of an accumulation of fat droplets in many of the

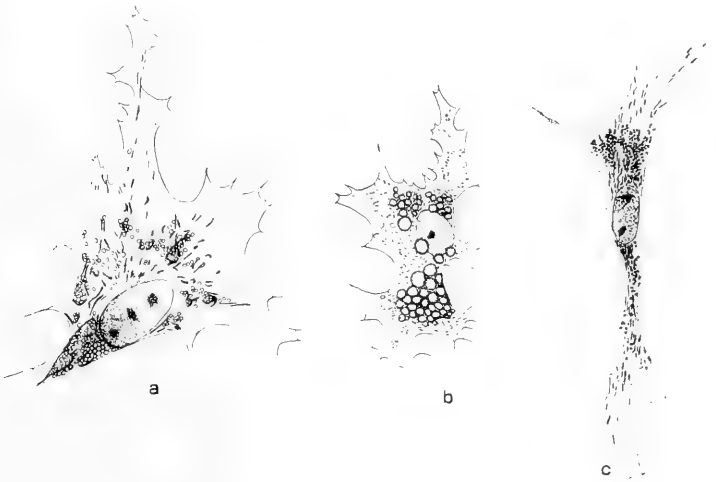

Fig. 26 *A*, *b*, cells from a 2-day culture of heart from a 10-day chick in which fat is accumulating; *a* was studied while living, after staining with nile blue B extra, after osmic acid vapor, after nile blue B extra again, Sudan III and Bensley's aniline fuchsin methylene green. No relation could be found between mitochondria and the formation of fat; *b*, a typical wandering fat cell with many small granular mitochondria about the fat droplets; *c*, cell from a 2-day culture of heart from a 11-day chick, accumulating fat; prolonged fixation with osmic acid followed by iron hematoxylin; the fat appears as dark granules.

cells of a preparation due to some unknown cause (fig. 26 a, c). These cells accumulate fat droplets from day to day, and some cells may become crowded full of fat droplets within forty-eight hours. Such cells should show the relation between the mitochondria and the fat globules were such a relation present, but so far as our observations go none such could be established.

These three types of droplets are undoubtedly fat. It is possible to treat the same cell with various fat stains in succes-

sion and to compare the results. The cell shown in figure 26 a was first studied and drawn while living. The clear, refractive fat globules were easily recognized. A drop of nile blue B extra (1–100,000) was added without any change in the appearance of either the fat globules or of the mitochondria. After a few minutes this was washed off and the preparation was fixed in osmic acid vapor for a few minutes. The same cell was then examined and the fat globules were stained a yellow brown, while the mitochondria remained clear. A drop of nile blue B extra was then added. The fat globules took a dark blue stain and the mitochondria a pale blue. A drop of Sudan III was then added and the fat became yellow while the mitochondria stained a trace of blue. The specimen was then dehydrated and stained with Bensley's anilin fuchsin, methylene green. The fat droplets were dissolved and the mitochondria stained a brilliant red.

Early in the experimental work it was observed that the mitochondria under certain conditions became granules around a vesicle. This vesicle stained pink with the nile blue B extra in the living cell, and at that time it was supposed that this indicated the formation of a fat by the mitochondria since Lorrain Smith ('08) had shown that nile blue stained neutral fat pink in tissue cells. Later it was observed that nile blue B extra only stains fat in the dead and not in the living cell, and therefore there was no indication that the mitochondria are in any way connected with the formation of fat.

So far as our observations go they show no direct relation between the mitochondria and the formation of fat, although in some cases there is a coincident change in shape of the mitochondria with the accumulation of fat droplets.

Canalicular system

One of the interesting cytoplasmic structures, the canalicular system, found by other observers, has as yet not been observed in these living cells.

Bensley ('11) by means of neutral red observed the small canaliculi as clear spaces in the deeply stained pancreatic cell.

In the cells of tissue cultures neutral red stains only a few granules unless used in such strong solutions as to stain the entire cytoplasm. In such cases a few clear unstained spaces were seen, but a study of the living cell and of the same cell fixed after the neutral red stain by means of osmic vapor and stained with Bensley's anilin fuchsin, methylen green stain demonstrated that the clear space seen in the cells stained with strong neutral red solution are only the unstained mitochondria.

The description of the Binnennetz given by Perroncito ('11) certainly resembles in many ways the behavior of the mitochondria in the tissue culture cells. He finds a network which is like that sometimes seen in these cells, and the 'corona' of granules shown in some of his figures appears very much like the mitochondria granules radiating out around the central body. In some of our permanent preparations where vacuoles are present these spaces have all the appearance of the canalicular system.

Prolonged fixation in osmic acid did not reveal the canalicular system, although the mitochondria became slightly blackened by the action of the osmic acid. However, none of the special stains for the canalicular system were used, as we desire to deal only with the structures seen in the living cell.

Amitosis and giant cells

Many cells of these growths contain two or more nuclei and the membrane within the nucleus, which Childs ('07) described as connected with amitosis, is occasionally seen in such cells, but no definite relation between such cells and the mitochondria has been observed. Certainly in some giant cells containing many nuclei, the number of mitochondria present is far greater than that present in a normal cell of the same growth, in fact, it is so much greater that it seems to be definitely related to the amount of nuclear material and to the extent of the cytoplasm. These cells show clearly that there is some other method of increase in the number of mitochondria than that of division at the time of mitosis, for these giant cells appear to be formed

by an amitotic division of the nucleus without a coincident division of the cytoplasm.

In regard to the structures of the differentiated cell, such as muscle fibrillae, etc., we have no observations to offer. However, from the behavior of the mitochondria in various shaped cells it is quite evident that any change which affected the morphology of the cell might also change the position of the mitochondria in such a way that they might appear to be connected with the formation of the differentiating structure.

DISCUSSION

We have made no attempt to formulate a theory from the above observations in regard to the origin or function of the mitochondria. A review of the literature shows that the mitochondria have been found in almost every kind of cell. They are present in the oocyte and spermatocyte (Benda '97, Van der Stricht '00, Meves '11, and others) and are carried over by the spermatozoon into the egg cell in fertilization (Benda '11, Meves '11); they are abundant in cells of the young embryo (Meves '08, Rubaschkin '11); they occur in plant cells as well as in the cells of most animals, including certain of the Protozoa (Lams '09, Duesberg '10, Meves '04, Guilliermond '12). It is claimed that they form certain cytoplasmic structures such as the fibrillae of the connective tissue (Meves '10), the neurofibrillae in the growing neuroblast (Hoven '10), the myofibrillae (Duesberg '10, Torraca '14) the fibrillae of the epithelial cell (Herxheimer '89, Korotneff '09, Fauré-Fremiet '10, Firket '11); that they play a part in the process of cornification (Firket '11); that they form the secretory granules, directly or indirectly, in the salivary (Regaud and Mawas '09, Bouin '05), gastric (Schultze '11), mammary (Hoven '11) and other glands (Schultze '11). They are described in the rods of the urinary tubule cells (Schultze '11, Regaud '08), in the intestinal cells (Champy '10), in the liver cell (Policard '09). They may form the test of the foraminifera (Fauré-Fremiet '13). They are described in connection with the formation of the retina cells (Leboucq '09).

Numerous observers have claimed that they form the fat directly (Altmann '89–'95, Metzner '90, Zoja '91, Arnold '07, Russo '07, Loyez '09, Van der Stricht '05, Policard '09, Frissinger '09, Regaud '10, Fauré-Fremiet '10, Dubreuil '13); indirectly (Bluntschli '04, Van der Stricht '05, Van Durme '07, Lams and Doorme '08, Schoonjans '08). It is claimed that they form the leucoplastids, chloroplastids and chromoplastids and possibly the glycogen (Guilliermond '12–'13).

The above theories seem impossible to correlate. It seems evident that the mitochondria are too universal in all kinds of cells to have the function of forming any one of the above structures of differentiated tissue, and in the light of what cytological chemistry is known, it appears practically impossible for the mitochondria to form all the cell structures mentioned above. In view of the fact that the mitochondria are found not only in almost all animal cells but in plant cells as well it seems more probable that they play a rôle in the more general physiology of the cell. It may be possible that they are concerned with respiration. As suggested by Kingsbury ('12), they may represent the structural expression of the reducing substances concerned in cellular respiration, which process Matthews ('05) has described in his theory of protoplasmic respiration. According to Matthews, the activity of the cell causes reducing bodies to be formed in the cytoplasm for whose neutralization oxygen is necessary. The lipoid nature of the mitochondria makes it possible to consider them as reducing bodies and certainly the mitochondria exhibit activities which may be due to the fact that they are continually formed in the cytoplasm and continually oxidized. On the other hand, the mitochondria may have to do with assimilation or they may even be stored-up food-stuff themselves, which are continually used up and restored again. Beckwith ('14) holds that the mitochondria are unnecessary for the life of the cell or for the development of such a complicated structure as a Hydractinia ciliated planula. The fact that such a large group of observers should each have evidence to show that the mitochondria form some one structure of the differentiated cell shows that the mitochondria must be

intimately connected with all transformations of the cytoplasm. On the other hand, we must bear in mind the fact that many observers have neglected to identify the body which they had under observation in such a manner that one can be certain that they had the same body which another observer would term mitochondria. It is quite doubtful whether all the bodies called mitochondria are really the same.

The criterion for mitochondria in the embryonic cell, as stated by Duesberg after Montgomery, is one which the observer would hesitate to carry out, but some criterion in the sex cell, in the embryonic cells and also in the adult cells should be established for the mitochondria, which all workers will endeavor to fulfil, in order that there may be some common ground for discussion of the results obtained by the numerous observers at work in this field.

CONCLUSION

1. Tissue cultures afford an excellent method for observations upon an undisturbed cell as it lives, divides and grows in a medium of known chemical constitution; for experimental work on a living cell; and for the study of the process of fixation.

2. These living cells do not correspond to the usual conception of a cell obtained from the study of fixed material. Both cytoplasm and nucleus are finely granular, almost homogenous in appearance. There is no sign of a reticular or of an alveolar structure of either the cytoplasm or nucleus. Osmic acid vapor is the best fixative for these cells.

3. Mitochondria are present in all the cells of these growths as slightly refractive, large or small granules, rods and threads, similar to those of the chick embryo cell. The mitochondria can be followed and studied in the living unstained cell for hours.

4. The mitochondria may be scattered throughout the cytoplasm or they may be located around the nucleus or around the idiozome. Any one mitochondrium may change its position in regard to other mitochondria or in regard to the entire cell. Mitochondria located around the centrosome may later migrate

out and become scattered through the cytoplasm, or those scattered throughout the cytoplasm may become located around the nucleus. During mitosis the mitochondria become more evenly scattered throughout the cytoplasm, except in the spindle area, where they are usually absent.

5. Any and every shape granule from a minute to a large granule, from small short rods to long threads, loops, rings and networks of various shapes and sizes can be found. Any one type of mitochondria such as a granule, rod or thread may at times change into any other type or may fuse with another mitochondrium, or it may divide into one or several mitochondria. Every type of mitochondria is continually changing shape and may assume as many as fifteen or twenty shapes in ten minutes. The shape of all the mitochondria in a cell can be changed by experimental means such as heat or hyper- or hypotonic solutions.

6. The mitochondria vary greatly in size from minute granules to irregularly shaped, large granules, from short rods to long threads. The size of a single mitochondrium may change by the fusion of two or more granules or by the division of a single mitochondrium. They also appear to increase or decrease without such fusion or division.

7. The number of mitochondria in a single cell varies from two or three to over two hundred. The number of mitochondria is not constant for any one kind of cell or for any phase of any one kind of cell. Daughter cells contain about one-half the number of mitochondria present in the mother cell. The number of mitochondria increases from the daughter cell to the mature dividing cell, and apparently also at times during mitosis.

8. The quantity of mitochondria is not constant for any one kind of cell. Some cells with many small granular mitochondria contain less mitochondrial substance than other cells with a few large granules.

9. Degenerating mitochondria become first a series of granules; later the granules become vesicles and then separate into a number of small finely granular rings which stain like the cytoplasm rather than like mitochondria.

10. The mitochondria become more or less scattered throughout the cytoplasm in an indifferent manner and decrease in size during mitosis. About one-half the quantity of mitochondria is separated into each daughter cell by the plane of division. The individual mitochondria pass over entire into one or the other daughter cell and do not each divide into two halves, each going to one daughter cell, as usually described.

11. There are some characteristic differences in the mitochondria of different kinds of cells, but these are not constant enough to be sufficient to distinguish the kinds of cells.

12. The mitochondria are extremely plastic bodies and often react more rapidly than any other cell structure. They are easily influenced in shape and quantity by varous agents, such as heat, carbon dioxide, acids, alkalies, fat solvents, and potassium permanganate, or by changes in osmotic pressure of the surrounding medium.

13. The mitochondria are stained in these living cells by Janus green but not by nile blue B extra or brilliant cresyl blue 2 b except in the dead cell.

14. Other granules are present in the cells which are not related to mitochondria.

15. Mitochondria show at times a coincident change in shape with the formation of fat droplets or vacuoles in the cytoplasm, but there is no evidence in these cells of a direct relation between the mitochondria and the formation of either the fat droplets or the vacuoles.

16. In giant cells the number and quantity of mitochondrial substances is greatly increased above that of the normal cells, somewhat in proportion to the increase in the amount of the cytoplasm and nuclear material.

The mitochondria are extremely variable bodies, which are continually moving and changing shape in the cytoplasm. There are no definite types of mitochondria, as any one type may change into another. They appear to arise in the cytoplasm and to be used up by cellular activity. They are, in all probability, bodies connected with the metabolic activity of the cell.

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THE ORIGIN AND EARLY DEVELOPMENT OF THE POSTERIOR LYMPH HEART IN THE CHICK

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FOURTEEN FIGURES

INTRODUCTION

During the autumn of 1912 Professor McClure suggested to the writer the advisability of working out the early development of the posterior lymph heart in the chick, with especial reference to the source of its endothelium. Throughout the following winter the problem was carried on under Professor McClure's supervision at Princeton University, while during the past year it has been continued under the direction of Professor Huntington at Columbia University.

Sala (1) in 1900 described the development of the posterior lymph heart of the bird, and gave a review of the literature to that date. In the caudal sections of an embryo of six days and eighteen hours incubation he finds that:

> In the mesenchyme which stands in the lateral relation to the caudal myotomes and corresponds to the lateral branches of the first five coccygeal veins, a progressive excavation occurs of little spaces or fissures which soon enter into communication with the lateral venous branches themselves—one would say in fact that these fissures are only simple dilatations and ramifications of the veins themselves.

If the writer interprets him correctly, Sala states that the lymph hearts are formed by an addition of spaces to the veins, and then a few lines later intimates that these spaces might be considered as "ramifications of the veins themselves." He also states that the 'fissures' are at first few in number and are arranged in a linear series, parallel to the axis of the vertebral column, corre-

THE AMERICAN JOURNAL OF ANATOMY, VOL. 17, NO. 4
MAY, 1915

sponding to the point of penetration of each venous branch of the intermuscular septum, and that afterward they gradually increase in number and come to lie near each other. He points out that at the end of the seventh day many of the little 'fissures' have fused to give rise to larger spaces, so that the spaces, separate at first, have finally established irregular communications between themselves, by breaking down their mesenchymal partitions. He goes on to show that by the end of the eighth day *the ensemble* of the cavities is transformed into a kind of a sac, still communicating with the first five coccygeal veins and later with the general lymphatic system, which develops independently by fusion of intercellular mesenchymal spaces at first appearing along the veins of the hypogastric plexus. The cavities at this stage often contain red blood cells and sometimes appear quite full of them, and by a condensation of mesenchymal cells the wall of the lymph hearts are formed. The rest of this paper, which does not especially concern us, shows that the lymph hearts increase in volume up to the sixteenth day, that the first and fifth coccygeal veins lose their connections with the hearts during this period, and that the connection of the lymph hearts with the independently developed general lymphatic system occurs toward the end of the tenth day. During the remainder of embryonic life the lymph hearts persist, but shortly after the chick is hatched they commence to degenerate. Traces of the degenerating lymph hearts were found in a chicken thirty-five days after hatching.

Mierzejewski, in 1909 (2), published an article on the origin of the lymphatic vessels in birds, which was presented by M. H. Hoyer before the Academy of Sciences of Cracow. Concerning the origin of the posterior lymph hearts he agrees with Sala, except that he holds that the first anlagen appear in the middle of the sixth day of incubation, and not, as Sala states, in the first hours of the seventh day.

Stromsten (3) has published two papers in 1910 and 1911 on the development of the posterior lymph heart in turtles. He finds that their development is initiated in the logger-head turtle by the vacuolization of the post-iliac mesenchymal tissue during

the latter part of the second week of development, and that the spongy tissue thus formed is invaded by capillaries from the dorso-lateral branches of the caudal portion of the postcardinal veins. The capillaries do not communicate primarily with the mesenchymal spaces. Near the close of the third week, parallel veno-lymphatic channels are formed in this spongy area by the confluence of mesenchymal spaces with one another and with the invading capillaries. These veno-lymphatics anastomose freely with each other and communicate by two or three openings with the veins running along their mesial borders. Finally a condensation of mesenchyme and an invasion of muscle cells form the wall, while a confluence of the veno-lymphatic sinuses gives rise to the single sac-like cavity of the adult form of the lymph heart.

The subject rested at this point until 1912 when E. L. Clark (4) cited observations, based on injections, to show that in the chick of five days and twenty hours, in the region later occupied by the posterior lymph heart, there exists a lymphatic plexus connected with the coccygeal veins, but not with the haemal capillaries which bear a superficial relation to the lymphatic vessels. She also shows that the lymphatic plexus is of a different pattern than the blood capillary plexus and is filled with stagnant blood, which she considers as backed up from the coccygeal veins. This state of affairs undoubtedly exists in the chick of five days and twenty hours but the observation, aside from its morphological value, throws no light on the origin and mode of growth of the lymphatic plexus.

E. R. and E. L. Clark (5) in a paper in the same number of The Anatomical Record attempt to prove, by observing the first appearance and early growth of this blood filled lymphatic plexus in the living chick embryo of about five days, that it is formed by a purely centrifugal outgrowth from the coccygeal veins. To quote from their article:

> The first lymphatics in the tail region of the chick arise as direct lateral buds from several of the main dorsal intersegmental coccygeal veins, and not by the transformation of a previously functioning blood vessel plexus. From now on the lymphatic endothelium is

specific and spreads by a steady centrifugal extension * * * * The buds send out processes forming clusters. From the clusters, in turn, processes are sent out which anastomose with one another, forming a plexus. Simultaneously, processes grow toward the surface from the clusters, and give rise to the superficial plexus of peripheral lymphatics of the posterior part of the body. There is no essential difference between the manner of growth of the peripheral lymphatics and that of the plexus which is to form the lymph heart (p. 258).

In June 1913 Miller (6) in a preliminary note on the development of the thoracic duct of the chick states that certain aggregations of mesenchymal cells mentioned by Sala (1) "comprise developing blood cells which are differentiated *in situ* out of the indifferent mesenchymal syncytium, that these blood cells then gain access to the lymph channels making up the developing thoracic duct, and that finally the haemal cellular elements in question, reach the blood stream via the thoracic duct and the jugular lymph sac." He clearly recognizes that lymphatic channels may serve to transmit blood cells arising *in situ* in the mesenchyme to the haemal channels and distinguishes this function of the lymphatics by the term 'haemorphic.' He further states that "the lymphatics arise as isolated lacunae directly from mesenchymal intercellular spaces and are not in any sense derived from the veins, and subsequently coalesce to form the continuous channel of the thoracic duct." The possibility of venous origin of these lymphatics or of the backing up of their blood content from the veins is excluded by the total absence of the azygos system in the Sauropsida. In his completed paper of September 1913 Miller (6) gives his results in greater detail. He states that the lacunae in question are bounded at first by indifferent mesenchymal cells which become flattened to form cells which are morphologically equivalent to endothelial cells.

Hoyer in June 1913 presented Fedorowicz's "Untersuchung über die Entwickelung der Lymphgefässe bei Anurenlarven" (7) before the Academy of Sciences of Cracow. Fedorowicz, working on Bufo vulgaris, Bufo viridis, Rana esculenta, and Rana temporaria found cell strands developing from the surface of the lymph heart. In these strands intercellular spaces and

finally lumina, which could not be injected from the lymph heart, appeared. The lumen of each strand he found to be lined with endothelial cells. By the continuation of the space formation lymphatic vessels developed which connected secondarily with the similarly acquired lumina of other cell strands which had appeared within the heart. It was not until this connection was established that it was possible to inject the lymphatic vessels from the heart.

Allen (8) in a recent important publication on Polistotrema (Bdellostoma) describes the caudal lymph heart as arising from isolated mesenchymal spaces in the region of the anterior end of the two branches of the caudal vein, and the ultimate fusion of these spaces by the breaking down of their partitions. Incident to this process certain cells in the interior of the system of spaces become spherical and are transformed into red blood corpuscles. Secondarily the cavity of the lymph heart establishes connections with the caudal vein by the same process, that is a breaking down of mesenchymal partitions while peripherally the cavity is enlarged by the new formation of isolated mesenchymal spaces and their ultimate annexation. Coincidentally the mesenchymal cells bordering the cavity of the lymph heart flatten to form its endothelium. Allen in conclusion says that his

> * * * * studies thus far indicate that the most primitive form of lymphatic system are veins that function for both lymphatics and veins. Hence it would be expected that ontogeny would repeat the phylogeny of the lymphatics, and instead of having their origin directly from the veins, they would begin directly as the veins did, by the vacuolization of the original mesenchyme.

These vessels Allen has designated 'veno-lymphatics.' The recognition of haemopoesis in the vicinity of developing lymphatics and from their endothelium is of major morphological importance and the substantial agreement between the results of Allen and of Miller should go far to clear up some of the difficulties that have beset the study of the ontogeny of the lymphatic system. The term veno-lymphatic was used by Huntington and McClure (9) in their studies of the mammalian

jugular lymph sac to designate constituents of the sac which were found at first to contain blood and later to be devoid of blood content. The term veno-lymphatic was simply meant to cover these two conditions of the vessels; for at the time of their studies the criterion of content seemed most available to discriminate between lymphatic and haemal channels. The work of Miller and of Allen demonstrating the *in situ* formation of blood cells and their carriage by lymphatics affords a complete and satisfactory explanation of these earlier observations, and Miller's term haemophoric lymphatic satisfactorily describes the actual conditions, and it is to be hoped in interest of clarity will replace veno-lymphatic. This question was fully considered by Huntington (10) at the Thirtieth Session of the American Association of Anatomists.

The present investigation is concerned with the *earliest* appearance of the posterior lymph hearts in the chick. They are two in number and bilaterally symmetrical. Each one arises in the mesenchyme lateral to the caudal muscle plate and posterior to the hind limb bud. Before the lymph heart assumes the form of a single sac-like cavity there exists in this same area a plexus of lymphatic vessels which later coalesce to form the single cavity of the lymph heart. Both the completed lymphatic plexus and later the lymph heart are in connection with several of the most anterior coccygeal veins by means of their lateral branches which pierce the caudal muscle plate, drain the lymphatics, and then pass outward in the younger embryos to drain a haemal capillary plexus, which bears a superficial relation to the lymphatic plexus.

It is the purpose of this paper to show that the plexus of lymphatic vessels, which later enters into the formation of the posterior lymph heart arises by the confluence of independent mesenchymal spaces which connect secondarily with the veins; that these spaces are bounded at first by mesenchymal cells which become flattened to form an endothelium, and that both in the endothelial lymphatic walls and in the adjacent mesenchyme an active haemopoesis is taking place.

MATERIAL

Forty-one of the forty-five embryos used in this work were injected with India ink through the large vitelline blood vessels, the injection usually being pushed to the point of extravasation for the haemal capillaries. Of the four embryos not injected

TABLE 1

List of sectioned embryos

Length in mm. after fixation	*Age in days*	*Hours*	*Series*
6.75	4	12	371
7	4	16	32A
8	4	16	31A
8.5	4	18	5A
8.5	4	18	33A
8.5	4	20	21A
9	4	18	29A
9	4	20	27A
9	4	20	23A
9	4	21	22A
9	4	21	4A
9	5	1	7A
9.5	4	18	30A
9.5	4	18	28A
9.5	4	20	24A
9.5	4	20	8A
10	?	?	13A
10	4	21	9A
10.5	4	20	26A
10.5	4	20	25A
11	4	21	20A
11	5	0	12A
11	5	1	6A
11	5	13	2A
11.5	5	7	18A
11.5	5	7	19A
11.5	5	3	14A
11.5	5	1	10A
12	5	6	34A
12.5	5	10	326
13.5	6	1	1A
13.5	?		3A
14	5	20	17A
14.5	?		11A
15	5	20	15A
15	5	20	16A

through the vitelline vessels three were injected directly into the posterior lymph heart plexus and one (12 mm.) was not injected at all. All material was fixed in Zenker's fluid. Thirty-six of the embryos were cut into 10 μ and 7 μ serial sections and stained on the slide with eosin and methyl blue by Mann's method. One or two series were stained with Delafield's hemotoxylin and orange G, but this method gave a very poor differentiation of the blood cells. The nine embryos not sectioned were cleared by the Spateholz method and examined *in toto* under the binocular microscope (table 1).

OBSERVATIONS

A. FORMATION OF BLOOD CELLS FROM THE MESENCHYME AND THEIR ENTRANCE INTO THE CIRCULATION VIA THE DEVELOPING HAEMAL CAPILLARIES, PRIOR TO THE FORMATION OF LYMPHATICS

As the appearance of numerous blood cells in the mesenchyme and the extension of the haemal capillaries, previously referred to, is the first change which occurs in the mesenchyme lateral to the caudal muscle plate in the caudal region of the embryo, these processes will be considered first. When the lymphatic anlagen first appear, in the 10.5 mm. embryo, the haemal capillary plexus has reached a very high degree of complexity and from this time onward merely holds its own or develops comparatively slowly.

The youngest embryo examined was one of 6.75 mm. In this specimen the mesenchyme lateral to the muscle plate was uniformly loose, and very nearly indifferent. A few rather rounded eosinophile cells were observed in each section. Some of these cells contained one or two large eosinophile granules. Occasional venous branches pierced the muscle plate to drain the mesenchyme lateral to it.

The same area in the 7 mm. embryo presents several changes. The mesenchyme is much more compact, being equal in density to the mesenchyme which lies medial to the muscle plate. Groups of differentiating blood cells are much more abundant.

These cells are becoming rounded, with a diameter of 7 to 8 μ. Their cytoplasm is neutrophile or eosinophile and contains several strongly eosinophile granules. The nucleus is slightly more basophile than the cytoplasm. Eosinophile granules were also observed in the cytoplasm of some of the mesenchyme cells. There is usually a free space of 2 to 3 μ about each differentiating cell, which is not encroached upon by the surrounding mesenchyme. Lateral branches of the coccygeal veins pierce the caudal muscle plate at regular intervals but the capillaries which they drain are few in number.

The 8.5 mm. embryo presents a very similar state of affairs, except that the capillaries emptying into the lateral branches of the coccygeal veins are somewhat more numerous, and the differentiating blood cells also occur in greater numbers. As may be seen from figure 1, *5*, the haemal capillaries are injected to the point of extravasation, but the differentiating eosinophile cells (*7*) are absolutely independent of them, nor are there any eosinophile cells medial to the caudal muscle plate.

From this stage on until the embryo reaches the length of 10.5 or 11 mm. (fig. 2), the capillary plexus steadily increases in richness and complexity, while the blood cells differentiating from the mesenchyme become scarcer. The capillary plexus has invaded the area formerly occupied by differentiating blood cells, and blood cells in the mesenchyme have decreased until only a small fraction of those present in the 8.5 mm. embryo remain.

These blood cells have, then, either degenerated and disappeared, or have been drained off by the capillary plexus. The present investigation has not been of such a character as to warrant tracing the complete history of the blood cells which differentiate from the mesenchyme but representatives of both the red and white blood cell lines have been identified in the tissue spaces.

