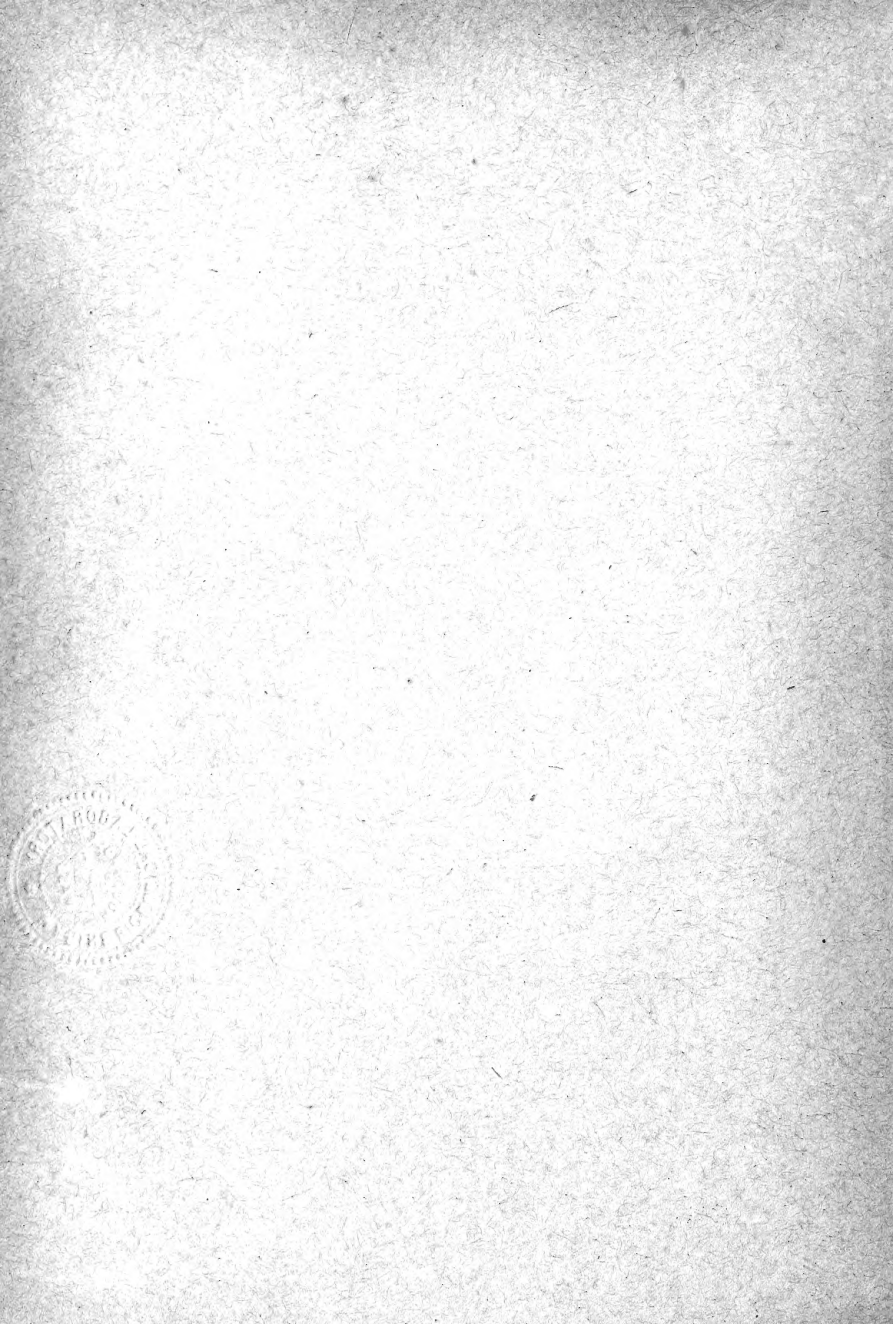

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WILLIAM A. LOCY AND OLOF LARSELL

TWENTY-TWO FIGURES

PART II[1]

3. THE AIR-SACS AND THE RECURRENT BRONCHI

Morphologically considered, the air-sacs and recurrent bronchi are parts of the bronchial tree, but on account of their importance in the avian lung and their unusual interest they are separately considered in this section. This plan also promotes clearness of description, since, at best, the bronchial tree is very complex. The recurrent bronchi, in particular, should receive special notice, because they have been recently recognized and are of capital importance in the physiological anatomy of the lungs.

The name 'recurrent bronchi' has been given to certain bronchial tubes that grow from the air-sacs into the lungs of birds to connect with the other air passages. In this sense they are 'recurrent.' They are outgrowths from the air-sacs, rather than extensions of the bronchial tree from within the lung, and the air-sacs and recurrent bronchi are so intimately related in their development that the two structures should be considered together. In the course of development they unite with twigs of the bronchial tree and thus establish complete circuits with the air passages within the lungs. In the adult lung the air passes from the air-sacs through these recurrent bronchi, entering the lung by a returning current, and, in this sense, the air circuit through these bronchi is a recurrent one.

The credit for the recognition of the morphological arrangement as well as for the part which recurrent bronchi play in the respiration of birds should be divided between Schulze ('09 and

[1] Part I of this paper appeared in the American Journal of Anatomy, vol. 19, no. 3, May, 1916.

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'10) and Juillet ('12) who, working independently, and without knowledge of each others observations, grasped the essential features of these very important and characteristic structures of the avian lung. Although they were figured by earlier observers (Campana, '75, Fischer, '05), Schulze was the first to observe carefully their arrangement and relation to the air-sacs and to the other bronchi in a number of different birds, and to appreciate their physiological rôle, while Juillet added some morphological facts and made observations on their development.

Review of the extensive literature on the air-sacs seems to us unnecessary, since the bulk of it relates to their position, size and anatomical relationships in the adult. In this respect the papers of Campana, '75, Bruno Müller, '07, and Schulze, '11, are especially good. As regards their development, except for the paper of Bertilli, little has been added to the embryology of the air-sacs since Selenka's paper of 1866 in which he described and figured their development in the chick.

The recurrent bronchi, however, have come into notice more recently and a brief account of the published observations on these structures should be given.

Campana ('75) in his extensive memoir dealing with the respiratory apparatus of birds and confined chiefly to a description of the adult structures, described the air-sacs and bronchial tree. He also made a careful analysis of the orifices connecting lung and air-sacs. Examination of his figures shows that some of the recurrent bronchi also stand out quite distinctly, but Campana considered them as the result of a reconstitution into a single trunk of several tertiary bronchi, without recognizing their true nature. He apparently considered them as merely a part of the network of air passages with no special significance attached to them.

On the other hand Campana used the term 'Bronche recurrente' in an entirely different connection, applying it to the curvilinear branch of the first entobranchus. This statement should be made to prevent confusion.

Guido Fischer ('05) likewise figures the recurrent bronchi of several of the air-sacs. The only reference he makes to these

features of his celloidin corrosion preparations, however, is in a note of explanation of one of his figures in which he calls attention to a bronchial trunk larger than the others in the network of air-tubes which extends to the dorsal surface of the lung on its lateral side. This bronchial trunk, he says, 'directs itself toward' the abdominal air-sac, but since it is the nature of recurrent bronchi to grow inward from the air-sacs, the language employed by Fischer shows that he had a wrong conception of these important structures.

F. E. Schulze in 1909, '10 and '11 recognized these bronchi as coming from the air-sacs and designated them both 'Rüchläufigen Bronchen' and 'Bronchi recurrentes sue Saccobronchi.' With sketches he describes their origin from basal pockets on the four posterior air-sacs, variations in the number of their branches as well as the nature of their connections with parabronchi. His comparative observations embraced a variety of birds including the chick, duck, goose, pigeon, Rhea, ostrich, Cassowary, etc. In the Cassowary he noted that recurrent bronchi are lacking on the abdominal air-sac. Schulze also points out that the recurrent bronchi carry air from the air-sacs into the lung parenchyma and play an important part in respiration.

Juillet ('12) made an extensive study of the recurrent bronchi, and since he was unacquainted with the observations of Schulze he claims rank as the discoverer of the true anatomical relations of these structures and of the part they play in the respiration of birds. In all this however he was preceded by Schulze, and to a limited extent he engaged in the study of the embryology of the recurrent bronchi, which was not touched upon by Schulze.

He found recurrent bronchi in all the twenty-four species of birds which he examined. By a study of sections he traced some stages of their development in the embryonic lungs of the chick, and although he does not give an extended account of their developmental history, he arrived at a true conception of their origin and of their nature.

No more important advance in the knowledge of the avian lung has been made since William Harvey, in 1651, discovered the perforations of the bronchi into the air-sacs and found them

"sufficiently conspicuous in the ostrich to admit the points of my fingers."

It will be advantageous to describe the development of air-sacs and recurrent bronchi by stages beginning with the seventh day.

The seventh day stage. There are five air-sacs in the lung of the adult fowl, and as will be shown later, one of these (the interclavicular) is the result of the fusion of four moieties, two from each lung, that arise independently. The names employed in the following descriptions are: cervical, interclavicular, anterior intermediate, posterior intermediate, and abdominal air-sacs. All the air-sacs, except the interclavicular of the adult, are paired. The cervical and interclavicular arise anteriorly, the other three upon the ventral and caudal surface of the lung.

The youngest embryo in which any of the air-sacs appear as projections beyond the lung wall are of about six days six hours incubation. As shown in figure 30, the abdominal air-sac of this stage projects as an extension from the lung proper. The primordium of this sac is the slightly expanded distal portion of the mesobronchus lying beyond the bend of the central lung tube.

In the same embryo may be seen the first indication of the cervical air-sac in the form of a bud projecting from the distal extremity of the first entobronchus. In its later development the entobronchus becomes much branched, and the orifice of the air-sac is not terminal, as in the embryo, but on the body of the cranial branch of the entobronchus.

The anterior intermediate air-sac, with the mesial moiety of the interclavicular united to it, is also foreshadowed in this specimen as a bud of the third entobronchus. The third entobronchus shows at this stage. The beginning of an unequal bifurcation which shortly (figs. 34 and 37) becomes well differentiated. The more caudad, and longer, branch of the bifurcation develops into the foliate division of the entobronchus, and the forward projecting bud becomes eventually differentiated into the anterior intermediate air-sac and the mesial moiety of the interclavicular sac. To avoid confusion, one should constantly keep in mind that the interclavicular air-sac arises from two moieties on each

side, and in subsequent references we should follow with care the development of a mesial and of a lateral moiety from different sources.

The ninth day stage. In the interval between the seventh and the ninth day the entire bronchial tree grows rapidly and the air-sacs enlarge.

Early on the ninth day of incubation carefully prepared air injections show important advances. The primordia of all five air-sacs now project beyond the lung surface.

The cervical sac (fig. 36, *Cerv.sc.*) is the forward prolongation of the cranial lobe of the first entobronchus and is little changed from the former stage.

In the meantime the first entobronchus has divided into several branches and from one of them (the transverse branch) may now be seen the beginning of the lateral moiety (*Lat. moi.*) of the interclavicular sac. At this stage it is small and does not project beyond the lung wall. As shown in subsequent development this lateral moiety fuses with the mesial moiety to form a part of the interclavicular sac of the adult.

The mesial moiety is well developed at this stage. It arises on an anterior branch of the third entobronchus. This branch bifurcates early on the seventh day of development (not figured). The smaller, and more cephalad, division becomes the mesial moiety of the interclavicular air-sac, the larger, and more caudad, division the anterior intermediate air-sac. As shown in figure 37, the mesial moiety, although very slender on the ninth day, is nevertheless sufficiently elongated to project beyond the lung wall.

Exceptionally the mesial moiety arises on a branch of the second entobronchus, in which case the third entobronchus gives origin only to the anterior intermediate sac. This condition is illustrated in figure 38 which represents a slightly earlier stage than the one sketched in figure 37.

When the development follows the usual rule the mesial moiety of the interclavicular sac is an offshoot of the anterior intermediate air-sac and the two are connected with the third entobronchus by a single orifice (the interclavicular canal).

By unequal growth the anterior intermediate air-sac (fig. 37) has increased relatively much faster than the mesial moiety of the interclavicular and forms, at this stage, a prominent landmark on the ventro-mesial part of the lung. It remains until the eleventh day of incubation the largest of the embryonic air-sacs.

Extending forward from its ventral anterior part may be seen three small papilla-like buds (*Rec.br.*) connected with the sac by a short stem. These buds are the beginnings of the recurrent bronchi of the anterior intermediate air-sac. They make their first appearance (not figured) as a single bud during the latter part of the seventh day of incubation, and by division of the distal end of this bud the three papillæ are formed. The proximal end remains as the stem and probably forms the basal pocket of Schulze.

The posterior intermediate air-sac (fig. 36, *P.int.sc.*) also makes its first appearance as a projection beyong the lung wall on the ninth day. It is the distal continuation of the third laterobronchus, and at this stage is but slightly distended and shows no indication of recurrent bronchi.

The abdominal air-sac, is on the ninth day of incubation greatly elongated. From its anterior end and point of union with the mesobronchus a pouch is developed which represents the beginning of recurrent bronchi of this sac. The distal end of the sac is but slightly more inflated than it was on the seventh day, but about two-thirds of it now project beyond the lung proper.

From the position of the pouch of the recurrent bronchi one would infer that the anterior limit of the abdominal air-sac of the embryo is more cephalad than is usually recognized. This also changes our idea of the position of the morphological tip of the mesobronchus.

The tenth day stage. From the beginning of the ninth day of incubation to the close of the tenth there is a steady growth of the various air-sacs and of their recurrent bronchi. It is not necessary to follow in detail the various steps between the two stages, since a description of the conditions found in the later stage will sufficiently indicate the changes through which the various structures have passed.

In an embryo of $9\frac{1}{2}$ days incubation (figs. 39 and 40) the cervical sac shows only an increase in size proportioned to the general growth of the lung.

The lateral moiety of the interclavicular sac (fig. 40, *Lat.moi.*) now shows a well defined extension outside the lung wall. The subsequent history of this sac indicates that some of the intrapulmonary part of the transverse branch of the first entobronchus, from which the sac arises, must be considered as a part of the air-sac primordium. The line of separation betwen the entobronchus and the lateral moiety is where the more dorsal buds of recurrent bronchi arise. Two groups of recurrent bronchi belonging to this division of the interclavicular have appeared. The bud which represents the more ventral group of these bronchi extends caudally and ventrally and is as yet undivided. The more dorsal group is represented by an already bifurcated bud projecting caudally and somewhat mesially. These buds develop into the only recurrent bronchi arising from the interclavicular air-sac and it is to be noted that they arise only on its lateral moiety.

The mesial moiety of this stage, a part of which is shown in figure 40, is scarcely changed from the condition described in the eight day embryo, except that it has increased in size.

The anterior intermediate air-sac (*A.int.sc.*) at the close of the tenth day of incubation has enlarged considerably, as compared with the preceding stage. The recurrent bronchi have not greatly changed, but the stem thereof has elongated to some extent.

The posterior intermediate sac now projects beyond the lung, and its distal end forms a flask-like swelling. The proximal part remains still constricted and lies within the lung. From this part two buds are seen projecting dorsad and cephalad. The more anterior of these buds is already divided at its tip. These branches indicate the beginnings of the recurrent bronchi of the posterior intermediate air-sac.

The abdominal sac (figs. 39 and 40) has expanded greatly since the eighth day stage, and now lies almost entirely outside the lung. Its recurrent bronchi, of which there are two sets, have

also made an obvious growth. The bud (fig. 39) previously described has divided into two main branches each of which has in turn bifurcated, as represented in the figure.

A second group of recurrent bronchi, also belonging to the abdominal sac, begins early in the tenth day of incubation. The

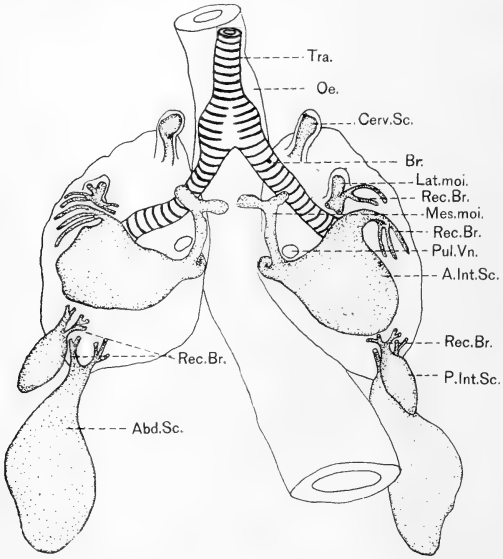


Fig. 47 Ventral view of the uninjected lungs of a ten and one-ha f day embryo. Shows the air-sacs and their recurrent bronchi. The relations of the mesial and lateral moieties of the interclavicular air-sac are well exhibited. *Rec.Br.*, recurrent bronchi, other reference letters as under figure 34.

bud for this group starts at a point just dorsal to the base of the first-formed group. At the close of the tenth day this outgrowth (fig. 39) has bifurcated so that there is an anterior and a lateral limb. The anterior limb curves gently upward. The lateral branch makes a more sudden turn posteriorly.

The eleventh day stage. The eleventh day stage is of some especial interest since it corresponds with Selenka's figure (78) in

which two moieties of the interclavicular sac are shown on the left hand of the figure (right lung). The air sacs are all clearly outlined at this stage and the recurrent bronchi have started.

The air-sacs as shown in figures 47 and 48 are relatively much larger than in the earlier stages.

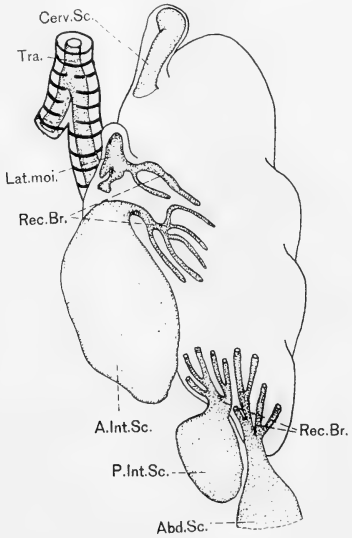


Fig. 48 Lateral view of the left lung of the same specimen. Showing the early condition of the recurrent bronchi of the four air-sacs possessing them. The mesial moiety of the interclavicular sac is hidden from view.

The cervical, the least modified of all the air-sacs, exhibits an expansion of the distal end, but no recurrent bronchi are developed from it.

The lateral moiety (fig. 47, *Lat.moi.*) of the interclavicular sac now projects well beyond the lung surface. The beginnings of the recurrent bronchi of this moiety (first seen in the tenth day stage) have elongated and undergone division. They are not

symmetrically developed, those of the right lung showing more branches. Schulze has pointed out that, after the union of the parts to form a single median sac, there are, commonly, recurrent bronchi only on one side.

The mesial moiety (*Mes.moi.*) of the interclavicular sac is forked at its extremity into two lobe-like branches, the more mesial of which extends towards the median plane and comes nearly into contact with the corresponding branch from the opposite lung. The laterally extending branch, passes ventral to the extra pulmonary bronchus, and partly engirdles the bronchus on the left hand of the figure.

The anterior intermediate air-sac (*A.int.Sc.*) has increased in size and its recurrent bronchi are much further developed. They occupy the antero-lateral border very close to those of the lateral moiety of the interclavicular sac.

Figure 47 shows also the connection between the mesial moiety of the interclavicular, and the anterior intermediate—both arising on a common canal (interclavicular canal) that opens into the third entobronchus (entobronchus not shown).

The posterior intermediate and abdominal air-sacs lie at the caudal extremity of the lung and have obviously increased in size. In figure 47, only the proximal ends of the recurrent bronchi have been sketched since these show on the surface. They exhibit the same relationships as in the tenth day stages.

Figure 48, which is a dorso-lateral view of the same specimen shows to greater advantage the recurrent bronchi from the lateral moiety and the anterior intermediate air-sac at ten and one-half days. The specimen in this position also shows the recurrent bronchi from the posterior intermediate and abdominal sacs. The enlargement at the base of the recurrent bronchi well exhibited in the posterior intermediate probably corresponds to the basal pocket of Schulze.

Although the general appearance of the air-sacs on the tenth day of development (figs. 36 and 37) have been described, it will be advantageous for comparison to insert at this point a separate sketch of the anterior intermediate air-sac and the mesial moiety of the interclavicular sac. Figure 49 represents these air-sacs as

removed from the left lung of an embryo of nine and one-half days incubation. (A) represents a view upon that surface of the anterior intermediate air-sac which is in contact with the lung. It is notable for showing the primordia of recurrent bronchi budding from the anterior lateral border of the air-sac. By comparison with figure 47 it will be observed that the recurrent bronchi of this sac lie close to those from the lateral moiety of the interclavicular sac.