That these cells are drained off by the extending capillaries is indicated by the fact that within five or six hours we find first a practically indifferent mesenchyme, a little later a very active haemopoesis taking place in it and finally a general vas-

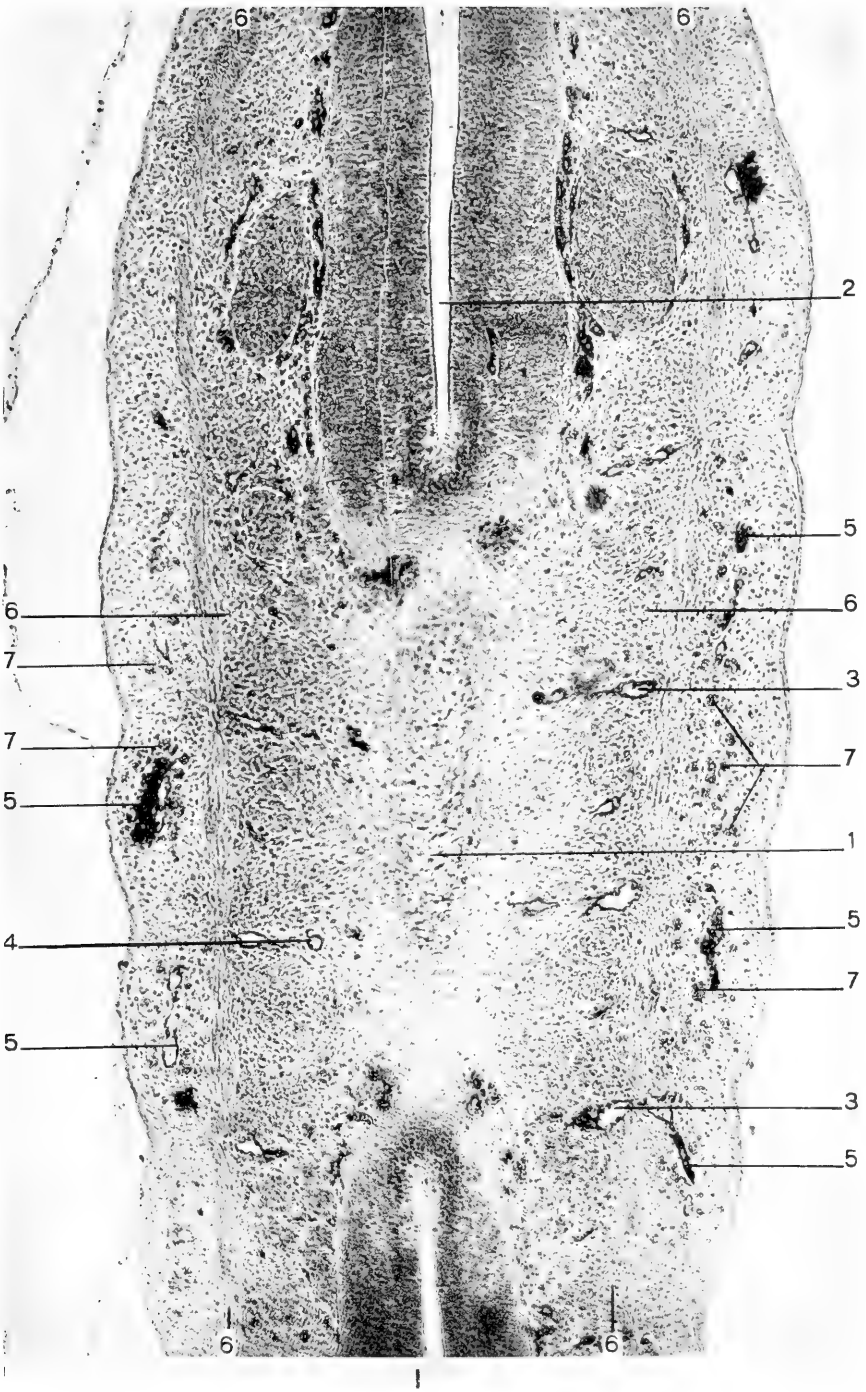
cularization of the tissue accompanied by a marked decrease in the number of blood cells in the tissue spaces. It seems highly improbable that decided haemopoesis should take place only to let the cells formed disintegrate three or four hours later without having entered a vessel, and moreover none of the blood cells observed in the tissues appeared to be disintegrating. McWhorter and Whipple (11) in their study of the chick blastoderm *in vitro* have observed a to-and-fro movement of the blood cells in the tissue spaces synchronous with the heart beat, and have also observed the entrance of these cells into the general circulation following their rhythmical movement. This phenomenon might be regarded as a plasmatic pulse, which would eventually force any blood cells lying free in the tissue spaces into the general circulation. In addition those cells having the power of amoeboid movement could enter the vessels by diapedesis through the capillary walls.

B. DEVELOPMENT OF THE LYMPHATIC PLEXUS AND ACCOMPANYING HAEMOPOESIS

The changes about to be described take place only in the mesenchyme lateral to the caudal muscle plates in the posterior region of the embryo, the mesenchyme lying medial to the muscle plates maintaining its compact indifferent character. For the sake of clearness we shall first consider the Histogenesis and then the Morphogenesis of the developing plexus of lymphatic vessels.

Fig. 1 Chick 8.5 mm., Series 21, Slide 1, Row 3, Section 2. × 200. Photomicrograph of transverse section of caudal end of the embryo.

1, Notochord
2, Neural tube
3, Coccygeal vein
4, Coccygeal artery
5, Haemal capillaries
6, Caudal muscle plate
7, Differentiating blood cells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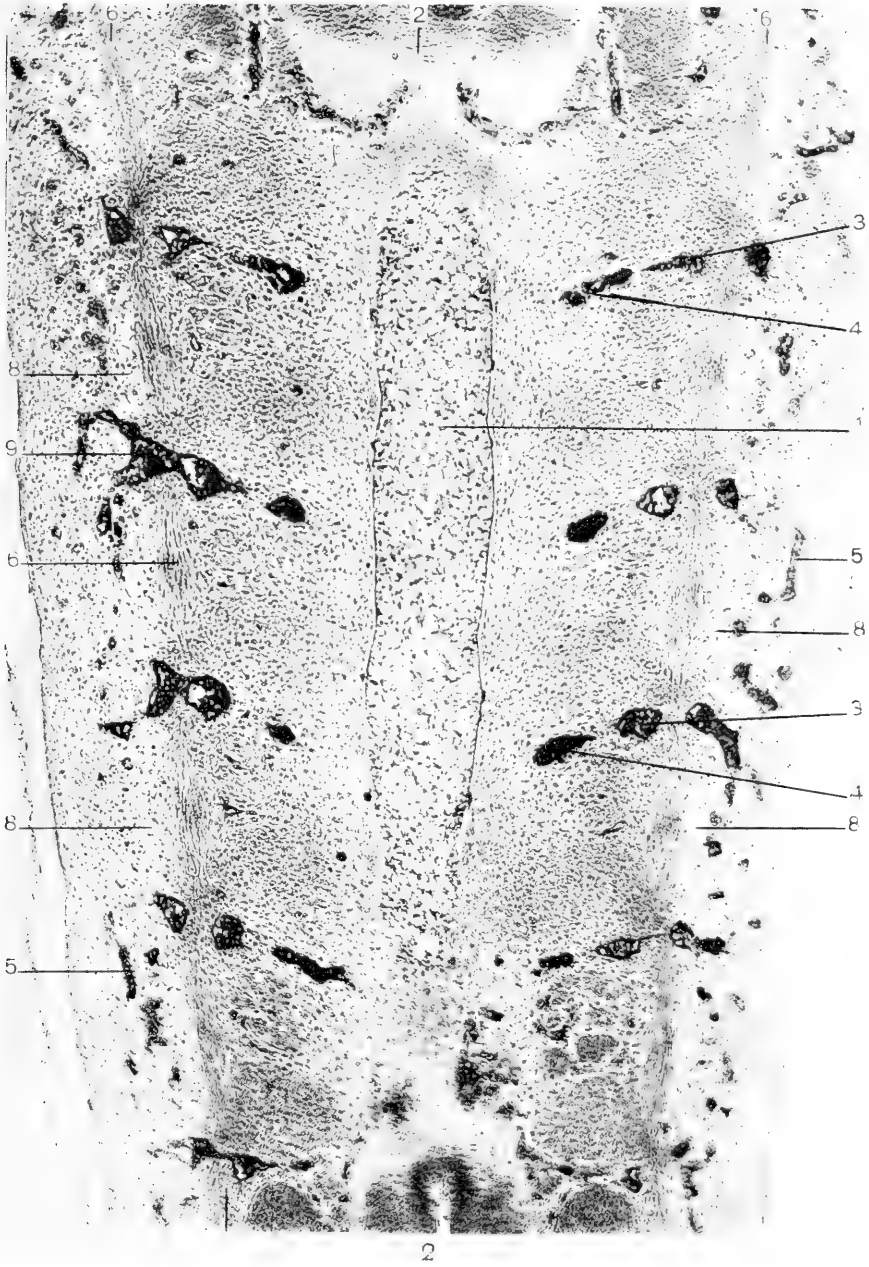
1. Histogenesis

In the embryo of 10.5 mm. (about 4 days and 22 hours) we observe two new phenomena; the formation of spaces bounded by mesenchymal cells which eventually become flattened to form an endothelium, and the appearance of certain strands of flattened cells in the mesenchyme. Haemopoesis continues to take place in the mesenchyme and also from endothelial cells of the lymphatic walls as soon as these are formed.

Throughout the younger stages until the embryo has reached the length of 10.5 mm. the mesenchyme lateral to the caudal muscle plate is of a uniform degree of compactness equal to that of the mesenchyme medial to the muscle plates. The 10.5 mm. embryo, however, shows a slight, but distinct loosening of the mesenchyme just lateral to the muscle plate, between the points of penetration of the lateral branches of coccygeal veins and at certain points the loosening of the tissue is more marked, giving rise to small mesenchymal spaces. The spaces still bounded by mesenchyme are more numerous in the 11 mm. embryo (fig. 2, *8*) and some differentiating blood cells have become included in them (fig. 5, *7*). Certain of the spaces nearest the veins have acquired a venous connection at this stage and in the injected embryos appear as small knob like processes (fig. 5, *10*) of a larger caliber than the veins with which they connect, filled with blood cells, and lined by endothelium. These knobs correspond in shape to the mesenchymal spaces mentioned. It is to be expected that when a space connects with a vein and is subjected to the pressure and friction of the general circulation, that the cells bounding it will tend to become flattened. And the fact that in later stages, when the still isolated spaces become larger and are under a greater plasmatic pressure

Fig. 2 Chick 11 mm., Series 20, Slide 1, Row 4, Section 3. × 200. Photomicrograph of transverse section of the caudal end of the embryo.

1, Notochord
2, Neural tube
3, Coccygeal vein
4, Coccygeal artery
5, Haemal capillaries
6, Caudal muscle plate
8, Mesenchymal space
9, Lateral branch of coccygeal vein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the bounding cells do flatten, renders it highly probable that a similar process takes place in the case of the smaller spaces which first acquire a venous connection.

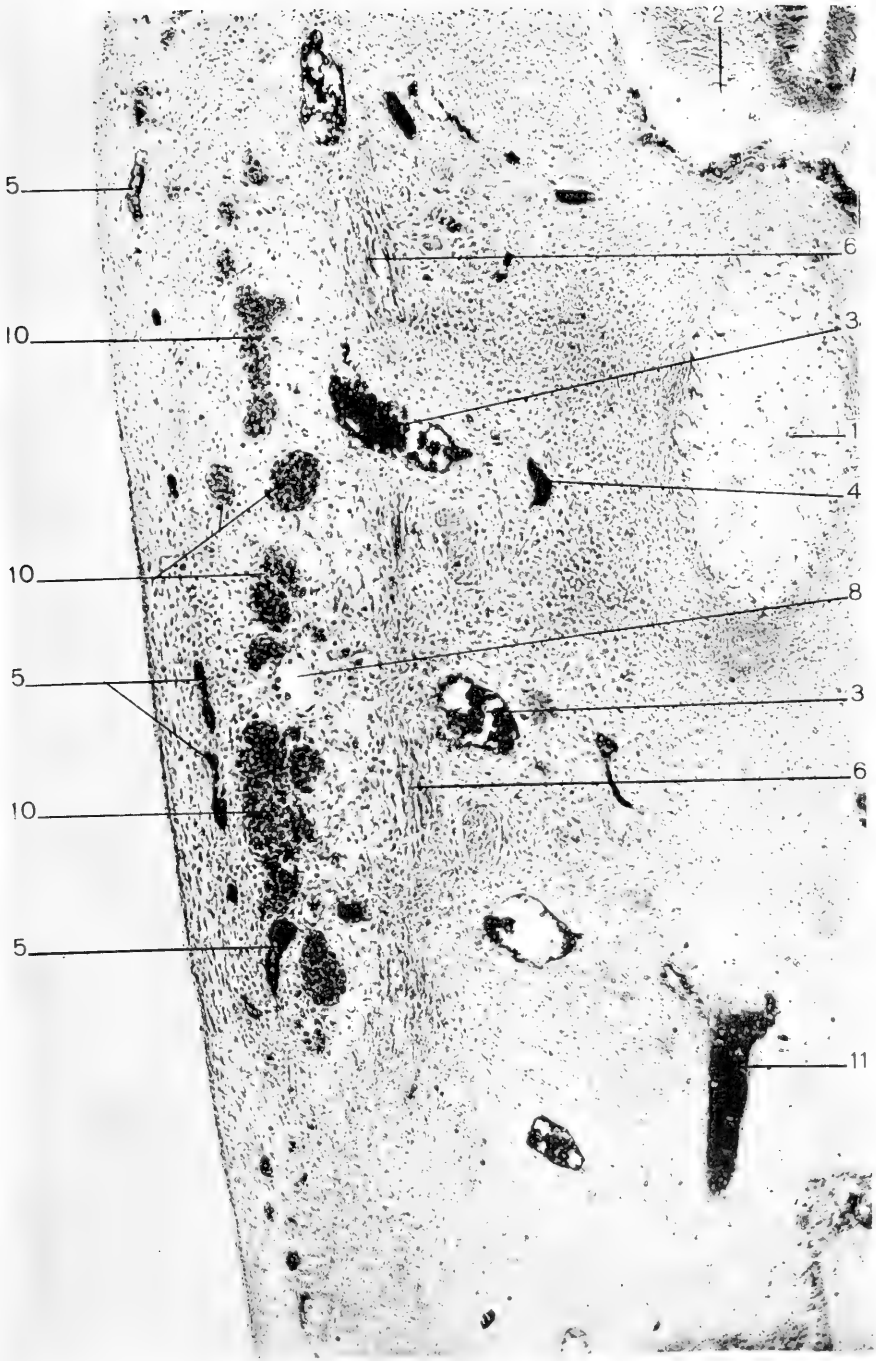
As was pointed out by E. R. Clark (12) at the Christmas meeting of the Anatomical Society in 1913, there are present in the mesenchyme lateral to the caudal muscle plate in the posterior region of the embryo certain strands of flattened cells which Clark holds to be outgrowths from the venous endothelium and to be always capable of being traced back to the veins. These cells, he says, contain nuclei which may be distinguished from the mesenchyme nuclei by their morphological and staining characters.

That strands of flattened cells, sometimes with continuous lumina, sometimes with an interrupted lumen or with no lumen at all occur in the chick as early as 9.5 mm. and more abundantly in the later stages, is true. But that they can be clearly distinguished from mesenchyme cells, and that they can always be traced back to a venous endothelium, are at least open questions.

E. R. Clark (12) describes the endothelial nucleus as being rather pale and elongated with one or two definite reddish discoid nucleoli, while the mesenchymal nucleus he holds to be darker, and more chromatic with one or two irregular bluish nucleoli, not sharply differentiated from the surrounding chromatin material. A careful examination, however, reveals a series of graduated stages between these two forms of nuclei. A slight change in the focus of the microscope will make a bluish nucleolus appear reddish, and vice versa, while a careful study of the tissue reveals great variance in the amount of chromatin

Fig. 3 Chick 15 mm., Series 16, Slide 2, Row 4, Section 8. × 300. Photomicrograph of transverse section of the caudal end of the embryo.

1, Notochord
2. Neural tube
3, Coccygeal vein
4, Coccygeal artery
5, Haemal capillaries
6, Caudal muscle plate
7, Differentiating blood cells
8, Mesenchymal space
9, Lateral branch of coccygeal vein
10, Lymphatic connected with vein
11, Aorta



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in the various nuclei. That the typical nucleus of the fully differentiated endothelial cell may be distinguished from that of the indifferent mesenchyme cell we do not deny, but that intermediate stages between the two exist, in the case in question we likewise hold to be true. And unless it be cut parallel to its long axis, it is practically impossible to distinguish even the fully differentiated endothelial nucleus from the mesenchymal nucleus.

As for the statement that these flattened rows of cells are always connected with a preëxisting endothelium it must be remembered that practically every cell in the embryo is, at this stage, in syncytial relation with every other cell, the blood cells excepted. So in a certain sense a *protoplasmic* connection between flattened cells and preëxisting endothelium may be demonstrated by passing over the protoplasm of indifferent mesenchyme cells. To assume that because all endothelium in the embryo is in syncytial relationship it is therefore derived from some preëxisting endothelium, appears unwarranted. Can it not be said with equal truth that since the embryonic vascular endothelium is in syncytial relationship with the mesenchyme it is therefore derived from the mesenchyme? This being the case, we know that there are in the mesenchyme certain flattened cells which are not connected with any preëxisting endothelium otherwise than by means of the protoplasm of the mesenchymal syncytium. The isolation of these flattened cells from any other endothelium and the fact that all possible gradations

Fig. 4 Chick 8.5 mm., Series 21, Slide 1, Row 3, Section 2. × 500. Photomicrograph of transverse section of the caudal end of the embryo.

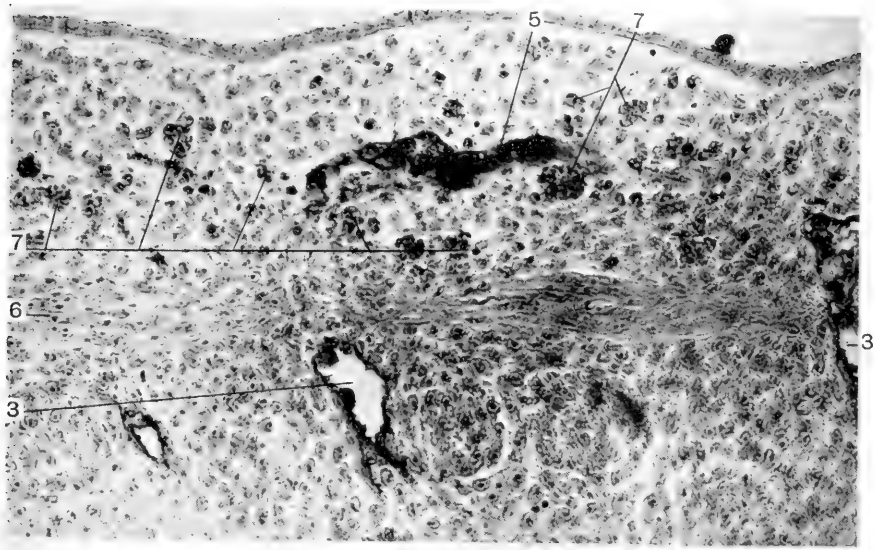
Fig. 5 Chick 11 mm., Series 20, Slide 1, Row 4, Section 4. × 600. Photomicrograph of transverse section of the caudal end of the embryo.

Figure 4.

3, Coccygeal vein
5, Haemal capillaries
6, Caudal muscle plate
7, Differentiating blood cells

Figure 5.

3, Coccygeal vein
5, Haemal capillaries
6, Caudal muscle plate
7, Differentiating blood cells
8, Mesenchymal spaces
10, Lymphatic connected with vein



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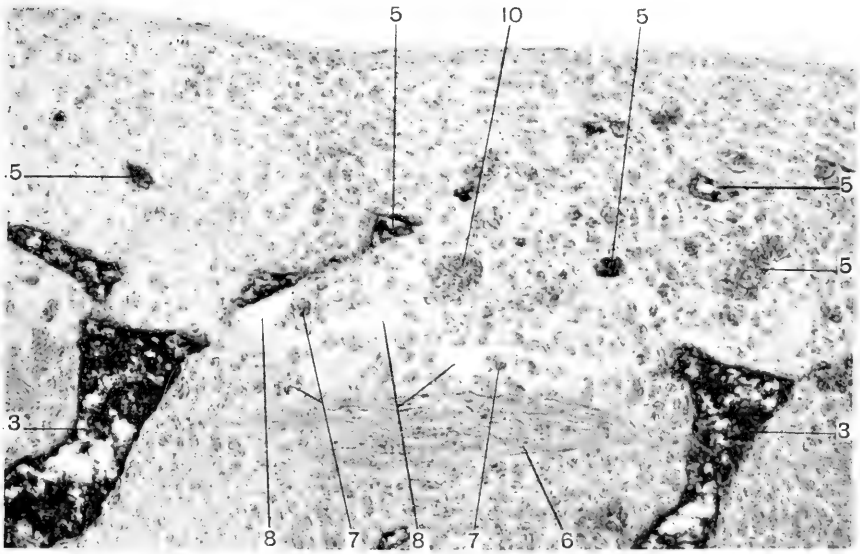

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exist between them and the typical mesenchymal cells shows clearly that an *in situ* differentiation of endothelial cells takes place (fig. 6, *13*, and fig. 7, *19*). The cells so formed may then bound isolated cysts filled with plasma (fig. 6, *12*) which sometimes enclose a differentiating blood cell. These plasmatocysts then proceed to grow together connecting up with one another and with the veins, and it is probable that they form in some instances a connecting link between the veins and the large lacunae in the mesenchyme. The early appearance of the blood-filled lymphatic plexus connected with the veins in the living chick, which E. R. and E. L. Clark (5) describe as follows, lends weight to such an interpretation of the facts:

> The first evidence of lymphatics in the tail region of the living chick is the appearance of separate knobs filled with stagnant blood just lateral to the coccygeal veins. Soon after these knobs appear similar ones develop about them which have fine connections with them. * * * * Their injection shows discreet tiny clusters, somewhat like bunches of grapes (p. 254).

Figure 6, a section of the caudal region of an 11 mm. embryo, shows an isolated plasmatocyst (*12*). This section and the adjacent sections were studied with the greatest care under the oil immersion lense, and the two elongated cells (*13*) with pale nuclei and distinct nucleoli bounding the cyst were not in connection with any other endothelium.

Figure 7, a section of the caudal region of a 15 mm. embryo, shows a structure which some might describe as a venous sprout. The injection mass has entered the lumen for a short distance in large amounts. Then the lumen becomes somewhat constricted, and beyond that point only occasional ink granules can be found. Finally the lumen terminates and a long flat cell (*13*) follows in which two distinct nucleoli are seen, beyond which is a space (*15*) bounded by a delicate strand of cytoplasm on either side. This space contains a differentiating red blood cell (*7*). The adjacent sections have also been examined with great care, and the one directly preceding shows one rather elongated flattened cell with a pale nucleus forming the floor and probably the end of the plasmatocyst containing the blood cell just described. Several

of the mesenchymal cells near by, in the direction in which this 'sprout' would extend, show a tendency to become elongated (*19*), but they are separated from the endothelial cell by indifferent mesenchymal cells, and their nuclei are quite chromatic. They probably represent cells which are about to flatten and to limit a plasmatocyst.

Since disconnected plasmatocysts have been found; since all gradations between an indifferent mesenchymal cell and a typical endothelial cell have been observed; and since, in the section just described, we find most distally an uninjected plasmatocyst, containing a differentiating blood cell, then a single endothelial cell enclosing no lumen, and finally a lumen connected with the veins into which the injection mass has entered, it does not seem justifiable to call this structure a venous sprout. It should rather be considered as a plasmatocyst which has differentiated *in situ*, and connected secondarily with the vein. Whether the endothelial cells between the plasmatocyst and the vein arise by an *in situ* differentiation, or by a mutual growth of the plasmatocyst and the vein toward each other, it is impossible in this particular case to determine definitely by the study of sections or injections. The latter interpretation would in no way invalidate the fundamental conception that endothelium arises *in situ* from mesenchyme. It merely implies that endothelial cells once formed are capable of proliferation, as cells in general are. It should be noted that discontinuity of the lumen of the 'sprout' present in figure 7 shows clearly the utter inadequacy of the injection method for demonstrating all of the endothelium in the embryo.

As regards the further development of the blind spaces in the mesenchyme, we have seen that in the 10.5 and 11 mm. embryos there exist a number of spaces in the mesenchyme just lateral to the caudal muscle plate, and that these spaces are bounded by mesenchymal cells. Some of these spaces are connected at this stage with the lateral branches of the coccygeal veins, and certain blood cells, differentiating from the mesenchyme, have become included in some of the disconnected spaces. In the 12.5 mm. and 13.5 mm. embryos more and

more spaces continue to connect with the veins, either directly or by means of the delicate hollowed 'cell strands' already described, and as the spaces acquire venous connection, they may become filled with blood backed up from the general circulation especially in injected embryos. The spaces which have not as yet attained a venous connection, increase in size, several smaller spaces coalescing by a breaking down of their cell boundaries to form a single larger space (fig. 9, *8;* fig. 8, *8;* fig. 3, *8*). As the plasmatic pressure becomes greater, the indifferent mesenchyme cells which bounded these spaces become flattened to form cells which are identical in appearance with endothelial cells (fig. 9, *8*). The first spaces about which endothelial cells were detected were in a 13.5 mm. embryo, although the cells bounding the spaces were somewhat flattened in the 11.5 mm. and 12.5 mm. embryos. The fact that the cells about a single isolated space may be in part endothelial and in part mesenchymal, with many intermediate stages between the two, indicates that an *in situ* differentiation of endothelium from mesenchyme is taking place.

The haemopoesis, which was described as taking place before the lymphatic anlagen appear, continues, but much less rapidly than formerly. We have seen that the mesenchyme lateral to the caudal muscle plate was first practically indifferent and non-vascular. Then came a wave of haemopoesis, followed

Fig. 6 Chick 11 mm., Series 20, Slide 1, Row 3, Section 7. × 500. Photomicrograph of transverse section of caudal end of the embryo.

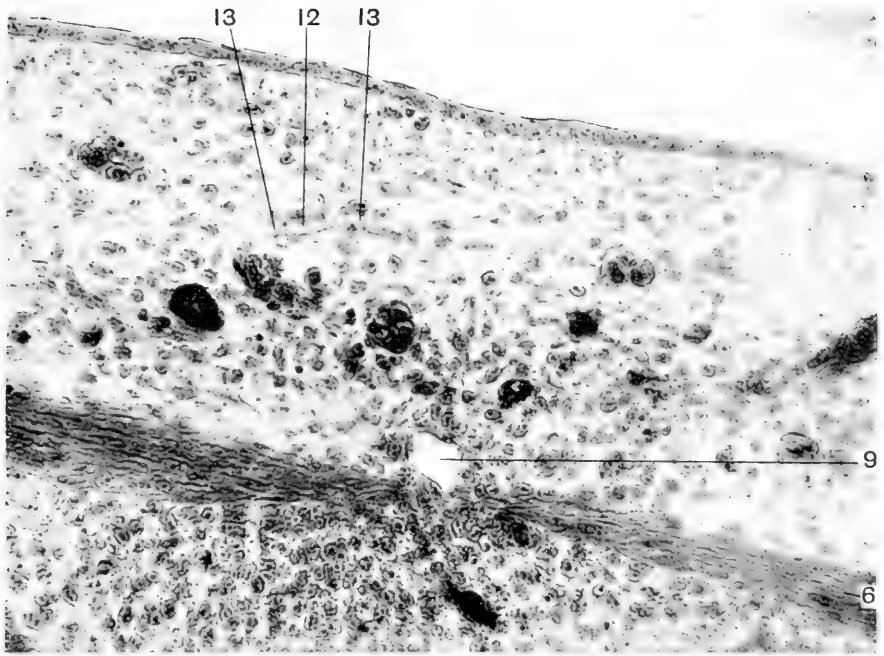
Fig. 7 Chick 15 mm., Series 16, Slide 2, Row 4, Section 6. × 500. Photomicrograph of transverse section of caudal end of the embryo.

Figure 6

6, Caudal muscle plate
9, Lateral branch of coccygeal vein
12, Isolated plasmatocyst
13, Elongated cell with pale nucelus and distinct nucleoli
7, Differentiating blood cell
8, Mesenchymal space
9, Lateral branch of coccygeal vein
13, Elongated cell with pale nucleus and distinct nucleoli
14, Lumen continuous with vein
15, Lumen not continuous with vein
19, Isolated flattened cell, with pale nucleus and distinct nucleoli

Figure 7

3, Coccygeal vein
6, Caudal muscle plate



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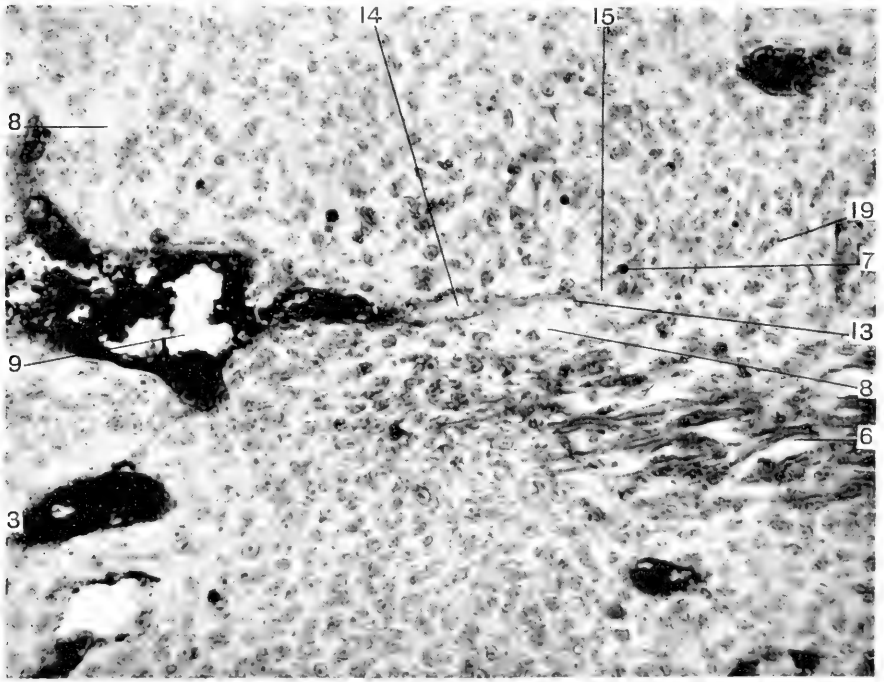

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quickly by a vascularization of the tissue and a decrease in the number of blood cells in the tissue spaces. This takes the embryo up to the 10.5 mm. stage, when the lymphatic anlagen first appear. From this time onward certain mesenchyme cells still seem to become rounded, break away from the surrounding syncytium, and acquire eosinophile granules. In other cells the cytoplasm becomes eosinophile more evenly, forming erythrocytes. These cells, which lie in the tissue spaces, for the most part become included in the lymphatic anlagen, and as these anlagen acquire a venous connection, reach the general circulation.

For the first time in the 12.5 mm. embryo groups of rounded strongly basophile cells, may be observed to be differentiating from the endothelium near the junction of the lymphatics and veins. Small clumps of rounded cells, more strongly basophile than the mesenchyme or endothelial cells, are seen forming and apparently splitting off from the endothelium of the lymphatics (fig. 10, *16*). In some of the older embryos the cytoplasm of these cells acquires an eosinophile tinge. These cells are identical with the erythroblasts described by Dantschakoff (13). Finally, in the 13.5, 14.5 and 15 mm. embryos large aggregations of slightly basophile cells with conspicuous eosinophile granules (fig. 11, *17*) are seen differentiating and splitting off from the lymphatic endothelium.

One final point must be noted, although it does not concern the endothelium of the lymphatic plexus. In the 14.5 mm. embryo strands of three or four myoblasts appear in the now

Fig. 8 Chick 14 mm., Series 17, Slide 2, Row 1, Section 6. × 300. Photomicrograph of transverse section of caudal end of the embryo.

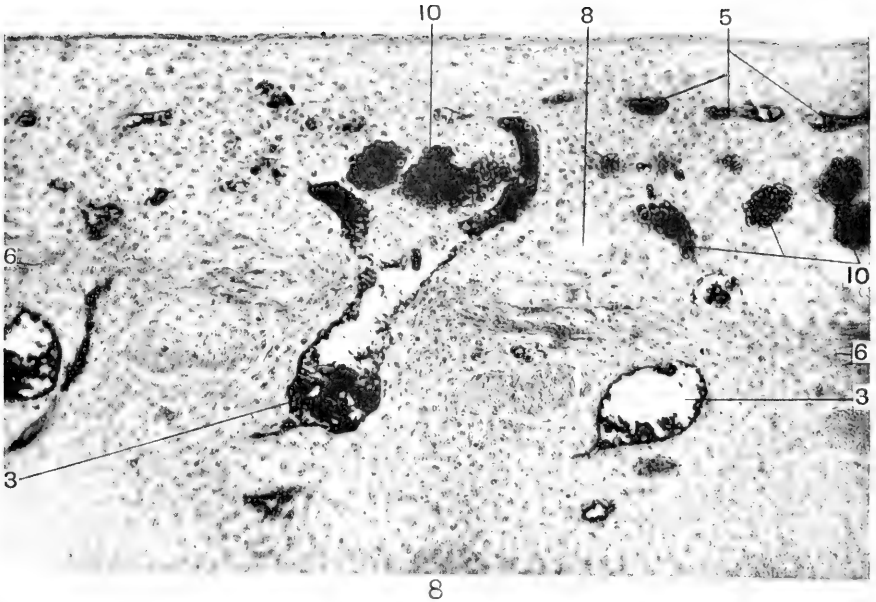
Fig. 9 Chick 15 mm., Series 16, Slide 2, Row 4, Section 6. × 600. Photomicrograph of transverse section of caudal end of the embryo.

Figure 8

3, Coccygeal vein
5, Haemal capillaries
6, Caudal muscle plate
8, Mesenchymal space
10, Lymphatic connected with veins

Figure 9

3, Coccygeal vein
6, Caudal muscle plate
8, Isolated space, bounding cells becoming flattened
9, Lateral branch of coccygeal vein
10, Lymphatic connected with vein



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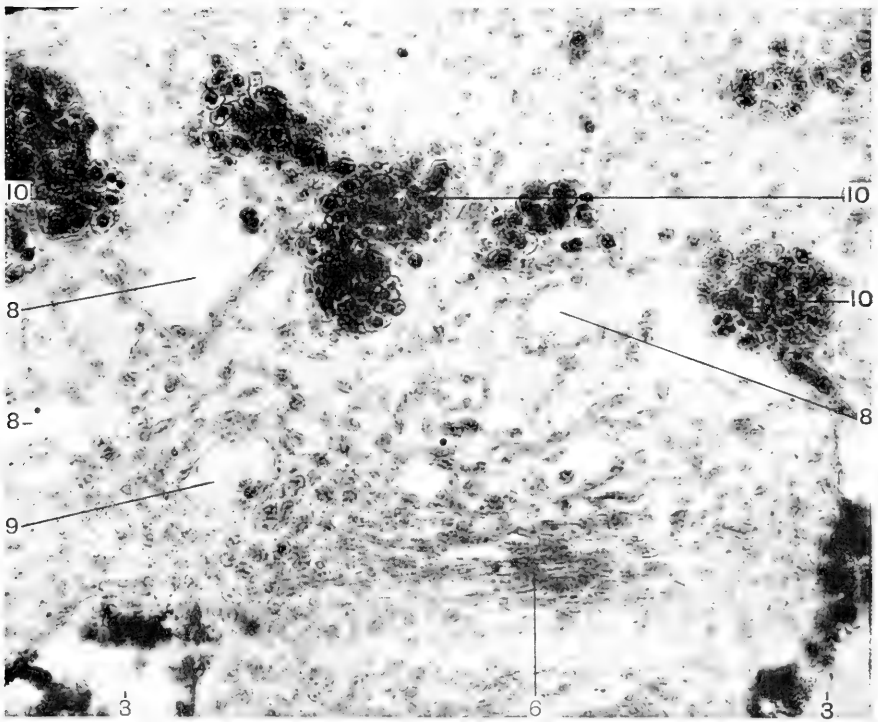

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vacuolated mesenchyme just lateral to the caudal muscle plate and parallel to the axis of the notochord, and occasional very small longitudinal spaces may be seen in the most lateral portion of the caudal muscle plate. In one or two sections, one end of the strand of myoblasts was seen to be in connection with the muscle plate. Whether these cells were splitting off from the muscle plate by delamination, or whether they were forming from the mesenchyme and being added to it by accretion, it was not possible to determine in the material available.

2. Morphogenesis

Up to this point we have considered the histogenetic changes which take place in the developing lymphatic plexus, and we shall now consider the morphogenesis of the plexus. For this purpose four wax reconstructions have been made by the method of Born, three of which are here reproduced.

Chick of 11 mm. Reconstructions of vessels and isolated spaces of the caudal region. × 150. Figure 12: Arteries black, veins and capillaries white, isolated spaces yellow. The postcardinal vein and the aorta run a few sections above the upper level of this reconstruction, but the coccygeal branches of the aorta (fig. 12, *4*) and a little more externally the coccygeal veins (*3*) which drain into the postcardinals, are seen running downward at right angles and dorsal to the axis of the vertebral column. All of these structures are medial to the caudal muscle plate, which has been omitted from this reconstruction for the sake of simplicity. This muscle plate extends in a plane, parallel to the ecto-

Fig. 10 Chick 12 mm., Series 46, Slide 1, Row 4, Section 4. × 500. Photomicrograph of transverse section of caudal end of the embryo. Uninjected.

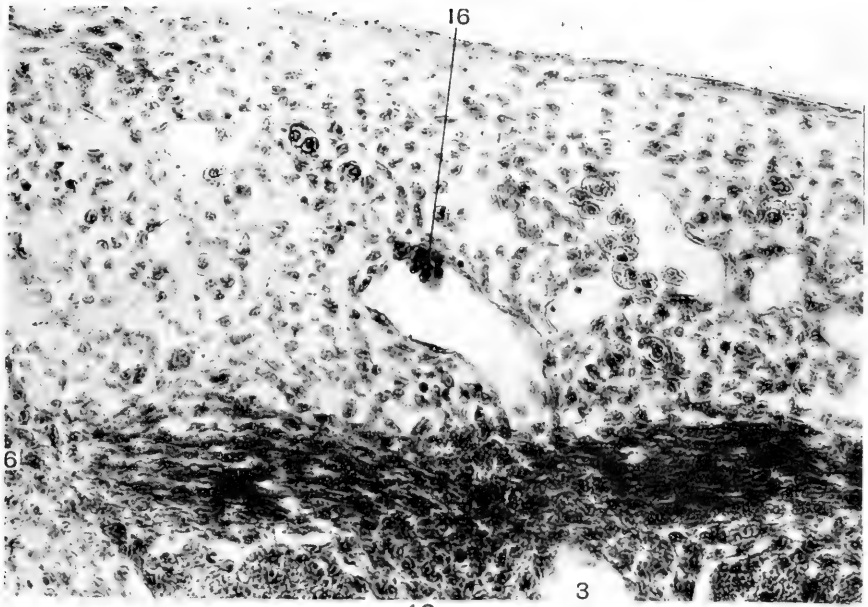
Fig. 11 Chick 15 mm., Series 16, Slide 2, Row 4, Section 7. × 600. Photomicrograph of transverse section of caudal end of the embryo.

Figure 10

3, Coccygeal vein
6, Caudal muscle plate
16, Blood cells differentiating from endothelium

Figure 11

5, Haemal capillaries
17, Blood cells differentiating from lymphatic walls



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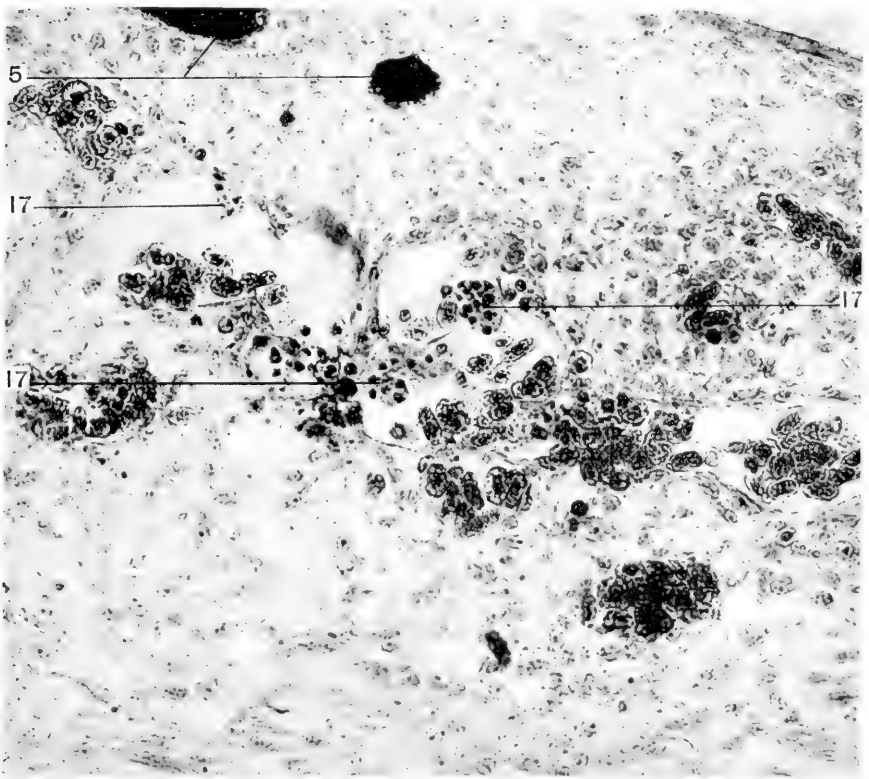

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derm, just lateral to the coccygeal veins. Two or three lateral branches of each coccygeal vein (fig. 12, *9*) pierce the muscle plate and proceeding directly outward terminate in a plexus of haemal capillaries which lie directly beneath the ectoderm.

The lymphatic plexus, which later forms the lymph heart, develops in the mesenchyme between the caudal muscle plate and this superficial plexus of haemal capillaries. A number of isolated spaces, bounded by mesenchyme cells which are still practically unflattened, are seen (fig. 12, *8;* fig. 5, *8*) to occupy the position just alluded to. They have been studied very carefully with oil immersion lenses and are absolutely independent of any vascular connection, either with the lateral branches of the coccygeal veins or the haemal capillaries; they occur only caudal to the level of the hind limb bud and only lateral to the muscle plate.

Chick of 14 mm. Reconstruction of the blood vessels of the caudal region, and the lymphatic plexus in so far as it forms a continuous channel connected with the veins. × *150. Figure 13: Arteries black, veins and capillaries white, lymphatics connected with veins, green.* The isolated spaces have been omitted from this reconstruction in order that the lymphatic plexus connected with the veins might be more clearly shown. The reconstruction has been drawn from the side and somewhat from above and the aorta and postcardinals have been shown in the drawing as folded upward and outward. We have in this reconstruction practically the same arrangement of arteries, veins and haemal capillaries as was described for the 11 mm. embryo. The two postcardinal veins (fig. 13, *18*) are seen above and somewhat lateral to the aorta; they anastomose above that vessel, and receive the coccygeal veins both cranial and caudal to their anastomosis. The coccygeal veins (fig. 13, *3*) as before, pass downward, at right angles to the axis of the vertebral column, close to the caudal muscle plate, and give off lateral branches (fig. 13, *9 a, b*) which pierce the muscle plate. It will be seen that a plexus of lymphatic vessels connected with the coccygeal veins has been established between the haemal capillaries and the muscle plate, which is characterized by the irregular size

of its vessels, prominent knob-like enlargements occurring wherever a large independent space previously existed. This plexus, as has been noted, usually fills with stagnant blood, backed up from the venous circulation. There is no connection between the lymphatic plexus and the haemal circulation except at the point where the lateral branches of the coccygeal veins have just pierced the muscle plate.

We now see that the lateral branches of the five or six most cranial coccygeal veins pierce the muscle plate, drain the lymphatic plexus and then pass outward to drain the haemal capillary plexus (fig. 13, *9a*). Soon that portion of the lateral branches of the coccygeal veins distal to the lymphatic taps degenerates, thus severing the connection of these veins with the haemal capillaries, so that those lateral coccygeal branches which drain the lymphatic plexus, cease to function otherwise than for the lymphatic drainage (fig. 13, *9b*). An examination of several injected embryos cleared by the method of Spateholz showed this point clearly; the haemal capillary plexus being drained in the 15 mm. embryo by the most dorsal portions of the coccygeal veins with only two of the lateral coccygeal branches assisting them, although in the embryo of 11.5 mm. five or six lateral coccygeal branches drained the plexus of haemal capillaries. One 17.5 mm. embryo which was examined in cross sections, showed no connection between the lateral branches of the five or six coccygeal veins which drain the lymphatic plexus and the haemal capillaries.

Chick of 15 mm. Reconstruction of the caudal vessels. $\times$ *150. Antero-lateral view. Figure 14: Arteries black, veins and capillaries white, lymphatics connected with the veins green, isolated spaces yellow.* In this reconstruction the coccygeal veins (*3*) are seen extending downward from the postcardinals (*18*) and the coccygeal arteries (*4*) from the aorta (*11*). The coccygeal veins give off lateral branches (*9*) which pierce the caudal muscle plate—which has been omitted from this reconstruction—and then proceed laterally to drain the lymphatic plexus (green) and at the points where the lymphatics are not as yet formed to any extent, the haemal capillary plexus (white). The lym-

phatic plexus may be clearly seen to occupy the area which in the reconstruction of the 11 mm. chick was filled only by isolated mesenchymal spaces. A great number of these isolated spaces (yellow, *8*) still exist, not connected as yet with the lymphatic plexus. They occur in greater numbers medial to the lymphatic plexus which is connected with the veins (green, *10*) that is between it and the caudal muscle plate, than they do lateral to the lymphatic plexus, although quite a number, as may be seen from the figure, occupy the latter position. It is especially interesting to note that the isolated spaces lie on all sides of the lymphatic plexus, seeming to precede it and form in an area which an hour or two later is occupied by the continuous plexus of lymphatics, connected with the coccygeal veins. Such outlying isolated spaces are clearly shown at the cranial end of this reconstruction.

Fig. 12 Reconstruction of caudal vessels of a chick of 11 mm., Series 20. × 150. Antro-lateral view; arteries in black; veins and capillaries in white; isolated spaces in yellow.

Fig. 13 Reconstruction of caudal vessels of a chick of 14 mm., Series 17. × 150. Antro-lateral view; arteries black; veins and capillaries white; lymphatic plexus connected with veins, green. The disconnected mesenchymal spaces have been omitted from this reconstruction.

Fig. 14 Reconstruction of caudal vessels of a chick of 15 mm. Series 16. × 150. Antro-lateral view; arteries black; veins and capillaries white; lymphatics connected with veins, green; mesenchymal spaces yellow.

Figure 12

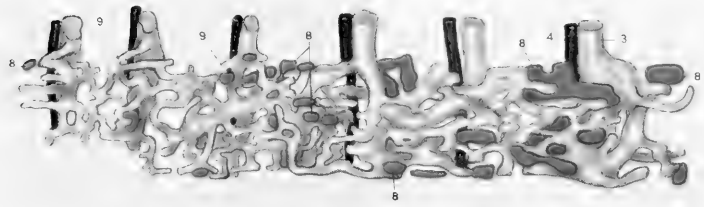
3, Coccygeal vein
4, Coccygeal artery
8, Mesenchymal spaces (green)
9, Lateral branches coccygeal veins draining haemal capillaries
9b, Lateral branches of coccygeal vein draining lymphatics only
10, Lymphatic plexus connected with the veins (green)
18, Postcardinal veins

Figure 13

3, Coccygeal vein
4, Coccygeal artery
5, Haemal capillary plexus
9, Lateral branches of coccygeal vein
9a, Lateral branches of coccygeal veins draining haemal capillaries and lymphatics

Figure 14

3, Coccygeal vein
4, Coccygeal artery
8, Disconnected mesenchymal space
9, Lateral branch of coccygeal vein
10, Lymphatic plexus connected with veins
11, Aorta
18, Postcardinal vein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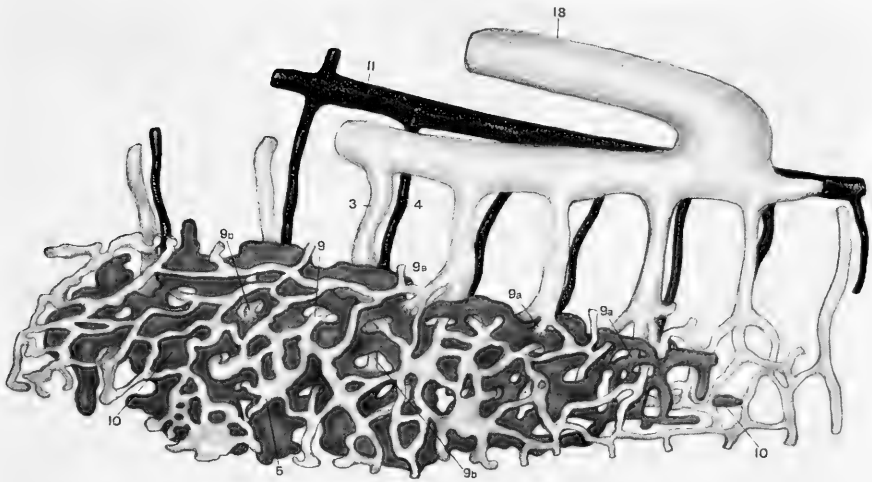
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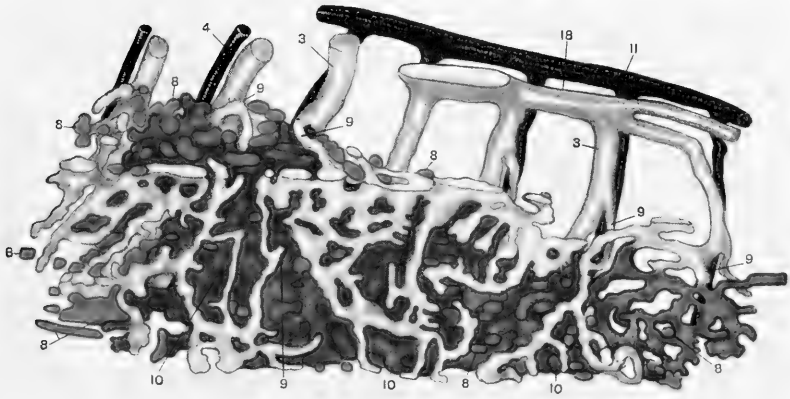
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As this investigation has been concerned solely with the origin of the lymphatic plexus which later forms the lymph heart, the later history of the lymph heart has not been studied. A cursory examination of a 16, 16.5, 17 and 18 mm. embryo would indicate that the conclusions of Sala are in the main correct, and that the plexus coalesces to form the single cavity of the lymph heart. The formation of the musculature, valves and the number of venous taps in the stages later than 15 mm. has not been studied.

GENERAL DISCUSSION

Of the previous investigators of the posterior lymph heart in the chick, Sala (1) and Mierzejewski (2) have not committed themselves as to the origin of the lymphatic endothelium, while E. R. and E. L. Clark hold that the lymphatics are outgrowths from the veins and that the endothelium is specific. E. R. and E. L. Clark (5) have studied the growth of the lymphatic plexus in the living chick under the binocular microscope, using the stagnant blood backed up in the growing lymphatics from the veins as the index to lymphatic growth. To quote from their paper:

> Since stagnant blood in the interior of the lymphatics is the index on which these studies are based, it was important to determine whether the blood always fills the lymphatics to their tips. This was tested in two ways, by pressure over the part filled with blood to see if it could be forced farther; and by injection. As a result of numerous tests by both of these methods it was found that in these early stages, practically all of the lymphatics, save very fine connections are filled with blood. * * * * Hence, since the blood fills the successive extensions of the lymphatic as soon as formed, the use of the stagnant blood as an index for the study of lymphatic development is justifiable (p. 255).

This method has overlooked even the possibility of the presence of disconnected mesenchymal spaces entering into the formation of this lymphatic plexus. How, extravasation excepted, could pressure over the blood-filled plexus or injections into it, reveal disconnected lymphatic anlagen in the form of blind spaces in the mesenchyme? It is obviously impossible to detect small

mesenchymal spaces filled with colorless lymph by examining a living chick under the binocular microscope. The tests just described would serve to show that the blood fills the lymphatics to their tips only *in so far as they formed a continuous channel connected with the veins*, and would utterly fail to reveal any disconnected anlagen in the form of independent mesenchymal spaces. The *appearance* of a centrifugal outgrowth of the lymphatic plexus from the veins is *simulated* if stagnant blood or any other form of injection be used as an index to the lymphatic development, for the mesenchymal spaces lying next to veins are the first to make the venous connection and fill with blood backed up from the general circulation. Then the spaces a little more distal join the spaces already connected, in turn are filled with blood, and so on until the entire blood-filled lymphatic plexus is formed. Thus, while the development is proceeding by the centripetal addition of disconnected anlagen, the stagnant blood in the plexus is extending in a centrifugal direction. In discussing the blood-contents of the early lymphatic plexus, which later forms the posterior lymph heart, the active haemopoesis in the surrounding mesenchyme is the only factor of morphological and genetic significance. The accidental or normal backing up of circulating blood into the lymphatic plexus, after it has secondarily established a connection with the veins, is of no significance as far as the genesis of the lymphatic structures is concerned. But the *in situ* origin of blood cells from the mesenchyme and their conveyance, via the lymphatics, into the general haemal circulation is of great importance, and at once places the posterior lymph hearts in the chick in the category of *haemophoric lymphatics*, such as are met with in the thoracic duct of the same form and in other vertebrates in various degrees of development, as has been brought out in Huntington's paper of July 1914 (10).

Until it can be absolutely proven by some other method than that of injection that all lymphatic development is centrifugal growth in continuity, with invariable continuity of lumen as well, such methods as this will seem to beg the question; for they can afford evidence only of the degree of the centrifugal exten-

sion of the lymphatics and by no means serve as a test of the process by which this extension is effected once the question of annexation of mesenchymal spaces has been raised. They serve simply as a measure of the process and do not indicate its nature.

It may be argued that the spaces here described are due to the action of fixing fluids. But if this were so they certainly would not appear *only* in the region of the embryo in which the lymphatics are developing, and *only* during the short period of embryonic history during which the lymphatic vessels are formed nor would the border cells of an artefact be flattened to form endothelium. That the spaces exist in the fixed and sectioned embryos, is clearly shown in the accompanying photomicrographs, and it is safe to conclude that they exist in the living embryo as well.

But, is there any evidence that the venous endothelium does not invade this vacuolized tissue and grow out to line these independent spaces? There is: The spaces are in the younger embryos (10.5 mm.) bounded by mesenchyme cells, but as the embryo becomes larger and the spaces increase in size the bounding cells become flattened and gradations between mesenchymal cell and endothelial cell are found bounding the spaces (fig. 9, *S*). Nor is there ever found an endothelial tube within the flattened cells. The idea just discussed has been suggested by Knower (14) without, so far as the writer is aware, the slightest objective evidence in its support. The spaces form and acquire a venous connection so rapidly that in only a few cases are isolated spaces found bounded by fully developed endothelial cells, which are disconnected with any preëxisting endothelium. But many spaces are found surrounded more or less completely by endothelium and in the remainder of their periphery by cells ranging from unmodified mesenchyme to almost typical endothelial cells.

Mesothelium has been produced experimentally from connective tissue, by introducing the factors of pressure and friction, by W. G. Clark (15). He has used non-irritating solid and fluid foreign bodies; celloidin and paraffin, injected into the cornea and subcutaneous tissue, and mucus which was allowed

to flow through a fistula. He concludes that "the fact that connective tissue cells are changed in form by physical agents into flat closely disposed cells, the outline of which may be defined by silver salts makes tenable the conclusion that the exposed connective tissue cells * * * * may become flattened by pressure or friction or both." Therefore, mesothelium and endothelium, both being tissues of mesenchymal origin, owe their production to identical mechanical factors.

To summarize: The evidence found from the study of injected embryos indicates that the lymphatic plexus which later enters into the formation of the posterior lymph heart, arises by the confluence of independent mesenchymal spaces which connect secondarily with the veins; that these spaces are bounded at first by mesenchymal cells which later become flattened to form an endothelium and that both in the endothelial lymphatic walls and the adjacent mesenchyme an active haemopoesis, the products of which reach the general circulation via the lymphatic plexus, is taking place.

In conclusion, I wish to thank Professor McClure and Professor Huntington who have directed this work for their constant guidance and criticism; Professor Schulte and Professor Miller for many valuable suggestions; Dr. McWhorter for the care that he has expended on the microphotographs, and Mr. Petersen for his drawings of the very complex reconstructions.