Figure 49 shows further the mesial moiety of the interclavicular sac spinging from a common canal (the interclavicular canal) into which the anterior intermediate sac also opens. The interclavicular in turn opens into the third entobronchus. No recurrent bronchi arise from the mesial moiety. (B) shows a view upon the cephalad surface of the anterior intermediate air-sac and brings into prominence the forked extremity of the mesial moiety of the interclavicular air-sac, and, also shows the common origin from the third entobronchus of the mesial moiety and the anterior intermediate sac.

The twelve and fifteen day stages. The subsequent history of the air-sacs presents little difficulties except as regards the formation of the azygous condition of the interclavicular sac of the adult from the union of four parts which arise separately in the embryo. The changes in this air-sac are so unusual that they will be described more in detail.

The condition of the air-sacs at the close of the twelfth day of incubation is represented in figure 50. This is the camera outline of a dissection and in finishing is made only slightly diagrammatic. The external aspects of lungs and air-sacs are represented but the recurrent bronchi have not been sketched. By comparison with figure 47 (the eleventh day stage) it will be seen that the medially directed forks of the mesial moieties of the interclavicular sac have expanded and approach each other more closely in the interbronchial region. The cranially directed prongs have also lengthened and extend forward nearly parallel to the trachea.

In this figure the connection between the mesial moiety of the interclavicular and the anterior intermediate air-sac is clearly

shown as well as the single orifice by which they open into the lung.

The lateral moiety of the interclavicular is relatively larger than in earlier stages but otherwise shows no marked change.

Between the twelfth and fifteenth days occur relatively rapid expansions of the moieties of the interclavicular sac. The mesial moieties have fused with each other along the median line

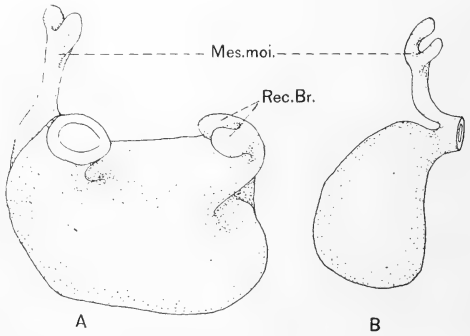


Fig. 49 Anterior intermediate air-sac of the left lung of an embryo, nine and one-half days incubation. (A) View upon the surface that is in contact with the lung. Notable for showing the primordia of the recurrent bronchi springing from the anterior intermediate air-sac The mesial moiety of the interclavicular air-sac is also shown. *Ent.* 3, opening into the third entobronchus; *Bd.*, buds of recurrent bronchi; *Mes.moi.*, mesial moiety of the anterior intermediate air-sac. (B) the same as seen from the cephalic end. Illustrates the connection between the anterior intermed ate and the mesial moiety of the interclavicular air-sacs, and also the forked extremity of the mesial moiety.

between the two lungs. They have apparently also united with the greatly expanded lateral moieties of the interclavicular (fig. 51). The dividing membranes remain for several days subsequent to the fusion of these different parts. The wall between the mesial moieties does not disappear until the first day after hatching. The septum between the mesial and lateral moieties is less persistent and, so far as dissections indicate, breaks down during the eighteenth day. In attempting to designate the time

at which particular morphological changes occur one must, as previously indicated, take into account that individual variation is very common in embryonic development.

The lateral moieties (fig. 51) have greatly expanded so as to unite in the median line. At this period they are the most prominent part of the interclavicular sac. The stalks connecting this portion of the interclavicular with the first entobronchus are

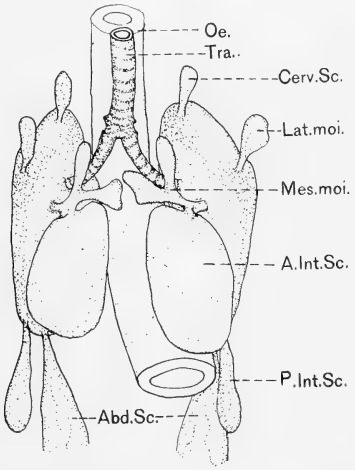


Fig. 50 Ventral view of the lungs and air-sacs of a twelve day embryo.

clearly indicated but the recurrent bronchi have not been shown. At the extreme antero-lateral margin of this division of the sac there is a narrow neck opening into the axillary sac.

The method of formation of the single interclavicular sac of the adult is now clearly foreshadowed. The four parts from which it is formed (two moieties from each lung) are in contact but still separated by partition walls.

The sixteenth day stage. The recurrent bronchi are now sufficiently advanced to observe the main features of their distribution. Figure 52 represents a partly diagrammatic camera trac-

ing of a dorso-lateral view of the lung and shows the relations of the recurrent bronchi of the two posterior air-sacs at the close of the fifteenth day of incubation. The recurrent bronchi are represented black and the other air passages in stipple.

The distal tips of the longest recurrents of the abdominal air-sac have anastomosed with the latero-ventral parabronchi of

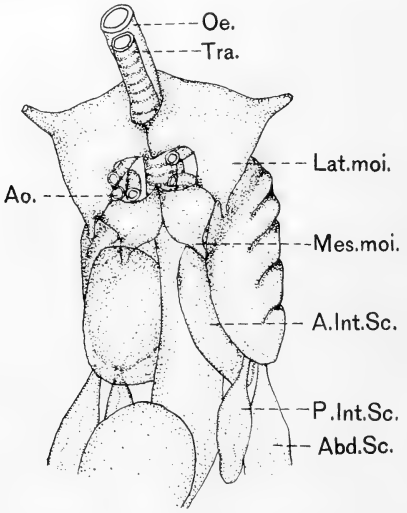


Fig. 51 Latero-ventral view of the lungs and air-sacs of a fifteen day embryo. Figures 48, 49 and 50 should be compared to show the progressive development of the air-sacs and the way in which the four moieties of the interclavicular become united.

the first entobronchus. The more dorsal branches unite with parabronchi of laterobronchi. It is worthy of notice that the main stems of the first and second groups of recurrent bronchi of the abdominal sac have united so that the second group appears to be a branch of the first. By reference to figures 39 and 40 it will be seen that the two groups were originally separate. By

uniting into a single trunk there is a single orifice (*do.*) opening from the abdominal sac into the recurrent bronchi.

The recurrent bronchi of the posterior intermediate air-sac do not extend so far forward as do the branches of the preceding group. They occupy the extreme ventral part of the lung. Their anastomoses (not sketched) are principally with parabronchi of the first and second laterobronchus.

The recurrent bronchi of the two other air-sacs anastomose during the sixteenth day with parabronchi in parts of the lung adjacent to them. The distal ends of the recurrents from the interclavicular anastomose chiefly with the more ventral parabronchi of the first entobronchus and the recurrents of the anterior intermediate sac unite with parabronchi of the latero-ventral part of the lung. It results that the anterior intermediate sac comes into communication with the air circuits in the latero-ventral lung region and the interclavicular sac comes into communication with passages in the anterior part of the lung.

As already pointed out, the anastomosing twigs are at first very slender, but, by the eighteenth day of incubation have increased in diameter so as to be practically the same size as the branches which they connect. The recurrent bronchi have by the eighteenth day of development assumed the relations to other parts of the bronchial tree which they bear in the adult lung.

Transition to the adult. In showing how the adult condition is reached it will be advantageous to summarize the principal changes subsequent to the eleventh day after which period the sacs grow more rapidly.

The abdominal sacs expand so as to fill the abdominal cavity, and partly surround the viscera therein contained. About the fourteenth day the walls of these sacs begin to fuse with the peritoneum and this fusion is apparently completed sometime before the eighteenth day of development. The left abdominal sac is somewhat larger than is the right.

The history of the posterior intermediate sacs after the eleventh day is closely parallel to that of the abdominals and does not require detailed description.

The same general course is followed by the anterior intermediate sacs. Their walls fuse with the lining of the thoracic cavity.

The cervical and interclavicular sacs attain their most rapid growth after the twelfth day of development. The cervical sacs grow forward toward the neck of the chick and between the fifteenth and nineteenth days of incubation their walls fuse to some degree with the pleura. They give rise to several subdivisions in the cervical and axillary regions.

The later stages of the interclavicular sacs require a more extended description than the others because of marked differences in their formation.

Returning to figure 47, the representation of the condition in the ten and one-half day embryo, we note again that the mesial moiety is bifurcated at its distal extremity. The more mesial lobe thus produced expands in such a manner that its walls come into contact with the walls of the corresponding lobe of the interclavicular sac of the opposite lung. This phase is reached on the fifteenth day of incubation (fig. 51). By the nineteenth day fusion of the walls has taken place, but there appears to be no breaking down of the septum thus formed. This appears also to be the case with the fused walls of the more anterior portion of the sac. This condition was demonstrated both by dissections and by Wood's metal casts of the adult lungs and air-sacs.

On the sixteenth day of development portions of the mesial moiety of both sides grow ventrally over the bronchus, and come into contact with the lateral moiety of the interclavicular sac. The membranous walls subsequently begin to fuse, and on the eighteenth day union is approximately completed. The single septum thus formed disappears sometime between the nineteenth day and the end of the first day after hatching, so that the two hitherto independent moieties coalesce to form one sac (fig. 53).

Thus the single large interclavicular sac of the adult is the result of the union of two moieties on each side which arise from different entobronchi. As diagrammatically illustrated in figure 53, the union of the sacs and the disappearance of the septum is completed by the close of the first day after hatching.

So far as we are aware a similar history of the formation of the interclavicular sac has not been given. It has been known since the time of Sappey ('47) that the single interclavicular sac of the adult was produced by the union of two parts, but the formation of two moieties on each side from independent sources

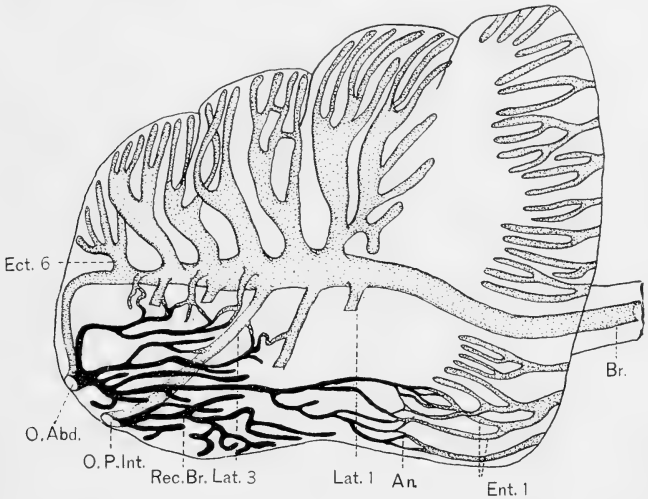


Fig. 52 Diagram of the lateral surface of the right lung of a fifteen day embryo, to show the recurrent bronchi of the abdominal and of the posterior intermediate air-sacs. The outlines of the recurrent bronchi (in black) so far as represented were traced with the aid of the camera lucida from an air injected preparation. Dotted portions diagrammatic. *O.abd.*, orifice of the abdominal sac; *O.P.Int.*, orifice of the posterior intermediate; *An.*, anastomosis of recurrent bronchi. Other reference letters as before.

and the details of their union had not, we believe, been anticipated.

We turn now from the air-sacs to the recurrent bronchi, the embryonic history of which has been outlined from their earliest appearance to the time when they have established their union with other branches of the bronchial circuits.

Since the orifices of the recurrent bronchi are usually close to the direct opening into the air-sacs, attention should first be called to Campana's ('75) analysis of orifices of the air-sacs: He divides them into two groups, the monobronchial and the polybronchial, according to the number of openings which they exhibit. The polybronchial orifices are further separated into two categories, the simple and the mixed polybronchials, the simple polybronchial being composed of several openings of one grade (parabronchi), and the mixed polybronchial embracing openings of two grades, or, as in the case of the interclavicular and the anterior intermediate, uniting two neighboring sacs. Juillet accepts Campana's terminology but substitutes a clear basis of distinction for his confused account of polybronchials. This distinction consists in recognizing direct and recurrent orifices as the components of the polybronchials. A monobronchial orifice must necessarily be direct, but a polybronchial group may be composed of both direct and recurrent orifices (mixed polybronchial or of recurrent orifices only (simple polybronchial).

According to this analysis, which is in harmony with the developmental history, the cervical sac has a direct monobronchial orifice only, since it does not possess recurrent bronchi. A number of branches of the first entobronchus ramify posteriorly into the lung from a point nearly opposite the direct orifice of the cervical sac. These possibly serve the same purpose as the recurrent bronchi of the other air sacs, but do not appear to be developed from the sac itself as are the tubes which have been called recurrent bronchi.

The interclavicular air-sac is connected with the lung by two groups of orifices. The more mesial orifice, which is of the monobronchial direct type, opens into a short tube, the interclavicular canal, which also receives the direct orifice of the anterior intermediate sac. This short tube in turn communicates with the third entobronchus.

The more lateral group of interclavicular orifices is mixed polybronchial, having both a direct and several recurrent orifices. The direct orifice is the opening of the ventral tip of the lateral branch of the first entobronchus, from which as already described

the lateral moiety of this sac has its origin. The recurrent orifices are three or four in number, and are the proximal openings of the recurrent bronchi. Reference to figure 47 will show that on the eleventh day of incubation there were but two recurrent openings connected with the sac. The change to the adult condition is brought about by the extension of the proximal ends of the original recurrent bronchial buds.

Juillet insists that there is no direct connection of the interclavicular with the first entobronchus, and accordingly, classifies this lateral group of orifices as simple polybronchial. We conclude, however, that the group is a mixed polybronchial and will return to the question under our general considerations.

The anterior intermediate air-sac also has two groups of orifices. The monobronchial direct orifice (figs. 47, 54) has already been mentioned in connection with the corresponding orifice of the interclavicular air sac. The other and more ventral group is of the polybronchial recurrent type. The number of orifices varies, but is usuall five or six.

The posterior intermediate sac communicates with the lung by a polybronchial group of orifices made up of both direct and recurrent tubes (polybronchial mixed). There is one direct orifice (figs. 53, 54), which is the opening of the third laterobronchus from which this sac has its origin. The recurrent orifices (figs. 53) represent the lungs of a one-day chick, but the relation of the orifices is the same as in the adult lung), three or four in number, as the openings of the recurrent bronchi and have a history very similar to that already described in connection with the two preceding sacs. The anterior ends of the posterior intermediate recurrent bronchi have for the most part anastomosed with the first and second laterobronchi.

The orifices of the abdominal air-sac are also of two kinds, direct and recurrent, which are so arranged as to form a mixed polybronchial group. The direct orifice (figs. 53 and 54) is the opening of the mesobronchus into the sac. The recurrent orifices arising as previously described are four or five in number. Schulze has shown that variations as to number exist in different birds being six to nine and sometimes reduced to one.

Figure 45 shows the surface aspects in the adult of the recurrent bronchi of the abdominal and posterior intermediate air-sacs to the bronchial tree and the way in which their stems connect with the air-sacs represented. In this metallic cast the abdominal air-sac was only partly injected so that the proximal end only is shown. It will be seen that the main recurrent bronchi of the abdominal air-sac extend more than one-third of the way

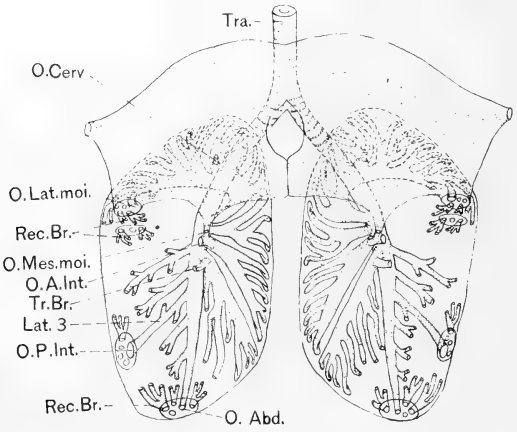


Fig. 53 Diagram of a ventral view of the lungs on the first day after hatching. The moieties of the interclavicular sac have united on each side and those of the right and left lung have come into contact, being separated only by a temporary partition wall. Shows also the nature of the orifices into the air-sacs. Cervical sac not represented.

toward the ventral anterior border of the lung before branching to any marked extend. The rami into which these recurrent bronchi finally break up anastomose with the numerous air-passages in the lateral facet of the lung.

Summary. Summarizing our observations we conclude that:

1. The recurrent bronchi are offshoots from the air-sacs leading into the lung parenchyma, and the air-sacs in turn, except the abdominal (from the mesobronchus), are the expanded ter-

minal portions of secondary branches of the bronchial tree. Thus the recurrent bronchi sustain the same relation to the air-sacs that the parabronchi do to the respective secondary branches from which they have their origin.

2. By means of the recurrent bronchi and their anastomoses with other branches of the bronchial circuits the air-sacs are brought into communication with all parts of the lung. They

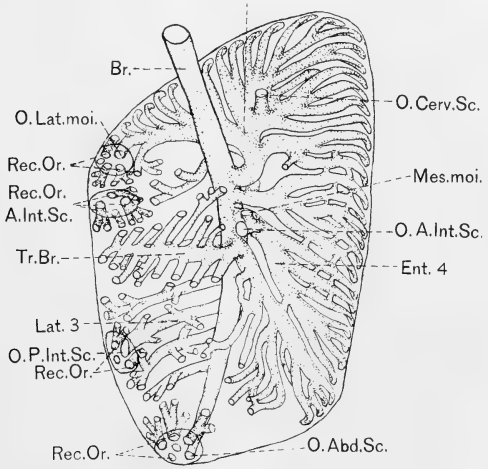


Fig. 54 Diagram of the ventral aspect of the adult lung constructed from studies of the prepared lung and from Wood's metal casts. Shows the nature of the seven orifices into the air-sacs as described in the text. *DO.*, direct orifices, *Rec.O.*, orifices of recurrent bronchi.

have direct communication with bronchus and central lung tube through their 'direct orifices' and a recurrent communication through the recurrent bronchi.

3. The unpaired interclavicular air-sac of the adult fowl is the result of fusion of four embryonic outgrowths: two moieties from each lung which first unite and then undergo fusion across the median line to form the single interclavicular air-sac.

4. THE DEVELOPMENT OF THE PULMONARY ARTERY

In describing the external appearance of the embryonic lung the pulmonary artery and pulmonary vein were noted as a part of the surface view. We shall now give a more detailed account of the method of origin of the pulmonary blood vessels and of the embryonic changes that they undergo.

The pulmonary artery is formed by the union of two parts, one of which, the proximal end, sprouts from the sixth aortic arch, and the other, the distal end, begins in the lung wall and grows towards the sprout from the aortic arch.

The vascularization of the walls of the lung precedes the formation of the pulmonary artery and the distal extremity is the first formed. Examination of sections of the fifty-two hour stage shows the presence of rounded vascular spaces in the mesenchyma of the lung primordium. When first formed these vascular spaces are of small extent and can seldom be traced through more than two sections, but the examination of numerous specimens of this age shows that they are fairly constant in appearance and as to their position in the median and dorsal portions of the lung parenchyma. These represent the rudimentary condition of the vascular area of the lungs.

As development proceeds, the vascular spaces assume greater definiteness, and by extension come together, and fuse forming an incipient network of capillary-like canals. Between the seventy-fifth and the eighty-second hour, in particular, these spaces show with increasing definiteness, and by the eighty-second hour of development the longer vascular spaces can be traced through twelve or more sections. These are intermingled with other spaces of less extent, all occupying the area of the lung in which, at a little later period, the pulmonary artery is formed. In the early formed vascular spaces it is not obvious which are destined to give rise to the artery and which to the pulmonary vein. This account agrees in essential particulars with Evans' observations of the development of blood vessels from capillary spaces.

These vascular changes in the mesenchyma of the lung wall begin before the formation of the sixth aortic arch. In many of

our injected specimens the pulmonary vein takes the injection before the artery as shown in figure 6, part I. This injected specimen gives a view of the pulmonary veins as seen from the ventral aspect of the lung. A single well defined blood vessel

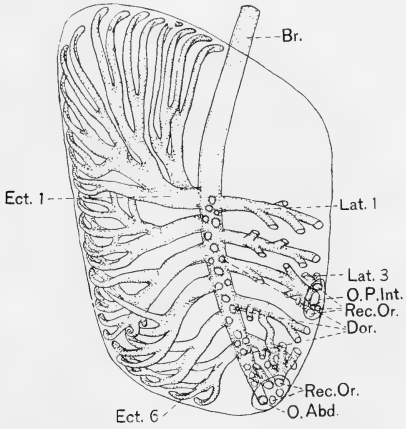


Fig. 55 Diagram of the dorso-lateral aspect of the adult lung. Exhibits openings into the mesobronchus of the dorsobronchi (*Dor.*) as seen when their stems are severed.

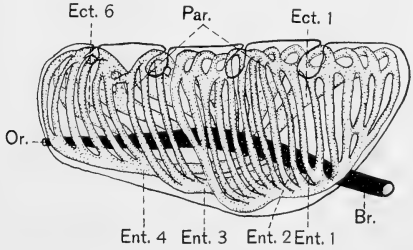


Fig. 56 Diagram of the mesial face of the adult lung to show parabronchi connecting ento- and ectobronchi.

passes on the ventral surface of each lung; these two unite into a trunk vessel situated in the median plane, and this, in turn, passes to the left atrium of the heart. Anteriorly is situated another vein that runs along the median line of the laryngo-tracheal groove and also unites with the trunk vessel that leads into the left atrium. In this specimen the pulmonary artery was not seen.

There is considerable individual variation in the time of formation of the blood vessels, so that the precise time and the degree of development is not identical in corresponding specimens. Some of this observed variation may be owing to imperfect injection. Nevertheless, the method of formation of the pulmonary artery is sufficiently definite to leave little room for doubt. By the beginning of the fifth day there is a stem vessel in the lung wall and a short spur from the ventral end of the sixth aortic arch. These are directed towards each other but they are separated by a very obvious interval, they constitute the proximal and distal ends of the future pulmonary artery.

Sections of the 96-hour stage (fig. 58) show on each side a short spur from the ventral part of the sixth arch. About twelve hours later ($4\frac{1}{2}$ days) we find in surface views (fig. 57) an almost completed pulmonary artery. There is, however, satisfactory evidence in the injected specimens that the spur from the sixth arch is not the only growing point in the formation of the artery. On the contrary, it meets a forward growing vessel from the lung, which has been formed through the medium of the vascular spaces already described, and which precede its appearance. Fourteen injected specimens of the middle of the fifth day ($4\frac{1}{2}$ days) were dissected. All showed the complete outline of the pulmonary artery, but in every specimen it was noticeable that the pulmonary artery was not of the same calibre throughout its course. Both ends were well developed, but about midway between the two ends the diameter was reduced, so that it presented the appearance of a slender tread. This is well shown in figure 57 which represents the dissection of an injected specimen of four and one-half days development. The distal division of the artery, from the sixth arch, is shorter than the proximal di-

vision from the lung. The slender thread-like portions shows the region of junction between the two ends. It would appear, therefore, that the first rudiment of the pulmonary artery begins in the mesenchyma of the lung walls in the form of vascular spaces, which by extension and union form a network of connecting passages, and from these arise the distal end of the pulmonary artery. The proximal division arises slightly later, springing from

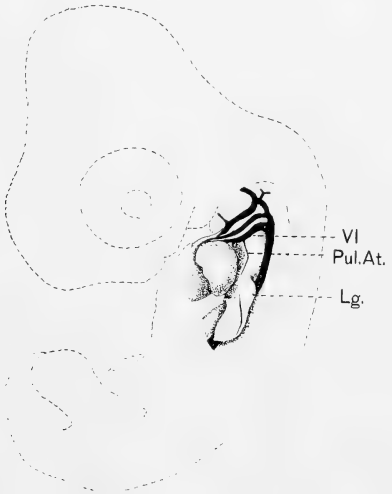


Fig. 57 Dissection of the lung territory of an embryo incubated four and one-half days. Shows the pulmonary artery not yet completed in its middle course. Drawn by G. H. A. Rech.

the sixth aortic arch, and then growing to meet the distal division from the lungs. When their union is effected the pulmonary artery is established. Figures 12 and 13, part I, show the surface appearance at five and one-half days after union of the two parts of the pulmonary artery.

Marshall ('92) was, we believe, the first to maintain that the "pulmonary arteries appear in the walls of the lung about the

middle of the third day before the two hinder pairs of aortic arches are formed. On the appearance of the fifth (sixth!) pair of aortic arches the pulmonary arteries become connected with their ventral ends."

The spur from the sixth aortic arch arises before the arch is completed and at its first appearance it is on the ventral part of the arch near the truncus arteriosis. The sixth arch, in common with the others, is formed by a dorsal moiety from the dorsal aorta (fig. 5), and a ventral moiety from the truncus arteriosus.

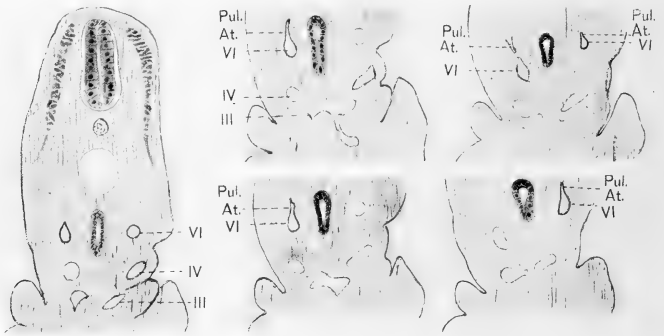


Fig. 58 Cross section of an embryo of the 96-hour stage to show the distal part of the pulmonary artery arising from the sixth aortic arch.

The ventral moiety is the longer and as it grows the relative position of the arterial spur changes. At the $4\frac{1}{2}$ day stage the position of the pulmonary artery is nearer the truncus arteriosus than the dorsal aorta. On the second half of the sixth day (fig. 12) it emerges at about the middle point of the sixth arch. The change in position is continued until, in later stages (fig. 22) the base of the pulmonary artery is nearer the dorsal aorta.

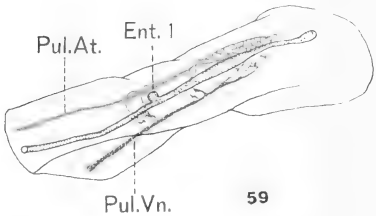
While the figures just described convey a good idea of the appearance of the pulmonary artery from surface views, the study of injected specimens as transparencies gives an idea of the internal distribution of vascular loops. Such a specimen of five days nine hours incubation is represented in figure 59. This is

the stage at which the first entobronchus (*Ent. 1*) is given off and the internal changes are to go on rapidly, accordingly, we may expect, a good development of blood vessels. The artery enters the lung substance and passes dorsally as well as nearly parallel to the lung tube. Loops of blood vessels pass from the dorsal side around the lung tube and unite with the vein below. These capillary loops are more abundant near the bud of the first entobronchus and the expanded portion of the lung tube.

At five days, twenty hours, the pulmonary artery is seen (fig. 60) running nearly parallel to the course of the extra-pulmonary bronchus and entering the lung dorsal to the bronchus. The pulmonary vein runs through the ventral region below the central lung tube.

As shown in figure 60 A, a sketch from the ventral aspect, and figure 60 B, from a lateral view, the artery branches and divides into capillary networks around the entobronchi, and, more caudally, around the embryonic vestibulum from which the ectobronchi are soon to arise. The capillary network of the dorsal side, having surrounded the lung tube and its outgrowths, passes ventrally, and comes into communication with the capillary network of the pulmonary vein.

In the course of a few hours the branching of vessels within the lungs has obviously increased. Figure 61 A is the sketch of the left lung and 61 B of the right lung of an embryo incubated five days, twenty-two hours. In the left lung are represented the chief branches of the pulmonary vein and in the right lung both veins, arteries and capillaries are sketched, but the capillary network has been simplified. The pulmonary vein is split into two great divisions that form a fork over the bronchus (fig. 61 B, fig. 21). One arises from venules situated deep within the lung substance, dorsal to the bronchus, and is designated the internal branch, the other is the external branch. In examining figures 60 and 61 it is to be understood that the capillary network is more complex than shown in the sketches, at the same time, the network was sketched with the aid of a camera, and embraces those minute vessels that carried the injection and which could be readily made out.



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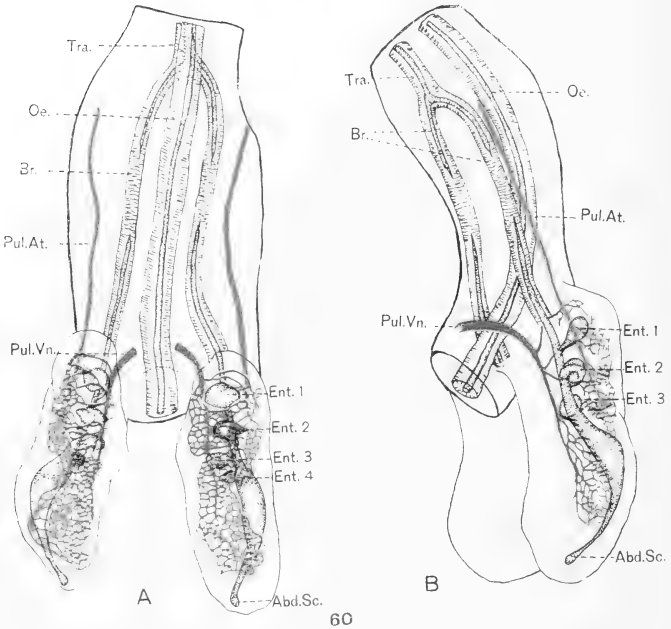

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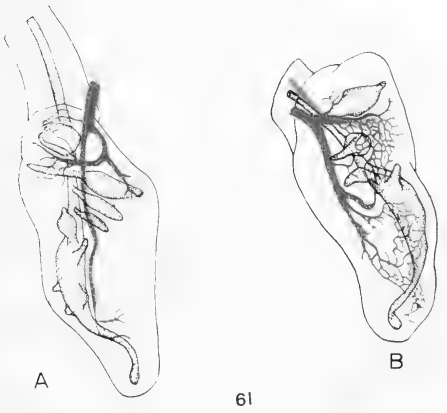
Fig. 59 Transparency of the lung of an embryo of 5 days 9 hours incubation to illustrate capillary loops surrounding the mesobronchus and connecting the pulmonary artery and the pulmonary vein.

Fig. 60 Lungs of an embryo incubated 5 days 20 hours to show the capillary network connecting pulmonary artery and vein. The four entobronchi have been established. The bronchus is occluded in its anterior portion. (A) dorsal aspect, (B) lateral aspect.



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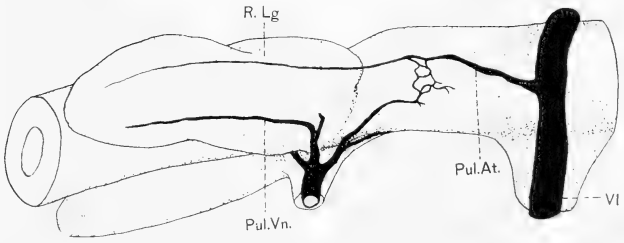

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Fig. 61 Transparency of the lungs of an embryo of 5 days 22 hours incubation. (A) illustrates the chief branches of the pulmonary vein of the left lung. (B) exhibits the pulmonary artery and the pulmonary vein of the right lung with parts of the capillary network connecting the two. Buds of ectobronchi are formed at this stage.

Fig. 62 Dissection of the lung territory of an embryo of 6½ days incubation to show especially the capillary plexus connecting the pulmonary artery and the laryngo-tracheal vein. In other specimens a small vessel leaves the ventral wall of the pulmonary artery and breaks into a capillary network (figs. 12 and 14). Shows also the connections of the pulmonary veins and the laryngo-tracheal veins with the trunk vessel leading into the left atrium of the heart.

Cross-sections of these stages were also studied under the microscope and in so far as the territory of distribution is concerned, bear out the observation on the injected specimens studied as transparencies.

The surface study of a dissected specimen of $6\frac{1}{2}$ days incubation (fig. 62) shows very well the connection between the pulmonary artery and the laryngo-tracheal vein. In part I, in a number of instances attention was called to a perpendicular branch (figs. 11, 12 and 14) emerging from the pulmonary artery upon its ventral border. Figure 62 shows that this short artery breaks into a network that is recombined into the vein running along the ventral border of the laryngo-tracheal region. This specimen was imperfectly injected so that the network of blood vessels on the anterior dorsal region of the lung did not show as in figure 14, but the connections of the arterial branch and of the laryngo-tracheal vein were well exhibited. The veins from the two sides join into a median vessel which, in turn, unites with the trunk vessel opening into the left atrium of the heart. The branches of the pulmonary vein also unite with this trunk.

On the eighth day of development, as indicated in part I (figs. 14 and 17), surface views of the injected lung show a capillary network occupying the antero-dorsal region of the lung, and, by the ten day stage the entire latero-dorsal surface is covered by a network of blood vessels. In addition to this there is a well defined denser network of capillaries upon the dorsal surface. Figure 63 from a specimen of the ninth day, shows this denser area of capillaries from the dorsal aspect. In this figure the central portion of the dorsal region is occupied by a distinctly limited network of capillaries extending in the form of a longitudinal stripe from the cranial to the caudal part of the lung. This vascular development corresponds in position to the lane-like area (before mentioned) between the ends of the ento- and ectobronchi as they curve towards each other. It is a characteristic anatomical landmark of all later stages.

In this sketch an oblique view of the lateral face is exhibited and, on that surface, the capillary network is obviously more scattered than on the dorsal surface.

We shall now give attention to the transformations of the sixth aortic arch by means of which the pulmonary artery becomes separated from the aorta so as to form an independent blood vessel coming from the right ventricle of the heart.

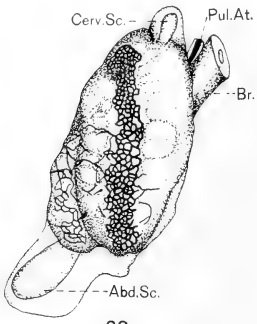
In the embryonic bird, after the sixth day, each pulmonary artery arises from one-half of the sixth arch. In the adult bud this has changed to the condition of right and left pulmonary arteries connected with a single trunk issuing from the right ventricle. Not only has there been a complete separation of systemic and pulmonic circulation, but, also, the distal extremities of the sixth arch have atrophied. The way in which this is brought about may now be followed.

About the middle of the fifth day ($4\frac{1}{2}$ day stage) the fourth, or systemic arch, the rudimentary fifth, and the sixth, or pulmonic arch are present. The fifth arch frequently fails to appear, and when it is present, it is extremely transitory. The disappearance of the rudimentary fifth leaves the fourth and sixth as the two posterior arches.

The left half of the fourth, or systemic, arch atrophies, the right half alone remaining, which becomes much enlarged.

In the meantime a septum develops that separates the right systemic from the base of the pulmonic, the systemic arch becoming connected with the left ventricle and the stem of the pulmonic with the right ventricle. When this has been accomplished the right and left halves of the sixth arch are still present and undiminished in size.

Figure 64 represents the condition at the close of the fourteenth day of incubation. The lung of the chick does not become functional as an organ of respiration until shortly before hatching. Accordingly, most of the blood from the right ventricle passes through both the left and the right divisions of the sixth arch to join the aorta with which these are connected. The pulmonary arteries springing from these divisions remain small (fig. 64). Near the heart the fourth and sixth arches are separated but the persistent right half of the fourth, and both right and left halves of the sixth arch, join the aorta. The fourth arch issues from the left ventricle, as the aortic arch, and the two halves of the



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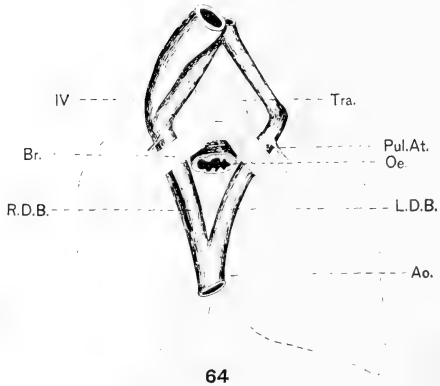

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Fig. 63 Dorsal aspect of the lung of an embryo of the ninth day, showing the lane-like area of capillaries extending from the cranial to the caudal part of the lung.

Fig. 64 Sketch from an embryo at the close of the fourteenth day of incubation to show the way in which the persistent right half of the fourth aortic arch and the two halves of the sixth arch join the aorta. The pulmonary artery is small. Drawn by G. H. A. Rech.

sixth united into a common trunk are connected with the cavity of the right ventricle.

Although the pulmonary arteries are relatively small, the right and left halves of the sixth arch are large, and, since they join the aorta, the principal function of the sixth arch at this time is in connection with the systemic rather than with the pulmonic circulation.

The pulmonary arteries remain relatively small up to the time of hatching, but those portions of the sixth arch lying behind them become constricted. This condition is represented on the nineteenth day of development in figure 65. At this time the pulmonary arteries are somewhat larger and the portions of the sixth arch behind them has undergone an obvious constriction. The parts of the sixth arch behind the pulmonary arteries are designated each ductus Botalli or ductus arteriosus. In the sketch the carotids and the pulmonics have been cut transversely so as to show in each case the cut ends of two vessels instead of single trunks into which they are united nearer the heart.

The subsequent steps in the formation of the adult pulmonary arteries involve the disappearance of the ductus Botalli on each side. When respiration begins, the increase of blood flow through the lungs reacts on the growing tissue so that the pulmonary arteries become enlarged, and the ducti Botalli being deprived of blood rapidly diminish in size and soon become reduced to strands of connective tissue which usually disappear in the adult, although in some species of birds they are persistent but not functional.

The progress of affairs is represented in figure 66 sketched from a chick newly hatched. The pulmonary arteries have increased in size and the ducti Botalli are much reduced.

After hatching the ducti Botalli become occluded and reduced to slender cords of connective tissue. Figure 67 represents the condition as seen from the right side, three days after hatching and figure 68 shows the heart and arteries of a young chick of the same age. In figure 68 the connections of the arterial trunks

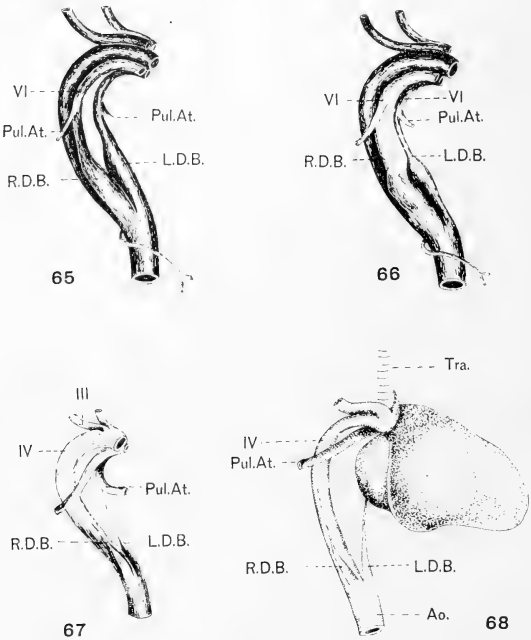

Fig. 65 The arterial trunks on the nineteenth day of development. The sixth aortic arches are constructed behind the pulmonary arteries into the ducti Botalli. Drawn by G. H. A. Rech.

Fig. 66 Condition of the pulmonary artery and of the ductus Botalli of the newly hatched fowl. Drawn by G. H. A. Rech.

Fig. 67 The atrophied ductus Botalli and the enlarged pulmonary artery on each side, on the third day after hatching. Drawn by G. H. A. Rech.

Fig. 68 The heart and the arterial trunks on the third day after hatching. The ductus Botalli of each side is reduced to a slender cord and the pulmonary arteries have attained the diameter of the sixth arch of which they are continuations. Drawn by G. H. A. Rech.

with the heart is exhibited. The pulmonary arteries are now large and the ducti Botalli are atrophied.

The effect of the obliteration of the ducti Botalli is that the blood from the right ventricle can no longer pass through the sixth arch to the aorta but is sent entirely through the pulmonary arteries to the lungs.

COMMENTS

The morphology of the avian lung can be made clear only by observations of its development. It is through this channel alone that one becomes acquainted with the nature of the modifications of the bird's lung that place it in a class by itself. It can be safely said that the facts of morphology separate the avian lung from the lungs of all vertebrates, with the possible exception of the reptiles. The excellent papers of Milani ('94) and of Hesser ('05) on the embryology of the reptilian lung are classics, but the embryology of the air-sacs is insufficiently known. On account of the development of air-sacs, of recurrent bronchi and labyrinthine connections between all air passages, the avian lung exhibits a special architecture and upon our understanding of this architecture will depend our conception of its physiology. While we have described what we conceive to be its physiological anatomy, we have not engaged in experiments that entitle us to make any special comment on its physiology.