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THE DEVELOPMENT OF THE THYMUS IN THE PIG

II. HISTOGENESIS

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THREE PLATES (EIGHT FIGURES)

CONTENTS

INTRODUCTION

There is perhaps no organ in the body whose mode of development has given rise to so bitterly contested and so widely divergent views as has that of the thymus. This is particularly true of its histogenesis. The source and nature of the small round cells that make up the greatest mass of the organ in its fully developed condition; the origin and nature of its intralobular supporting structure; the origin and significance of its granular cells and of the Hassall's corpuscles; the extent to which red blood-cells and granular leucocytes are formed in it; have during the past thirty-five years attracted the attention of many investigators, and yet the only point upon which they unanimously agree is that the thymus is, in part at least, of epithelial origin. This disparity of views cannot be due to differences in the development of the thymus in different animal forms, for some workers

who have extended their investigations over a comparatively wide range of species and classes of animals have found that the developmental processes involved are practically the same for the different types of animals investigated.

While the investigation presented in this paper deals with the histogenesis of the thymus as a whole, special consideration is given, (1) to the origin and nature of the small round cells, and (2) to the origin of free erythrocytes and eosinophile cells that are present in both the interlobular septa and the parenchyma of the thymus in later developmental stages. Though comparatively little attention has been paid to the development of the reticulum and the thymic bodies, they nevertheless have received a consideration sufficient to determine their origin.

MATERIAL AND METHODS

The material used for the histogenesis of the thymus was collected in great abundance at a packing house. Often the embryos still showed signs of life while they were being measured and prepared for the fixing fluid. The upper jaw, the cranium, and the posterior thoracic wall were removed from embryos from 10 to 20 mm. in length. The part containing the thymus was thus made comparatively small and fixed well. Embryos from 20 to 55 mm. in length were treated in a similar manner and in addition the sides were trimmed and the cervical vertebrae removed in order to reduce the size of the piece. From embryos ranging from 60 to 165 mm. in length only portions of the thymus with some of the surrounding tissues were removed. From all these stages the entire superficial thymus and thymus head, and parts of the mid-cervical and thoracic portions were procured. From embryos 180 to 280 mm. in length (full term) the superficial thymus and portions of the thymus head and mid-cervical segment were removed. The left thymus was usually selected in those stages from which only a portion of the organ was removed. The lengths in millimeters of the different developmental stages of which the thymus was prepared for a study of its histogenesis are as follows: 17, 20, 23, 25, 26, 27,

28, 30, 33, 35, 36, 37, 40, 42, 45, 50, 50, 55, 60, 65, 68, 78, 85, 100, 110, 115, 125, 135, 140, 165, 180, 190, 210, 230, 270, and 280. These figures represent the length of the embryos while in a fresh condition.

Helly's fluid (Zenker-formol) was almost exclusively used for fixing the material. This fixer does not destroy the basophilic character of the cytoplasm of the lymphocytes and apparently produces no appreciable alteration in the hemoglobin of the red blood-cells found in the thymus. A few embryos of different developmental stages were fixed in Zenker's fluid, mainly to check up the results of the work done by investigators who employed this fixer for a histogenetic investigation of the thymus. It was found that Zenker's fluid is not at all suitable for work of this nature since, to a large extent, it destroys the basophilic character of the lymphocytes, the preservation of which is of inestimable value in tracing out the origin of the first lymphocytes found in the thymus. The tissue was imbedded in paraffine and cut in sections 3 to 5 μ in thickness. Only such sections as were desired were spread on slides. These always included sections of the superficial thymus, the thymus head, and cervical and thoracic segments.[1] For the preservation of cells with basophilic granules the material was fixed in 95 per cent alcohol.

Hasting's modification of Nocht's Romanowsky blood stain proved to be of the greatest value for this work and was almost exclusively used in the investigation of the histogenesis of the thymus. In properly differentiated sections the cytoplasm of the lymphocytes, which has a distinctly basophilic character, stains a light blue, while the cytoplasm of the epithelial cells of the thymus and that of the mesenchymal cells stains a light red color. The lymphocytes can thus be distinguished from the epithelial nuclei with comparative ease. Erythrocytes and the granules of eosinophile cells are stained intensely red, while the granules of the cells with basophilic granules (fixed in 95 per cent alcohol, are stained blue. In tissue fixed in Zenker's

[1] For a discussion of the different regions of the thymus in the pig reference should be made to Part I of this investigation ('14).

fluid (used by Bell) the basophilic character of the cytoplasm of the lymphocytes is lost, thus rendering it difficult to distinguish a large or medium sized lymphocyte from some of the smaller epithelial nuclei of the thymus. Mallory's connective tissue stain was also used for staining the connective tissue fibers of the thymus.

HISTORICAL

Only a brief historical sketch will be given to outline, in a general way, the views regarding the histogenesis of the thymus. For a comprehensive review of the literature on this subject reference should be made to Hammer's work of 1910.

The investigations that have been made of the histogenesis of the thymus of various classes and species of animals have led to the formation of two general theories, viz., the pseudomorphosis theory and the transformation theory, each of which has been more or less modified by the different investigators of this subject. As it is beyond the scope of this work to give a detailed discussion of each theory and its modifications they will be discussed only in a general way. The pseudomorphosis theory will be first considered. In its original setting this theory held that the epithelial anlage of the thymus is gradually invaded by mesenchymal and adenoid tissue. This process displaces the epithelial cells and the only remnants of them in the fully developed thymus are the Hassall's corpuscles. This view was held by Maurer for the thymus of teleosts ('86) and for the thymus of Urodela and Anura ('88). He gives no detailed description of the development of the reticulum, or of the invasion of the epithelial anlage of the thymus by the lymphocytes.

Von Ebner held a somewhat modified view of the pseudomorphosis theory as set forth above. According to him, the reticulum and Hassall's corpuscles of the medulla are derived directly from the cells of the original epithelial anlage, while the entire cortex with its reticulum, lymphocytes, and blood vessels, and also the lymphocytes of the medulla, are of mesenchymal origin.

The latest modification of the pseudomorphosis theory which is now generally accepted by investigators belonging to this school, was set forth by Hammer in his investigations of the thymus of human embryos ('05). According to him, the reticulum of both the cortex and medulla and the Hassall's corpuscles are of epithelial origin. He then was in doubt as to the origin of the lymphocytes in the thymus, but called attention to the presence of 'wanderzellen' (lymphocytes) in the immediate vicinity of the organ before and some time after they were present in it. He also observed darkly stained cells in the thymus of early developmental stages which, with only moderately high magnification, could easily be mistaken for lymphocytes, but with very high magnification could readily be recognized as degenerating epithelial cells. He thus cast a doubt on the origin of lymphocytes from the epithelial cells of the thymus, as is still held by some investigators, and pointed out as probable an infiltration of the thymus with extrathymic lymphocytes which have migrated into it from the surrounding mesenchyme. This last view he was unable to prove conclusively on account of a lack of sufficient range of developmental stages. His investigations on numerous developmental stages of the Teleostean thymus ('08) also led him to conclude that the fixed elements of the thymus are of epithelial origin. He fully believed, however, that the lymphocytes first present in the thymus of the Teleost migrate into it from the mesenchyme and there, through repeated division, give rise to the numerous lymphocytes found in it in its fully developed condition. In his latest work ('11) on the development of the human thymus he makes no mention of a migration of lymphocytes into the thymus from the surrounding mesenchyme.

Maximow in his work ('09 b) on the developing thymus of mammals (rabbit, guinea-pig, cat, rat and mouse) and on the thymus of the Axolotol ('12) also holds that the fixed elements of the thymus are of epithelial origin, while the lymphocytes first present in that organ have migrated into it from the mesenchyme and, through repeated division, form the numerous small lymphocytes of the thymus in later developmental stages.

His views of the developing mammalian thymus are, therefore, similar to Hammer's views of the developing Teleostean thymus.

The chief exponents of the transformation theory are Prenant ('94), Maurer in his later work ('99), Bell ('06), Stöhr ('06), and Dustin ('11). The main point of this theory which they most strenuously defend is that the lymphocytes arise from transformed epithelial cells of the thymus. The epithelial cells proliferate rapidly. A part of the daughter cells transform into lymphocytes while the undifferentiated portions continue to proliferate and are the source of succeeding generations of epithelial cells and lymphocytes. All hold that the epithelial cells give rise to the reticulum and Hassall's corpuscles, excepting Dustin ('11) who claims that the reticulum is of mesenchymal origin. These investigators regard the small round cells of the thymus as real lymphocytes.

Stöhr also derived the small round cells of the thymus from epithelial cells, but claimed that except for their similarity in structure to the small lymphocytes in the blood, they have nothing in common with them. They never enter the blood stream, they remain epithelial cells as long as they exist, and have the power to enlarge and change back to typical epithelial cells. In the medulla, according to Stöhr, are found real lymphocytes that have entered from the blood. He, however, fails to explain how they can be distinguished from the small round cells (epithelial cells) when they lie side by side. He also derives the reticulum and Hassall's corpuscles from the epithelial cells of the thymus.

It is now generally accepted that the thymic bodies are derived from the epithelial cells of the thymus. Hammar and Bell have given a thorough description of their development.

From this brief historical sketch it can be seen that the nature of the development of the thymus is by no means a settled question. I wish to state at this point that the results of my investigation of the histogenesis of the thymus agree with these of Hammar ('08) and Maximow.

A brief historical sketch relative to the origin and development of the free erythrocytes and granular cells of the thymus will be given in connection with their consideration.

HISTOGENESIS

To determine the origin of the different cellular elements that are found in the fully developed thymus it is necessary to begin with stages in which the thymus is purely epithelial, and to use a differential stain by which one can definitely distinguish a lymphocyte from an epithelial cell. The latter fact was emphasized by Maximow ('09 b) who accomplished this differentiation by fixing the tissue in Helly's fluid and staining with eosin-azure. A similar differential staining was accomplished by me by fixing the tissue, as stated above, in Helly's fluid and staining with Hasting's Nocht's blood stain. The material prepared for the histogenesis of the thymus begins with an embryo 17 mm. in length. Of the many stages that were prepared and examined there are chosen for description only a few series of successively older stages, each of which is decidedly advanced in development over the previous stage and yet closely enough connected with it so that the developmental history will be continuous.

The histogenesis of the thymus may conveniently be divided into epochs, each of which is characterized by more or less distinct developmental features. They are: (1) a purely epithelial epoch which extends from its earliest development as an outpocketing from the third pharyngeal pouch and the formation of the cervical vesicle to the appearance of the first lymphocytes in the epithelial anlage of the organ; (2) The epoch of lymphocyte infiltration and lymphocyte proliferation, and the formation of the reticulum. This epoch begins with the appearance of the first lymphocytes in the thymus. The invasion of the thymus by lymphocytes from the surrounding mesenchyme continues probably up to stages 180 mm. in length while the proliferation of the lymphocytes in the thymus still continues in full term embryos and doubtless after birth. During this epoch the cortical and medullary portions of the lobules appear first in stages ranging from 65 to 75 mm. in length. The reticulum, which according to the nature of its development is formed gradually, is fully developed in embryos 180 mm. in length;

(3) the epoch of the formation of red blood-cells and the development of granular leucocytes. Although an occasional red blood-cell is formed in the thymus shortly after the appearance of the first lymphocytes, this epoch properly begins in embryos about 55 mm. in length for it is at this developmental stage that erythrocytes are beginning to be formed in comparatively large numbers. Granular cells appear first in appreciably large numbers in embryos 125 mm. in length. The formation of both erythrocytes and granular cells in the thymus still continued in the full term embryo.

1. The purely epithelial epoch

A 23 mm. embryo is the first stage in which the histological structure of the thymus will be described. The thymus at this stage is a purely epithelial structure. It has the form of a greatly elongated mass of protoplasm and is a syncytium. No cell walls are present. The cytoplasm of the superficial thymus, the thymus head, and the mid-cervical and thoracic segments contain many vacuoles which vary in size anywhere from the (apparent) size of a small pinhead to that of an epithelial nucleus when magnified 1300 diameters. The vacuoles in the intermediary and cervico-thoracic cords are comparatively few in number. Fine, branching, and rather deeply stained protoplasmic threads give the syncytium a distinctly reticular appearance. These protoplasmic threads, however, must not be confused with the reticulum in later developmental stages. The outer surface of the enlarged portions of the thymus is already quite irregular, being studded over with blunt epithelial buds which are the beginnings of lobules. No basement membrane is present.

The form and size of the epithelial nuclei vary considerably. While the majority are slightly ellipsoidal in shape, some are spherical and others slightly irregular in outline. They are quite regularly distributed through the different segments of the organ, lying farther apart in the more vacuolar regions than in

the intermediary and cervico-thoracic cords where only a few vacuoles are present. Those lying near the surface are quite regularly arranged. The long axis of the oval ones is usually perpendicular to the surface. The more centrally located cells have no regular arrangement. A very distinct nuclear membrane is present. They possess a quite rich supply of chromatin which is distributed mostly in the form of fine threads, giving it a reticular structure. About one-half of the nuclei possess two nucleoli while the other half contains but one. Occasionally one can be found with three nucleoli. They are large and occupy no definite position in the nucleus. In the oval nuclei they may lie near the ends or near the center, while in the round nuclei they may occupy an eccentric position. The nuclei at this stage do not all stain with the same intensity. In the enlarged segments of the thymus some stain much more intensely than the majority and with only moderately high magnification could easily be mistaken for transforming stages leading to the development of lymphocytes, which, however, is not the case. A consideration of their real significance will be given in connection with a discussion of the origin of the lymphocytes in the thymus. Nuclear division at this stage goes on rapidly in the enlarged segments of the thymus. Even with a magnification of 1300 diameters often three nuclei in mitotic division can be brought into a microscopic field.

To determine whether or not cells migrate into the thymus it is necessary to make a study of the connective tissue, at least in the earlier stages, as painstaking as the study of the thymus itself. The mesenchymal cells are of the spindle or stellate type. Their protoplasmic processes often unite with those of neighboring cells. Many fine fibers are scattered in the meshes between the cells giving the appearance of a network. The mesenchyme so closely invests the thymus that in places the cytoplasmic processes of the mesenchymal cells appear to be fused with the cytoplasm of the epithelial cells. The nuclei are large and spherical or oval in shape, and contain about as much chromatin as the epithelial nuclei of the thymus.

Large lymphocytes[2] are found scattered here and there throughout the mesenchyme of the neck and upper thoracic regions which were the only regions examined. They are characterized by a wide rim of basophilic, nongranular cytoplasm and a large nucleus containing a generous amount of chromatin. Their shape varies; some are nearly spherical while others have an irregular outline with one or more projecting pseudopodia. When treated with Hasting's Nocht's blood stain the cytoplasm takes on a distinct bluish hue the deepness of which may vary in different lymphocytes found in a single section, thus indicating that some are more basophilic than others. The relation of faintly stained to the more deeply stained cells will be considered later on in this paper. The nucleus is sharply defined from the cytoplasm by a distinct nuclear membrane. The chromatin is in the form of irregular and deeply stained granules which vary much in size. Some of the granules adhere to the nuclear membrane, while others are scattered in the less deeply stained nucleoplasm. In most nuclei only one nucleolus is present but some contain two. The shape of the nucleus often conforms to the shape of the cell body. In round lymphocytes it usually is round while in the irregular shaped lymphocytes it may also be irregular in shape. It is impossible to mistake an irregularly shaped lymphocyte with its blue stained cytoplasm and its nucleus rich in chromatin for a spindle or stellate shaped mesenchymal cell with slightly reticulated and lightly red stained cytoplasm and a nucleus containing considerably less chromatin.

While the lymphocytes are scattered singly throughout the entire mesenchyme, local accumulations are also found. These are most pronounced in the upper thoracic region near the large blood vessels and the thoracic segments of the thymus. Most profound growth activity is apparent in the mesenchyme surrounding the thoracic segments and it is here that transition stages from mesenchymal cells to lymphocytes frequently occur. Large lymphocytes are quite numerous. Even with

[2] The 'Wanderzellen' of Maximow and other investigators are regarded as being identical to the large lymphocytes.

a magnification of 1300 diameters five were found in a single microscopic field. Those with only a slightly basophilic cytoplasm are quite numerous.

Other cellular elements such as giant cells, the megaloblasts of Maximow, normoblasts and definitive erythrocytes also occur. These elements and the granular cells found in the thymus in later developmental stages are discussed further on in this paper.

The occurrence of lymphocytes in the mesenchyme before they are present in the thymus is of the greatest significance from a histogenetic view point, for Bell ('06) in describing the thymus of a 45 mm. pig embryo says: "There are no lymphocytes in the connective tissue around the thymus or in the blood at this stage," and of a 70 mm. embryo he says: "There are a few lymphocytes outside the thymus in the interlobular tissue in this region; * * * * I have never seen lymphocytes outside the thymus where there were none inside it, but they appear outside shortly after they are formed here." The results of my observations are contradictory to those of Bell. In a 17 mm. embryo an occasional lymphocyte can be found in the mesenchyme while in the 23 mm. embryo an occasional lymphocyte was found in the blood. His failure to detect lymphocytes in the mesenchyme in stages less than 70 mm. in length was perhaps due to the use of Zenker's fixing fluid which, as stated above, destroys the basophilic character of the cytoplasm. Beard ('02), in his work on the smooth skate, positively asserts that the first lymphocytes in the body are found in the thymus.

Embryo of 26 mm. In this stage the lobes of the superficial thymus, the thymus head, and the thoracic segments have greatly enlarged. Lobules are beginning to grow out from them. The cervical segment, which enters its period of rapid development a little later than the thymus head and the thoracic segment, is now quite pronounced and is studded over with short, blunt epithelial buds. No lobules have yet started to grow out from the intermediary and cervico-thoracic cords. The surface of the thymus is quite definitely marked from the mesenchyme but no basement membrane is present. The vacuoles

in the syncytium are somewhat more numerous than in the preceding stage. They vary greatly in size. Some are in contact with the epithelial nuclei while others have no connection with them. No consideration was given to the mode of their formation. They will again be considered in a later developmental stage.

Large lymphocytes, as in the previous stage, are found plentifully in the mesenchyme surrounding the thoracic segment of the thymus. They are more numerous in the region of the larger blood vessels of the thorax than in those parts of the connective tissue containing only smaller vessels where, however, they can be found without much searching. Around the superficial and head thymus local accumulations now occur. In general, they are more numerous than in the preceding stage.

2. The epoch of lymphocyte infiltration and lymphocyte proliferation and the formation of the reticulum

30 mm. embryo. The thymus of this stage is decidedly in advance of the 26 mm. stage just described. The lobules of the superficial and head thymus and the cervical and thoracic segments have greatly enlarged while those of the intermediary and cervico-thoracic cords have started to develop. The mesenchyme occupies all the spaces between the lobules and is somewhat denser than that surrounding the thymus. Blood vessels are numerous in the connective tissue septa but none are present in the lobules. At this stage the lumen of the blood vessels is comparatively large, and their walls are thin, being made up of large endothelial cells only.

The structure of the epithelial nuclei of the thymus is the same as in the preceding stage. Mitoses (fig. 1, *M.e.N.*, also fig. 2, 37 mm.) are quite frequent. The "large dark nuclei" and "small dark nuclei (lymphoblasts)," (figs. 1 and 2, *D.e.N.*), according to Bell's nomenclature, are present as in the 23 mm. embryo. These I regard as epithelial nuclei in the first stages of degeneration. A completely degenerated epithelial nucleus is also present (fig. 1, *D.e.N'*). Its chromatin has massed into

deeply stained clumps which lie in a clear space that has almost the same size and shape as a normal nucleus.

The cytoplasmic syncytium of the epithelial anlage has the same general structure as that in a 23 mm. embryo. The vacuoles (fig. 1, *V*) are, however, more numerous and the vacuolation of the cytoplasm has reached its greatest height in this stage. No basement membrane is present but the anlage is quite sharply defined from the surrounding mesenchyme which closely invests the thymus. As in earlier stages some of the protoplasmic processes of the mesenchymal cells are apparently fused with the outer surface of the cytoplasmic syncytium of the epithelium.

The point of greatest interest and importance in this developmental stage is the presence of lymphocytes (fig. 1, *L.L.*) in the thymus anlage. They are present in small numbers in the superficial thymus, thymus head, and in the thoracic segment. None were found in the mid-cervical segment which in this and younger stages is not as far advanced as the head and thoracic segments. Lymphocytes, however, occur in the thymus before the 30 mm. developmental stage. In a 25 mm. embryo a single lymphocyte was found in the thymus head. None were seen in the thymus of a 26 mm. embryo. In a 27 mm. embryo one lymphocyte was found in one of eleven sections prepared from the thoracic segment. In a 28 mm. embryo only a few could be demonstrated. In the stage being described they are present in small but appreciable numbers, hence, this stage was chosen for the discussion of the origin of the lymphocytes in the thymus. Their location in the lobules varies. Some are found in or near the center of the lobules while others lie near the periphery. All the lymphocytes that were found in the thymus in this and somewhat later stages are large lymphocytes. No small lymphocytes such as make up the bulk of the organ in late developmental stages, are present. The large lymphocytes are characterized by a generous amount of nongranular cytoplasm which is distinctly basophilic in its character. This basophilic character of the cytoplasm enables one to distinguish it unmistakably from the cytoplasm of the epithelial cells. On account of their power of undergoing amoeboid

movement they take on various forms. Some are round with a layer of cytoplasm of uniform thickness around the nucleus. Some are irregularly oblong with the bulk of the cytoplasm massed at one or both poles of the nucleus. Others are very irregular in outline, sending out one or more pseudopods. The size of the large lymphocytes varies somewhat but all possess a large amount of cytoplasm. In the smaller of the large lymphocytes the nucleus is proportionally smaller than in the larger ones.

The nuclei of the lymphocytes are large, but on the whole a little smaller than the epithelial nuclei, i.e., the nuclei of the large lymphocytes are smaller than the large epithelial nuclei while the nuclei of the smaller lymphocytes are smaller than the small epithelial nuclei. A distinct nuclear membrane is present. The form of the nuclei may vary considerably. Some are spherical, some oval in outline, while others have a very irregular shape. The varied forms of nuclei are undoubtedly brought about through the motile activity of the lymphocytes. The chromatin is generally distributed in the form of larger or smaller irregular granules some of which are attached to the nuclear membrane. These are often united with each other by fine irregular chromatin threads. The nucleoli are large, and usually very irregular in outline, having an extremely jagged surface. On the whole the nucleus of a large lymphocyte contains a more generous supply of chromatin than does an epithelial nucleus. The amount of chromatin present and the manner of its distribution in the two kinds of nuclei is, however, not the main feature by which one can readily distinguish a large lymphocyte from an epithelial nucleus. The basophilic character of the cytoplasm of the lymphocytes is the main distinguishing feature between them. At no stage in the histogenesis of the thymus is the value of a differential stain more appreciable than at the stage marking the appearance of the first lymphocytes, for, by the use of it, a lymphocyte can be distinguished unmistakably from an epithelial cell or nucleus.

The most critical developmental stages in which the source of the lymphocytes in the thymus is to be determined, are those in which only a few lymphocytes are found. The evidence indi-

cating a transformation of epithelial cells into lymphocytes, and the evidence indicating an infiltration of the epithelial thymus by lymphocytes from the mesenchyme surrounding it or from the blood, were carefully followed out. The transformation theory will be first considered.

In describing the structure of the thymus of a 23 mm. embryo it was stated that not all of the epithelial nuclei stain with the same intensity. In the enlarged portions of the thymus—superficial thymus, thymus head and mid-cervical and thoracic segments—are found normally shaped nuclei (spherical and ellipsoidal forms; fig. 1, *D.e.N.*), of both the larger and smaller types, the nucleoplasm of which stains much more intensely than it does in the great majority of epithelial nuclei present. The nucleoplasm, however, does not stain with the same degree of intensity in all of the darkly stained nuclei for, in some the deeply stained nucleoplasm almost completely masks the chromatin fibrils and granules while in others the chromatin is seen some what more clearly. Thus the transition forms, observed by Bell, occur between the more usual clear type of nuclei (which I regard as the normal nuclei) and the more deeply stained ones. In the stage (30 mm.) being described they are less numerous in the intermediary and cervico-thoracic cords than in the enlarged segments of the thymus. In a single section through the thoracic segment nineteen were found. In a 23 mm. embryo only an occasionally one was found in the intermediary and cervico-thoracic cords, while in a section through the superficial thymus ten were counted, thus indicating that they appear first in those portions of the organ that develop most rapidly. In a 17 mm. embryo none of the intensely deeply stained nuclei were present although a few were found that were stained somewhat more deeply than the great majority of normal nuclei present.

A consideration of these darkly stained nuclei is of the greatest importance, for Prenant ('94), Bell ('06), and others apparently have taken these cells to be the forerunners of the first formed lymphocytes in the thymus. A close examination of them, therefore, is necessary in order to reveal their fate. Be-

sides the normal shaped dark nuclei others are found that are very irregular in outline and greatly distorted. Varying degrees of deformed nuclei can be found between the more deeply stained normally shaped types and the greatly distorted ones. Others are again found that are, without doubt, undergoing degeneration. Figures 2 and 5 represent each a small portion of a section through the thymus head of a 37 and 36 mm. embryo respectively and were drawn specially to show the degenerating cells.[3] In some of the degenerated epithelial nuclei (*D.e.N'.*) represented in figure 5 the chromatin is massed together in one, two or three clumps that stain intensely deep blue or blue-black. These chromatin masses usually lie in clear spaces around some of which the distorted nuclear membrane apparently still persists. Occasionally a slightly elongated nucleus can be found in one end of which is a clump of chromatin lying in a nuclear vacuole while in the opposite end the nuclear threads and the nucleoplasm are stained as deeply as that in the nuclei described above. Again, here and there in the cytoplasm of epithelial cells may be found larger and smaller deeply stained particles which are evidently the débris of degenerated epithelial nuclei (fig. 5, *D.d.e.N.*). To the succession of microscopic pictures—deeply stained and normal shaped nuclei, deeply stained and distorted nuclei, and nuclei that have fallen to pieces—described above and drawn in figures 1, 2, and 5, only one interpretation, it seems to me, can be given, namely, that the deeply stained normal shaped epithelial nuclei are on their way to degeneration and do not transform into lymphocytes. Some of the epithelial cells show signs of degeneration in comparatively early stages as stated above. They are, however, most numerous and most pronounced in stages from about 30 mm. to about 45 mm. in length. They are also found in later stages and will be referred to again.

With a proper fixer, a suitable stain, and a high magnification, such as was used for this work, the plump lymphocytes stand

[3] Judging from the greater number of lymphocytes present and the great number of completely degenerated epithelial cells, the thymus of the 36 mm. embryo is slightly farther advanced in its development than that of the 37 mm. stage.

out in sharp contrast to the dark epithelial nuclei. Now, what structural features of the dark epithelial nuclei give them a semblance to small lymphocytes? As the amount of cytoplasm around the nucleus of a small lymphocyte is meager and often difficult to demonstrate, a comparison between the two must be confined largely to the structure of the nucleus. Prenant observed that the nucleoplasm of the small dark epithelial nuclei stained so intensely as to mask its internal structure. Bell makes no mention of the affinity of the nucleoplasm for basic stains. They apparently contain no greater amount of chromatin than do the normal epithelial nuclei. Their diffused dark color is due to the affinity of the nucleoplasm for a basic stain. This is not the case of the nuclei of the small lymphocytes. Their dark color is due to the deeply stained chromatin granules which, in proportion to the size of the cells, is much greater in amount than that found in the epithelial nuclei. The nucleoplasm of the small lymphocytes, which is meager in amount, but with a high magnification easily demonstrable, is quite as clear as that ordinarily found in nuclei, for example in normal epithelial nuclei of the thymus anlage. This fact alone, namely, clear nucleoplasm and an abundance of chromatin in the nucleus of a small lymphocyte in contrast to the deeply stained nucleoplasm and a smaller amount of chromatin in the small dark epithelial cells, is sufficient to cause one immediately to doubt the identity of the two kinds of cells. Prenant writes of degenerated epithelial nuclei in the thymus of a 28 mm. and later stages of sheep embryos but apparently saw no connection between them and the darkly stained nuclei. Bell does not mention degenerating nuclei.