The steps in arriving at a conception of the bird's lung have been gradual and dependent upon increasing definiteness in the knowledge of its internal architecture. Since the observations of the early investigators have been a factor in molding this conception, and since ideas of lung anatomy have been so dominated by their results, they may be briefly summarized. Moreover, the sketches of Selenka ('66) are still used in text-books to illustrate the development of air-sacs (vide Lillie, Development of the Chick, '08) Hertwig-Kingsley, Manual of Zoology, Revised Edition, '12), accordingly a summary will not deal with obsolete matters.

The traditional view, that somehow the bird's lung should be swung into line with the others, and ought to be compared part by part with the mammalian lung, persisted for a long time and created difficulties of interpretation. The idea that there are culs-de-sac on the ultimate twigs of the bronchioles, corresponding to the alveoli of the mammalian lung, has so often been tacitly assumed in the descriptions of the anatomy of the bird's lung that confusion has resulted. The conception, so fundamentally different from this, of labyrinthine passages, all intercommunicating, and forming bronchial circuits instead of a bronchial tree, has been a matter of gradual growth.

Inasmuch as the pioneer observers examined the bird's lung with great care, by transmitted as well as by reflected light, it is pertinent to inquire to what extent the structure of the bronchial tree was anticipated.

Rathke's ('28) figures of the embryonic lung of the chick show an attempt to represent the internal anatomy of the lung. In his figure 15 of the seven day stage, the main bronchus is shown with hernia-like growths (entobronchi) coming from it. In one of his five figures of the eleventh day (his fig. 16) he shows the air-sacs and a more profuse branching of the bronchial tree. In his figures 11 and 21 he sketches details of the terminal twigs which he illustrates as ending in grape-like clusters. These he compares directly with the alveoli of the sheep's lung of which he gives a similar picture.

Von Baer ('28) gives no picture of the embryonic lung but his descriptions show that his observations were carefully made. While Rathke's are tinctured with a subjective bias, Von Baer's are objective.

The next figures of importance for our review are those of Remak ('55). He was an excellent observer and his sketches are of high quality. His illustrations show clearly two entobronchial buds on the fourth day of development (his fig. 78). This shows well the mesenchymic sheath and the endodermal tube. His pictures of the lung at $3\frac{1}{2}$ days (fig. 72), at the end of the fourth day (fig. 74), and on the fifth day (fig. 75), show two entobronchial buds. His sketch of the early sixth day (fig. 88)

shows three such buds and of the seventh day (fig. 79) shows four. These figures are evidently not drawn with the camera since although the number represented is correct, the buds are too widely separated and cover too much territory of the central lung tube. The sketch of the condition on the eighth or ninth day (fig. 80) shows both ento- and ectobronchi as well as the expanded end of the mesobronchus. It should be said that Remak's figures represent the essential features of the anatomy of the embryonic lung. They are somewhat out of proportion and they show the great difficulty of study by transmitted light without the aid of some especial method of injection.

Selenka's studies ('66) of the development of the air-sacs of the chick added a number of points to the anatomy of the avian lung. His figure of the $3\frac{1}{2}$ day stage shows the occluded bronchus (fig. 2). In the fifth day stage he illustrates for the first time, the beginning of the embryonic vestibulum (his fig. 3). In his sketch of the sixth day stage he represents the bud of the first entobronchus, not quite in position, however, in reference to the expansion of the lung tube, being in his figure posterior instead of anterior to the expanded part. His sketch of the condition on the seventh day (his fig. 5) shows seven or eight outgrowths (buds of ento- and ectobronchi) of the central lung tube.

His figure of the lungs and air-sacs of the eleven day embryo is very interesting and, as already stated, has been extensively copied in text-books. On the right lung is sketched (2′) the sac that we have designated the lateral moiety of the interclavicular sac and which he designates "cellula infra laryngeus, on the left, not yet united"—"stetz sich später in die cellula axillaris fort." Although this figure needs attention, and some correction, it is for the methods at his command a good figure. This figure also shows on the right lung the mesial moiety of the interclavicular sac, but it is represented on the wrong side of the bronchus. As shown in our figures 21 and 47 it arises on the mesial side and extends across the bronchus ventrally to the lateral side.

The sketches of Selenka and of Remak, made by talented observers show the limitations of observing the internal structures of the lung by transmitted light without the help of injections.

The reconstruction method has the advantage of giving relatively large models, but it is difficult and protracted. Injection with air, described under 'technique,' is simpler, it can be repeated indefinitely with the same specimen and gives clear pictures of the minuter details.

The researches of Sappey ('47) laid the foundation for those of Campana ('75) which, in particular, mark the next advance in our knowledge of the bronchial tree. These investigations were carried out on the adult lung and resulted in accurate figures and extended descriptions. The quality of Campana's work has been spoken of before, but too great emphasis can not be laid on the thoroughness of his anatomical analysis. It is now fifty years in the past and on that account, coupled with the fact that his memoir is not easily obtainable, it is likely to be slighted. The memoir is complete and critical for the adult stage embracing the intra- and extrapulmonary features, the bronchial tree, the air-sacs, their orifices and the bronchial circuits. Campana was apparently the first to fully grasp the idea of bronchial circuits. The earlier observers referred to, indicated the beginning of bronchial branches but their conception was apparently that of a true bronchial tree comparable to that of mammals. Schulze ('71), in giving a picture of the histology of the bird lung showing among other features the air-capillaries evidently interprets them more as alveoli than as a network of connecting passages.

Fischer ('05) studied the bronchial tree by injections and published many figures of wax casts. His descriptions are terse, more general than critical and are somewhat burdened with a new terminology.

It is through the investigations of Schulze ('11) and Juillet ('12) that we arrive finally at our present conception of the architecture of the avian lung. Their discovery of the recurrent bronchi has been sufficiently commented upon in previous pages. Juillet's investigations introduced some innovations besides recurrent bronchi, as sketches of the embryonic tree of six and eight day stages. He was also the first to show the method of development of the recurrent bronchi, and after Schulze, to recognize their significance.

Some particulars in which our results differ from those of previous observers may now be mentioned.

Comparison of embryonic stages. On account of the method employed, of air injection, we were able to carry our studies of the embryonic bronchial tree to later stages of development than those previously sketched. One who compares our figures of the embryonic tree with those of Juillet will note several discrepancies. The development of the bronchial tree in his reconstruction of the six day stage (his fig. 4) is more advanced than in our sketch of five day twenty hours which is well along in the last half of the sixth day. His reconstruction of the bronchial tree of the eighth day (his fig. 5) is also in advance of our sketches of the same structure in the early part of the ninth day. There is substantial agreement as to the number of main branches but differences in detail including one of importance, viz., the relation of the transverse branch of the first entobronchus to the lateral moiety of the subbronchial sac.

It is also to be noted that Juillet omits the laterobronchi in his reconstruction of the eight day stage. His plastic reconstruction of that stage embraces, besides the mesobronchus, only ento- and ecto-bronchi. In his text, as well as in his sketches, he gives little consideration to the laterobronchi, and sets to one side the dorsobronchi. He states clearly his reasons for so doing. Nevertheless, after observing them carefully in embryonic stages as well as in the metalic casts of the adult lung, we are inclined to attach considerable importance to the dorsobronchi, and also to the laterobronchi, on account of the part they play in helping form the network of the bronchial circuits. As Campana pointed out, the dorsobronchi (approximately twenty-five in number) form a fine network in the middle of the dorsal face of the lung that can be detected by surface studied of untreated specimens. The laterobronchi, besides giving rise to an air-sac, form many anastomoses through their branches in the ventral part of the lateral ahd caudal regions of the lungs and also with the recurrent bronchi of the two posterior air-sacs.

The interclavicular air-sac. It is on the question of the embryonic development of the interclavicular sac that our observa-

tions are more at variance with previous results than on any other point. The method of development of this sac is very remarkable. In late embryonic stage, as in the adult, it is an unpaired medial sac but it arises from four separate moieties, two from each lung. The lateral moiety springing from the transverse branch of the first entobronchus and the mesial moiety coming (with the exception noted above) from the third entobronchus.

The chief anticipation of the simultaneous existence of lateral and mesial moieties of this sac is found in Selenka's sketch of two separate sacs on the right lung (see reproduction of the sketch in figure 191 of Lillie's development of the chick. He says that the sac which we have designated the mesial moiety later unites with the cellula axillaris. Only one moiety of the interclavicular (the lateral moiety) is sketched on the opposite side. As indicated above his sketch of the mesial moiety shows its origin on the wrong side of the bronchus.

On account of its position on the lung, the lateral moiety is the one that has usually been sketched in the published drawings of embryonic stages of the lung and the mesial moiety has commonly escaped notice. However, when the moieties have united, as in late embryonic stages and after hatching, the more obvious opening of the interclavicular, which is the mesial, has been correctly identified and that part of the sac that is derived from the lateral moieties has been regarded merely as an extension of the mesial moieties. Thus Juillet's diagram of the lung of the embryonic chick represents only the mesial moiety and this as expanded and bearing recurrent bronchi on its lateral border. It is only by following the embryonic development from the eleventh to the sixteenth days that the complex relations of this sac are cleared up.

Guido Fischer ('05) maintains that the lateral branch of the first entobronchus of the adult opens into the interclavicular sac. He gives a figure of a plastic cast to show this but does not name the bird in which it is found. While Campana does not mention it, his figure 11 is suggestive in showing the termination of the branch in question close to the lateral orifices of the interclavicular sac.

Juillet ('12) is very drastic in his criticism of several of Fischer's observations and of this one in particular he says: "G. Fischer ('05) a donnè des bronches diaphragmatiques une discription assez confuse et à laquelle il y'a plusieurs reproches à faire . . . . Le sac interclaviculaire s'ouvre d'après lui sur ce rameau" (lateral or transverse branch) "bronchique: l'orifice qu'il signale ici est très certainement l'orifice récurrent de ce sac dont il a mal saisi les rapports exacts," etc.

However defective Fischer's observations may be on other structural matters (as Juillet claims), on this point, in particular, our observations indicate that Fischer is probably correct. At any rate, we have found the orifice in question in the embryo chick and also in the adult. In our preparations there are several that show both direct orifices, the lateral and the medial, of the interclavicular sac as in the Wood's metal cast of which figure 45 is a photograph. Moreover, the existence of a separate lateral moiety implies, at least in the embryo, a direct opening from that moiety into the lung. In this criticism Juillet overlooks the fact that the direct connection between the curvilinear branch of the first entobronchus and the interclavicular sac has been indicated by a number of observers, as Huxley ('82), Baer ('96), Lillie ('08), Schulze ('10). Accordingly, the claim of Fischer was neither novel or unique. A further claim of Fischer, as Juillet points out in the same paragraph, is that the curvilinear branch of the first entobronchus has also a direct opening into the anterior intermediate sac. On this point of the criticism our observations lead us to agree with Juillet that such an orifice is lacking.

Juillet's Schema (fig. X, p. 313), to show the relations of the air-sacs and their recurrent bronchi with the ventral face of the right lung of a chick embryo of the tenth day is faulty in showing the interclavicular sac with a wide lateral extension crossing the lung transversely and giving rise to recurrent bronchi. The interclavicular sac at this stage of development (and for several days thereafter) has separate lateral and mesial moieties as shown in figures 47 and 49. The recurrent bronchi of the interclavicular sac sprout from the lateral moiety, while, so far as we have been able to determine, the mesial moiety never has any.

Excepting the analysis of Campana and of Juillet, there is much confusion among authors regarding the orifices of the air-sacs. In the chick we find seven groups of orifices, agreeing with Juillet except in regard to the nature of the lateral orifice of the interclavicular sac. This we find to be mixed polybronchial instead of simple polybronchial as claimed by Juillet. On the basis of our observations the seven groups of orifices are: one direct monobronchial orifice for the cervical sac; one medial direct monobronchial, and one, laterally placed, mixed polybronchial for the interclavicular sac, one direct monobronchial and one simple polybronchial for the anterior intermediate sac; one mixed polybronchial for the posterior intermediate and one mixed polybronchial for the abdominal sac.

As Juillet ('12) has shown in admirable comparative studies, much variation exists as to numbers (6 to 9) and arrangement of orifices in the twenty-five species of birds which he studied. Since our observations are limited to the chick, the reader is referred to Juillet's analysis of the different types of orifices (pp. 340–351) which can not be satisfactorily abbreviated.

As regards the method of growth and the type of branching within the lungs, we shall limit ourselves to the brief remarks—that the excellent observations of Moser indicate the general method of growth and on the question of branching by monopodial or dichotomous formation, our observations incline us to adopt the view of an unequal dichotomy.

Recurrent bronchi. The recurrent bronchi are the most important recently recognized structures connected with the lungs of birds. As already indicated the credit for the recognition of their structure, development and physiological function is shared by Schulze ('11) and Juillet ('12). These new structures are of especial interest. There is no doubt that they spring from the air-sacs and grow into the lungs where they establish numerous connections with the bronchial branches. This gives a new view of air sacs: They are expanded parts of the bronchial circuits; branches from the main bronchus lead into them, but they are not terminal sacs, they are air reservoirs on the course of the bron-

chial circuits. Conduits developed from them turn back into the lung and join the network of air passages, so that, the air, little changed, and warmed to body temperature, is carried back into the lung for aëration of the blood. The lung, although relatively small, is highly vascular and a very efficient organ of respiration. Rapid respiratory changes are favored by the structures described and by the very intimate relations between air capillaries and blood capillaries. Taken together they constitute a feltwork of vascular and air capillaries mingled together.

It is clear from Campana's text that he noticed the recurrent bronchi of the adult on the four air-sacs from which they have their origin. He makes comment upon them in each case. His reference to those of the abdominal sac shows that he thought of them as combinations of parabronchi. On page 54 of his memoir he says: "On voit à la face dorsale du poumon des grosses tertiaries, on pourrait presque dire des secondaires reconstituées par la reunion des tertiaries plus fines, aboutir sur les parois du septième infundibulum (fig. 13, C) ou, ce qui revient au même, s'ouvrir dans la termination des dernieres secondaires externes."

On the whole, Campana's observations afford a sort of prophetic anticipation of the full recognition of recurrent bronchi.

The discovery of the recurrent bronchi brings a new point of interest into the study of the lungs of Sauropsida, and it is much to be desired that extensive comparative studies may be entered into that will embrace a careful consideration of the air-sacs and their relations in reptiles.[2]

[2] The variations in chronology of chick embryos represented in the illustrations of the standard references is so great that an additional comment is appropriate. Comparison of the figures of Duval and of Keibel and Abraham shows variations sometimes exceeding twenty-four hours (cf. Duval, fig. 142, 140 hrs.; and Keibel and Abraham, fig. 27, 114½ hrs.). While it would be a satisfaction to embryologists to have the chronology standardized, the essential point is a correct analysis of anatomical conditions and the sequence of events.

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THE FUSION OF THE CARDIAC ANLAGES AND THE FORMATION OF THE CARDIAC LOOP IN THE CAT (FELIS DOMESTICA)

H. VON W. SCHULTE

From the Anatomical Laboratory of Columbia University

SIXTEEN FIGURES

In the transformation of the bilateral anlages of the heart into a single median organ, two processes are to be distinguished, the fusion of the myoepicardial mantles and the fusion of the endothelial tubes. For while the union of the mantles is a necessary condition of the coalescence of the tubes and its character determines the general features of the endothelial fusion, yet the latter process is relatively much retarded and evinces a considerable degree of independence in many details. For these reasons it will be convenient to follow the two processes separately.

This study is based upon the now numerous early embryos of the cat in the Columbia Embryological Collection. These were cut in transverse serial sections of 13.3 μ and variously stained. In selected embryos reconstructions by the Born method were made of the myoepicardium, of the endothelium and mesenchyme, and finally of the lumina of the heart tubes and angiocysts. By a comparison of the last two it is possible to ascertain the precise limits of the solid and hollow parts of the anlages.

The processes of approximation, of fusion, and of loop-formation take place in a short period of development as measured by the rate of formation of the mesodermic somites, the whole duration of these changes in the cat falling between the stages 8 and 21 somites. At 8 somites (fig. 1) ,the foregut in the cardiac region is widely open and the splanchnopleure is spread out

flat; the cardiac anlages lie in the same plane as the neural tube and are separated by a wide interval. In the embryo of 11 somites (fig. 3) they are ventral to the closed foregut and their proximal or bulbar ends are close together. At 12 somites

Fig. 1. Reconstruction of compact mesoderm of cat embryo of eight pairs of somites. Columbia Collection, No. 588. ×180. Reduced one-half. Dorsal view the parietal mesoderm has been exsected to show topography of parietal cavity. *1*, Parietal cavity; *2*, pericephalic cavity; *3*, parietal recess; *4*, myoepicardial mantle; *5*, oblique groove dividing mantle into mesal and lateral protions; *6*, retrocardiac plate; *7*, precardiac plate.

fusion of the mantles is complete (fig. 4); at 14 the formation of the loop is initiated (fig. 7); and at 16 it is completed. The fusion of the endothelial tubes begins in the embryo of 12 somites, in that of 14 it is all but complete, and the embryo of 20 somites is the oldest that shows a remnant of septum between the endothelial tubes (figs. 11, 13, and 16).

THE MYOEPICARDIAL MANTLES

There are many brief statements and illustrations of the myoepicardial mantles in the literature recording conditions in these structures immediately antecedent to and during fusion, from which nevertheless it would be impossible to compile a history of the process. The observations of Kölliker, His, Türstig, Schultze, Spee, Bonnet, Fleischmann, Selenka and Heape, are as familiar as they are important and have been adequately summarized by Mollier,[1] who has also included the less well known and less accessible work of Martin,[2] which is valuable for its excellent illustrations. Strahl and Carius[3] and Parker,[4] alone have given continuous and detailed accounts of the processes here considered. The former have illustrated by a series of diagrammatic cross sections two different types of fusion, one in which the formation of the ventral mesocardium antecedes that of the dorsal, the other in which the converse obtains. Two factors here come into play, first the width of the foregut and second the primitive position of the bilateral cardiac anlages. The area of visceral mesoderm which intervenes between the myoepicardial mantle and the mesal angle of the parietal cavity, they designate the retrocardiac plate, similarly that between the mantle and the lateral angle the precardiac plate

[1] Mollier, S. Die erste Anlage des Herzen bei den Wirbeltieren in Hertwig's Handbuch der vergl. und experiment. Entwickel. der Wirbeltiere, Jena, 1906, Bd. 1, which see for literature.

[2] Martin. Lehrbuch der Anatomie der Hausteire. Bd. 1, Stuttgart, 1902.

[3] Strahl and Carius. Beiträge zur Entwickelungsgeschichte des Herzens und der Körperhohlen. Arch. f. Anat. und Physiol., Bd. 15, 1899.

[4] Parker, Katherine M. The early development of the heart and anterior vessels in Marsupials, with special reference to Perameles. Proc. Zool. Soc., London, 1915, Pt. III.

(fig. 1). If the retrocardiac plate is narrow relatively to the foregut, the myocardial mantles will be widely separated upon the closure of the gut and the ventral mesocardium will precede the dorsal in its formation. This type obtains in the rabbit, and to it the cat conforms with some peculiarities in detail which are recorded below. If, however, the converse obtains, if the retrocardiac plate is broad relative to the width of the foregut, the formation of the dorsal mesocardium will be accelerated. Of this modification of the process the guinea-pig is an example. This in brief is the analysis of Strahl and Carius. Mollier comments justly that is it 'not quite correct to say that the closure of the foregut is the cause of the fusion of the anlages of the heart, because this occurs independently and later, a point which Kölliker had already made.' The possibility of a third type of approximation of the mantles must be admitted, such for example as obtains in the chick, where the retro- and pre-cardiac plates are of such proportions that ventral and dorsal mesocardiac are formed at approximately the same time. Further it is not necessary to assume that the retrocardiac plate is of the same breadth in its whole extent, and a difference in this respect might reach such a degree as to produce a mixed type of mesocardial formation.