Most of the investigators who have made a detailed study of the histogenesis of the thymus and who adhere to the transformation theory of the formation of the lymphocytes lay a great deal of stress on the vacuolation of the cytoplasmic syncytium as a factor in the histogenesis of the small lymphocytes. This is particularly true of Prenant and Bell. The latter calls the small dark epithelial nuclei, while still imbedded in the cytoplasm of the epithelial cells, lymphoblasts. During the

process of vacuolation some of the lymphoblasts become free and lie in vacuoles. They then are called lymphocytes. My observation on the histogenesis of the thymus in the numerous pig embryos examined warrants no distinction in the nomenclature between the two. Referring again to figures 1, 2 and 5, it will be seen that some of the small dark epithelial nuclei lie entirely in the cytoplasm and some in contact with vacuoles. Occasionally one can be found that lies free in a vacuole (none of the latter happened to be present in the portions of the sections from which the figures were drawn). The structure of the nucleus is the same whether they lie in the vacuoles or in the cytoplasm. This also is true of the large lymphocytes (*L.L.*). Some are entirely imbedded in the cytoplasm of the epithelial cells while others are in contact with or entirely in the vacuoles. The structure of all is the same and there is no reason why they should not all bear the same name. The significance of the vacuoles and their mode of formation in the thymus is unknown to me. They are already present in embryos of 17 mm. in length, and slightly increased in size and number in later stages (30 to 40 mm.).

In considering the genetic relationship of the small dark epithelial nuclei, the 'lymphoblasts' of Bell, to the large dark epithelial nuclei, Bell expresses a doubt by saying that "the large dark nuclei probably divide by mitosis and form the lymphoblasts." I was unable to find any of the dark epithelial nuclei in mitosis although I earnestly searched for them. To me it appears that the small and large dark nuclei are derived respectively from small and large normal epithelial cells, their degree of darkness in stained preparations depending on the extent of degeneration. Also it cannot be that the small dark nuclei are formed through a contraction of the large ones for the structure of both is the same. Prenant, in the developing thymus of the sheep, described and figured direct cell division. I was unable to find amitotic cell division in the thymus of the pig.

The true source of the lymphocytes first present in the thymus will now be considered. Reference has already been made to the presence of lymphocytes in the thymus anlage of a 30 mm.

embryo. Some are represented in figure 1 (*L.L.*). In slightly later stages they have become more numerous as represented in figures 2 and 5 (*L.L.*). All the lymphocytes present in the thymus of these early stages are large lymphocytes. No small lymphocytes are present. No transition forms from the normal epithelial nuclei to the large plump lymphocytes with a generous amount of basophilic cytoplasm can be seen. When first present in the thymus they are there in a fully developed condition. It is, therefore, evident that their source must not be sought in the thymus anlage. It was stated above that large lymphocytes were present here and there in the mesenchyme of a 17 mm. embryo. In successively older stages their numbers gradually increase until in stages ranging in length from 25 to 30 mm. they can be found in all parts of the mesenchyme without much searching. In these later stages, however, they are most numerous in the neighborhood of the thymus and the large blood vessels in the anterior portion of the thorax. Figure 1 represents a portion of a lobule of the thymus head and surrounding mesenchyme of a 30 mm. embryo. Three lymphocytes (*L.L.*) can be seen in the mesenchyme, two of which have a structure identical to those in the thymus. In one (lower corner to the right) the cytoplasm has a distinctly lighter hue, i.e., less basophilic, than the other two. Only very seldom can this latter type be found in the thymus anlage (fig. 2, *L.L.*; lower border to the left). These will be considered farther on in the paper. One of the lymphocytes (fig. 1) is in contact with the surface of the thymus. The microscopic picture, which is reproduced in the figure, is suggestive. Since lymphocytes are found in the mesenchyme in the neighborhood of the thymus before they are found in it, and since there are no transition forms between epithelial cells and lymphocytes nor any blood vessels in the thymus anlage, only one conclusion can be drawn in regard to the source of the lymphocytes first present in the thymus, namely, that they have migrated into the thymus from the surrounding mesenchyme. The lymphocyte bordering on the surface of the thymus was apparently about to enter it when the material was fixed. Many similar conditions exist, indicating

the entrance of lymphocytes into the thymus at the time of the fixation of the material.

Usually there are no indications on the surface of the lobules to mark the place where lymphocytes have entered it. On account of the plasticity of the cytoplasmic syncytium we can assume that the gaps formed in it by the entrance of the lymphocytes immediately close up. Not infrequently, however, places can be found where the surface of a lobule is dented in and a lymphocyte located in the thymus near the depression (fig. 2, *L.L.*, lower border to the left). Also occasionally a lymphocyte can be found in a lobule some distance away from the periphery with a trail (fig. 2, *T.*) leading to the surface of the lobule. This trail apparently marks the path that a very active lymphocyte took in its migration from its place of entrance to the position it now occupies. To similar microscopic pictures as represented above, Maximow has given a like interpretation. The first lymphocyte present in the thymus then must come from the mesenchyme and not from transformed epithelial nuclei.

Another type of cells which are comparatively few in number and found only in the earlier developmental stages deserves mention before passing on to a later developmental stage. These cells (fig. 1, *X.*) are characterized by rather deeply stained nuclei which resemble closely the degenerating epithelial nuclei discussed above. The cell wall, if present, is indistinct. Their cytoplasm can be distinguished from the cytoplasm of the epithelial cells only by its darker color. The majority of the cells are long and drawn out and usually lie near the surface of the thymus anlage with the long axis of the cell nearly perpendicular to the surface. These were most numerous in the 17 mm. stage and entirely absent from the 40 mm. and later developmental stages. This type of cells was also observed by Maximow ('09 b) who derived them from epithelial cells which for a time assume such form then revert to the usual type of epithelial cells. Their origin and significance are unknown to me.

Embryo 42 mm. (fig. 3). The lobes of the superficial thymus, the thymus head, and of the mid-cervical and thoracic segments have greatly increased in size. A few lymphocytes are now

present in the intermediary and cervico-thoracic cords. No blood vessels are present in the thymus. The walls of the blood vessels of the interlobular connective tissue septa are made up of endothelium only. The mesenchyme around the superficial and head thymus and the thoracic segment is much looser in its structure than in the corresponding regions in previous stages. Around the intermediary and cervico-thoracic cords and the mid-cervical segment it has a somewhat denser structure than around the above named regions of this stage.

As in previous stages completely degenerated epithelial nuclei (fig. 3, *D.e.N'.*) are present. Epithelial nuclei (*D.e.N.*) in the first stages of degeneration are also present but they are not as numerous as in the 36 mm. embryo. Mitoses of epithelial nuclei (*M.e.N.*) are quite numerous. The vacuoles are not as numerous as in stages ranging from 25 to 37 mm. in length. The most striking feature of this stage, however, is the large number of lymphocytes that are present in the thymus. They no longer all belong to the type of large lymphocytes but now and then a small lymphocyte (*S.L.*) is found. These are characterized by a rather small nucleus which is richly laden with chromatin and surrounded by only a very thin layer of cytoplasm which is often difficult to demonstrate. Intermediate stages between the large and small lymphocytes make up a relatively large proportion of all present. Some in mitotic division can be found without much searching. Mitosis of epithelial nuclei and large lymphocytes can be distinguished from each other without much difficulty. The chromosomes of the lymphocytes are shorter, somewhat thicker, and more closely packed together than those of epithelial cells. The basophilic cytoplasm of the lymphocytes also is sharply outlined in contrast to the cytoplasm of epithelial syncytium. The absence of blood vessels in the thymus at this stage, the absence of transition forms between epithelial cells and lymphocytes, the unbroken series of intermediate stages between the large and small lymphocytes, and the frequent mitoses found among them all, are evidences that undoubtedly point to the conclusion that the large lymphocytes through repeated

division give rise to the small lymphocytes. This view of the origin of the small lymphocytes in the thymus is in accord with that of Hammer for teleosts ('08) and with that of Maximow for mammals ('09 b).

The mesenchyme of the interlobular septa (*S.i.*) in the head and thoracic segments contains a large number of large and intermediate sized lymphocytes. A few small lymphocytes are also present. The mesenchyme surrounding the above named regions also contains a relatively greater number of lymphocytes than it does in corresponding regions of the previous stage described.

An occasional nucleated red blood-cell can be found lying free in the thymus. At this stage they are scarcely more numerous than in the previous stage. None were present in that part of the section from which the figure was drawn. Eosinophile cells also can be found occasionally in any part of the mesenchyme. They were first found in embryos 35 mm. in length. None were seen in the thymus.

Embryo of 65 mm. (*fig. 4*). The lobules are more numerous than in the previous stage along the entire extent of the organ. Those of the enlarged regions of the thymus are much more voluminous than those of the intermediary and cervico-thoracic cords. An almost interrupted layer of greatly attenuated mesenchymal cells closely invests the outer surface of the lobes and apparently forms a limiting membrane (fig. 3, *L.M.*) for the outer surface of the thymus. This membrane is present in slightly earlier stages. Blood vessels (*Bl.V.*) are numerous in the interlobular septa. No thick walled vessels are yet present. The walls of most of them are made up of endothelium only. A few small blood vessels of an essentially capillary nature can now be found in the center as well as in the periphery of the thymic lobules.

The thymus now contains many lymphocytes. The small lymphocytes (*S.L.*) are more numerous than in the previous stage. The medium-sized lymphocytes have also greatly increased in number while the number of large lymphocytes has remained about the same. Mitoses of both the lymphocytes (*M.L.*) and the epithelial nuclei (*M.c.N.*) are of frequent occur-

rence. Only an occasional deeply stained epithelial cell can be found. Completely degenerated epithelial nuclei can be seen scattered here and there throughout an entire section. The connective tissue of the interlobular septa now contains numerous lymphocytes of all sizes. The deep portions of some of the septa are so completely gorged with them that it is difficult to distinguish clearly where the septa end. An especially favorable place for lymphocytes to collect seems to be along the course of blood vessels of the septa. They can be found strung along in rows on one side of the vessels or the accumulation may extend entirely around it. The vacuoles of the syncytium are not as numerous as in the preceding stage. Most of them have become occupied with lymphocytes. In later stages they are altogether absent.

An almost uninterrupted zone of epithelial syncytium (*Z.pr.*) extends around the periphery of the thymus. It is from one to three epithelial nuclei deep and, on account of the few lymphocytes which it contains, appears quite clear in contrast to the deeper portion of the syncytium in which are found many lymphocytes. It is not pronounced along the interlobular septa. Mitoses of the epithelial nuclei are more numerous in this zone than they are in the deeper portions of the lobules, hence, Prenant called it the zone of proliferation. According to him both lymphocytes and reticulum cells are formed from this zone. This, however, cannot be the case for the transition forms from epithelial nuclei to lymphocytes are not present. Considering the fact that the epithelial zone is most pronounced only on the convex peripheral surface of the lobules, and that it is present only during the period of rapid growth of the thymus, it can rightly be regarded as a zone of proliferation for epithelial cells but not for lymphocytes. It is mainly from this zone that the reticulum of the peripheral margin of the cortex is formed while the thymus is rapidly growing in thickness. This interpretation of the significance of this zone is in accord with that of Maximow.

This develomental stage marks the appearance of the medulla. Longitudinal sections through the thymus head show that the epithelial syncytium of almost the entire central stem has under-

gone changes. The medulla of the lobes, in some of which at this stage it is but slightly developed, is continuous with that of the central stem. The deep portions of some of the interlobular septa are almost in contact with it while others are separated from it by a cortical layer of considerable thickness. The medulla is formed directly from the epithelial syncytium. In sections stained with Hasting's Nocht's stain it is easily distinguished from the cytoplasmic syncytium of the cortex by its brighter red color. The initiative changes marking its appearance are apparently chemical in their nature as pointed out by Bell, for the syncytium in some parts of the central stem and in the center of some of the lobules is stained a bright red even before any morphological changes have set in. The morphological changes of the epithelial structure occur very soon after, or almost simultaneously with, the chemical changes. The epithelial cells hypertrophy. The nuclei become large and relatively clear when compared with those of the cortex. The cytoplasm of the syncytium also increases in amount. Its anastomosing processes are no longer thin and attenuated as they now appear in the cortex but have become more or less massive bands. Although the cortex and medulla are quite sharply defined the cytoplasmic processes of the epithelial cells lying along the line of demarcation between these two structures are continuous with each other.

Soon after the medulla has started to develop some of the epithelial nuclei contained in it greatly increase in size, growing much larger than the majority of hypertrophied nuclei. These may be found singly or in groups of two or three and mark the beginning of Hassall's corpuscles.

All the different types of lymphocytes (large, medium-sized, and small) found in the cortex are also found scattered in the meshes of the reticulum of the medulla where they are, however, much less numerous than in the former place. According to Maximow ('09 b) the disappearance of the lymphocytes from the medulla, when it is first formed, is due to their migration into the cortex and to degeneration. His interpretation does not seem to explain similar conditions existing in the thymus of the

pig, for only very seldom can degenerated cells be found which may be degenerated epithelial nuclei, and as to whether they migrate into the cortex it is indeed difficult to establish in fixed material when no circumstantial evidence is present indicative of their migration. A more plausible interpretation seems to be that during the hypertrophy of the epithelial cells the medullary portion of the thymus greatly increases in volume through the enlarging of both the nuclei and anastomosing processes of the syncytium thus separating the lymphocytes farther apart. Just as many are present in the rapidly newly formed medulla as there were in the syncytium from which the medulla was formed only they are scattered over a larger area making them to appear less numerous. This interpretation is made plausible when the great rapidity of its initial development is considered, e.g., in the thymus of a 60 mm. embryo no traces of the medulla were present while in a 65 mm. embryo it has reached a stage of development as described above.

The reticulum of the cortex also is formed from the cytoplasmic syncytium of the epithelial cells. Its development, unlike that of the medulla, is gradual. The change from the rather coarse syncytial network of younger stages to fine and greatly attentuated threads making up the reticulum in the fully developed thymus is due to the lymphocytes constantly increasing in numbers in its meshes thus gradually separating the cell bodies of the reticulum farther apart. In all of the developmental stages studied mitosis of the epithelial nuclei could be found, being, however, more numerous in younger than in later developmental stages.

In this and slightly earlier stages (55 mm.) nucleated and non-nucleated red blood-cells lying free in the parenchyma of the thymus are of frequent occurrence. While some are scattered about singly they usually occur in groups. An occasional eosinophile cell can also be found. In the interlobular septa phagocytes can be found without much searching.

The thymus head and superficial thymus were so oriented on the microtome that sections of both of these regions were made by a single stroke of the knife. This made a comparison

of their histological structure easy as they lay side by side on the slide. No difference in structure could be distinguished between the two, thus indicating that the histogenetic processes of that portion of the thymus derived from the ectoderm keep pace with that portion derived from the entoderm.

Embryos 85, 100, 125, 165, and 180 mm. in length. In these developmental stages all the structures found in the fully developed thymus are laid down and will, therefore, be considered only briefly. The average size of the lobules and the thymus as a whole increases in the successively older stages. In the first four stages the thymic septa have still a very loose structure while in the 180 mm. embryo they are quite narrow and correspondingly denser. Many of the septa are broadly expanded where the larger interlobular blood vessels are harbored. The thymic septa of the 85 and 100 mm. stages are characterized by the large number of all types of lymphocytes (large, medium-sized, and small) which they contain. The presence of so many lymphocytes in the septa is a feature that is most marked in developmental stages from 65 to about 115 mm. in length. The septa of the 125 and 165 mm. stages contain many lymphocytes but on the whole they are less numerous than in the earlier stages cited. In the 180 mm. embryo the lymphocytes are mostly confined to the deep expanded portions of the septa which are often gorged with them. The number of lymphocytes in the connective tissue immediately surrounding the thymus is relatively small when compared with the number present in the septa. Mitoses of all types of lymphocytes are of frequent occurrence.

A marked feature of the cortex in these stages is the large number of small lymphocytes which it contains. Excepting the 85 mm. stage they make up the largest proportion of all the lymphocytes present. Mitoses of all types of lymphocytes are of frequent occurrence while mitoses of epithelial nuclei can occasionally be found. The epithelial (reticulum) nuclei are, in general, smaller than those found in younger stages but their structure has remained unchanged. The reticulum composing the strands are greatly attenuated and only in very thin sections can it be satisfactorily demonstrated. Its meshes are filled with lymphocytes. Vacuoles are no longer present.

The clear epithelial zone around the periphery of the thymus is present in all the stages excepting the 180 mm. embryo. Around the thymus head of the 165 mm. embryo it is at its highest development. This is contradictory to the observations of Bell who states that this zone has disappeared in a 140 mm. embryo. Mitoses of epithelial cells in this zone are quite numerous and in no stage is it entirely free from lymphocytes. The limiting membrane could no longer be distinguished around the thymic lobules in the 180 mm. stage. It apparently has become blended with the thin capsule that invests the thymus of this and later stages.

In all these stages the medulla contains a relatively much larger number of lymphocytes than in the 65 mm. embryo, which makes it appear less conspicuous. This is especially the case in the 180 mm. embryo, but even in that stage in suitably stained preparations it is still quite sharply defined from the cortex. Mitoses of all types of lymphocytes occur here as in the cortex. In the 180 mm. embryo Hassall's corpuscles are more numerous than in the earlier stages, while some are still in the process of formation. The reticulum on the whole is much coarser than in the cortex and hence more easily demonstrable.

In the 180 mm. embryo deeply stained (degenerating) epithelial nuclei can be found only after prolonged searching while in the earlier stages they are of more frequent occurrence in both the cortex and medulla. Débris of degenerated cells, some of which in these stages is undoubtedly composed of nuclei extruded from normoblasts, also occurs.

A discussion of the red blood-cells and granular leucocytes will be considered later.

Embryo 270 mm. (full term). Since one of the main objects of the investigation of the developing thymus was to determine the origin and fate of the superficial thymus its histological structure will, therefore, be considered. The lobules are now closely packed together. The cortex has greatly increased in thickness over that of the 180 mm. embryo. The lymphocytes are no more closely packed together than in the previous stage, room having been made for the additional number by an increase in the volume of the organ. While the small lymphocytes are

by far the most numerous, large ones are still plentiful in all parts of the cortex. Even with a magnification of 1300 diameters eleven were counted in a single microscopic field. All gradations between the large and small ones are present. Mitoses of all the different types occur.

The medulla is still quite sharply defined from the cortex. It contains less lymphocytes than the cortex. In some places where the medulla of the lobules joins with that of the central stem it comes in contact with the deep portion of the interlobular septa. It contains all the different types of lymphocytes that are present in the cortex and mitoses among them can be demonstrated without much difficulty. Hassall's corpuscles are more numerous than in the 180 mm. stage and an occasional one can still be found in the process of formation.

In the medulla the strands of the reticulum are often wavy and in general are much coarser than those of the cortex. Also the epithelial nuclei are on the whole larger and clearer, and surrounded by a more generous amount of cytoplasm than those in the cortex. Mitoses of epithelial nuclei in both the cortex and medulla can only very seldom be found. In sections treated with Mallory's connective tissue stain fibrillae can be seen to come off from the interlobular septa and the capsule and extend a distance of from one to four cells deep into the cortex. In both the cortex and medulla the same condition prevails between the adventitia of the larger blood vessels (which are very few) and the reticulum. I was unable to determine whether the connective tissue fibers fuse with the reticulum. This intimate relation of the connective tissue of the septa and of the large blood vessels to the reticulum was observed by Mietens ('08) but denied by Maximow ('09 b). No connective tissue fibers aside from those mentioned above could be demonstrated in either the cortex or medulla. Bell, however, by using Jackson's modification of Mallory's stain states that he was able to demonstrate them thinly scattered through both the cortex and medulla in all late developmental stages.

The interlobular connective tissue septa are greatly reduced in thickness, being widest in those places which lodge the larger

blood vessels and at the points of intersection of two or more septa. In some places prolongations of the septa dip down into the cortex of the lobules. These secondary septa approach very nearly the medulla but seldom enter it and are usually expanded at their deeper ends where they may lodge larger blood vessels. The structure of the wider portions of the septa is usually looser than the thinner parts. Small blood vessels of a capillary nature are found through the septa and can often be seen entering the cortex. Lymphocytes are present only in comparatively small numbers. In the more compact portions of the septa they may be entirely absent.

A discussion of the red blood-cells and the granular leucocytes in full term embryos will follow.

3. The epoch of the formation of the red blood-cells and the development of granular leucocytes

Investigators disagree as to the extent of the formation of red blood-cells in the thymus. Many have observed red blood-cells lying free in the parenchyma of the thymus during both its growth and involution but to my knowledge no extended investigation through a wide range of developmental stages has yet been made of their origin. Afanassiew ('77) apparently was the first to consider their origin. He held that during the development of the thymus a rearrangement of some of the blood vessels took place resulting in the formation of the concentric (Hassall's) corpuscles. During this process some of the blood vessels are ruptured thus permitting erythrocytes as well as leucocytes to escape into the parenchyma where they then may be found singly or in groups. In mammals the red blood-cells usually underwent degeneration. He regarded the thymus a hemolytic organ.

Watney ('82) also observed erythrocytes, 'hemoglobin masses,' and cells containing fragments of hemoglobin in their cytoplasm in the thymus of mammals, birds, reptiles, and fishes. He does not state whether the erythrocytes and hemoglobin masses are derived from cells in the parenchyma or whether they have

passed into it from blood vessels but regards the thymus as a source of some of the "colored blood corpuscles."

Prymac ('02) holds that during involution of the teleostean thymus numerous red blood cells are formed from the small round cells. Erthrocytes also escape into the parenchyma from the blood vessels. All undergo degeneration. The products of degeneration of the greater number of red cells are granules which are taken up by indifferent thymus elements, while that of the smaller portion is in the form of pigment which accumulates in masses in the parenchyma.

Schaffer ('93) in the thymus of the rabbit and cat found red blood-cells in various stages of development, and transition forms between the leucocytes and nucleated red blood-cells. He believes that the thymus has a hematopoietic function.

Bell ('06) in the thymus of a 240 mm. pig found numerous erythrocytes, lying singly and in groups, in the cortex while free erythrocytes were rarely to be found in the medulla. He does not consider their origin.

Maximow ('09) was not able to recognize definite erythroblasts or transition forms in the parenchyma of the thymus but thinks that lymphocytes have been confused with them. In the interlobular septa, however, he found collections of erythroblasts and normoblasts, or briefly, all transition forms from large lymphocytes to erythrocytes. He does not believe that red blood-cells are formed in the thymus.

Other investigators have observed free erythrocytes in the thymus in various stages of its development. Some make no mention of their origin while others suggest a possible origin but do not trace out their cytomorphosis.

Granular cells have been observed in the thymus by many investigators but the views regarding their origin and nature, which will be only briefly summarized, are conflicting. During the latter part of fetal life and the remaining period of growth and involution, Watney ('82) found in the medulla of the thymus of birds, reptiles, and mammals many granular cells. He divided them into four classes which were connected with each other by intermediate forms. He found them especially numerous along

the course of blood vessels to the outer tunic of which many were attached. He derived them all from connective tissue cells.

Schaffer ('91) apparently was the first investigator to observe eosinophile cells in the thymus. In various developmental stages of the human thymus he found large numbers of eosinophile cells in the connective tissue surrounding the thymus, in the interlobular septa, and along the course of some of the capillaries in the medulla. A few also were found in the cortex. The size of the granules vary and the nucleus he described as being round. His investigations of the eosinophile cells in the thymus were then too incomplete to say anything definitely regarding their origin but he believed that they were not identical with the 'granular cells' of Watney. In the medulla of the involuting thymus of the mouse he ('09) found many eosinophile cells and numerous free granules that stained intensely with eosin. These granules he regarded as products of degenerated epithelial cells. Within the lobules and in the interlobular septa of the involuting thymus many plasma cells were also present. These he derived from the small lymphocytes of the thymus. Many had the appearance of undergoing degeneration.

Goodall ('05) is of the opinion that the pseudo-eosinophile cells in the region of Hassall's corpuscles in the thymus of the guinea-pig, are derived from the blood.

Maximow ('09) claims that different types of granular cells were found in the thymus in different species of mammals. In the more advanced stages of rabbit embryos an appreciable number of pseudo-eosinophile myelocytes were found in the interlobular septa, cortex and medulla. Only a few mast cells were found, most of which were in the septa. In guinea-pig embryos psuedo-eosinophile myelocytes were seldom found. In cat embryos 35 to 50 mm. in length special myelocytes and leucocytes were found in quite large numbers, while in embryos 120 to 130 mm. long numerous mast cells were found in the deeper portions of the cortex and in the medulla. In the septa they were less numerous. Only an occasional eosinophile cell was

found in the interlobular septa of the thymus in the above named animals. A few were found in the parenchyma of the thymus in a rat embryo 19 mm. in length. The different types of cells named above are derived from lymphocytes and the granules in all of them are products of the cell in which they are contained.

In the description of the later developmental stages of the thymus mention was made of free nucleated and non-nucleated red blood-cells and eosinophile cells in both the parenchyma and interlobular septa of the thymus. Through the investigations of Maximow ('09 a), and others, who have traced the development of the blood from early developmental stages in which the cells of the blood islands were still undifferentiated to later stages in which all the different types of blood-cells were found in the circulating blood, the view of a common ancestor for all the different types of blood-cells has been quite generally accepted. This primitive or undifferentiated blood-cell is structurally very much like a large lymphocyte and by some regarded identical with it. Also, Maximow and others have shown that erythrocytes, and granular cells develop from mesenchymal cells of the intra-embryonic mesenchyme.[4]

Since erythrocytes were already present in the blood streams of the youngest embryos collected for this work, the blood islands and other hematopoietic regions were not investigated. In the mesenchyme, however, the development of the free erythrocytes and granular cells was traced apparently from their source. A consideration, therefore, of the source of the above named cells found in the interlobular septa (mesenchyme) of the thymus will be made first, for a knowledge of their origin will aid in determining the origin of free erythrocytes and granular cells

[4] For a detailed account of the development of free blood-cells in the mesenchyme, reference should be made to Maximow's work of 1906. With the methods of technic used for this work I was able to confirm most of his conclusions regarding the origin of mesenchymal blood-cells. Hence, I have adopted tentatively the nomenclature employed by him. A detailed account of my observations would unnecessarily lengthen this article. The descriptions and drawings, therefore, will be only sufficiently detailed to be within the limits of clearness and accuracy. The primitive blood-cells or 'Wanderzellen' have been termed 'large lymphocytes' throughout this work.

in the parenchyma of the thymus which, to anticipate, develop from the same type of undifferentiated cells as those in the mesenchyme.

In early stages the development of the free blood-cells can be demonstrated in most any portion of the mesenchyme in the neck and upper thoracic region of young embryos. There are, however, localized regions where this process is carried on even in later developmental stages. The interlobular septa of the thymus are particularly favorable places to study the development of blood-cells in well advanced embryos. The thymus of an embryo 125 mm. in length was selected for the cytomorphosis of the erythrocytes, eosinophile cells, and phagocytes, although somewhat later stages could have been used. The connective tissue of the septa is loosely arranged and contains many transitional forms leading from the connective tissue cells to the above named elements. A few stellate-shaped connective tissue cells are still found but the spindle shaped type is most numerous. In figure 7 is a series of diagrams representing a suggested 'cell lineage' between connective tissue cells and their derivatives, i.e., erythrocytes (blood-plastids of Minot), phagocytes, and granular cells. Diagram *a* represents a connective tissue cell. The cytoplasm is finely granular and is only very slightly basophilic. In some small vacuoles occur. No cell membrane could be demonstrated. The nucleus may be round or oval and has a distinct nuclear membrane. The chromatin is in the form of small irregularly shaped granules many of which are joined together by very fine chromatin threads. The nuclei vary in number from one to three. Diagram *c* represents a transformed mesenchymal cell. Its protoplasmic processes are retracted and now lie free in the septa. The cytoplasm has slightly but appreciably increased in amount and has become more basophilic and now is homogeneous. The nuclear changes are represented apparently by a slight massing of the chromatin, the granules becoming slightly coarser and less numerous. In some connective tissue cells the cytoplasm becomes more basophilic and the nuclear changes occur even before its processes have been retracted (diagram *b*). This type of connective

tissue cells is interesting in that its transformation to the free cell can be easily followed. Now and then a transformed mesenchymal cell, *d*, can be found, the cytoplasm of which is quite pale, being no more or only slightly more basophilic than that in the ordinary connective tissue cells. Its cytoplasm, however, is homogeneous and its nuclear structure similar to that of ordinary large lymphocytes. This type of cells evidently has the power to wander about in the mesenchyme for occasionally (very seldom) can one be seen in the epithelial anlage of the thymus (fig 2, lower border to the left). These were observed by Maximow who claims that their cytoplasm soon turns basophilic after they are formed. Judging from their structure and the small number present his interpretation is correct and they must therefore be considered as belonging to the same type of cells as those in which the cytoplasm is more basophilic. In some transforming mesenchymal cells, *c*, the cytoplasm becomes basophilic and the chromatin increases in amount while the protoplasmic processes are being retracted, i.e., the cytoplasmic and nuclear changes take place simultaneously. This process results in the formation of a cell in which the cytoplasm is less basophilic and the nucleus contains less coarse granules than in a fully developed large lymphocyte. These cells (young lymphocytes) transform into the typical large lymphocytes as represented in diagram *e*. Since this type of cells is of more frequent occurrence than those represented in diagrams *b* and *d* it is assumed that this is the most usual manner by which a mesenchymal cell transforms into a lymphocyte. The type of cells under consideration and represented in diagrams *c*, *d* and *e* are the primitive mesamoeboids of Minot and the primary wandering cells of Maximow and others. With Maximow and others, I agree that they are essentially identical with the large lymphocytes which term was given them in the account of the histogenesis of the thymus in the early stages of its development.

The power of the lymphocytes to develop in different directions is clearly manifested in the interlobular septa of this stage in which many lymphocytes of all types are found. Judging

from the mitoses that some are undergoing, the large lymphocytes through repeated divisions become smaller and form small lymphocytes, *f*. Whether or not the small lymphocytes have the power to grow and again form large lymphocytes, as claimed by some investigators, is difficult to demonstrate. The point of interest and importance is the development of erythrocytes and granular cells from the lymphocytes. In some of the transition stages between the large and small lymphocytes, or for convenience, the large and medium-sized lymphocytes, changes occur in both their nucleus and cytoplasm. The latter stains a faint brick-red indicating the presence of hemoglobin while the nucleus becomes granular. These are the megaloblasts of Maximow or erythroblasts, *g*. In some cells, *h*, the nucleus has the characteristic granular structure of the erythroblasts while the cytoplasm still retains its basophilic character, or is dimmed only slightly by a faint trace of hemoglobin. These are the younger forms of erythroblasts and aid in tracing the source of the older ones. They may be found lying singly but usually occur in groups. Mitoses of erythroblasts can be found without much searching. Diagram *i* represents a normoblast. The cells of this type are on the whole a little smaller than the erythroblasts from which they are derived. Through the extrusion of their nuclei they are transformed into erythrocytes, *j*. That the nuclei are extruded is indicated by deeply stained degenerating nuclei or fragments of them lying free among the cells in a group made up of a mixture of both erythrocytes and normoblasts. Thus the free erythrocytes of the interlobular septa, as stated by Maximow, are derived from the lymphocytes, their ultimate source being from transformed mesenchymal cells. Whether or not they enter the circulation will be considered presently. While they are found in the septa in quite early stages they are most numerous in this region in embryos ranging from 115 to 165 mm. in length, the greatest number being present at about the 125 mm. stage. The superficial and head thymus of a 270 mm. (full term) fetus contained a few, singly and in groups, in the deeper and looser portions of the septa.

With this brief review of the origin of the erythrocytes in the interlobular septa we are prepared to consider their source in the cortex and medulla of the thymus. In every stage from the 55 mm. to the full term embryo that was examined, red blood-cells were found singly and in groups in the thymus. In the developmental stages approaching maturity a larger number of the lobules contain groups of red cells than in younger stages. Some lobules contain two or three groups some of which are quite large. Also red cells lying singly in the thymus are more numerous in the later than in the earlier stages. The superficial thymus and the thymus head were found to contain a relatively larger number than the mid-cervical segment. Unfortunately, the thoracic segment of late developmental stages was not collected, so I was unable to make a comparison of their number with that of the other segments of the thymus. The superficial thymi and the thymus heads of two full term fetuses (270 and 280 mm in length) contained a comparatively larger number of red blood-cells than the corresponding segments of somewhat earlier stages. In the thymus of the 280 mm. fetus the red blood-cells were about equally distributed in the two segments while the red cells in the superficial thymus of the 270 mm. embryo were much more numerous than in the thymus head. In full term embryos groups of red blood-cells are found in both the cortex and medulla of the thymus. In the younger stages no groups of red blood-cells were found in the medulla although they may be found lying singly in that region.

An occasional nucleated red blood-cell can be found in the thymus of embryos 35 to 50 mm. in length. Erythrocytes in these stages are very seldom found. They do not come from the blood for blood capillaries have not yet penetrated the lobules at this stage. In an embryo 55 mm. in length, in which only a few capillaries are found in the lobules, they are much more numerous than in the preceding stages. Nucleated and non-nucleated red cells can be found singly among the lymphocytes which at this stage are already quite numerous but the striking feature is that they are present in groups (fig. 6). They vary somewhat in size as do those in the interlobular septa but the

majority in the thymus have a smaller average diameter than those in the latter place. Their contour is often very irregular which is due to their lying closely together when found in groups or wedged in between lymphocytes and epithelial cells when they occur singly. The nuclei of some have the characteristic coarsely granular structure of erythroblasts (*Erb.*) and young normoblasts, while in others the nuclei are pyknotic. The reddish hemoglobin-containing cytoplasm of the nucleated red cells varies in its amount in different cells but is easily recognized even with moderately high magnification in the larger and medium sized cells. Some of the nucleated red cells have two nucleoli (*A. Erb.*) which stain intensely, are round, and of equal size. These apparently are undergoing amitotic division. Erythrocytes (*Erc.*) are quite numerously scattered among the erythroblasts. Aside from the irregular outline of some of the nucleated red cells found in the thymus of this stage they compare favorably in all other respects with the free erythroblasts and normoblasts found in the septa. Also they have the same origin, namely, from the lymphocytes.

The lymphocytes in the thymus in which the origin of the red blood-cells was just considered belong almost entirely to the large and medium-sized type. Only a very few small ones are present. It is, therefore, necessary to consider the origin of the numerous free erythrocytes in the thymus of late developmental stages in which the large majority of all the lymphocytes belong to the small type. The superficial thymus of a 270 mm. (full term) fetus was selected for this purpose because the red blood-cells in the thymus of this stage are more numerous than in any other examined. The great majority lie in groups which, in a section, appear as smaller or larger bright red irregular patches or as long tortuous streamers. The proportion of lymphocytes to the red cells varies in different groups. In some the lymphocytes are most numerous, in others they are about equal in number, while in still others the red cells greatly predominate. Also, in some groups the erythrocytes make up nearly the entire number of red blood-cells, only a few nucleated red cells being present. In some groups many nucleated

red cells are found among the erythrocytes, while other groups are composed almost entirely of nucleated red cells. Both the erythrocytes and nucleated red blood-cells vary in size, but the small ones greatly predominate over the medium sized and larger ones. They are usually irregular in outline. The structure of the nuclei vary as those in the 55 mm. stage. An interesting and most helpful feature in tracing out the origin of erythrocytes in late developmental stages is the presence of lymphocytes with granular nuclei, the structure of which is the same as that of the erythroblasts and normoblasts found in the interlobular septa, the only difference being their smaller size. Only a few small lymphocytes with this type of nucleus were found in the thymus of the 55 mm. stage and in the interlobular septa of the 125 mm. embryo. They stain intensely and when examined with lenses of low magnification appear as black dots in contrast to the other small lymphocytes among which they lie. Maximow ('09 b) in writing of the erythropoetic function of the thymus, which he denies, makes mention of this type of lymphocytes but on the ground that they contained no hemoglobin he does not consider them to be normoblasts. It is true that in many lymphocytes with this type of nucleus, sometimes entire groups, no traces of hemoglobin can be detected in their cytoplasm even when highly magnified ($\times$ 2000). But, many small cells can also be found with similarly granular nuclei and with a distinct reddish tinge which indicates the presence of hemoglobin in their cytoplasm. These are small erythroblasts that are derived from small lymphocytes and the small cells referred to by Maximow, and so plentifully found in the thymus of this developmental stage, are transition forms between the ordinary small lymphocytes and the small erythroblasts. On account of the meagre amount of cytoplasm in these erythroblasts they appear much as if the nucleoplasm was stained slightly red. But that is not the case for the red stained cytoplasm, of those in the late normoblast stage in which the nucleus has become shrunken and pyknotic, stands out sharply, although it is small in amount. Transition forms are often scattered along the border of groups of red cells containing many

erythrocytes, and in groups of nucleated red cells they are almost invariably found scattered among the erythroblasts and normoblasts. Typical large erythroblasts and normoblasts such as occur in the thymus of the 55 mm. stage are also present in the thymus of late developmental stages; but in these stages they make up only a small proportion of the erythroblasts. This is accounted for by the fact that the small nucleated red cells are derived from the small lymphocytes while the large ones are derived from large and medium-sized lymphocytes which are comparatively few in number in late developmental stages.

The débris of degenerated nuclei extruded from the normoblasts can often be found scattered among erythrocytes. It is not uncommon for this débris to collect in heaps which appear in sections as deeply stained dark structureless patches in a group of erythrocytes. Why the degenerated nuclei have a tendency to flow together can only be conjectured; also why the red blood-cells are mostly formed in groups instead of uniformly throughout a lobule is unknown to me.

The blood stream of a 55 mm. embryo contains nucleated red blood-cells and since capillaries are already found in the thymus in this developmental stage it might still be argued, as is held by some, that the free erythrocytes in the thymus are derived from the blood. This, however, cannot be the case for in a full term fetus in which many erythrocytes and nucleated red blood-cells are found in the parenchyma of the thymus no nucleated red cells were found in the blood stream.

From the observations cited above I must conclude that the erythrocytes in the meshes of the reticulum of the thymus are derived from the lymphocytes. Furthermore, on this conclusion are hinged three important features two of which strongly reflect on the nature of the small round cells of the thymus, while one furnishes additional proof for the pseudomorphosis theory of the histogenesis of the thymus. They are: (1) Since both the lymphocytes of the mesenchyme and the small round cells of the thymus have the power to transform into erythrocytes they are potentially alike. The small thymic cells are, therefore, lymphocytes and not epithelial cells as held by Stöhr

('06); (2) The lymphocytes can be regarded as undifferentiated blood-cells and under certain conditions are, in some organs, potentially like the primitive blood-cells, and (3) The like potentiality of the lymphocytes in both the thymus and the mesenchyme is additional evidence that the lymphocytes first present in the thymus have migrated into it from the mesenchyme.

Whether or not any of the erythrocytes formed in the thymus or in the mesenchyme surrounding it enter the circulation is difficult to determine in fixed material. Some undoubtedly undergo degeneration. In the mesenchyme of early stages and in the interlobular septa of later developmental stages some erythrocytes are present the cytoplasm of which is granular instead of homogeneous. In some cells the granules are small, round, and of a quite uniform size while in others the granules vary greatly in size. Some are apparently about to break up into a small number of irregularly shaped fragments. I am confident that these erythrocytes are degenerating forms and are not artifacts, for in the same microscopic field may be found numerous other cellular elements (connective tissue cells, lymphocytes, nucleated and non-nucleated red cells) all of which have the appearance of a good preservation. Also not infrequently erythrocytes can be found that have completely fallen to pieces, the débris of degeneration being in the form of varying sized globules and irregularly shaped fragments, or irregular groups or long drawn out rows of small deeply red stained (eosinophile) granules. The latter may be derived directly from the disintegration of granular erythrocytes or from the further disintegration of large fragments of them. Some of the red cells undergo degeneration while still in the normoblast stage. These are characterized by a pyknotic nucleus and more or less granular cytoplasm. Except for their small size and the type of nucleus they contain, some could easily be mistaken for small eosinophile cells.

Degeneration of some of the free erythrocytes in the lobules of the thymus takes place in a manner similar to that described above. Groups of free eosinophile granules can be found in the thymus of all developmental stages in which red blood-cells

are formed in that organ. The free eosinophile granules are never very numerous but the thymus of late developmental stages in which the erythrocytes are comparatively numerous contains more than the thymus of early stages. Erythrocytes and normoblasts with granular (degenerating) cytoplasm are also present. The red blood-cells in the thymus usually have an irregular outline. This is not a sign of degeneration but is brought about by purely mechanical factors as stated above. In late developmental stages phagocytes ingested with erythrocytes and other degenerated products can occasionally be found in the lobules of the thymus. These, however, appear first and are more numerous in the interlobular septa. In some groups of red cells in the superficial thymus of the 270 mm. fetus some of the erythrocytes are apparently fused, forming as seen in section, irregularly and deeply red stained and quite homogeneous patches which contain only a few lymphocytes. Whether or not the fused erythrocytes undergo degeneration could not be determined with the material at hand. The thymus of post-natal pigs needs to be investigated to determine the fate of the numerous free erythrocytes present in that organ. It is evident, however, that not all, if any, enter the circulation.

In all the developmental stages examined eosinophile cells[5] are, in general, more numerous in the connective tissue of the interlobular septa than in the lobules of the thymus. A consideration first of their origin in the former place will, therefore, aid in determining their origin in the lobules. Eosinophile cells are already present in the mesenchyme of quite young stages (20 to 25 mm.). In all these and in somewhat older stages (55 mm.) they are not found in localized areas but may be found in almost any part of the connective tissue. In the 55 mm. embryo they are somewhat more numerous than in the younger stages but can be found only after considerable searching. From embryos more than 55 mm. in length only the

[5] The value of the distinction of eosinophile myelocytes (myeloid eosinophiles) and eosinophile leucocytes is not considered and cells containing eosinophile granules are therefore simply designated as eosinophile cells regardless of the shape of their nucleus.

thymus was removed. In late developmental stages, therefore, only the eosinophile cells in the interlobular septa will be considered. The septa of the thymus in embryos from 65 to 85 mm. in length contain only a few eosinophile cells. In some sections none were found. In the 100 mm. stage they can be found without much searching while in the 110 mm. stage a single group was found in the sections prepared from the thymus head while those lying singly are more numerous than in the previous stage. In a 125 mm. embryo groups of eosinophile cells are of frequent occurrence and lie usually along the course of blood vessels but some are also present in the deep looser portions of the septa. They are also found lying singly in the septa. The greatest numbers occur in stages 165 and 180 mm. long. In the former stage they were more numerous in the superficial thymus than in the thymus head or cervical segment, and on the whole more numerous than in the latter stage in which their distribution was about equal in the different segments examined. In the full term fetus (270 mm.) they are much less numerous in the septa than in the 180 mm. stage. In the last three stages many eosinophile cells are found lying singly in the deep looser portions of the septa but the large majority are found in groups which almost without exception are found in the immediate vicinity of the larger blood vessels where the structure of the septa is comparatively loose. Some groups extend entirely around blood vessels (fig. 8, *Eo.C.*) while others lie only to one side of them. In some groups the eosinophile cells lie closely together while in others they are more loosely arranged. Without exception a greater or less number of large and medium-sized lymphocytes are promiscuously scattered among the eosinophile cells.

The eosinophile cells vary in size from very large to medium-sized lymphocytes. The outline of the greater number is spherical but when they lie closely together or in close contact with other cellular elements they may have an irregular shape. The eosinophile granules are coarse, round, and of a nearly uniform size. Their number varies greatly in different cells. In some a few granules may be found in a group to one side of the nucleus,

in others they are thinly and quite evenly scattered throughout the basophilic cytoplasm, while still others are completely gorged with them.

A striking peculiarity is that the large majority are mononuclear. Only very seldom can one of the polymorphonuclear type be found. The nuclei are round, slightly indented, or crescentic in outline and are usually eccentrically located in the cell. In those cells that are gorged with granules the nuclei are crowded to one edge of the cell and stand out conspicuously among the eosinophile granules. The structure of the nuclei is identical with that of the nuclei in the large lymphocytes which have been described.

The thymus of a 125 mm. embryo was chosen to consider the origin of the eosinophile cells. In this stage the interlobular septa, loose in structure, contain numerous lymphocytes, red blood-cells, and many eosinophile cells lying both singly and in groups. Also in a single group can be found eosinophile cells containing varying numbers of granules, as stated above. An interesting and instructive feature often to be observed is the presence of large lymphocytes containing only from one to three or four eosinophile granules which are of the same size and shape as those found in cells completely gorged with them. Often in very limited areas—covered by very slightly moving the slide under high magnification—can be found large lymphocytes and a series of eosinophile cells with gradually increasing numbers of granules (fig. 7, *l.m.n.*). Only one interpretation can be given to microscopic pictures of this kind, namely, that the eosinophile cells are derived from lymphocytes. This conclusion also accounts for the large numbers of eosinophile cells along the course of blood vessels in late developmental stages, for it is along the blood vessels—in the loose portions of the septa—that the lymphocytes are most numerous.

I believe that the groups of eosinophile cells in the septa are identical with the granular cells of Watney which he found in the interlobular septa of the thymus in various classes of animals, although none of the cells were attached to the tunica externa of the vessels, as was observed by him. The ultimate

source of the eosinophile cells in the interlobular septa of the thymus of the pig is the same as that of the granular cells of Watney, the only difference is that he derived them directly from connective tissue cells while in the pig thymus they are derived from transformed connective tissue cells, the large lymphocytes.

Of course, in fixed material it is difficult to determine whether all the lymphocytes along the blood vessels are derived from the loose connective tissue in which the vessels lie or whether some come from the blood. Two features are in favor of the former view; (1) transition forms from connective tissue cells to lymphocytes are of frequent occurrence. The lymphocytes thus formed through division also increase in number; (2) diapedesis of the leucocytes is thought of as taking place only through thin walled blood vessels, but the lymphocytes and eosinophile cells are as numerous along the course of thick walled vessels as along those of a capillary nature. Another possible source of the lymphocytes in the septa is from the parenchyma of the thymus. However, in late stages that portion of the thymus contains mostly small lymphocytes and judging from the small number of small lymphocytes present in the septa very few have migrated into them from the parenchyma. Only a few eosinophile cells were found undergoing mitosis, so the number of this type of cells formed through their proliferation is almost neglible.

The source and nature of all the granules in eosinophile cells is difficult to determine. There is, however, no evidence indicating that the granules are débris of degenerated epithelial cells, as held by Schäffer ('09), but ample evidence that not all are products of the protoplasmic activities of the cells containing them, which view is held by Maximow for the origin of the granules of the myelocytes found in the thymus of various animals. Mention was made of free eosinophile granules (fig. 8, *Eo.G.*) in the interlobular septa where free red blood-cells also occur. These can be traced directly to degenerated red blood-cells, but the free granules usually observed in the septa of any developmental stage do not seem to be numerous

enough to account for all of the granules in the numerous eosinophile cells even though all should be ingested by lymphocytes. However, lymphocytes with only a few granules in their cytoplasm and lying among free eosinophile granules suggests that some eosinophile cells are simply lymphocytes ingested with débris of degenerated erythrocytes. This view of the origin of the granules in eosinophile cells is held by Weidenreich ('08, '08, mammals), and by Badertscher ('13, amphibia) in a somewhat modified form in that some of the granules are also formed from the débris of degenerated muscle tissue. Also circumstantial evidence indicating the formation of eosinophile granules from erythrocytes is not wanting and may be enumerated as follows: (1) The free red blood-cells appear in the interlobular septa in advance of eosinophile cells; (2) The red blood-cells appear in large numbers in earlier developmental stages than do large numbers of eosinophile cells, e.g., in the septa of the thymus of a 125 mm. embryo the red blood-cells are more numerous than in any other developmental stage while the largest number of eosinophile cells occur in the septa of the thymus of a 165 mm. fetus; (3) As the free red blood-cells in the septa of late stages begin to decrease in number the eosinophile cells decrease in number in correspondingly later stages, e.g., the red blood-cells in the thymic septa of 165 and 180 mm. fetuses are not as numerous as in the 125 mm. embryo but the eosinophile cells in the 270 mm. embryo are less numerous than in the 165 and 180 mm. fetuses. These facts can be stated in a general way by saying that the height and decrease of erythrocyte formation in the septa are followed respectively by the height and decrease of eosinophile cell formation in somewhat later stages. If the granules in eosinophile cells are products of degenerated erythrocytes this apparent relationship existing between these two types of cells can be accounted for only on the assumption that the majority of free red cells in the septa undergo dissolution and the products of degeneration taken up by the lymphocytes, possibly in soluble form, and in them transformed into granules.

Cells of a peculiar type (fig. 7, *k*). are quite frequently found among lymphocytes and eosinophile cells in the thymic septa. They are derived from large lymphocytes and are characterized by a part of or the entire superficial layer of the basophilic cytoplasm staining a deep red similar to the erythrocytes or the granules in eosinophile cells. Their nuclei have the characteristic structure of those in the lymphocytes or eosinophile cells. They cannot, therefore, be erythroblasts which have granular nuclei but must be classed with the eosinophile cells. The cells of this type are never very numerous and the youngest stage in which they were found was in the body mesenchyme of a 25 mm. embryo. They occur most frequently in the thymic septa of quite late developmental stages.

The origin of the eosinophile cells in the lobules of the thymus can now be discussed briefly. Their structure is the same as of those in the interlobular septa. They belong to the mononuclear type. They were first found in the lobules of the thymus of a 42 mm. embryo. In this stage they are very rare and can be found only after prolonged searching. Their number increases in successively advanced developmental stages. In the 125 mm. embryo they are readily found in both the cortex and medulla. In the 180 mm. embryo a group of them was found in the medulla of the mid-cervical segment while those lying singly are more numerous than in younger stages. In the full term fetus they are present in appreciably greater numbers than in the previous stage, groups of them being found in both the cortex and medulla and many can be found lying singly. Since the red blood-cells were considered particularly in the superficial thymus of a 270 mm. (full term) fetus the eosinophile cells also in that region will be emphasized. Some groups of eosinophile cells are found in the immediate vicinity of blood vessels but as many are found that are not associated with the vessels. The groups occur most frequently along the border of or near the vicinity of groups of erythrocytes but some groups are isolated and as far as position is concerned their origin does not seem to bear any relation to erythrocytes. Here as in the interlobular septa the origin of some is, undoubtedly, from the

large lymphocytes that have ingested eosinophile granules (débris of degenerated erythrocytes) which as was stated above can be found lying free in the meshes of the reticulum among the lymphocytes. The free eosinophile granules do not seem to be numerous enough, as in the case of the septa, to account for all granules found in eosinophile cells. However, an apparent general relationship exists between the latter type of cells and the erythrocytes which indicates that at least some of the granules of eosinophile leucocytes are derived from degenerated erythrocytes. The features indicating this relationship may be expressed as follows: (1) As in the thymic septa, the red blood-cells are present in advance of the eosinophile cells; (2) The eosinophile cells increase in numbers in successively advanced developmental stages as do also the red blood-cells; (3) They are most numerous in the thymus of a full term fetus in which developmental stage the red blood cells are also most numerous; (4) In the thymus of a 270 mm. embryo the eosinophile cells are more numerous in the superficial thymus than in the thymus head, the difference in the numbers corresponding favorably to the difference in the numbers of red blood-cells which are much more numerous in the former than in the latter segment. Here also it must be said that if all the granules of the eosinophile leucocytes in the lobules are derived from degenerated erythrocytes it must also be assumed that their degenerated products are taken up in soluble form by the lymphocytes in which it is transformed into granules.

Phagocytes (fig. 7, *o.* and *p.*) are found in the interlobular septa of the thymus in a wide range of developmental stages. They are most numerous in those stages in which the septa have a loose structure and contain many lymphocytes. They possess a large amount of cytoplasm which in some cells is vacuolar. Some are gorged with ingested material which consists mainly of lymphocytes (apparently) in various stages of degeneration. Occasionally one can be found in which an entire erythrocyte or a part of one makes up a part of the ingested material. They arise directly from connective tissue cells some of which contain ingested particles even before their

protoplasmic processes have been withdrawn. The phagocytes vary greatly in size. Some are from two to three times as large as the largest lymphocytes. Only a few were found in the lobules of the thymus of late developmental stages.

Cysts were found in the thymus in embryos 55, 65, 110, 125, 165 and 180 mm. in length. They vary in size and shape and all are lined with simple cuboidal or low columnar epithelium which is ciliated only in patches. The cilia are long and slender. No consideration was given to their origin.

CONCLUSIONS

1. The lymphocytes first present in the thymus are all large lymphocytes and have migrated into it from the mesenchyme.

2. The numerous small round cells of the thymus are formed by the repeated division of the large lymphocytes which thus become small, and also by their own proliferation.

3. Judging from the source and structure of the small round cells they are small lymphocytes and are identical with the small lymphocytes of the blood. The thymus, therefore, may well be considered as a source of some of the small lymphocytes found in the circulating blood.

4. The reticulum of the thymus is of epithelial origin and is formed passively by its meshes becoming filled with lymphocytes which separate the nodal nuclei farther apart and thus greatly attenuate the protoplasmic processes of the syncytium.

5. The Hassall's corpuscles are of epithelial origin.

6. The free red blood-cells and eosinophile cells found in both interlobular septa and the thymic lobules are derived from lymphocytes *in situ*.

7. Whether or not any of the erythrocytes formed in the thymus enter the circulating blood is difficult to determine in fixed material. Some of the free erythrocytes undoubtedly undergo degeneration and the products of disintegration of those existing in the form of eosinophile granules are taken up by the lymphocytes which thus become transformed into eosinophile leucocytes.

8. It was impossible to trace the origin of all the eosinophile granules in the eosinophile cells directly to degenerated red blood-cells. However, the fact, that the height and decrease of the formation of red blood-cells in the septa is followed by the height and decrease of the formation of eosinophile cells, is circumstantial evidence that a relationship exists between the disappearance of the free erythrocytes and the formation of free eosinophile cells.

9. The histogenesis of the thymus may be divided into epochs each of which is characterized by more or less distinct developmental features. They are:

(1) The purely epithelial epoch which extends from its origin as an outpocketing from the third pharyngeal pouch and the formation of the cervical vesicle to the appearance of the first lymphocytes in the thymus.

(2) The epoch of lymphocyte infiltration and lymphocyte proliferation and the formation of the reticulum. The infiltration of the thymus by extrathymic lymphocytes from the mesenchyme surrounding it begins in embryos from 25 to 30 mm. in length and probably continues up to stages 180 mm. in length, while their proliferation in the thymus undoubtedly continues after birth. The reticulum, which according to the nature of its development is formed gradually, differentiates into the cortex and the medulla in developmental stages 65 to 75 mm. in length, and is fully formed in embryos 180 mm. in length.

(3) The epoch of the formation of red blood-cells and the development of granular cells. An occasional red blood-cell is found in the thymic lobules shortly after lymphocytes are found in them. They are, however, first present in appreciably large numbers in stages of about 55 mm. in length and are most numerous in the thymus of full term embryos. In the interlobular septa of the thymus the greatest number occurs in stages of about 125 mm. in length while only a few are found in embryos of 180 mm. in length to full term.

Eosinophile cells were first found in the thymic lobules of a 42 mm. embryo but occur first in appreciably large numbers

in embryos of about 180 mm. in length and are most numerous in the parenchyma of the thymus of full term embryos. In the interlobular septa they are seldom found in embryos from 65 to 85 mm. in length. They occur first in appreciably large numbers in the septa of embryos of about 125 mm. in length and are most numerous in embryos 165 to 185 mm. long but are still present in the septa in full term embryos.

I wish to thank Prof. B. F. Kingsbury for the aid and encouragement given me on this work, and Prof. S. H. Gage for many suggestions.