In marsupials Parker describes a developmental process resembling but not identical with the conditions present in the cat. Like that species and the rabbit there is no ventral mesocardium. On closure of the foregut the mantles are widely separated, the intervening remnants of the precardiac plates forming a median plate between them. Associated with this middle cardiac plate are mesenchyme cells, later capillaries. Subsequently the middle cardiac plate is inconspicuous in marsupials not forming a thicked ridge as in the cat (Cf. Parker, fig. 17, with fig. 12 of this paper), nor are the angiocysts of the region assigned a rôle in effecting the fusion between the endothelial anlage. These first fuse in the region of the bulb. In embryos of 15–16 somites a constriction between bulb and ventricle is present only on the right side, which as a whole exceeds the left side in size. Caudad the ventricle is limited by a constriction,

and the following dilatation where endothelium and myocardium laterale are closely approximated is interpreted as auricle. The actual stages of fusion other than in the bulbs and the early stages of the loops are not represented in Miss Parker's material.

In the cat from the first the myoepicardial mantle has an obliquely sagittal direction in consequence of which the retrocardiac plate broadens somewhat as it is followed caudad, and tapers in the opposite direction, (embryos of 4–7 somites). The topography of the parietal cavity in an embryo of 8 somites is shown in figure 1, which agrees closely with Fleischmann's figure of a total view of a cat embryo of this stage.[5]

Before the infolding of the splanchnopleure is begun, the topography of the mantles is such as would seem to entail their earlier approximation caudad where the retrocardiac plates are broadest. The direct contrary is the case; cephalad where these plates are narrow the bulbar segments are quickly brought into apposition, while the ventricular portions diverge and are widely separated caudad, nor is there sufficient difference in the width of the gut opposite these two segments of the heart to account for their difference in position. The problem thus presented is not easy of solution and its difficulty is increased by the rapidity of the process, for the heart passes in the period required for the development of three somites from the position shown in figure 1 to that in figure 3. Thus in the interval between the appearance of the eighth and eleventh somites the formation of the foregut is completed as far as the atrial extremity of the heart, and the anlages of that organ have been moved through a dorso-ventral arc of nearly 180° and become approximated ventral to the pharynx.

It is not possible on the basis of the material in hand to attempt a complete solution of this problem for which several processes extrinsic as well as intrinsic require minute investigation. Primarily there are the changes incident at this period in the general shape of the region, notably the shortening, associated with the beginning ventral flexion of the forebrain, which

[5] Fleischmann, A. Embryologische Untersuchungen. I, 1889.

together result in such a remodelling as is hardly to be attributed to unequal growth. In this shortening is to be found an explanation of the kinking of the heart tubes prior to their fusion. In the second place the acceleration of infolding of the splanchnopleure cephalad tends to bring the cardiac anlages into an oblique position with their arterial ends near together, their venous extremities divergent. The supposition of accelerated growth in the cephalic portion of the retrocardiac plate is not supported by a conspicuous increase of mitotic figures, but the perceptible diminution of its thickness and its rectilinear position at the end of the process suggest the action of a moulding force. Finally Mollier's comment must be borne in mind—the closure of the gut is not the cause of fusion between the cardiac anlages. It determines their approximation, but when this is complete the mantles are separated by an interval, which is bridged by a plate of mesoderm derived from the precardiac plates. By compression and ultimate absorption of this into the mantles fusion is accomplished, and the process as a whole is evidently one of remodelling which cannot be explained simply as a cessation of growth in this district. Similar forces may therefore come into play in effecting the early and close approximation of the arterial ends of the mantles notwithstanding the narrowness of the cephalic portion of the retrocardiac plates.

In an embryo of 9 pairs of somites the cardiac anlages have a position intermediate between those shown in figures 1 and 3. The mantles are obliquely placed, their arterial ends close together though separated by a deep cleft, their venous ends widely divergent. Each is indented in its lateral contour by an angular incisure which marks the junction of their approximated and divergent portions. The differentiation of the heart tube into segments prior to fusion was observed by His and the portions have been variously designated bulbar and atrial or ventricular and atrial. Their subsequent history in the cat show the first segment to be the bulb continuing forward into the truncus, the second the ventricle later expanding at its caudal end into the atrium, but this only after the loop is initiated and fusion well under way. The angulation is therefore the bulbo-

ventricular sulcus. Caudad the mantles diverge and pass into the lateral mesocardia with a gentle curve. A distinct angulation at this point is not apparent until the stage of 11 somites.

On account of the obliquity of the myoepicardial mantles, a triangular interval with its base at the anterior intestinal portal is left between them. This is bridged by the residue of the

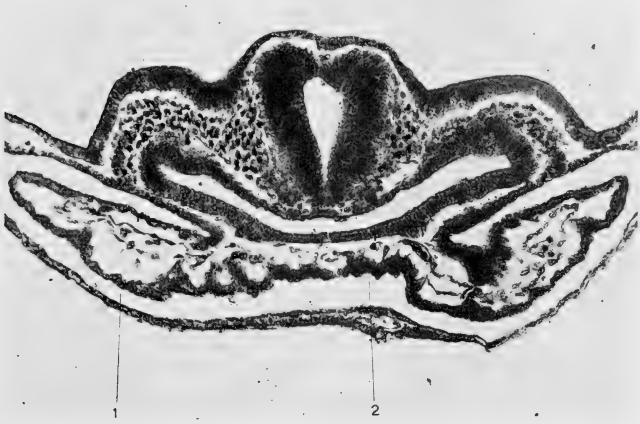

Fig. 2 Photomicrograph of a cat embryo of nine pairs of somites. Columbia Collection, No. 532. × 125. Reduced one-half. *1*, Ventricular segment of mantle; *2*, middle cardiac plate.

precardiac plates after the closure of the foregut and the disappearance of the ventral mesocardium. It may be designated the middle cardiac plate.

Between the bulbar segments it is narrow in this differing from the rabbit (Strahl and Carius), but caudad attains a considerable width (fig. 2). It is seen to agree in thickness and in the character of its cells with the myoepicardial mantles and like them to project entad in numerous longitudinal ridges, here shown in cross-section. These ridges as elsewhere in the splanchnopleure are intimately related to the formation of mesenchyme

and differ in no important character here in the middle cardiac plate and in the myoepicardial mantles from those observed in the splanchnopleure at large. I shall recur to this field of mesenchyme and endothelium in connection with the description of the endocardium. The middle plate is demarcated from the mantle on each side by a sulcus which gradually becomes shallow and is effaced as it is followed caudad.

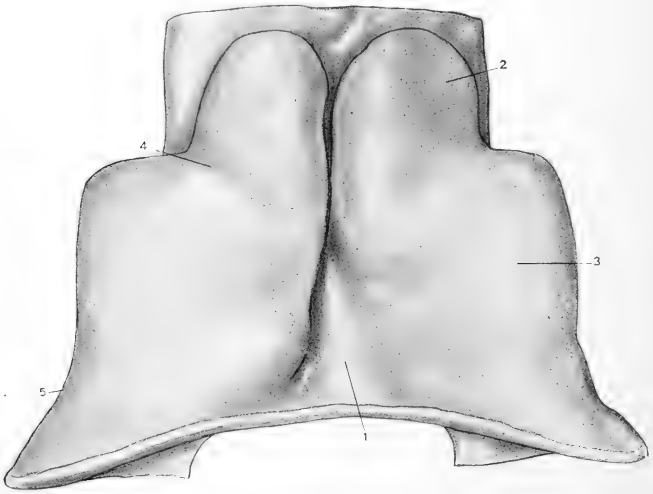

Fig. 3 Myoepicardial mantle of a cat embryo of eleven pairs of mesodermic somites. Columbia Collection, No. 534. × 300. Reduced one-half. *1*, Middle, cardiac plate; *2*, bulbus; *3*, ventricle; *4*, bulbo-ventricular sulcus; *5*, atrio-venous angle.

The mantles in their ventricular segments are dorso-ventrally flattened and of great transverse extent. Between them in ventral view the middle field forms a flat depression. The bulbar segments are close together but separated by a deep cleft, the roof of which is continuous with the middle plate. They are much narrower and less flattened than the ventricles.

Embryos of 11 pairs of somites show some variation in development. Some have hardly progressed beyond the one of 9 somites, but in the most advanced of this stage the mantles are approximated in the greater part of their length and the middle plate is considerably reduced. Fusion has not yet occurred (fig. 3).

The mantles present two angulations in thier ectal contour. The first bend is the bulbo-ventricular sulcus already described, which becomes accentuated on the left side in the later stages as the flexion of the tube develops. In this embryo it is nearly rectangular and is approximately symmetrical on the two sides. I take the deep incisure in Martin's cat embryo of 4 mm. to be its equivalent. It is also to be recognized in Duval's figure[6] of a chick of 8 somites, though obscured by the outline of the amnion. The second bend is a more gradual change of direction at the junction of the heart tube with the omphalomesenteric vein and may accordingly be designated the cardiovenous angle. It is not to be distinguished in Martin's figure, but in the heart of the chick as shown by Duval it is a deep cleft. These angulations are important in that they determine the earliest points of fusion in later stages between the endothelial tubes.

The middle plate is much reduced. Its cephalic portion is concealed in the deep cleft between the bulbs; caudad it forms a convex triangle exposed in its whole extent. Throughout the mantles are markedly dorso-ventrally flattened.

The fusion between the mantles and the formation of a dorsal mesocardium is effected in embryos of 12 and 13 pairs of somites. The dorsal mesocardium is very short, set off by a sulcus from the mesoderm covering the foregut and ventrally by a deeper sulcus from the mantles. Cephalad its leaves separate to give passage to the forming aortic roots and caudad it expands and is continuous with the lateral mesocardia.

[6] Both these cuts are given by Mollier. Op. cit. figures 713 and 715. Allen Thompson illustrates this form of the heart in the chick in his article 'On the development of the vascular system in the foetus of vertebrated animals.' Edinburgh new philosophical journal, 1830, Pl. 2, fig. 12.

The mantles are clearly demarcated from the lateral mesocardia by deep sulci so that the junction between omphalomesenteric vein and heart tube is deeply indented on each side. From this point the mantles gradually expand again to be in-

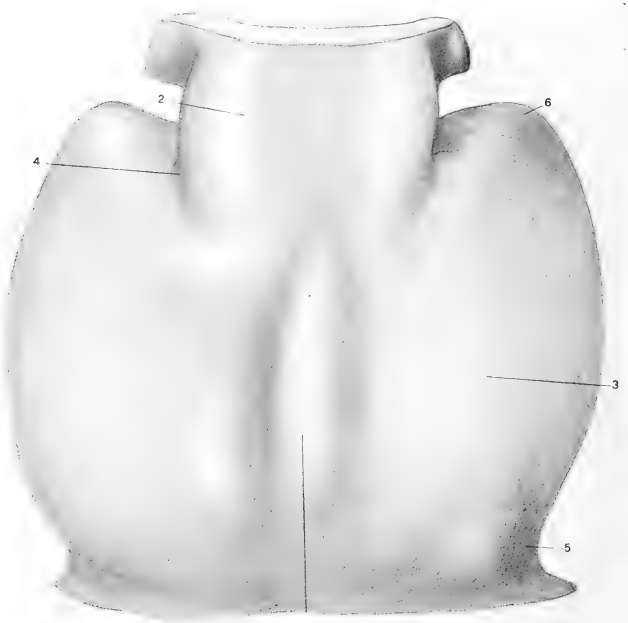

Fig. 4 Myoepicardial mantle of a cat embryo of twelve pairs of somites. Columbia Collection, No. 547. × 300. Reduced one-half. *1*, Middle cardiac plate; *2*, bulbus; *3*, ventricle; *4*, bulbo-ventricular sulcus; *5*, atrio-venous angle; *6*, shoulder of mantle.

dented by the deep oblique bulbo-ventricular clefts. Beyond them the truncus has the form of a cylinder, slightly flattened dorso-ventrally, emerging between the high shoulders of the mantles (fig. 4), In this stage the heart is approximately symmetrical and the bulbo-ventricular sulci of the two sides are in

all important respects identical. The ental projections which they occasion are shown in figure 5.

The mid-region of the fused mantles requires some comment. Here the middle-plate has been compressed to a longitudinal ridge bounded by well defined sulci. These are expressed entally by ridges, which in an embryo of 13 somites extend far into the bulbus. In the heart of this 12 somite embryo they are reduced in this segment and the middle region of the bulbus is slightly concave. The fusion is here complete. As the bulbo-

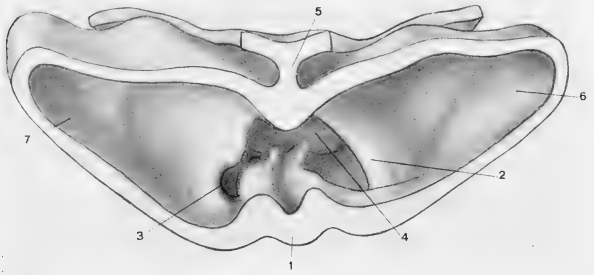

Fig. 5 Cephalic portion of same model as figure 4, ental view. *1*, Middle cardiac plate; *2*, fundus of right bulbo-ventricular sulcus; *3*, fundus of left bulbo-ventricular sulcus; *4*, interior of bulbus; *5*, dorsal mesocardium; *6*, right ventricle; *7*, left ventricle.

ventricular fissures are approached the ridges begin as low elevations which increase in height to about the middle of the ventricular segment fading out towards the terminal constriction of the heart. Between the ridges projecting from the middle plate entally are occasional small processes of mesoderm, evidently remnants of the more numerous projections of earlier stages.

As compared with the heart of the 11 somite embryo this heart has lengthened somewhat, but its striking changes in contour are associated with the deepening of the two pairs of incisures at its lateral margin. The bulbar segment has diminished absolutely in breath and so in less degree has the ventricular especially at its caudal end where it joins the lateral meso-

cardia and septum transversum. The dorso-ventral increase in diameter is also marked (fig. 12).

Upon this condition follows so rapidly the development of the cardiac loop that in only one embryo of 14 somites was an

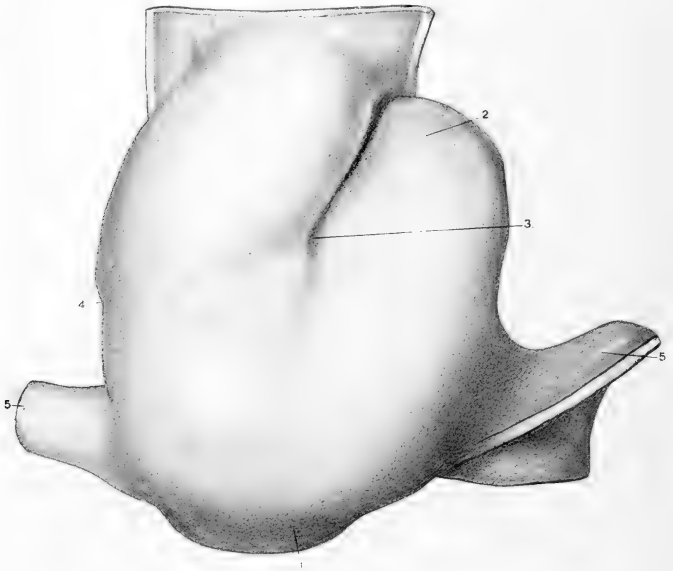

Fig. 6 Myocardium of a cat embryo of fourteen pairs of somites. Columbia Collection, No. 188. × 300. Reduced one-half, ventral view. *1*, Interventricular sulcus; *2*, shoulder of left mantle; *3*, left bulbo-ventricular sulcus; *4*, right bulbo-ventricular sulcus; *5*, lateral mesocardia.

intermediate stage observed. The model of this myocardium is shown in figures 6 to 8. In figure 6 in which the reconstruction is viewed from in front it presents resemblances to Mall's[7]

[7] Mall, F. P. On the development of the human heart. Am. Jour. Anat., vol. 13, 1912, fig. 1. Cf. Dandy, W. E. A human embryo with seven pairs of somites, measuring about 2 mm. in length. Id., vol. 10, 1910. Also Evans, Keibel and Mall, Manual human embryology, vol. 2, figs. 408–9, and Mall, Ibid., vol. 1, figs. 382–6.

freely-treated model of the heart of a human embryo of 7 to 8 pairs of somites. In both there is a bulbo-ventricular cleft on the left and there is little in this view of either model to suggest a mode of formation of the loop different from that given by Mall, which is simply the deepening of this sulcus between bulb

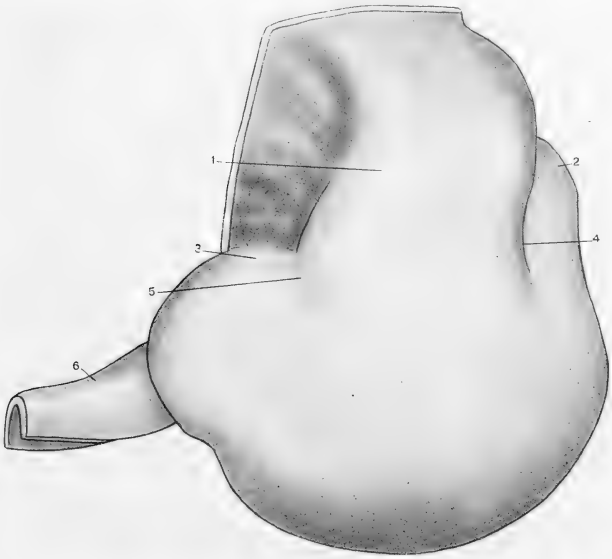

Fig. 7 The same model as in figure 6 viewed somewhat from the right and above. *1*, Bulbus; *2*, shoulder of left mantle; *3*, shoulder of right mantle; *4*, bulbo-ventricular sulcus; *5*, right bulbo-ventricular sulcus; *6*, right lateral mesocardium.

and ventricle. The heart of the cat is rather more plump, its contour more convex, but this may well be due to a greater distension with blood. Two details small in size but not in morphologic significance are present in the cat, which are not shown in the figure of the human heart. The right margin has a small indentation which is the remnant of the right bulbo-ventrical

cleft as is readily seen in the view from the right and above shown in figure 7, and caudad there is a slight nearly transverse depression furrowing the apex of the cardiac loop, associated with an ental ridge which marks the beginning of the septum ventriculorum.

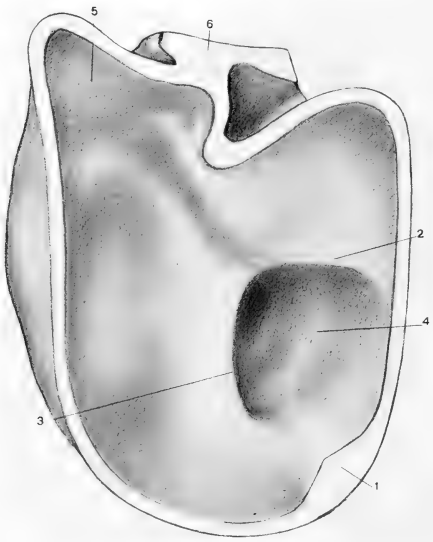

Fig. 8 Cephalic portion of same model as in figures 6 and 7, ental view. *1*, Ridge representing middle cardiac plate; *2*, fundus of right bulbo-ventricular sulcus; *3*, fundus of left bulbo-ventricular sulcus; *4*, interior of bulbus; *5*, atrial region; *6*, dorsal mesocardium.

The changes that have supervened to transform this heart in the period between appearance of the twelfth and fourteen pairs of somites are easily appreciated on the comparison of the figures (figs. 4 and 7). The most striking changes affect the shoulders of the mantles, that of the left side is greatly elevated, that of the right correspondingly depressed. This entails a deepening of the left bulbo-ventricular sulcus and an opening out on

the part of the right. There is also axial rotation. The shoulder of the left mantle thrusts ventrad displacing the bulbus to the right, and the accompanying rotation displaces the depressed shoulder of the right mantle dorsad so that it is concealed in ventral view. The loop is directed to the right, ventrad and slightly caudad, and the left lateral mesocardium comes to occupy a slightly more cephalic position than the right. As a whole, the dorso-ventral depth of the myocardium is increased at the expense of its transverse breadth (fig. 15) and its extremities are a little approximated, the sagittal distance between the end of the bulbus and the junction of the mantles with the lateral mesocardia being absolutely diminished. The dorsal mesocardium is retained unbroken in its whole extent.

The middle plate is undergoing reduction by being absorbed into the mantles. It is represented by a ridge projecting entad and at the sides gradually diminishing in thickness as it fades into the mantles. It can be followed into the beginning of the bulb where it lies opposite the partition between the endothelial tubes (fig. 14). It then runs along the convexity of the loop occupying the same position relative to the fusing endocardial anlages as in the bulb (fig. 15). On reaching the caudal contour of the loop it becomes continuous with the ental elevation produced by the interventricular sulcus. Thus by the ridge and interventricular sulcus the primitive median line of the heart is represented, a conclusion which is borne out by their location in their whole course opposite the line of fusion, as yet incomplete, between the endothelial tubes.

In the heart of an embryo of 16 pairs of somites the loop has increased and projects more strongly. The left bulbo-ventricular sulcus is nearly horizontal and the right has been reduced, appearing only as a slight furrow ectally and a slight angle within the myocardium. The middle plate is again represented by a low ridge extending from the end of the bulb to the beginning of the interventricular septum. This now has an obliquely transverse direction. In an embryo of 18 to 19 pairs of somites the ridge of the middle plate disappears. The interventric-

ular sulcus is still oblique. It is only as the venous end of the heart begins to move towards the right that the septum assumes a dorso-ventral direction. Its transverse direction in early stages is rendered possible by the primary displacement of this extremity to the left as will be demonstrated in the consideration of the endothelial analges.

To summarize, the fusion of the myoepicardial mantles is accomplished with the aid of a middle cardiac plate, which subsequently becomes reduced to a ridge marking the primitive median line during the formation of the loop. It is continued caudad as the interventricular septum, which thus forms in the line of original fusion of the heart anlages and by its appearance separates again, so far as the ventricles are concerned, the primitive bilateral anlages. The myocardium in the early stages of fusion is bilaterally symmetrical with a well marked bulbo-ventricular sulcus on each side. In the formation of the loop the left sulcus deepens and the right opens up and gradually is obliterated. It is possible that the middle plate now reduced to a ridge and located at the convexity of the forming loop, is less plastic than the thinner portions of the mantles and failing to lengthen to a sufficient degree exerts a traction which occasions the appearance of the interventricular sulcus.

THE ENDOTHELIAL TUBES

The origin of the endocardium differs in nowise from the origin of endothelium elsewhere in the cat. It develops from mesenchyme which is formed in loco, first by migration of cells from the compact visceral mesoderm, second by delamination of groups of cells from the same source. In this process ridges and projections are formed by the mesoderm from which the mesenchyme loosens itself. A means of migration is afforded the amoeboid cells by the presence of interdermal cytodesmata, delicate protoplasmic bridges stretching between the mesoderm and entoderm. The early mesenchyme consists of single cells and scattered groups which are arranged in plates or even cords. Within these groups vacuoles appear and enlarging flat-

ten the containing cells to endothelium.[8] The resulting vesicles Bremer[9] has termed angiocysts.

In the heart the conformation of the myoepicardial mantle confines the mesenchyme and angiocysts lodged within its concavity and entails their transformation into a longitudinal channel. In the embryo of 4 pairs of somites the projecting ridges of the mantle still intervene between the angiocysts and delay the formation of a continuous lumen. In embryos, of 7 to 8 somites this is nearly complete, and is so from omphalomesenteric vein to ventral aorta in the embryo of 9 somites. In all of these however, and to a less degree in still older embryos there are present unannexed angiocysts and abundant mesenchyme about and especially between the endothelial tubes. The mesenchyme is so gradually transformed into endothelium that it is not easy to define the limit between the two stages, but the endothelium is the dominant tissue by the time fusion begins. The cat thus conforms to Mollier's[10] observation of the late fusion relative to condition of tissue in amniotes, the fusion occurring when the heart is mesenchymatous in sauropsids, when it has become endothelial in mammals. His recognition of a stage of solid cords antecedent to the mesenchymatous stage, in which the accelerated fusion of anamniotes occurs, is theoretically and terminologically not altogether fortunate, for the undifferented cells first moving into the mesostroma and subsequently giving rise to a variety of products are properly termed mesenchyme on grounds of morphology. The term does not necessarily connote any theoretical prepossessions. It simply designates a position and arrangement of cells other than that obtaining in the three germ-layers. In immediately subsequent stages the descendants of some of these cells retain this character, while other become flattened in response to the

[8] Cf. for interdermal cytodesmata, v. Szily. Anat. Anz., Bd. 24, 1903, p. 417; and Studnička, Ibid., Bd. 40, 1910, p. 33. For origin of endothelium in the cat. Fleischmann, A. Embryologische Untersuchungen I, 1889; Schulte, Mem. Wistar Inst. Anat. and Biol., no. 3, 1914.

[9] Am. Jour. Anat., vol. 13, 1912.

[10] Mollier, Op cit., p. 1051.

collection of fluid and are modified to endothelium. I should prefer, therefore, to term Mollier's first stage of solid cords, so far as the mammal is concerned, simply mesenchyme. His second 'mesenchymatous' stage is really a mixed condition of mesenchyme and endothelial vesicles; it might be termed the stage

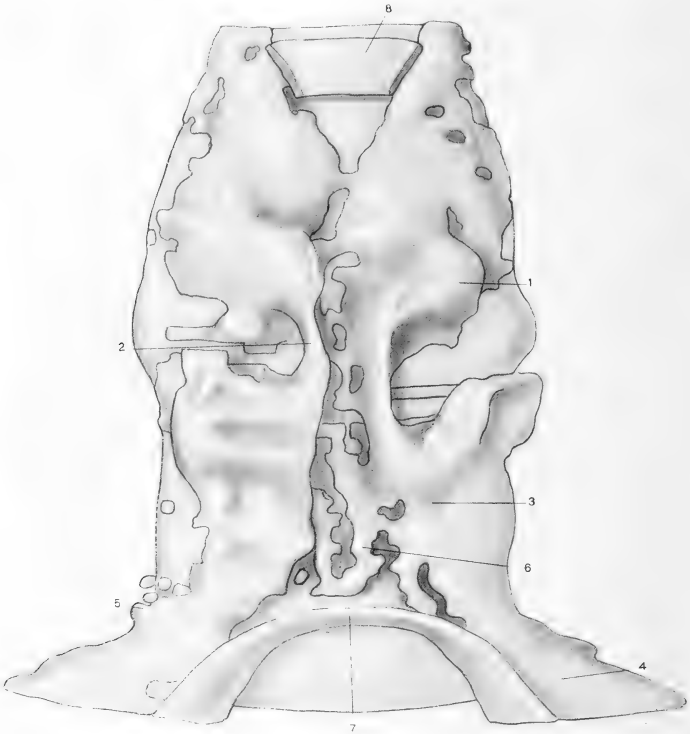

Fig. 9 Reconstruction of endothelial heart tubes and adjacent mesenchyme of a cat embryo of eleven pairs of somites. Columbia Collection, No. 534. × 300. Reduced one-half. *1*, Bulb; *2*, isthmus, corresponding to bulbo-ventricular sulcus; *3*, ventricle; *4*, omphalo-mesenteric vein; *5*, atrio-venous angle; *6*, mesenchyme between tubes; *7*, anterior intestinal portal; *8*, oral plate.

of angiocysts. The third is well designated as that of the endothelial tube with a continuous lumen but in this stage there may also be separate angiocysts and mesenchyme adjacent to the tube.

The heart of the embryo of 9 pairs of somites is an example of this last mentioned state. In it the endothelial tube on each side has a continuous lumen not of equal diameter throughout, it is true, for it has two constrictions and its walls still show traces of their component angiocysts. The constrictions are located at the bulbo-ventricular sulcus and at the cardio-venous angle. The former reduces the lumen to a narrow dorso-ventral cleft, the latter produces a smaller but quite perceptible diminution of the lumen. The bulb and ventricle are on the contrary dilated, and markedly so in their transverse diameter.

Between the bulbs corresponding to the narrow middle cardiac plate there is room for but little mesenchyme. Between the divergent ventricles, however, it is more abundant and stretches across between the endothelial tubes in plates and anastomosing strands, among which are scattered small angiocysts (fig. 2).

Conditions in the embryo of 11 somites (figs. 9 and 10) differ only in that the tubes are approximated and nearly parallel in their whole length. The slight asymmetry of the two sides is due mainly to the collapse of the shoulder of the left ventricle and in the cast of the lumen to a similar collapse of the right omphalo-mesenteric vein, which in other embryos of about this stage is actually a little larger than the left.

The bulbs are dilated and merge into the ventral aortic roots and the plexus forming about the foregut. As yet fusion has taken place across the median line only at one point situated well forward towards the oral plate. Elsewhere between the bulbs are strands of mesenchyme in which are two small angiocysts.

Corresponding to the bulbo-ventricular sulcus on each side is a narrow isthmus compressed laterally.

The ventricles are wide, prolonged into the shoulders of the mantles and diminishing caudad to their junction with the omphalo-mesenteric veins. The right in four places shows rem-

nants of the partitions separating its component angiocysts. On the left but one minute one is present. The separated lumina at the shoulder of the left ventricle are due to collapse of the tube. Between the two ventricles is a net work of mesenchyme strands which have for the most part a longitudinal direction. In the model of the lumina but two angiocysts are found in this area, one median in position, one close to the left endo-

Fig. 10 Lumina of endothelial tubes of embryo shown in figure 9, same scale. *1*, Bulb; *2*, isthmus; *3*, ventricle; *4*, omphalo-mesenteric vein; *5*, atrio-venous angle; *6*, angiocysts.

thelial tube. The irregular contours of both tubes mesad suggest the addition of imperfectly assimilated angiocysts.

In embryos of 12 and 13 somites the mesenchyme of the middle cardiac plate is condensed into a single strand which occupies the concavity of the plate between the ridges which demarcate it from the mantles (figs. 11 and 12). In the model of the endothelium (not illustrated) this strand extends from the constricted regions or isthmi between bulbs and ventricles as far as

the anastomosis between the omphalo-mesenteric veins. In it are three angiocysts. Two are very small; the more caudal of these is in process of annexation to the right ventricle, the other is attached by its wall to the left ventricle but does not communicate with its lumen. The largest angiocyst is elongated and has important connections. It communicates cephalad with the isthmian region of each bulbus and so establishes the first

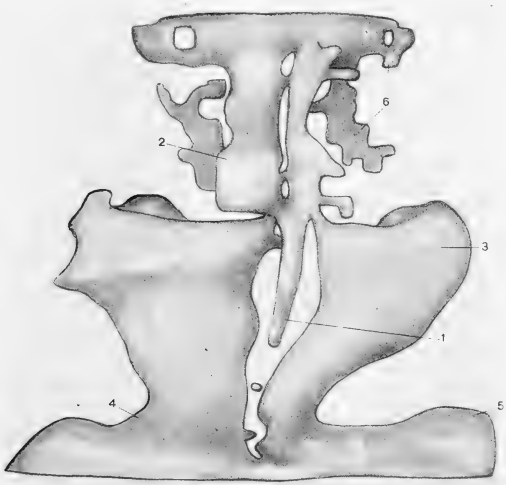

Fig. 11 Reconstruction of the lumina of the endothelial heart tubes and adjacent vessels of a cat embyro of twelve pairs of somites. Columbia Collection, No. 547. × 300. Reduced one-half. *1*, Median angiocyst; *2*, bulb; *3*, ventricle; *4*, atrio-venous angle; *5*, omphalo-mesenteric vein; *6*, forming aortic arches.

continuity of lumen between the endothelial tubes. In addition it has a small connection with the right ventricle. Thus the median angiocysts play a rôle in the coalescence of the endothelial anlages analogous to that of the middle plate in the fusion of the myoepicardial mantles. For the rest the changes accomplished in this stage are easily appreciated on comparison with the heart of the embryo of 11 somites (fig. 10). The cardio-

venous angles are greatly deepened and the omphalo-mesenteric veins are brought close together. Between their approximated portions a small communication has formed. As a whole the right tube is larger, perhaps more distended than the left. The right omphalo-mesenteric vein is certainly larger than that of the opposite side.

Fig. 12 Photomicrograph of section of heart shown in figure 11. × 125. Reduced one-half. *1*, Middle cardiac plate; *2*, median angiocyst; *3*, ventricle.

In the heart of the embryo of 14 somites as marked changes have supervened in the endothelial tubes as in the myocardium. The loop is well formed but in its entire length is double, being composed of two parallel tubes as yet only at the beginning of fusion. The general configuration is shown in figure 13, in which the model is seen from behind and slightly from above, bringing its caudal aspect prominently into view and to a less

degree its ventral surface. The drawing is greatly foreshortened. The bulbar segments are approximated but their lumina are still completely separated by an endothelial partition. The right bulb has a slightly greater cross-section than the left (fig. 14) and in its terminal segment the left is slightly irregular in contour as can be seen in the illustration of the model. These changes, though small, foreshadow the reduction of the left bulb

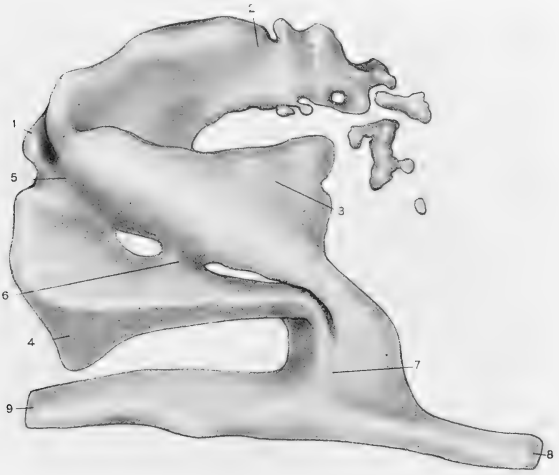

Fig. 13 Reconstruction of the lumen of the heart in a cat embryo of fourteen pairs of somites. Columbia Collection, No. 188. × 300. Reduced one-half. *1*, Right bulb; *2*, left bulb; *3*, left ventricle; *4*, right ventricle; *5*, fusion between isthmi; *6*, fusion between ventricle; *7*, fusion at cardio-venous angles; *8*, left omphalo-mesenteric vein; *9*, right omphalo-mesenteric vein.

in later stages. The isthmus of the left side is strongly kinked by the deepening left bulbo-ventricular sulcus, while the opening out of the sulcus of the right side places that bulb under more favorable conditions as regards flow. The area of fusion between the isthmi has greatly increased.

The ventricles are pyramidal in form, tapering toward the isthmus and caudad and prolonged in strong angular projec-

tions into the shoulders of the mantles. The projection on the left side is long and pointed, that of the right blunt and short conformably to the alteration in shape of the mantles at this

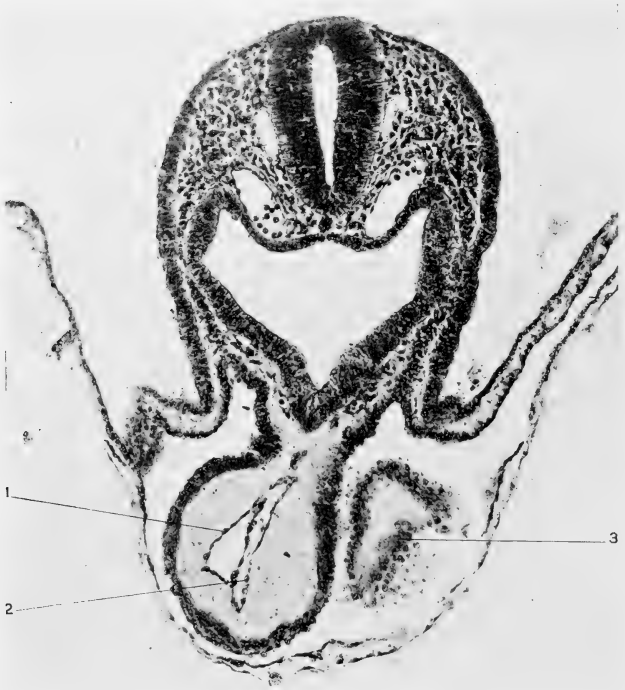

Fig. 14 Photomicrograph of section through the bulbs of embryo shown in figure 13. × 125. Reduced one-half. *1*, right bulb; *2*, left bulb; *3*, shoulder of left ventricle myocardium.

period. Coalescence has been effected at about the middle of the ventricles. Immediately ventrad of this area is a region where the endothelium of the tubes is not yet in contact and a perforation extends through the heart from the convexity to the

concavity of the loop (fig. 15). Elsewhere only a partition of endothelium separates the ventricles.

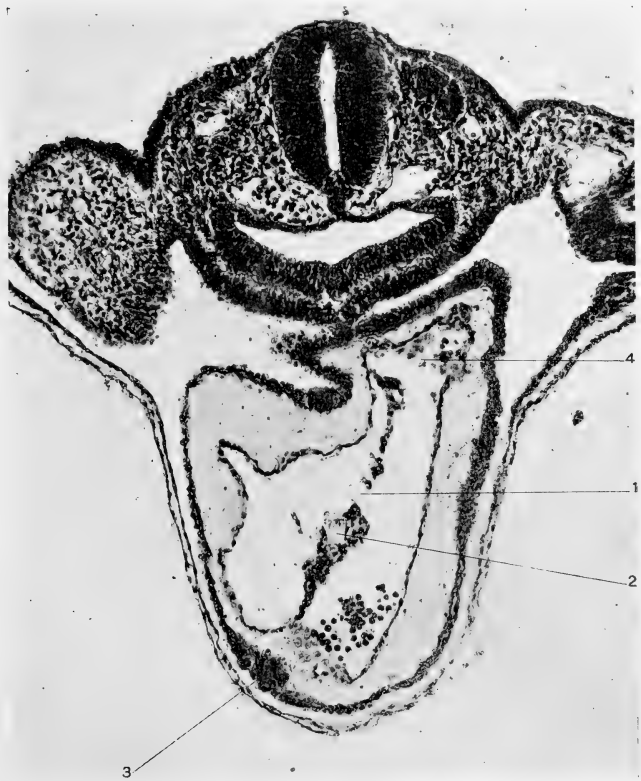

Fig. 15 Section through ventricles of the same embyro. × 125. Reduced one-half. *1*, Area of coalescence of ventricles; *2*, interval between endothelial tubes; *3*, middle cardiac plate; *4*, atrium.

Caudad the area of union between the omphalo-mesenteric veins has increased and extends upon the terminal portion of

the heart tubes, which here are dilated. This enlargement represents the atrium. It is displaced well to the left of the median line. This preliminary excursion in a direction opposite to that occurring in later stages probably depends on the larger size of the right omphalo-mesenteric vein and seems capable of play-

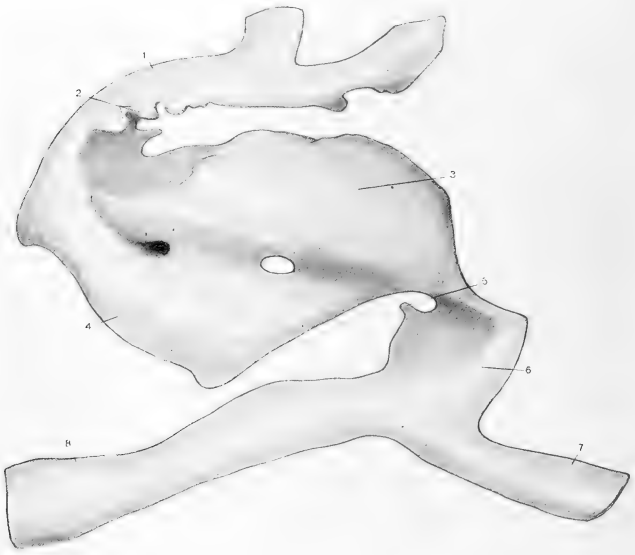

Fig. 16 Reconstruction of the lumen of the heart in a cat embryo of sixteen pairs of somites. Columbia Collection, No. 551. × 300. Reduced one-half. *1*, Right bulb; *2*, remnants of left bulb; *3*, left ventricle; *4*, right ventricle; *5*, interruption of right tube; *6*, atrium; *7*, left omphalo-mesenteric vein; *8*, right omphalo-mesenteric vein.

ing a decisive rôle in determining the direction which the cardiac loop will take. For the movement of the venous confluence to the left favors flow into the left ventricle as against the right. This becoming engorged thrusts itself strongly against the shoulder of the left mantle, and the direction of the flow be-

ing ventrad as well as cephalad tends to throw the left mantle ventrad and so initiates the axial rotation begun in this stage. These changes of position on the part of the left heart would seem to entail as consequences the observed displacements of the right, the opening out of the right bulbo-ventricular sulcus, the reduction of the shoulder of the right mantle, and its rotation dorsad.