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REFERENCES ON PLATES

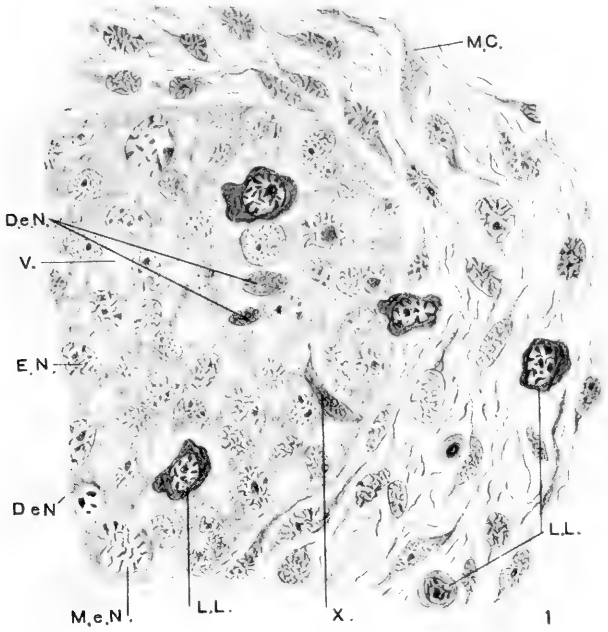
A.Erc., amitosis of erythroblasts
bl.v., blood vessel
D.d.e.N., débris of degenerated epithelial nuclei
D.e.N., degenerating epithelial nucelus
D.e.N'., completely degenerated epithelial nucleus
E.N., epithelial nucleus
Eo.C., eosinophile cells
Eo.G., free eosinophile granules
Erb., erythroblast
Erc., erythrocyte
L.L., large lymphocyte
L.M., limiting membrane
M.C., mesenchymal cell
M.e.N., mitosis of epithelial nuclei
Me.L., medium sized lymphocyte
M.L., mitosis of lymphocyte
Nmb., normoblast
Pc., phagocyte
S.i., interlobular septa
S.L., small lymphocyte
T., trail
V., vacuole
X., cell of unknown origin and significance
Z.pr., zone of rapid proliferation of epithelial cells

PLATE 1

EXPLANATION OF FIGURES

1 Camera lucida drawing of a portion of a lobule of a section of the right thymus head in a 30 mm. embryo. The infiltration of the thymus by extrathymic lymphocytes from the surrounding mesenchyme has just begun. In order to reduce the size of the drawing the very large lymphocyte represented in the outer border of the mesenchyme was drawn a little nearer the thymus than it really is. 1.5 mm. Zeiss App. objective, × 12 Comp. ocular; reduced one-half.

2 Camera lucida drawing of a portion of a lobule of a section of the left thoracic segment of the thymus in a 37 mm. embryo. The thymus in this developmental stage is slightly more advanced in development than in the 30 mm. embryo. This drawing was made to show particularly the large number of epithelial nuclei that are in the first stages of degeneration and the trail that was apparently made by an active lymphocyte that migrated into the thymus from the mesenchyme surrounding it. 1.5 mm. Zeiss App. objective, × 12 Comp. ocular; reduced one-half.


M.C.
D.e.N.
V.
E.N.
D.e.N.
L.L.
M.e.N.
L.L.
X.
1

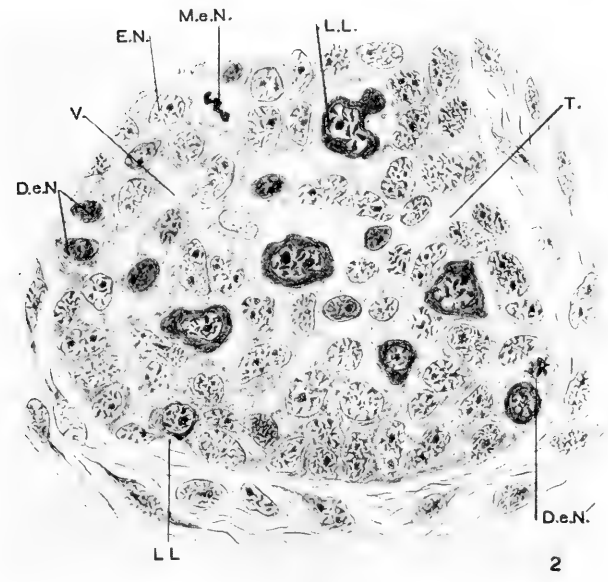
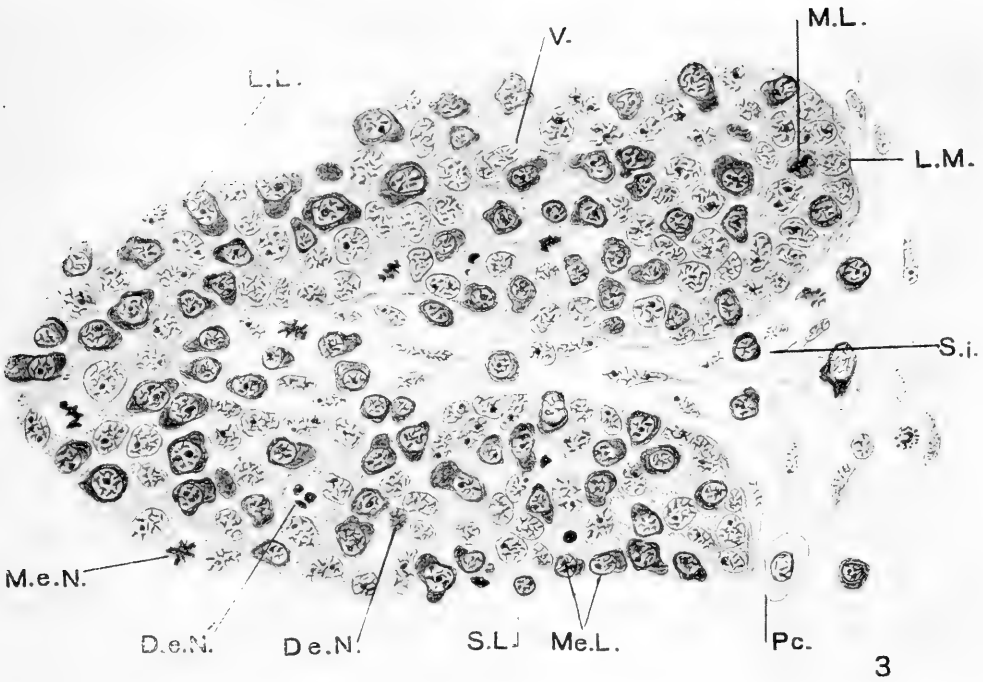

M.e.N.
E.N.
L.L.
V.
T.
D.e.N.
D.e.N.
L.L.
2

PLATE 2

EXPLANATION OF FIGURES

3 Camera lucida drawing of portions of two lobules of the left thymus head in an embryo 42 mm. in length. In the thymus of this stage many lymphocytes are present most of which are large and medium-sized. Only a few small lymphocytes are present. Mitoses of both epithelial nuclei and lymphocytes occur. The lymphocytes in the interlobular septa are quite numerous. 1.5 mm. App. objective, × 4 Comp. ocular; reduced one-fourth.

4 Camera lucida drawing of portions of two lobules of the left thymus head in a 65 mm. embryo. The thymus of this stage contains numerous lymphocytes most of which are small ones. Mitoses of lymphocytes are comparatively numerous. Many lymphocytes are found in the interlobular septum. 1.5 mm. App. objective, × 4 Comp. ocular; reduced one-fourth.


V.
M.L.
L.L.
L.M.
S.i.
M.e.N.
D.e.N.
De.N.
S.L.
Me.L.
Pc.
3

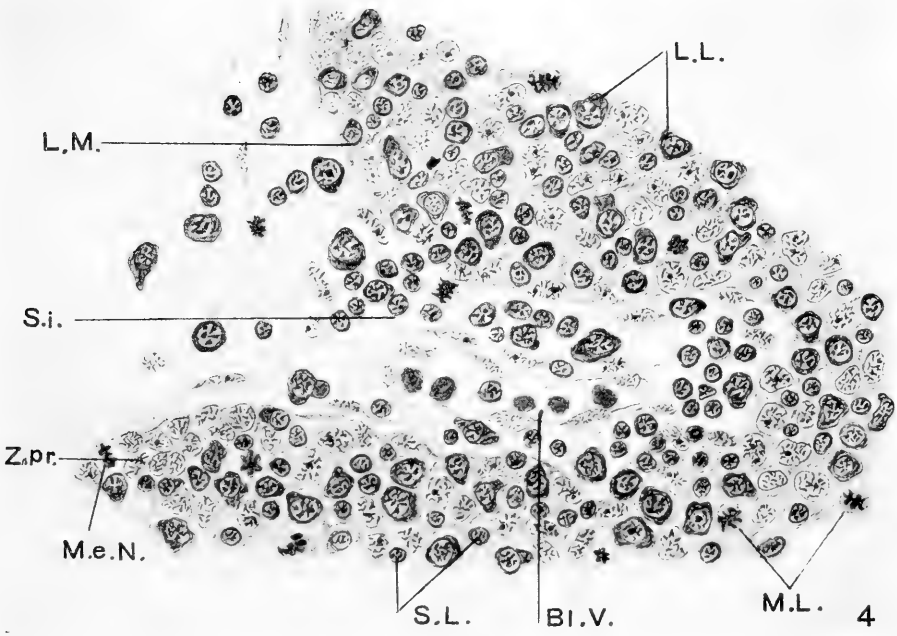

L.L.
L.M.
S.i.
Z.pr.
M.e.N.
S.L.
Bl.V.
M.L.
4

PLATE 3

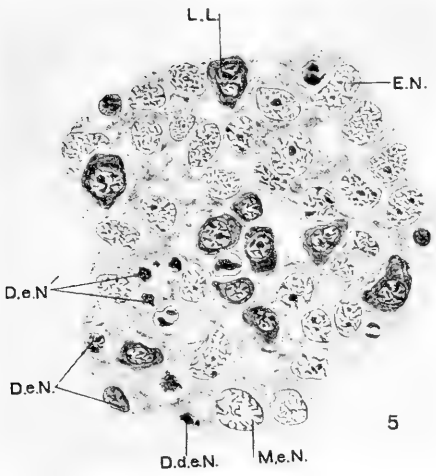
EXPLANATION OF FIGURES

5 Camera lucida drawing of a portion of a thymic lobule in a 36 mm. embryo to show specially epithelial nuclei in various stages of degeneration. 1.5 mm. Zeiss App. objective, × 8 Comp. ocular; reduced one-half.

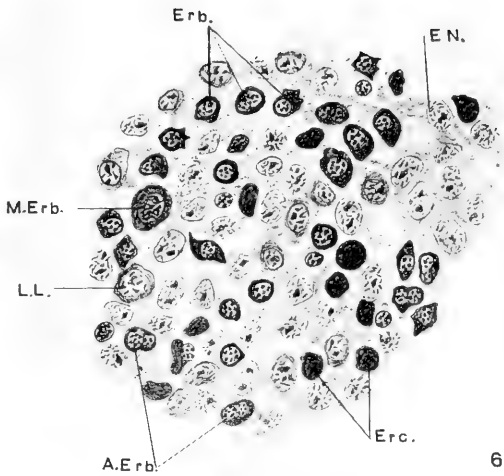
6 Camera lucida drawing of a portion of a lobule of the thymus in a 55 mm. embryo to show especially free nucleated and non-nucleated red blood-cells. In the portion drawn one erythroblast is in mitotic division while several are in amitotic division. 1.5 mm. Zeiss App. objective × 8 Comp. ocular; reduced one-half.

7 Diagrams showing the different types of cells that are derived from mesenchymal cells. The direction of the arrows shows the relation of the different types of cells to each other; *a*, mesenchymal cell; *b*, *c* and *d*, transforming mesenchymal cells; *e*, large lymphocyte; *f*, small lymphocyte; *g* and *h*, erythroblasts; *i*, normoblast; *j*, erythrocyte; *k*, lymphocyte capped with a layer of hemoglobin; *l*, *m* and *n* show the formation of eosinophile leucocytes, and *o* and *p*, phagocytes; *a*, *b*, *k* and *p* are camera lucida drawings while the remainder are free-hand drawings from actual specimens. All were drawn from specimens in the interlobular septa of a 125 mm. embryo excepting *o* and *p*, which were drawn from specimens in an interlobular septum of a 110 mm. embryo. 1.5 mm. App. objective, × 8 Comp. ocular; reduced one-half.

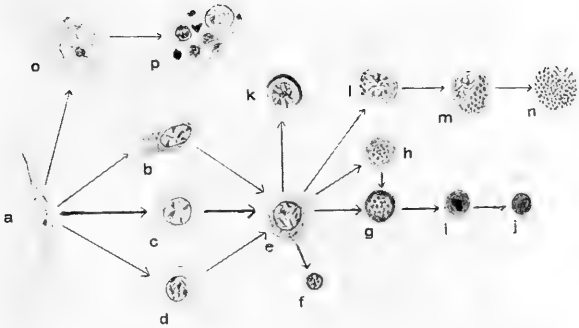
8 Camera lucida drawing of a portion of an interlobular septum of the thymus in a 165 mm. embryo showing specially eosinophile leucocytes and a few free eosinophile granules.


L.L.
E.N.
D.e.N.
D.e.N.
D.d.e.N.
M.e.N.

5


Erb.
E.N.
M.Erb.
L.L.
Erc.
A.Erb.

6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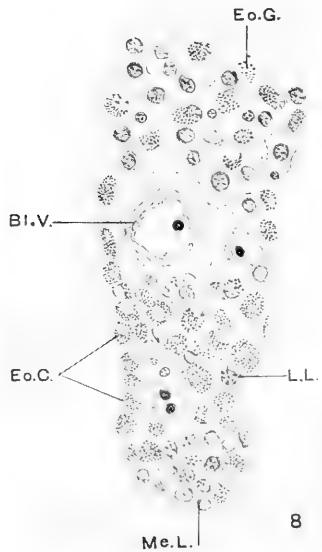
Eo.G.
Bl.V.
Eo.C.
L.L.
Me.L.

8

ON THE PREMATURE OBLITERATION OF SUTURES IN THE HUMAN SKULL

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INTRODUCTION

In the developmental history of the human skull, there is a period in which the phenomena of development are as yet fairly unknown to us: viz., the phase of life included between the third year and the adult state. The reason of this is quite clear. It is impossible to acquire a perfect knowledge of an object of such intricate structure as the human skull, unless investigation is made of a very great number of infantile skulls. Now, the number of non-adult skulls, except those belonging to children of one and two years old, found in the anatomical institutes is generally quite restricted. This was the case with the anatomical institute of the University of Amsterdam, until two years ago, when it became the possessor of about two thousand infantile human skulls. This collection may be utilized for investigations of many totally different natures and my intention is to communicate occasionally in this journal the results of some investigations worked out by myself or by my pupils on the material of this splendid collection.

The present paper will refer to the sutures of the cerebral part of the skull.

It is a well known fact, that the bones of the human skull coalesce either during the developmental period or in a more advanced phase of life. In the first case, coalescence takes place for the greater part during the foetal period, in the second case at a date varying extraordinarily for each suture. Therefore in human life a phase exists during which skull-bones do not unite, beginning about the fourth year, when the metopic suture

has closed itself and the different parts of the occipital bone are united with each other. The sutures still existing at this date of life, are the so-called persisting sutures remaining for a shorter or longer time after the individual has become full grown. Regarding the variability of age in which these sutures disappear there are already some extensive and carefully written communications, for instance by Frederieg and Nibbe. Yet it is a well-known fact that now and then one of these persisting sutures does close during childhood before the individual has reached the adult state. Of this fact, and its influence on the shape of the skull, the casuistic literature is already very abundant, but a systematical inquiry into this phenomenon is as yet wanting. In the present paper I wish to deal with the results of my examination of the premature concrescences of bones in the human skull, results acquired by the investigation of 1820 skulls of non-adult individuals. I regarded the obliteration of the occipito-sphenoidal synchondrosis as a criterion of the adult condition of the skull. The youngest skulls at my disposal already possessed their complete milk-dentition, therefore in this communication skulls of the first two years are not mentioned.

Before beginning my investigation I divided my collection into seven groups, in accordance with the developmental phase of the dentition. Of the different groups a brief description follows:

Group I. This first group is composed of the very young skulls, with complete milk dentition, and therefore with the following dental formula:

i. i. c. m. m.

These skulls, 725 in number, are those of children who died between the third and sixth year.

Group II. This group includes the skulls in which, besides the complete milk dentition, the first permanent molar tooth is also present. The dental formula of these skulls (of children aged 6–7 years) is as follows:

i. i. c. m. m. M.

This second set comprises exactly 400 skulls.

Group III. In this group the eruption of the permanent central incisor had taken place. There were 168 of such skulls belonging to children aged about 7 or 8 years. The dental formula is as follows:

I. i. c. m. m. M.

Group IV. This group contains 157 infant skulls in which the lateral permanent incisor has made its appearance corresponding with the age of 8 or 9 years. The dental formula of this series is to be written:

I. I. c. m. m. M.

Group V. In this set were included the 109 skulls in which the first milk-molar was lost, and the dental formula is the following:

I. I. c. P. m. M.

Such a set of teeth corresponds with an age of 9–11 years.

Group VI. Includes 203 skulls between 11 and 13 years, in which the second premolar and the canine are present. The order of eruption of both of these teeth is not a constant one. Although in a considerable majority of skulls the second premolar precedes the canine, yet there were a certain number in which the eruption of the second premolar evidently succeeds the eruption of the canine. Therefore I have included all these skulls in one set. Its dental formula is as follows:

I. I. c. P. P. M.

Group VII. This last group contains all the skulls (58 in number) with a complete set of teeth, save the third molar. These skulls, of which the dental formula is

I. I. C. P. P. M. M.

come from individuals who died between the age of 13 years and the adult state.

I have found in my whole collection but three skulls, not yet completely developed, in which the eruption of the third molar had already taken place. It must therefore be considered as a rule that the wisdom tooth makes its eruption after the

termination of the development of the skull. Exceptions to this rule are very infrequent.

Table 1 gives a brief résumé of the above described groups of my collection:

TABLE 1

GROUP	DENTAL FORMULA	AGE	NUMBER
I	i. i. c. m. m.	3–6	725
II	i. i. c. m. m. M.	6–7	400
III	I. i. c. m. m. M.	7–8	168
IV	I. I. c. m. m. M.	8–9	157
V	I. I. c. P. m. M.	9–11	109
VI	I. I. C. P. P. M.	11–13	203
VII	I. I. C. P. P. M. M.	13–20	58

Soon after the beginning of my investigations, the fact struck me that the closing of so-called persisting sutures in skulls of non-adults occurs more frequently than I had supposed. However, this is not the case with all cranial sutures in the same degree. In some a premature concrescence is an unusual rarity, but on the other hand there are some in which the concrescence occurs so often, that it can scarcely be considered as an anomaly. Now in this communication, I will discuss first: those sutures which I found most frequently closed, and second: those in which coalescence appeared as a very exceptional variety.

PREMATURE OBLITERATION OF THE MASTO-OCCIPITAL SUTURE

The examination of this suture produced one of the most surprising results of my investigation. Frederieg, in his very extensive and valuable paper "On the obliteration of the cranial suture,"[1] asserts that the coalescence of the occipital and the temporal bones does not occur before the thirty-first year, it being a very rare exception when it has already occurred in the twenty-first or twenty-fourth year (loc. cit., p. 441). On another page in the same work the author strongly points out the

[1] Zeitschrift fur Morphologie und Anthropologie, B. 9, 1906.

fact that the masto-occipital suture belongs to those persisting the longest.

Now it is important to note that this author had at his disposal only a small number of skulls of 20 to 30 years and that his investigation was made principally on adult skulls. If the investigator had extended his examination upon a sufficient number of non-adult skulls, his conclusion would, no doubt, have been quite different. For the coalescence of the occipital and petrosal bone in the skull of infants is not at all a rare event. On the contrary amongst my material there even was a considerable and unexpected number of skulls, showing complete or partial closure of the masto-occipital suture, either on one side or on both. Moreover not in all cases was the coalescence restricted to this one suture, but in a large number of skulls two or three or even four sutures were totally or partially obliterated. Here I wish to treat separately the cases in which only the masto-occipital suture was closed and in which the obliteration was of a more extensive nature. I will begin with the first group.

It is scarcely necessary to particularly mention that in case of closure of the masto-occipital suture the coalescence of the two bones can be a total or a partial one. In the second table this fact is taken into consideration. As a rule the coalescence of the petrosal and occipital bones begins midway in the suture, passing in the majority of cases from this point towards the masto-parietal suture, in consequence of which, in a partial closure, it is most often the upper half which is obliterated. Table 2 shows the results of my examination on the masto-occipital suture. This table demonstrates at once the quite unexpected fact, that in the human non-adult skulls the masto-occipital suture is found closed so often, that one is inclined to consider this phenomenon no longer as an anomaly. Let us consider the frequency of this obliteration. It is not possible to recognize, with the aid of table 2 (p. 500), the absolute number of skulls in which the suture showed obliteration, a certain number being twice mentioned, viz. the skulls entirely closed on the one side and partially on the other; the skulls in which

TABLE 2

CLOSURE OF MASTO-OCCIPITAL SUTURE

GROUP	NUMBER	Both sides		Right		Left	
		entirely	partial	entirely	partial	entirely	partial
I.....	725	16	11	12	20	9	21
II.....	400	12	5	5	11	9	7
III.....	168	10	2	2	4	3	2
IV.....	157	7	1	2	4	2	1
V.....	109	6	0	0	1	2	1
VI.....	203	9	5	8	3	6	5
VII.....	58	3	1	4	1	3	3
	1820	63	25	33	44	34	40

on both sides the suture was partially or entirely closed are mentioned once, and also those in which the suture on one side only was partially obliterated. Moreover an uncertain number remains in which the suture is totally closed on one side. Taking this into consideration, I found amongst about 1820 skulls of non-adults at least the number of 63 + 25 + 44 + 40 = 172 with closure of the masto-occipital suture either on both sides or on one side only. Reckoning the number of skulls with a total closure on one side to be 10, we can conclude that in 10 per cent of our non-adults the said suture shows more or less signs of obliteration. Therefore Frederieg's conclusion is not right, when he writes that the coalescence of the petrosal and occipital bone in the third decennium of life rarely occurs. Even before the twentieth year the coalescence is not exceptional. The preceding table shows yet another phenomenon of no less importance. At what age does this obliteration take place? Our table includes skulls from about three years up to the adult state. Now two possibilities must be considered. Either the coalescence may begin at each date of this period, or the commencement of the process is limited to a shorter or longer phase of it. In the first case the number of synostotic skulls increases while the age advances; in the second case such a correlation is wanting. Now for the solution of this problem it is a happy coincidence that the number of skulls in Group I is considerable.

This group includes 725 skulls with a complete milk-dentition. And, proceeding in the same manner as before, it appears that in the whole collection the number of skulls showing a closure of the masto-occipital suture in this group must be at least as follows: Complete obliteration on both sides 16 times, partial on both sides 11. A partial closure on the right side only 20 times, and on the left side 21 times, amounting to 68 skulls out of 725. Resuming, we find the following remarkable result. In 1820 skulls varying in age between 3 to 20 years, the masto-occipital suture is obliterated wholly or partially 172 times, making about 10 per cent, and in 725 skulls of infants aged 3 to 6 years, the closure occurred 68 times, also coming to about 10 per cent. In this early stage of childhood, the obliteration is found in the same proportion as in the total number of skulls including the whole developmental period. Hence the following conclusion is obvious: The number of infantile skulls with closure of the masto-occipital suture does not increase after the age of six or seven years; the premature obliteration of the said suture is limited to a circumscribed period of infancy, beginning as a rule before the end of the sixth year.

This fact deserves our full attention in reference to its etiological interpretation. For the question arises whether this premature obliteration is a pathological phenomenon, or one of a purely physiological nature. When working out my statistical material I doubted at first the physiological nature. I took into consideration the possibility of this process being caused by some inflammation in the neighborhood of the suture, especially in the tympanic cavity. No doubt an otitis media will cause a hyperaemic state in the adjacent parts of the skull, and one can imagine that under the influence of the latter a coalescence of the occipital and petrosal bone may occur. The consideration, however, that certainly not 10 per cent of our children undergo an inflammation of the middle-ear is sufficient to reject the idea of this pathological cause for the obliteration. Moreover there was yet another circumstance pleading against such a supposition. As we will demonstrate in the following paragraph of this paper, the sagittal suture is also very often the

seat of a premature obliteration, and it is almost improbable that this process is effected by an influence originating from the middle-ear. Therefore it is necessary to explain the great frequency of the closure of the masto-occipital suture in infantile skulls in a quite different manner. We will return to this question after having discussed the premature obliteration of the sagittal suture, which resembles in many points the masto-occipital. The number of all non-adult skulls, showing a closure of the masto-occipital sutures amounts to about 10 per cent, and we have found the same proportion in infants' skulls aged 3 to 6 years. The process is therefore limited to the period before the commencement of the dentition and is not extended over the whole period of growth of the individual. This fact is proved by another statement given in table 2. It appears that the number of partial coalescences diminishes when the age of the skulls advances and that on the contrary the number of total coalescences increases with the age of the individuals. To prove this let us compare the first two and the last two groups with each other. In the first two groups are included the skulls of children from 3 to 7 years. The total number of these is 1125. In 28, or nearly 25 per cent, of these, a complete coalescence of the masto-occipital suture was seen on both sides. In the Groups VI and VII, including the skulls of 12 years and more to the adult state, there were 261 skulls, and of these there were 12, or about 5 per cent, with complete closure on both sides. The difference appears still more considerable by comparing the unilateral coalescence. In the first two groups there are $12 + 5 + 9 + 9 = 35$ completely closed sutures on one side and $20 + 11 + 21 + 7 = 59$ partially obliterated. Therefore, in the very young skulls (Groups I and II) the cases with partial obliteration greatly outnumber those with complete obliteration. After the twelfth year (Groups VI and VII) the correlation becomes reversed; total obliteration being then more common than partial, proved by the following addition: totally closed $8 + 4 + 6 + 3 = 21$ and partially closed $3 + 1 + 5 + 3 = 12$.

Summarizing the preceding results our investigation leads us to the following conclusions with reference to the masto-occipital suture. In the infantile skull there is found a premature closure of the suture between the mastoid and occipital bone either on one or on both sides in about 10 per cent of the cases. This process is not pathological but ought to be considered as merely physiological. The beginning of the coalescence between the two bones is restricted to earlier stages. After the child has reached its seventh year it has but little chance to be subject to the said premature synostosis.

In the preceding pages we only considered the skulls in which exclusively the masto-occipital suture was closed and all others were intact. There were, however, in my collection of infants' skulls a certain number showing a more complicated condition in which premature obliteration was seen in more than one suture. For the sake of brevity we will postpone the examination of these cases until after the description of the skulls with a single obliteration.

THE PREMATURE OBLITERATION OF THE SAGITTAL SUTURE

In this suture too my investigation resulted in unexpected results, the frequency of premature closing being more considerable than I anticipated.

The premature closure of this suture has attracted the attention of many anatomists, more so than the masto-occipital suture. The frequency of the latter's synostosis was till now an unknown fact in the anatomy of the skull. In general it was acknowledged that a premature closure of the sagittal suture occurred occasionally, although investigations with statements are as yet wanting. The cause of this difference between two homologous phenomena is near at hand. In case of closure of the sagittal suture, the possibility arises of a deformity of the skull, more considerable the sooner in life the process commences. This anomaly is known as scapho-cephaly (which name was introduced by von Baer), because when excessively deformed the skull becomes boat-shaped. A premature coales-

cence of the occipital and mastoid bones on the contrary does not cause a striking deformation of the skull or the head. In some cases of a premature union of these two bones I met with a somewhat peculiar form of the occipital region of the skull. But this peculiarity can scarcely be observed during life because the greater part of this region of the skull is covered by the muscles of the neck. Now on the contrary, the deformity becomes more visible when obliteration of the sagittal suture occurs in early life. The effect of this process is clearly visible and an extensive literature in all the principal languages has treated of this subject. We may distinguish two schools of method in this literature, the purely descriptive and the etiological. The investigations of the former simply reported the description of the scaphocephalic skulls, without referring to the origin of the deformity.

The naturalists of the latter school did not limit their subject to a simple description, but they went more to the bottom of the problem and tried to point out the genetical cause of the deformity. The opinions of this group were directed in that way principally by a work of Virchow. In it the author demonstrates that the anatomical details characteristic of the scaphocephalic skull, were due to the coalescence of the two parietal bones in an early stage of development. Before Virchow this hypothesis had been defended by von Baer, but the correlation between cause and effect was clearly demonstrated for the first time by Virchow.

However, though I intend to write about the genetical relation between skull deformation and premature obliteration of sutures in a following paper, still I wish to lay stress here upon the justness of an observation already made by Huxley, and which was confirmed by my investigation. This famous naturalist demonstrated infantile skulls, absolutely normal in shape and size, although the sagittal suture was entirely obliterated. One might observe that in such cases the individual died shortly after the synostosis of the suture and that the skull had therefore no time to deform. To this supposition I will reply that the number of skulls with premature obliteration and without

any sign of scaphocephalic deformation in my collection is too large to accept this point of view. But as mentioned before I will return to this question later on.

The number of skulls with premature closure of the sagittal suture was a fairly large one. After finding this fact the question arose whether this process should be considered either pathological or physiological.