But there is an additional factor to be considered. The accentuation of the left bulbo-ventricular sulcus increases the compression of the isthmus of that side and in so far impedes circulation through it. This condition favors the engorgement of the left ventricle and so participates in producing the effects enumerated above. However, prior to these events a connection has been formed between the two isthmi close to their junction with the ventricles. When the left isthmus is compressed this communication serves as a collateral channel leading the blood stream into the right bulb which from now on exceeds the left in development. The distension of the right ventricle is maintained by the interventricular communication already described. There is need of some arrangement of this sort for the communication of the atrium with the right ventricle is of smaller caliber than on the left side. This also depends upon the shift of the venous end of the heart to the left with a consequent marked accentuation of the right cardio-venous angle and a diminution of angularity on the left.

The excess of the right omphalo-mesenteric vein over the left seems then the efficient cause of the displacement of the atrium to the left and this joined with the configuration of the tubes and mantles at the beginning of the process is capable of affording a mechanical explanation of the formation and direction of the loop.

Conditions in the embryo of 16 pairs of somites are corroborative of the findings in the embryo just described. The lumen of the heart is shown in figure 16. The right omphalo-mesenteric is the larger, the atrium is strongly displaced to the left and joins the atrio-ventricular canal almost at right angles. Here an important change has been effected for the right tube, atten-

uated in the embryo of 14 somites is now interrupted and represented in the model of the lumen only by a pointed projection of the atrium. In the model of the endothelium, which was made of this embryo, there was also a solution of continuity at this point and in addition to the atrial protrusion shown by the lumen there was also a small projection of collapsed endothelium from the ventricle. The ends of the two processes were separated by a small but perfectly definite gap.

The fusion between the ventricles has progressed, especially caudad, and the two gaps in the line of luminal coalescence are filled with epithelium. There is no longer a foramen leading between the ventricles as in the 14 somite stage. At the isthmus also the communication between the two tubes is greatly increased, and from this point on the functional bulb is that of the right side. The left is interrupted in its continuity and represented only by irregular projection of the lumen and patches of cells adherent to the wall of the right tube.

In later stages there is no trace either of the left bulb or the right atrio-ventricular canal and nothing in these slightly older embryos as far as they have come under observation is indicative of the history of these regions. The remnants of the interventricular partition persist awhile but are entirely effaced in an embryo of 21 somites.

The coalescence of the endothelial tubes in the cat is thus seen to be delayed until the cardiac loop has been completed. It is begun just prior to the initiation of the loop at points where the tubes are bent mesad. In the actual coalescence angiocysts developed in relation to the middle cardiac plate are involved. Two segments of the primitive tube are in part sacrificed—the left bulb and the right atrio-ventricular canal. The factor seemingly responsible for the direction of the loop is the larger size of the right omphalo-mesenteric vein with the consequent displacement to the left of the region of venous confluence.

CONCERNING CERTAIN CELLULAR ELEMENTS IN THE COELOMIC CAVITIES AND MESENCHYMA OF THE MAMMALIAN EMBRYO

V. E. EMMEL

From the Anatomical Department, Washington University Medical School and the Institute of Anatomy, University of Strassburg

FOUR PLATES (FORTY-FOUR FIGURES)

I. INTRODUCTION

The following study is concerned with the free cellular elements in the pericardial, pleural and peritoneal body cavities. The results of recent morphological as well as experimental and clinical investigations regarding the nature and origin of these structures as they occur in the serous cavities of the adult mammal are of a divergent character. In view of this fact together with the apparently entire absence as yet of embryological data bearing on the problem, it has appeared desirable to obtain if possible more definite information as to the cytological conditions in the coelomic cavities of the embryo. The nature of the present subject has also necessitated the extension of the study to include certain cellular structures occurring in the embryonic mesenchyma.

Part of the following study was made while at the University of Strassburg during a leave of absence from the Washington University Medical School. I wish to express my indebtedness to Prof. G. Schwalbe for the generosity with which the facilities of the Anatomical Institute were placed at my disposal and the encouraging interest taken in the work by Prof. Franz Weidenreich. It is with regret that in consequence of the present disrupted political condition in Europe it has been necessary to forego the pleasure of Professor Weidenreich's valued criticism of the final results of the research.

II. OCCURRENCE AND DISTRIBUTION OF THE FREE CELLULAR ELEMENTS IN THE EMBRYONIC COELOM

The presence of free cellular elements in the embryonic body cavities were first noted in a 7.4 mm. pig embryo. In order to ascertain to what extent these elements were normally present, the observations were subsequently extended to include the following material: 5 to 12 mm. pig embryos, 9 mm. rabbit embryos, 5 to 9 mm. mouse embryos and several 9 mm. opposum embryos. The specimens were fixed in Zenker-formalin (Helly's modification), embedded in celloidin or paraffin and the serial

sections stained with Giemsa or Azur-Eosin in accordance with the technique developed by Maximow.

In the study of these cellular elements as they occur in the pericardial, pleural and peritoneal cavities, the following precautions are to be taken into account. In the case of early embryos in which the peritoneal cavity is still in communication with the extra embryonic coelom, if the umbilical cord has been cut previous to fixation there is present the possibility of an accidental entrance of external elements into the peritoneal cavity through the cut cord during the fixation and removal of the embryo from the uterus During the necessary manipulation of staining and mounting, extra coelomic blood and tissue cells may become accidentally detached and transferred to the coelomic areas of the section. In the present study in the case of a doubt as to confusion with such dislocated cells the data was either discarded or recorded with a question mark. In the majority of cases, however, erroneous data arising from such sources can be satisfactorily eliminated by confining the evidence to such cells or groups of cells which can be demonstrated to extend through two or more successive sections in the series, or are clearly embedded in the coagulum of the serous fluid. The results of such a critical study seem to leave no doubt as to the normal and constant occurrence of a considerable number of free cellular elements (somewhat variable perhaps in quantity) in the body cavities of the mammalian embryo.

These free cells are irregularly distributed throughout the serous fluid. At certain stages of development, i.e., previous to the closure of the pleuro-pericardial and pleuro-peritoneal canals, they may be especially abundant in the region of the developing pleuro-pericardial and pleuro-peritoneal membranes In general they are not infrequently found relatively more numerous and aggregated at one side or another of the cavity, a condition no doubt due in part to the settling of the coelomic fluids and their cellular content toward one side of the body during fixation, as is not infrequently observed in the case of the blood in the heart and blood vessels.

In the following account the coelomic cellular elements found in the present material may, on the basis of their cytological and functional characteristics, be conveniently described as falling into two groups: first, the basophilic staining and usually phagocytically active cells, which, as will become more evident in the ensuing description, may be designated as the coelomic macrophags; and second, cellular elements characterized by their eosinophilic staining qualities and nonphagocytic activity.

III. THE COELOMIC MACROPHAGS

1. Cytological characteristics

The majority of the free coelomic elements are embraced in the first of the above indicated groups. These basophilic cells may be further roughly subdivided into three types which may be described as follows:

The first of these types is illustrated in figures 1, 2, 9 and 14 *icm*. These cells are more or less spherical in form and somewhat smaller in size than those of the other two types. The nucleus varies from a central to an eccentric position within the cell, and may be either round or more or less indented on one side, so as to approximate a kidney shape. The cytoplasm is typically basophilic in staining reaction, is without any specific granular structure and may occasionally contain several small vacuoles. In form, size, nuclear and cytoplasmic structure these cells appear comparable to certain phagocytic cells occurring in the embryonic circulation of the same embryos. The cells of the second type are illustrated in figures 4, 5, 6, 8b, 10, 14 *pcm*. They are as a rule larger in size and more oval or irregular in contour. The nuclei are quite eccentric in position and as a rule more flattened and kidney shaped in form. The cytoplasm usually takes a much lighter basophilic stain. A distinguishing characteristic is the phagocytic inclusions contained in the cytoplasm. These inclusions consist almost entirely of nuclei and cell bodies at various stages of intracellular digestion. It is of interest to note that these inclusions consist largely of red staining or apparently erythrocytic elements. The majority of the

free cells of the coelomic cavities belong to this second class. Cells of the third type are illustrated in figures 14 *vcm.* and 13. The chief characteristic is that of the highly vacuolated condition of the cytoplasm. The rounded or flattened nucleus is eccentric in position. Not infrequently cytoplasmic knobs or buds are observed projecting from the surface of the cell (figs. 14 and 44). These cytoplasmic buds may vary considerably in intensity of stain but in all the cases observed they were basophilic in their stain reaction. The relation of some of these buds as partially if not entirely detached from the cell (fig. 44*b*), together with the occasional occurrence of apparently similar basophilic bodies free in the coelomic fluid, indicates the possible constriction off of these cytoplasmic processes from the parent cell. If such is the case the phenomenon appears comparable to the liberation of detached portions of the cytoplasm as described by Weidenreich, '12, p. 2602 (also Downey and Weidenreich, '12, and Downey, '12).

The question now arises as to whether these cell types represent genetically distinct kinds of cells or whether these cells are more or less closely interrelated structurally and functionally. In attempting to answer this question from sectioned material, we are necessarily largely dependent upon such evidence as can be obtained by a comparative cytological study, and endeavoring to ascertain the occurrence or absence of data indicative of structural intergradations between the cells in question. In the course of such a study the following results were attained. Attention has already been directed to the fact that a chief distinguishing characteristic of the second type of cells is that of the phagocytic ingestion of other cell bodies. In comparing cells *icm* and *pcm* in figure 14 as representative of the first two groups just described, a considerable size difference is at once evident. It may be questioned, however, whether this is not largely a result of functional activities. The ingestion of cell bodies may be expected to increase the cell size and this is what is actually found, for the coelomic macrophags containing three, four or more cellular inclusions are larger than those having only a single inclusion, as may be observed for example in

figures 4, 5 and 6. Cells with a single inclusion usually do not differ greatly in size from those of the first group. Reddish staining bodies, corresponding apparently to centrospheres, may be found in both types (figs. 1 and 8*a*) although less frequently observed in the more highly phagocytically active cells. The flattened or more kidney shaped form and more eccentric position of the nucleus in the cells of second type is evidently largely due to the bulging out of the cytoplasm, as the result of the ingestion of the cellular inclusions and a correlated eccentric displacement of the nucleus toward the opposite side or pole of the cell body. Between these two cell types a striking difference in the intensity of basophilic stain may also be frequently noted. The present data, however, warrants the conclusion that these staining differences are correlated to an important degree with functional activities. All gradations are to be observed between the strongly basophilic cells, figures 1, 2, 9, 14 *icm* and the pale staining cells in figures 6, 10, 14 *pcm*. Furthermore, it appears of significance that the macrophags containing the larger number of cellular inclusions are almost always the paler in cytoplasmic stain. After the study of a large number of these cells one is impressed with the suggestion that this may, in part at least, be a direct result of intracellular digestive functions. Certainly there can be no question but that the digestive chemical interactions between the phagocyte and cellular inclusion, whatever their exact nature may be, are of such a character as to change the ingested erythrocyte from a bright red staining cell to a practically colorless non-staining mass (figs. 5, 6, and 8). In case of an ingested erythroblast the erythrocytic nucleus may first become fragmented (figs. 4 and 5) or else manifest progressive changes from center toward its periphery (figs. 6*n* and 33*e*, also p. 95), but in either case it eventually experiences a practically complete loss of its staining properties. On the other hand, however, may not this digestion also involve correlated changes in the staining substances of the phagocytic cell? The frequent association of a pale staining cytoplasm with maximum phagocytic activity strongly indicates that this is the case. If this conclusion is correct it offers an explanation for the basophilic differences between the two types of cells under consideration.

No evidence was obtained as to the exact nature of this change in the staining qualities of the cytoplasm of the phagocyte—whether it is the result of an internal absorption of digestion products or whether it is due to a modification or reduction of cytoplasmic elements of the phagocyte itself as they participate in the digestive processes. In either case it is remarkable how constant and sharp is the demarkation between the cytoplasm of the phagocyte and that of the ingested cell, and that one is able to detect little if any difference in the cytoplasm of the phagocyte in the immediate vicinity of the inclusion as compared with that in the more remote parts of the same cell body. It is to be observed that these color changes in the phagocyte are apparently not confined exclusively to its cytoplasm for the nucleus also may become much lighter in the highly active macrophags, a change involving apparently the nucleoplasm rather than the chromatin (cf. figs. 1, 2, 9 and 14 *icm*, with 5, 6, 10, and 14 *pcm*). These cytoplasmic and nuclear differences appear especially well demonstrated in figure 8. These two cells from the pericardial cavity of a 9 mm. pig embryo were lying side by side in the manner drawn. Consequently there can be no question of variation in fixing or staining technique as factors in their staining differences. In (*a*) both nucleoplasm and cytoplasm are quite basophilic, while in (*b*) which is at a comparatively much higher stage of phagocytic activity, nucleoplasm and cytoplasm are both much paler in color. The same observation also applies to figures 9 and 10 which were taken from two consecutive sections of a second 9 mm. pig embryo. In examining the literature bearing upon this subject it is of interest to note that similar changes in the basophilic character of the cytoplasm is described by Downey ('10) in the phagocytes of the lymphorenal tissue of the ganoid fish, Polyodon spatula, as indicated in the statement that "as seen in Polyodon these cells are strongly basophilic right after phagocytosis (fig. 2). As the phagocytosed erythrocyte breaks down the cell gradually becomes pale and often metachromatic" p. 85.[1]

[1] Kyes ('15, p. 546) in a recent paper also describes in the liver of birds variations in nuclear structure and cytoplasmic staining reaction of endothelial cells as correlated with the intra-cellular digestion of ingested erythrocytes.

Turning finally to the cells of the third group it will be observed that their most distinguishing characteristic is the presence of cytoplasmic vacuoles. The nuclei may be round or flattened, are usually more or less eccentric in position and do not differ materially from the nuclei of the phagocytically active macrophags. The vacuoles vary greatly in number and size, so that while in some cases only one or two may be observed, in other instances they are sufficiently numerous to fill almost the entire cell body (figs. 14 *vcm.* and 13). While many of these vacuoles are filled with an apparently homogeneous material which is non-staining with Giemsa, others contain remnants of hemoglobin and nuclear staining elements. In comparing such cells as figure 5 with its almost entirely digested erythrocytic inclusion, figure 6 and 14 *pcm.*, with large vacuoles containing just a trace of ingested material, and figure 8*b* in which one of the vacuoles contains a cellular remnant while the two others are practically clear, there can hardly be any question but that many if not the majority of these vacuoles have arisen in connection with intracellular digestive processes. Upon the complete transformation of the chromatic elements of the ingested erythrocyte there may thus still remain, for a time at least, a non-staining vacuole-like structure in the cytoplasm of the phagocyte.

In resume it appears, therefore, that the size, form, nuclear and cytoplasmic difference between these three types of cells are to be regarded as correlated with variations in the degree of differentiation and functional activity rather than as indicative of differences of a more fundamental character. The most prominent function of these cells being that of phagocytosis, they may not inadequately be designated as the coelomic macrophags of which the cells of the first type present the least differentiated and least active stages and the cells of third type end stages in functional activity. That these coelomic cells are not only manifesting normal functional changes and cytological differentiation but are also undergoing cell multiplication is positively demonstrated by the not infrequent occurrence of mitosis (figs. 3 and 7). Indeed in some instances it appears that

the macrophags may undergo mitosis even while still retaining incompletely transformed remnants of previously ingested material in its cytoplasm (fig. 11).

2. Evidence as to their origin from the coelomic mesothelium

a. Certain characteristics of the mesothelium in general. Are these macrophags of the coelomic cavities cells which have migrated into these body spaces from the neighboring blood vessels or are they tissue cells which have become detached or liberated from the tissue walls surrounding these cavities where they have undergone further differentiation and assumed phagocytic and possibly other functional activities in the serous fluids? As to the first view, while no conclusive evidence of such a migration of macrophags from the blood vessels and adjacent tissues was obtained, it is to be recognized as not improbable that such cells may enter the embryonic coelom in this manner as has been maintained by Maximow to occur in the serous cavities of even the adult mammal. But the crucial question still remains as to whether this is to be regarded as the only source of the coelomic macrophags in the embryonic body cavities.

The embryonic mesothelium consists of cells which are rather flattened in form. The nuclei also have a correlated flattened oval shape and cell walls are not clearly evident. Typically these cells form a single epithelial layer lining the coelomic cavities (*mes* in figs. 15 to 18 and 41 to 44). Such a layer is, however, by no means always sharply defined, for in various regions the surface cells are in such an intimate syncytial association with the deeper lying cells that characteristic structural differences between them are not readily evident, indeed in certain regions the conditions are such as to suggest that the surface mesothelial cells may have given rise to many deeper lying cells comparable to the endothelial growths described by Mall ('12, pp. 258, 261) in certain endocardial regions of the heart. In a careful study of the mesothelium as seen in serial sections, it may be noted that the form and structure of its component cells are not always constant throughout the body cavities. In various regions the flattened mesothelial cell body, as well as

its nucleus are observed to have assumed a more rounded form and not infrequently a more basophilic stain reaction. Instances of such changes involving only single cells or small groups of cells are especially evident in the visceral epicardium. Cells may be observed in which the nuclei instead of being oval or flattened are more rounded or spherical in shape and not infrequently indented in a kidney shaped manner (figs. 17 and 42). The cytoplasm also is rounded up, the cell as a whole projects from the mesothelial surface and in some instances is attached by only a slender basal cytoplasmic process (fig. 41). It may be observed that as a rule the cytoplasm of such cells also presents a more basophilic stain. Many of these cells have every appearance of being destined to become subsequently detached from the coelomic wall, and it appears evident that when liberated into the body cavity they would be practically indistinguishable in either nuclear or cytoplasmic structure from the macrophags already present in the serous fluid. The important point that the mesothelial cell may function in a phagocytic manner seems clearly demonstrated in figure 42 showing erythrocytic inclusions in the mesothelial cytoplasm. Figure 44 represents a section through a region of the visceral epicardium in which the proliferative activities involve a larger number of cells. Many of the apparently recently liberated cells are phagocytically active, present vacuoles and bud-like cytoplasmic processes and appear identical with the typical coelomic macrophags.

Are we to conclude from such data that the coelomic mesothelium is really giving rise to coelomic macrophags? In view of the close approximation of many of the mesothelial cells to the macrophags in both their cytological characteristics and potential phagocytic functions, as observed with the present technique, it would not appear a great step to their differentiation into such cells. But a substantial proof of such a process is a more difficult matter especially from fixed material where all the intermediate stages in a given case cannot of course be directly observed. In evaluating the present data it seems clear, however, that the form and surface relations of the cells

just described are at least not to be discarded as being merely misleading appearances due to tangential planes of section as can be ascertained from the serial sections. The possibility was also considered as to whether such cell forms may not be due to a shrinkage or folding of the coelomic walls or artificial ruptures of the mesothelial surface. All of these artificial conditions may of course occasionally occur in consequence of the histological technique employed but the rounded projecting cells do not have the appearance of elements artificially torn from the living mesothelium. If they are the products of histological shrinkage, it is not easy to understand how single isolated cells could be made to assume the present forms, for such cells may be found in regions of the coelomic wall where the regular surface curvature is not indicative of any artificial irregularity in its reaction to fixing agents. When found in regions where the surface of the wall appears more irregular, the cell types in question may occur on both convex areas of the slight surface elevations as well as in the concave areas of the mesothelial depressions. What appears a criticism of a more serious character, however, is the possibility that the cell forms under consideration are either instances of cells from the adjacent mesenchyma and blood vessels migrating through the mesothelium to reach the coelomic cavities or else coelomic macrophags themselves merely resting upon or in close proximity to the mesothelial surface. That a coelomic macrophag may occasionally be caught in the coagulum of the serous fluid and fixed in close contact with the coelomic surface must be granted. Furthermore since one is apparently forced to admit that erythrocytic elements, as will be subsequently described, must enter the coelom from extra coelomic regions it seems necessary to admit the possible migration of other cellular elements into the embryonic coelom. Much of the evidence also tends to be vitiated by the absence of decisive cytological differences between many of the mesothelial cells and either the coelomic macrophags or similar cells occurring in the vascular channels.

In view of the above difficulties it becomes necessary to ascertain whether there exists any other sources of evidence condu-

cive to a more convincing conclusion regarding the problem. The results of such a further study may be presented in the form of two groups of data: the first referring to the character of certain cellular masses found free in the coelomic cavities and the second to the structure of the mesothelium in certain regions of the coelomic walls.

b. Free cell masses in the coelomic cavities. In addition to single free coelomic cells there are also found certain interesting groups or masses of cells (figs. 15 and 16). Such masses are of fairly constant occurrence. Some of them may consist of only two or three component cells (fig. 15) or of a much larger number of cells as in figure 16 which is a section of a mass extending through as many as five sections.