To justify the putting of this question some observations may precede upon the variability of the closure of this suture in the adult. The opinions of the writers diverge greatly as to the age in which the normal obliteration of the sagittal suture commences. According to Tapmord the process begins normally at the age of 40 to 45 years, a conclusion also accepted by Ribbe. In the text book of human anatomy the average age of the closure is given as about 50 years. Dwight, on the contrary, lays stress upon the fact that the obliteration commences between the twentieth to thirtieth year, although the individual variability is considerable, while the process can occasionally be postponed till a fairly old age. In his admirable paper, already mentioned, Fredericg shows that in 22 out of 34 human skulls, varying between 20 to 30 years, the suture commences to disappear. In this connection the author cited an observation of Schwalbe, who always found the sagittal suture either partially or entirely coalesced after the fortieth year.

The process of obliteration however can proceed very slowly, and it even happens that in skulls 80 years of age, the two parietal bones are not yet totally united. Based upon the results of the investigations of Schwalbe and Fredericg, the following point of view presents itself. It is proved, and we need not doubt the reality of the fact, that the beginning of the obliteration of the sagittal suture is seen fairly often between the twentieth and thirtieth year. But this fact was found by merely examining skulls older than 20 years. Until the present time young skulls have not yet been investigated as to the occurrence of the closure of the sagittal suture. And if it becomes clear in the course of such an examination, that such a closure in infantile skulls is not an exception, then I must

say a doubt ought to arise whether such cases have been rightly considered as pathological. It is true that it is premature, for the individual has not attained his adult stage, but why pathological? Could it not be possible that the normal variability is even more extensive and that the age at which the obliteration may begin, which as Frederick truly found, reaches the threshold of manhood, may also include a restricted period of childhood?

The problem will be thoroughly examined later on.

The results of my researches upon the said suture are arranged systematically in the following table 3.

TABLE 3

Obliteration of the sagittal suture

GROUP	DENTAL FORMULA	NUMBER	OBLITERATED	
			partially	entirely
I	i. i. c. m. m.	725	10	2
II	i. i. c. m m. M.	400	8	4
III	I. i. c. m. m. M.	168	4	2
IV	I. I. c. m. m. M.	157	3	3
V	I. I. c. P. m. M.	109	2	2
VI	I. I. C. P. P. M.	203	1	.6
VII	I. I. C. P. P. M. M.	58	0	0
		1820	28	19

I wish once more to emphasize that in this table only those skulls are referred to in which the process of obliteration was limited to the sagittal suture.

This table shows that in 47 skulls out of 1820 there was a partial or total closure of the sagittal suture, making $2\frac{1}{2}$ per cent. I had not expected to find such a considerable number. The cases of partial obliteration outnumber those of entire closure, a condition which is in no way surprising. For the majority of the skulls are those of children, who died early in life, so that the process of uniting had scarcely time to be extended along the whole suture.

In truth the fact that an entire obliteration was found in 19 skulls, making 1 per cent, surprises us as highly as the large

frequency of the obliteration in general. For by the investigation of Frederick and Ribbe it is made clear that total obliteration of the sagittal suture in the adult required a fairly long period. Taking this fact into consideration the large number of entirely closed sutures in infantile skulls awakes a strong suspicion that the obliteration, beginning in an early period of life, proceeds more quickly than those taking place in the more advanced phase of life. The increased intensity of all physiological and histological processes natural to youth, evidently influenced also the process of premature obliteration.

Now we will enter into the problem whether the obliteration of the infantile skull is pathological or not. It is clear that this problem is not solved by observing that the union of the two parietal bones, when occurring at an early date in life, causes deformity of the skull to a certain extent, for the effect of an intrinsically normal process may become under circumstances an abnormal one, while the proper nature of the process is not altered by it. One must distinguish formal and causal genesis.

Moreover one may not conclude that the closure must be of a pathological nature only because it occurs before the full development of the body is reached. For (1) many sutures in the skull disappear during this stage of life and, (2) I call attention to the result of my investigation in which I showed, after examination of about 800 skulls of apes and monkeys,[2] that in a large number of genera of primates, and especially in anthropoids, synostosis of the sagittal suture happens before the individual is full-grown. Thus, in forms with which the human being stands in close phylogenetical connection, the premature synostosis of the two parietal bones appears to be normal. Here the process bears a purely physiological character. Why should we refuse then to consider it also physiological in man? These arguments however are purely theoretical and through them a decisive answer to the question proposed is not possible. Let us try to find it, by examining more closely the contents of table 3. It showed us that in infantile skulls

[2] Zeitschrift fur Morphologie und Anthropologie, B. 15, 1912.

the obliteration appeared in $2\frac{1}{2}$ per cent. As I pointed out, there are two possibilities. The process is either confined to a definite period of development, or it can happen during its whole course. To determine which of the two possibilities really occurs, we have only to observe the frequency of premature obliteration appearing in the two groups of youngest skulls, containing those of children from 3 to 6 years. Their total number is 1125. Amongst these skulls there were 24 with partial or entire closure of the sagittal suture, amounting to 2.1 per cent. The conclusion therefore is quite simple and lies close at hand.

Amongst 1820 skulls of non-adult individuals (aged 3 to 20 years) there are found 47, or 2.5 per cent, in which the parietal bones are united, and amongst 1125 skulls of children, less than 7 years of age, I count 24, or 2.1 per cent, in which coalescence had taken place. Consequently the number of skulls with synostosis of the sagittal suture scarcely increases after the seventh year.

The period during which the obliteration of this suture in infancy begins reaches a limit therefore in the seventh year. The tendency to premature closing is not extended over the whole period of growth, but practically stops after the seventh year. I recall the fact that exactly the same relation was found in the masto-occipital suture.

Referring to the suture just mentioned, still another circumstance presents itself, proving that the number of prematurely closed sutures do not augment after the seventh year, *i.e.*, the proportion between the partially and totally closed sutures. The former diminish as the skulls reach a more advanced age. To demonstrate this I beg the reader to look at the last two rows on table 3. In Groups I, II and III (skulls up to 8 years of age) the partially closed sutures exceed in number those entirely closed; in the Groups IV and V (skulls up to 9 and 10 years of age) an equal number of each is found, and finally in Groups VI and VII the entirely obliterated surpass the partially closed ones. I may conclude, therefore, that the process once commenced is of a progressive nature.

ON THE GENETIC SIGNIFICANCE OF THE PREMATURE OBLITERATION OF THE SAGITTAL AND MASTO-OCCIPITAL SUTURES

The facts, demonstrated in the foregoing paragraphs as to the sagittal and masto-occipital suture, exhibit so much resemblance in some principal points, that it is desirable to treat these sutures together from a more general point of view. My reason for intercalating these considerations here and for not waiting till the description of the premature closure of all the sutures is given, is founded on the circumstance that in the other sutures premature obliteration is very seldom seen, and does not occur with the regularity which characterizes the two sutures above mentioned. The following points of resemblance between the two sutures may be mentioned. Firstly, the frequency of premature obliteration. Especially in the masto-occipital suture this is so often found, that one may well question why this phenomenon has remained unknown in literature until now. The synostosis of the masto-occipital suture is more frequent than that of the sagittal suture. On the other hand one should not forget that the former suture is paired and the chance of a premature closure therefore is doubled. Secondly, both sutures have the fact in common that the commencement of the process of closure is confined to a circumscribed phase of the development ending approximately in the seventh year. By this limitation in time the process attains a peculiar character. There is, as one might say, in the development of man a stage, during which he exhibits an intensified tendency to obliteration of some sutures. Beyond this stage, this disposition seems to be lost. The weight of this tendency is not at all a small one, as is proved by the fact that a premature obliteration of the masto-occipital suture is seen in more than 10 per cent of the skulls, and of the sagittal suture in 2.5 per cent. This unexpected large number of cases with premature synostosis gives a predominant significance to the problem of the etiology of this anomaly. This question has already been mentioned, *i.e.*, is this synostosis of skull bones a pathological phenomenon?

In the literature on this subject generally the opinion is advocated that premature synostosis of skull bones is a symptom

of some constitutional disease. And as a rule rhachitis or heredity syphilis are accused of being the primary causes of the anatomical anomaly.

It is clear that the literature on this subject principally refers to the sagittal suture, because the deformity, which in some cases results from the premature closure of the latter, has long since attracted the attention of anatomists. Concerning this deformity, scaphocephaly as von Baer first called it, an extensive literature exists, in which the question is widely discussed whether scaphocephaly can be acquired after birth, or is in each case already present in the foetal skull. Although we shall not enter into this question, it seems necessary to state the fact that in all the skulls described in the preceding paragraphs, the synostosis of the skull-bones had undoubtedly taken place after birth and in the majority of the cases at the age between the third and seventh year.

Still I cannot agree with the opinion of the investigators, who consider the premature obliteration as the result of rhachitical or syphilitical disposition of the individual and will give some arguments against this theory.

My first objection is based on the large number of skulls with premature closure. If rhachitis or syphilis is the cause of it, one must not shrink from the conclusion that one or the other of these diseases has affected more than 15 per cent of the individuals.

I admit this argument to be purely theoretical and therefore of a problematical value. Still there are other reasons why the pathological nature of the premature obliteration should be denied. In my collection of skulls there were, as need scarcely be mentioned, a certain number with evident symptoms of rhachitis: Hydrocephaly, flattened occipital region, defective development of the enamel of the teeth, etc. A special examination has shown to me that the positive rhachitical skulls were characterized in no manner by an increased tendency to premature synostosis of the skull-bones. Amongst these rhachitical skulls there were naturally a certain number with premature closure of the sagittal or masto-occipital suture, but the

proportion in which this happened was not larger than in the normal skulls. This fact is further strengthened by the circumstance that in most cases of premature obliteration no other symptoms of rhachitis were visible, they possessed a normal structure of the bone tissue and of the tooth-enamel.

Another argument pleading against the rhachitical character of the premature obliteration is the great regularity with which the process commences and proceeds. In all the skulls described in the foregoing paragraph, it was clear that the synostosis of the sagittal suture regularly commenced at the very point where in normal cases the obliteration begins, *i.e.*, in the obelion. Should the process be of a pathological nature the starting-point of the synostosis should be very inconstant.

Finally, if the obliteration is really the effect of some general constitutional disease, how can we understand that the process confines itself to the whole length of one suture only? In the sagittal suture the synostosis is often complete, extending from the bregma to the lambda point. Why, one may ask, does not the process continue along the coronal and lambda sutures? Is such an anatomically strictly confined extension of the process in accordance with a supposed pathological origin? I must admit that these arguments prove nowhere decisively that the premature synostosis cannot be caused by some constitutional disease. But on the whole I think that they form a strong evidence against it. On the other hand, I will by no means absolutely deny all genetical correlation between anomalies in the system of sutures of the infantile skulls and constitutional diseases. I willingly admit the possibility of such a relation, but I wish to reserve it for those cases in which an entire or partial closure of several sutures is seen in an often very irregular manner.

Now the question arises as to the real significance of the premature closure. If it is not, as I just made clear, the result of some pathological cause, from which point of view is the phenomenon to be explained? I believe I am able to give such an explanation, and I wish to give in the following pages a brief account of my opinion upon this subject.

Some years ago I published an extensive investigation upon the normal obliteration of the sutures in Primates. The results of this inquiry were based upon the examination of more than 800 skulls of platyrrhine and catarrhine monkeys and a considerable number of skulls of anthropoid apes, all present in the anatomical museum of the University of Amsterdam. As to the problem interesting us in the present paper, we may limit ourselves to the conclusions relative to the anthropoid skulls.

There are striking differences in the process of obliteration between man and apes. These differences concern the age in which normal obliteration takes place and the order of succession in which the closure in the different sutures begins. In man, as a rule, the principal sutures persist for a longer or shorter time after the complete formation of the skull. The same happens in some genera of American monkeys; but in apes the sutures can close immediately after the skull is full grown. At this moment the general growth of the individual is not yet finished, and though it is, for reasons near at hand, impossible to know the age in which the obliteration begins, it is sure that the process commences, and perhaps in some sutures is even finished, before the animal has attained its adult state.

The significance of this premature synostosis of the skull bones in apes may be found in the strong development of the muscles of mastication, arising from almost the whole surface of the braincase, and moreover in Gorilla and male Orangs from strong crests developing exactly in the line of union of the parietal and occipital bones.

Now it is obvious that in the apes, as well as in man, there exists a relation between the growth of the brain and the braincase. In apes, as a rule, the different bones of the skull cannot unite together before the brain has attained its final volume. This is so clear and simple that it is altogether unnecessary to enlarge upon it. As it is, the conclusion lies close at hand that the sutures in the braincase of apes disappear immediately after their physiological function is finished. The physiological

function of the sutures is to produce new osseous tissue along the margin of the skull bones for the sake of the enlarging of the braincase. This function is continued as long as the braincase needs enlarging, *i.e.*, as long as the brain increases in volume. Summarizing, I think it is clear, that in apes the sutures commence to obliterate as soon as the enlargement of the brain has ceased. And in this respect there is a remarkable difference between man and apes. In the former the sutures often persist a long time after the brain has ceased growing.

We can now return to our starting point and consider the question whether there is some relation between the normal progress of suture-obliteration in apes and premature obliteration in man. There is no doubt about the fact that man stands in nearer phylogenetical relation to the anthropoids than to any other representative of the primate stem. Therefore, since, as a rule, the sutures begin to disappear in apes shortly after the brain is full-grown, which happens in youthful animals, we have the right to conclude that the condition in man is of a progressive nature. This condition, *i.e.*, the persistence of the sutures during a certain period of the adult state, must be considered as a peculiarity acquired by man during the earliest phase of his phylogenetic evolution.

This conclusion gives rise to the following question. Should not the premature obliteration of the sutures in the braincase of man be considered an atavistic phenomenon? This hypothesis deserves our full attention. If the statement is accepted as true, that in human ancestors the sutures closed as those of the anthropoids of today, *i.e.*, at an early stage of life, then the occasional premature obliteration in recent man loses its non-proved pathological character and becomes more intelligible. For we know that each quality newly acquired in the evolution of beings often requires a long space of time before it becomes absolutely fixed. During this period the antecedent condition reappears individually now and then. For my part I think I may conclude that the premature closure of sutures in infant-skulls is such an atavistic phenomenon.

ON THE OBLITERATION OF ONE OF THE OTHER SUTURES OF THE SKULL

In considering the occurrence of premature obliteration a striking difference is observed between the sagittal and masto-occipital suture on the one hand and all the other sutures on the other. A special discussion therefore upon the sutures just mentioned seems desirable in every respect. The frequency of premature closure in the other sutures being very small, there is no ground to describe each of these in a special paragraph. I will subsequently communicate the results of my investigation on each of these sutures. I wish to point out that for the present only those cases are being discussed in which the obliteration is limited to one single suture.

I shall begin with the coronal suture. There is a notable difference between the coronal and sagittal suture concerning the starting point from which the obliteration begins. This point is always the same in the sagittal suture, it is the so-called obelion. I have found no cases in which the frontal half of this suture was closed, while the occipital was left open. In the coronal suture on the contrary this regularity does not exist at all and the synostosis between the parietal and frontal bones may commence at any point of the suture. Moreover the synostosis in most cases is asymmetrical and only proceeds more symmetrically when starting at the bregma-point. These differences clearly show that the process in the coronal suture in some ways is of another character compared with the sagittal suture.

In table 4 I gathered the cases in which the coronal suture was partially or totally obliterated.

A comparison of the contents of this table and the former, referring to the sagittal and masto-occipital suture, shows immediately that here one has to reckon with a different category of phenomena. For a non-complicated obliteration of the coronal suture only appeared in 6 of the 1820 infantile skulls. Therefore one can surely consider a premature obliteration of this suture to be exceptional. Once more I lay stress upon this fact, because it is of great importance for the general question concerning the etiological nature of the pre-

TABLE 4

Obliteration of the sagittal suture

GROUP	NUMBER	OBLITERATION	
		entire	partial
I	725	1	2
II	400	0	1
III	168	0	1
IV	157	0	0
V	109	0	0
VI	203	0	1
VII	58	0	0
	1820	1	5

mature concrescence. In the preceding paragraph I demonstrated my view on the significance of the premature closure of the sagittal and masto-occipital suture, and in particular I objected there to the conception of a pathological process, result of a general constitutional disease, causing the obliteration of these sutures. For when in two sutures (which possess as to the development of the skull identical significance, as is the case with the sagittal and coronal sutures) a premature obliteration appears in the former 47 times and in the latter only 6 times, then one must conclude that other and more special influences have to be regarded as causing the difference. If the obliteration was caused by a general and constitutional disease, one would expect the number of premature obliterations in both sutures to be almost the same. Here I repeat that I do not wish to deny that general diseases of the skeletal system can evoke an unfavorable influence on the sutures of the skull. Then still there is no reason why the osteogenesis, which can be disturbed in all other subdivisions of the skeleton by diseases of the bony tissue, should remain always normal in the skull. The abnormal process should present a character of generality and irregularity and the suture-system should show different signs of the disturbing influence. In the sagittal and masto-occipital suture the obliteration shows too clearly a sharply defined morphological character.

In the coronal suture, however, it appears to be of a more irregular character, as follows from the fact that in cases of partial concrescence at one time a certain point of the suture is obliterated, at another time again a different point. In the five cases of partial concrescence I found the following conditions: once the right half was totally obliterated and of the left half the lower part; once only the right half was totally obliterated; once the upper part of the right half, once the upper part of the left half and once the lower part of the left half. The contrast with the sagittal suture in which the synostosis regularly appears in the occipital half is indeed very manifest.

Rarer still than the non-complicated synostosis of the coronal suture is that of the parieto-temporal (squamosal) sutures. On the whole I only encountered three infantile skulls of my collection in which this was the case: *i.e.*, two in Group I and one in Group II. Twice the middle part of this suture on the left side of the skull was obliterated, and once the hindmost part of the suture on both sides. These cases do not call for a special consideration.

A premature synostosis of the fronto-sphenoidal suture I found four times in infantile skulls belonging to the first, second, fourth and sixth groups. It was a remarkable thing that the process appeared symmetrically, in all these cases the synostosis being noted on both sides. This does not seem to me to be of a special significance, for the suture between the sphenoidal and parietal bone I only found obliterated once unilaterally (left side) in an infantile skull from Group III. I found, moreover, in this same group a skull in which a part of the left half of the lambdoid suture has disappeared.

These are the cases in which a synostosis, total or partial, of only one single suture was seen. Before we pass to the examination of the more complicated cases I will give a table (5) containing the facts heretofore stated.

This table shows very clearly the typical place occupied more particularly by the masto-occipital suture but also by the sagittal suture with regard to the premature obliteration.

TABLE 5

Premature synostosis in one suture only
Number of skulls 1820

SUTURE	NUMBER OF OBLITERATIONS
Masto-occipital	180
Sagittal	47
Coronal	6
Squamosal	3
Fronto-sphenoidal	4
Spheno-parietal	1
	241

THE PREMATURE OBLITERATION OF TWO SUTURES AND MORE

We will begin with the discussion of the more simple cases in which only two sutures were prematurely closed. It is quite natural that amongst this group the coincidence of a synostosis in the sagittal and masto-occipital suture appears most frequently. One will remember that the obliteration of the masto-occipital suture, either unilateral or bilateral, has been found in no less than 10 per cent of the infantile skulls. The probability therefore that such an instance can be complicated with an obliteration of the sagittal suture is not small. Now, the same possibilities may occur in these cases, as in the non-complicated synostosis of the masto-occipital suture. Together with the sagittal suture the masto-occipital can be obliterated bilaterally or unilaterally, totally or partially. It does not seem necessary for me to describe all these cases in detail, as no principle is involved. I will restrict the communication to those cases in which the premature obliteration appeared in both sutures. A summary of this is seen in table 6.

As this table shows, we find among 1820 skulls 19 in which at the same time the sagittal and masto-occipital sutures were no longer intact, this making 1 per cent. The absolute numbers are too small to decide whether the frequency increases according to the age of the individuals. One can demonstrate however that a predisposition of these two sutures toward a premature closure is revealed by the relative large number of cases

TABLE 6

Obliteration of the masto-occipital suture in skulls in which also the sagittal suture is totally or partially closed

GROUP	BILATERAL	LEFT ONLY	RIGHT ONLY	TOTAL
I.....	2	1	2	5
II.....	1	0	1	2
III.....	3	0	0	3
IV.....	2	0	0	2
V.....	0	0	0	0
VI.....	4	1	0	5
VII.....	1	0	1	2
	13	2	4	19

in which this combination coincides. It can be proved in the following simple way. The frequency of the premature obliteration exclusively in the masto-occipital suture is 10 per cent, that of the sagittal suture 3 per cent. Therefore, when both phenomena were totally independent of each other a combination of both should then according to the rules of probability never come to 1 per cent, as we have been able to determine. Thus this very frequent coincidence can only be explained on the assumption that the cause of the premature obliteration in one of the two sutures at the same time increases the predisposition to a simultaneous obliteration in the other suture. In one of the preceding paragraphs I have developed my view as to the cause of the obliteration. This too is sufficient to explain the relative large frequency of the simultaneous obliteration in the sagittal and masto-occipital sutures.

The other cases in which together with the masto-occipital suture yet another was obliterated were the following. In three cases the masto-parietal and in one case the coronal suture was obliterated simultaneously with the masto-occipital suture. Finally I found a case in which the posterior half of the squamosal suture and the whole masto-parietal suture were obliterated.

On the whole there were found in my collection 24 infantile skulls in which two sutures were coalesced.

Finally there were amongst my material a small number of skulls in which the premature obliteration had assumed a more

extensive character and showed a more irregular form. It is impossible to divide these cases according to a presumed point of view in groups. Therefore I will give only a simple description of them.

Amongst the infantile skulls with milk-dentition (Group I) I found the following cases of complicated closure:

1. On the right side: the posterior half of the squamosal suture, the masto-parietal and the masto-occipital suture; on the left side: the masto-parietal and masto-occipital suture.

2. On the right side: the whole of the squamosal suture, the masto-parietal and the masto-occipital; on the left side only the masto-occipital suture.

3. On the right side: the spheno-frontal and spheno-parietal suture, the lower half of the coronal suture, the posterior half of the squamosal suture, the masto-parietal and masto-occipital suture; on the left side the spheno-frontal, the spheno-parietal, the lower part of the coronal, the lower part of the lambdoid and the masto-occipital suture.

4. The occipital half of the sagittal and the right half of the lambdoid sutures.

5. On the right side: the lower half of the coronal, the hinder part of the squamosal, the masto-parietal and the masto-occipital suture. On the left side the coronal suture.

6. The sutura sagittalis totally. On the right side: the spheno-parietal, the squamosal, the masto-parietal and masto-occipital and the lower half of the lambdoid suture. On the left side: the spheno-parietal and the masto-occipital sutures.

7. The occipital part of the sutura sagittalis. On the right side: the spheno-parietal and masto-occipital suture. On the left side: the squamosal, masto-parietal and masto-occipital sutures.

8. The sutura sagittalis. On the right and left side the occipital half of the squamosal, the masto-parietal and the masto-occipital suture.

9. The sutura sagittalis. On the right and left side the mesial part of the coronal suture.

Amongst the skulls of Group II the following cases were found:

10. On the right side: the lower half of the coronal, the whole of the masto-occipital suture. On the left side: the coronal suture and partly the masto-occipital suture.

11. On the right side: the masto-occipital and masto-parietal suture. On the left side: the hinder part of the squamosal, the masto-parietal and masto-occipital suture.

12. The sutura sagittalis. On the right side: the masto-occipital suture; on the left side the lower half of the coronal, the masto-parietal and masto-occipital suture.

Amongst the skulls of Group III, the following case was found:

13. On the right side: the whole of the squamosal and the masto-occipital sutures. On the left side: the masto-occipital suture.

Amongst the infantile skulls of Group IV, the following cases were found:

14. On the right side: the hinder part of the squamosal and the masto-occipital suture; on the left side: the squamosal, masto-parietal and masto-occipital sutures.

15. The sutura sagittalis. On the right and left side: the spheno-parietal, the masto-parietal and masto-occipital sutures.

Amongst the skulls of Group V I found the following cases:

16. The sutura sagittalis partially on the right and left side: a hinder part of the squamosal and the masto-parietal sutures.

17. On the right side: the posterior half of the squamosal, the masto-parietal, the masto-occipital sutures. On the left side as on the right, and moreover the lower half of the lambdoidal suture.

Amongst the skulls of Group VII I found the following case:

18. The sutura sagittalis partially. On the right side: the masto-occipital suture. On the left side: the posterior half of the squamosal, the masto-parietal and the masto-occipital suture. The number of non-adult skulls in which the premature obliteration of the sutures assumes an irregular character on a larger scale amounts to 1 per cent (18 skulls amongst 1820).

When examining these cases more closely, and seeking to determine the question in which suture the greatest amount of ob-

literation occurs in cases of more extensive premature closure, the very interesting fact presents itself that it is the squamosal suture. To demonstrate this fact, I recall that amongst 1820 infantile skulls there were only three in which only the suture mentioned showed signs of obliteration, forming a striking difference with the sagittal suture, in which this appeared 47 times. On the other hand I find amongst 18 skulls with more extensive and irregular premature obliteration not less than 11 in which the suture squamosa was no longer intact, and only 9 in which the sutura sagittalis was partially or totally obliterated. I call attention to this fact because it speaks in favor of my opinion that the isolated obliteration of the sagittal suture is caused by a special cause.

Finally I shall proceed to give a general view of the sutural obliteration in our collection of skulls considered as a whole. In this collection, consisting of 1820 skulls, I found a premature obliteration either in a single suture or in more, in no less than 343 skulls. This amounts to 19 per cent. This result, due largely to the very frequent obliteration of the masto-occipital suture, I did not expect, neither should it have been expected by anybody.

In the preceding paragraphs I pointed out how frequently only a single suture in a skull showed a more or less extensive obliteration, while all others remained intact. This, of course, did not give a real idea as to the number of times in which each of the sutures amongst the 1820 skulls really was obliterated, because amongst these, the cases in which more than one suture showed signs of obliteration, were not counted. In the table 7 I give a short summary which shows how many times each suture was obliterated either totally or partially. If this occurred, as is possible in paired sutures, on both sides, the case is only once counted.

The extraordinary frequency of the obliteration in the masto-occipital suture is very obvious, and no less that of the sagittal suture. To estimate the frequency correctly one has to compare, of course, the bilateral sutures with each other, and also the unpaired. Then the difference between the sutura masto-

TABLE 7

Absolute frequency of premature obliteration in 1820 skulls

	times
Sut. masto-occipitalis	272
Sut. sagittalis	71
Sut. squamosa	17
Sut. parieto-mastoidea	16
Sut. coronalis	12
Sut. parieto-sphenoidalis	5
Sut. fronto-sphenoidalis	5
Sut. lambdoidea	5

occipitalis with 272 cases or 15 per cent and the sutura squamosa with 17 cases, or 1 per cent is no less surprising than that of the sutura sagittalis with 71 cases or 4 per cent and of the coronal suture with 12 cases or 0.6 per cent. Once more it is demonstrated by these relations that a premature obliteration of the sagittal suture occurs more often than was formerly believed, while that of the masto-occipital suture occurs so often that it can scarcely be considered an anomaly.

From the annotations, collected during my investigation, I finally will communicate a very interesting observation. As generally known it may happen in the skull of man that the sutura frontalis persists. According to the communications of the authors, this should be the case in about 6 per cent. In Dutch skulls the persistence of this suture is found in not quite 9 per cent. It has struck me, however, that I did not find in my collection of non-adult skulls the coincidence of a persisting sutura frontalis and premature obliteration in one of the other sutures. This seems not inconceivable in case of the sagittal suture, for this suture and the frontal can be considered as two parts of one, extending from the nasion to the lambda. But also nearly the same was stated as to the masto-occipital suture. There were, as mentioned, 272 skulls with premature obliteration of this suture, but according to the general relation, one should expect to find amongst these skulls, 9 per cent or 24 with a persisting metopical suture. In reality I only found two cases.

This fact however is not altogether inconceivable. When the metopical suture, obliterating normally between the second and third year, persists this fact points to a decreasing tendency of the sutures of the skull to coalesce. And that in such skulls a premature obliteration does not take place, seems to me a very natural phenomenon. This fact can be considered as a new proof of the justness of my opinion that premature obliteration of the sutures is not caused by a general pathological influence, but that it is a phenomenon of which the origin has to be looked for in the sutures themselves and in the process of growth localized in them.