These masses cannot be said to represent merely a loose aggregation or agglutination of otherwise free coelomic macrophags, for upon closer study it may be observed that as a rule their component cells are organized into a definite peripheral or epithelial layer with an occasional cell more centrally situated. Consequently as seen in section, the larger masses present the appearance of epithelial rings surrounding a lighter and less cellular core. The majority of the cells in these masses, especially the more peripheral ones, are indistinguishable in both nuclear and cytoplasmic structure from the mesothelial of the adjacent coelomic walls. In other words there is every indication that these masses are groups of mesothelial cells in which the mesothelial character of the cells are still clearly evident. Upon the careful examination of serial sections it can also be definitely established that the majority of these masses (such as shown in figs. 15 and 16) are entirely free in the coelomic cavity, nor do they present the appearance of having been artificially separated from the coelomic walls. Occasionally such masses are, however, still attached to the coelomic wall and their relations in this case are such as to indicate that the mesothelium has grown out into the lumen of the coelom in the form of a papillary projection which may subsequently become detached. We have therefore, what appears to be a clear case of the actual separation of mesothelial cells from the coelomic wall and lying free in the coelomic cavity.

The important question next arises as to the fate of these mesothelial cell masses. How long they may persist as definitely organized structures the present data does not indicate. While mitotic figures were not observed neither was there any evidence of degeneration noted. That their component cells may assume phagocytic activities is indicated in the smaller of the two masses in figure 15. But the point especially pertinent to our present purpose is the fact that some of these masses show evidence of further modification and subsequent disintegration into isolated free cells. In the smaller of the two groups shown in figure 15 the nuclei are more rounded in shape and the epithelial character of the component cells is no longer so clearly evident. The single isolated cell in the same figure is apparently of the same type except that the nucleus has assumed a kidney shape and the cytoplasm a deeper basophilic stain. These changes seem strikingly shown in figure 16 in which several of the peripheral cells have become more spherical in form and the cytoplasm takes a darker stain. Of the two approximately spherical cells in the lower part of the figure, one is partially and the other almost entirely free from the main mass. Such cells appear quite comparable to the basophilic cells or macrophags found free in the coelomic cavities.

Granting the correctness of the above conclusions, these disintegrating mesothelial cell masses, therefore, furnish a type of evidence indicative of the cytological transformation of mesothelial cells into coelomic macrophags which appears to obviate the possible objections previously noted with reference to similar changes at the surface of the coelomic wall. For it is evident that the rounded cells at the periphery of these masses can certainly not be regarded as migratory cells from mesenchymal or vascular sources and it is highly improbable that they represent free coelomic macrophags incidentally resting or fixed at the surface of the mass.

c. The mesothelium of the pleuro-pericardial and pleuro-peritoneal membranes. The second of the two sources of evidence referred to on page 84 is found in the regions concerned with the subdivision of the embryonic coelom by the development of the

pleuro-pericardial and pleuro-peritoneal membranes. These membranes as can be directly observed, are localized centers of increased mitosis and cellular growth. Without taking into account here the deeper lying causes it appears evident that this increased cellular proliferation is an important factor in the gradual outward extension of the coelomic walls or membranes, the final fusion of the juxtaposed surfaces of which is destined to effect a closure of the pleuro-pericardial and pleuro-peritoneal canals. In such regions of fusion the free mesothelial surfaces necessarily disappear. Consequently, if mesothelial cells can differentiate into coelomic macrophags such regions might be expected to furnish valuable evidence of such a process.

Figure 43 is one of fourteen sections of such a region in a 7 mm. pig embryo, showing the embryonic pleural cavity (*pc*) and the pleuro-pericardial canal (*pplc*). At the center of the figure is seen a section through the distal border of the pleuro-pericardial membrane (*pp*) for the left pleuro-pericardial canal (the embryo having been cut in the sagittal plane). Figure 14 shows the same central mass drawn at a higher magnification. If this cellular mass is traced back through the fourteen sections in which this membrane is present to its attachment to the parietal wall, its component cells are found to merge and become continuous with the mesothelial and mesenchymal elements at the juncture of the parieto-pleural and parieto-pericardial walls. Directing attention more especially to the present object of inquiry it is important to note that toward the more peripheral or distal margins of these advancing membranes the superficial cells have a more nearly spherical shape and that a definite flattened mesothelium is no longer evident. In the more central portion of the section shown in figure 14 there may be recognized a somewhat more definite layer of mesothelial cells (*mes*) surrounding a central core (*c*). Toward the periphery of the section, however, the cells are no longer so intimately united and many of them are partly if not completely detached as free rounded cells. The structural characteristics of such an area is not indicative of degenerative changes. On the contrary the frequent mitotic figures (*m*) furnish ample proof of active cell multiplication and

there is abundant evidence of phagocytic activities (*pcm*), intra cellular digestion and vacuole formation (*vcm*). It may be further observed in these regions that the more superficial cells are not only modified in form, but that the cytoplasm also tends to take a deeper basophilic stain. Such cells as *icm* and *pcm* are certainly identical in structure with the coelomic macrophags. At the same time there are present all transitional stages between these cells and the original mesothelial elements. Finally beginning at these proliferating areas, detached free cells of the same type may be traced through successive sections out into the adjacent body spaces as they become scattered throughout the pericardial, pleural and peritoneal cavities where they appear indistinguishable from the free cells normally present in these regions. Evidently here again, just as in the case of the free mesothelial cell masses previously described, the proliferating and disintegrating mesothelium is giving rise to free cells functioning as macrophags in the coelomic cavities.

In resume it may be stated, therefore, that the data derived from both the pleuro-pericardial and pleuro-peritoneal membranes and the free mesothelial cell masses support in a substantial manner the conclusion suggested by the cytological conditions observed at the surface of the coelomic walls. Namely, that the coelomic mesothelium is an important source of the phagocytic cells found in the embryonic coelom, and that as these mesothelial cells round up and become detached from various regions of the coelomic wall, they assume structural and functional characteristics identical with that of the coelomic macrophags typical of these embryonic body cavities.

IV. ERYTHROCYTIC ELEMENTS IN THE COELOMIC CAVITIES

As already indicated (p. 76) the second group of cellular coelomic elements are characterized by their eosin staining qualities and non-phagocytic activity. These eosin staining elements again fall into two sub-groups, the one consisting of small non-nucleated bodies and the other of larger nucleated cells.

1. Small eosin staining bodies

In figures 14 and 20 several small round red bodies (*e*) are to be observed about a third or less than a third the size of an average erythrocyte. Such bodies were found more or less constant in young pig, mouse and rabbit embryos although they may vary considerably in number in different specimens. These structures may be observed lying free anywhere in the coagulated coelomic fluid or, as is more frequently the case, in contact with the coelomic walls. As a rule they are quite round, sharply defined and take a brilliant red stain with Giemsa and Azur-eosin. Upon careful focus they sometimes present the appearance of a slightly clearer central area. They are non-granular in structure. Occasionally similar bodies are also found in the circulating blood.

As to the nature of these bodies, the first suggestion to present itself is that of cytoplasmic fragments of disintegrated erythrocytes. Upon closer examination, however, it may be observed that a narrow basophilic rim can in many cases be detected at the periphery of structures in question (figs. 21, 24 to 26). Consequently, without excluding the possibility of their partial or even entire cytoplasmic character in some instances, the latter observation necessitates the identification of the majority of these bodies as elements other than merely erythrocytic cytoplasmic fragments, as will be presently more fully discussed. A second possibility to be considered is that of their identification with cytoplasmic buds constricted off from coelomic macrophags. Downey (13 p. 42) in a description of the detachment of cytoplasmic buds from lymphocytes and large mononuclear cells in the lymph gland of the rabbit, found that these detached bodies may vary greatly in their basophilia and concluded "that after separation from the cell the irregular masses assume a spherical shape and that they gradually lose their basophilia." It was thought that possibly similar changes might account for the eosinophilic bodies in question, but no satisfactory evidence was obtained demonstrating transitional stages between such basophilic bodies and these intensely eosin-

stained structures. Some of the bodies may be much paler than others, but the sharply defined basophilic rim which can still be observed in many cases seems to render them structurally different from the hyalin bodies derived from lymphocytes and mononuclear cells.

While the present data is consequently negative as to the derivation of these eosinophilic bodies from the cytoplasm of either erythrocytes or macrophags, it does, however, furnish evidence of a more positive character as to another conclusion concerning their origin. Concerning the basophilic material already described it appears significant to note that aside from the form of a narrrow peripheral ring it may present pronounced additional accumulations in the form of either irregular masses (figs. 22, 23), one or more delicate crescentic masses (fig. 21) or small, compact and more or less centrally situated spherules (figs. 25, 26). In studying erythrocytic nuclei (p. 597) conditions are met with which are strongly suggestive with reference to the present question. Not infrequently both free and ingested erythrocytic nuclei are observed undergoing changes in which instead of becoming more or less compact in a single pyknotic body or broken up into several compact nuclear fragments the nucleus becomes lighter colored at its center and has a dark staining periphery (figs. 34 and 36). In such cases it appears that the dissolution or chemical modification of the chromatin proceeds from the center towards the periphery in such a manner that a stage may be reached in which the basophilic staining material remains as only a very thin peripheral envelope, the interior of which may take an eosin stain of such a character as to render it practically indistinguishable from the hemoglobin containing cytoplasm of an erythrocyte. Jolly ('07, p. 245) has further shown that under certain circumstances these changes may proceed to a complete tinctorial transformation of the entire chromatin content to oxychromatic staining material. The close approximation of the cytological appearance of such highly modified nuclei to that of the eosin staining bodies in the coelomic cavities appears to justify the conclusion that the latter are also of a similar character. Concerning the

occurrence of such degenerating erythrocytic nuclei in the coelomic cavity two possible sources of origin may be noted: first, through the disintegration of the erythroblasts occurring in the coelom itself as will be presently described, and second, through the occasional passage or elimination into the coelomic cavities of the degenerating erythrocytic nuclei frequently found in various regions of the coelomic walls. The latter possibility would be in accord with the fact that these eosin staining bodies, as already noted, are frequently found in intimate contact with the surface of the coelomic walls.

2. Nucleated erythrocytic cells

Figure 18 illustrates the second sub-group of coelomic cellular elements. In contrast to the coelomic macrophags, these cells have an eosinophilic instead of a basophilic cytoplasm. The nucleus also may be more irregular in form, lobulated or even subdivided into two or more almost wholly if not entirely separated segments (fig. 19). The cytoplasm is frequently vacuolated. The cell as a whole may be either round or more irregular in shape with the peripheral cytoplasm presenting a fragmented appearance. In the material studied such cells were most frequently observed in rabbit embryos although they were also present in both mouse and pig embryos. They may occur as isolated elements or in groups consisting of two or three to a dozen cells.

Upon first examination the polymorphonuclear character of some of these cells is suggestive of leucocytic elements. On the other hand in no instance was there any special leucocytic granulation detected. On the contrary the cytoplasm is of a homogeneous structure and in many instances (figs. 18, 19) the staining reaction of both cytoplasm and nucleus is apparently identical with that of the typical erythrocytes in the same embryo. Concerning the origin of these cells the possibility was considered as to their representing a partial or abortive tendency toward erythrocytic differentiation on the part of the coelomic macrophags. There can be no question but that such cells as

(*a*) in figures 18 and 19 present nuclear and cytoplasmic characteristics readily comparable to the erythrocytes to be found in the adjacent blood vessels, although this is by no means so clearly evident in such cells as (*c*), figure 18. In view of the recent observations of Haff ('14) and Bremer ('14) indicative of a participation of mesothelial cells in the origin of blood islands and blood cells it would indeed not appear so remarkable if the same cells liberated into the coelomic cavity would here be found to manifest a potentiality for erythrocytic differentiation. However, it must be admitted that no convincing evidence of such a differentiation was obtained. The irregular form and structural character of the nuclei of these cells is not typical of developing erythroblasts, the nuclei of which during the earlier phases of their differentiation are normally more or less spherical in shape (Emmel '14). No evidence of phagocytic activity as a characteristic which might justify associating these cells with the coelomic macrophags was observed, nor is the vacuolated and fragmented condition of the cytoplasm in many of these cells suggestive of progressive erythrocytic differentiation.

On the contrary the following considerations support a different conclusion. As already indicated wherever these eosin staining cells are found in groups, some of the cells in such a group can almost always be clearly identified as erythrocytic. It is equally evident that in the case of erythrocytic degeneration, the nuclei (i.e. of erythroblasts) may undergo form changes identical with those to be observed in these eosin staining cells. Such a lobulation and subdivision of erythrocytic nuclei can not infrequently be found even in the circulating blood (fig. 37 and the subsequent description on p. 100). Weidenreich ('03, p. 420) has fully described degenerative changes in which the erythrocytic nucleus becomes irregular in form, indented, bilobed, dumb-bell and clover leafed shaped, and finally constricted into two or more parts connected by a small thread-like strand or entirely separated from each other and thus give rise to a so-called double nucleated cell. In some cases such degenerating nuclei may become smaller, more compact and take a much darker stain, in other instances, however, the nucleus may main-

tain a comparatively open chromatin network, as is well shown in figure 33 of Maximow's ('09) work. Such modified nuclei are indistinguishable from the irregularly lobulated nuclei of the eosin staining cells in the coelomic cavity. In other words on the basis of the present data the cells in question are evidently correctly interpreted as degenerating nucleated erythrocytes, in which the nuclei are greatly changed in form, the cytoplasm having become deficient in hemoglobin, stains a paler color with eosin and presents the vacuolated condition described by Minot ('12, p. 511) as preliminary to further changes in certain types of erythrocytic disintegration. Here and there fragments of such disintegrated cells can be readily found. Occasionally a cell is observed with a striking peripheral fringe of eosin staining material. In some cases this material has the appearance of fragments of disintegrated cells incidentally resting against or adhering to the cell in question. In other instances the union with the cell body is so complete that a question arises whether it may not represent a phase in the degeneration of the hemoglobin containing cytoplasm of the erythroblast (fig. 12).

How these degenerating erythrocytes come to be situated in the coelomic cavities is more difficult to determine. That cells with erythrocytic characteristics are normally present constantly in the embryonic serous cavities appears positively demonstrated by the character of the cellular inclusions in the macrophags. In the absence of conclusive data as to their differentiation in situ there remains the alternative assumption that under various conditions they may escape from the blood vessels and pass through the coelomic mesothelium into the coelom. Very young erythroblasts may possibly do this by an active migration although evidence of such a migration was not obtained. It may be noted that erythroblasts may also be found within such extra vascular spaces as the lumen of the Wolffian tubules, occasionally in the lumen of the developing lung buds, and in mesenchymal spaces (p. 603) throughout the embryo.

V. ERYTHROCYTIC DISINTEGRATION IN THE MESENCHYMA

1. Degenerative changes in erythrocytic nuclei with reference to:

a. The question of the mesenchymal secretion of erythrocytic elements. The fact that degenerating erythroblasts, especially their nuclei, may undergo changes resulting in the production of small red spheres or masses with either central or peripheral accumulations or remnants of basophilic material as observed in the coelomic cavities, merits further consideration with reference: first, to the bodies interpreted by Maximow as mesenchymal secretions, and second, to the ring bodies of Cabot.

Concerning the possible relation of these eosin bodies to mesenchymal activities it may be noted that Maximow ('09, p. 513) describes the observation in the blood of the embryonic rabbit of apparently similar eosin-basophilic-droplets which he states soon disintegrate in the circulating plasma. Maximow inclines to the conclusion that these bodies are secretion products of mesenchymal cells and represent an abortive or precocious differentiation of erythrocytic or hemoglobin containing elements. Evidence is advanced for the occurrence of such a secretion in various regions of the mesenchyma, such as that of septum transversum, in the head region and adjacent to the distal ends of growing blood vessels. In these regions he records the observation of many large and small red and blue stained spherical or angular bodies generally embedded in clear vacuoles in mesenchymal cells. These bodies are described as consisting of red spheres containing one or more central blue spherules, red bodies furnished with one or more deep blue peripheral crescents or caps, or blue rings filled with a clear eosin stained content (p. 500). After the consideration of several possibilities as to their origin it is decided that they are probably elaborated in situ in the mesenchymal cytoplasm and it is stated that one can observe how the inclusion in the cytoplasm of the mesenchymal cell develops from a few initial small erythrocytic granules and how it grows in size, and the basophilic substance appears within it or on its surface.

Since these structures are so closely similar to the small eosinophilic elements occurring in the coelomic cavities it became necessary to reexamine the evidence for an intra-cellular origin of such bodies. For the writer the subject had also an additional interest in consequence of a previous study of the cytological differentiation of erythrocytes in which there was occasion to consider the possible origin of erythrocytic or hemoglobin containing elements in the cytoplasm of the mesenchymal cell (Emmel '14).

As already indicated, the preceding results of the present investigation with reference to the eosinophilic bodies in the coelom were negative as to their origin as intra-cellular secretions. Furthermore after careful study I have been unable on the following grounds to convince myself that the bodies described by Maximow in the mesenchyma necessarily represent intracellular secretions of mesenchymal cells. In the first place these eosin staining bodies are found equally as abundant and indeed frequently even more so within the ectodermal tissue of the brain wall (fig. 33*e*) cranial and spinal ganglia (fig. 28) and the entoderm of the growing lung buds and digestive tube, in situations where they would be least expected if they are derivatives of mesenchymal cells. They also occur in inter- as well as intracellular situations.

Figure 31 is from the mesenchyma in the ventral thoracic wall of a 9 mm. pig embryo and figure 32 from the mesenchyma of the septum transversum of a 7 mm. pig embryo. It will be observed that in both cases practically all the eosinophilic bodies in question are clearly situated in inter-cellular mesenchymal spaces. Similar relations can also be readily demonstrated in the brain wall and the cerebrospinal nerve ganglia (fig. 28). On the other hand similar bodies can also be found which appear unquestionably situated within the cytoplasm of cells in the mesenchyma. Since these bodies are both inter- and intra-cellular in position it seems clear from this aspect of the subject that they may be as adequately interpreted as either extra-cellular elements, some of which may have become phag-

ocytically ingested, or originally intra-cellular elements, some of which had been subsequently extruded from the parent cell.

The point then to be established is the correctness of one or the other of these two alternatives. The abundant occurrence of these elements in such tissues as the brain wall and nerve ganglia which are relatively deficient in mesenchymal cells is a fact in itself sufficient to raise a question as to their intra-cellular origin. Again upon examining the bodies in question it will be observed that they may present a variety of structural forms, among which may be noted single small basophilic spherules surrounded by just a trace of eosin staining material (fig. 31*a*), several basophilic spherules situated in a larger red staining body (fig. 31*b*), a crescent, or less frequently, a spherule of basophilic substance peripherally located (figs. 31, *c*, and *d* and 32), or a peripheral basophilic ring completely surrounding the red stained material (figs. 31*e*, 33*e*, and 28). Now if one turns to the circulating blood of these same embryos, degenerating nucleated erythrocytes are occasionally found, as is well known, in which the disintegrating nuclei have become broken up into several small more or less rounded fragments. Again in other instances such degenerating nuclei, especially in the mouse embryo, present the appearance of a red stained central area surrounded by a peripheral basophilic ring as shown in figures 34*a*, 35 and 36. It may also be observed in the same figures that portions of these rings may be very thin while other areas are much thicker and present the form of basophilic crescents. Similar degenerating nuclear changes can also be demonstrated in phagocytically ingested erythrocytic nuclei as partially shown in figures 4, 6 and 8*b*. Now it is well known and can be readily verified that erythrocytes not infrequently escape from the blood vessels and become isolated in the mesenchymal and other tissue spaces of the embryo where they may undergo various types of disintegration (Minot, '12, p. 509). From the data derived from the erythrocytes occasionally degenerating in the circulating blood it becomes evident that these degenerating corpuscles may assume structural appearances identical in char-

acter with the bodies under consideration. Perhaps the most constant difference between the bodies in the tissue spaces and the degenerating corpuscles in the vascular channels is the presence of only a small amount or frequently the entire absence of any cytoplasm peripheral to the basophilic rings or spherules in the case of the tissue spaces as compared with the conditions in the degenerating corpuscles of the blood. But this appears readily accounted for on the basis of a more rapid and earlier disappearance of the peripheral cytoplasm of the erythrocytes degenerating in the environment of the inter-cellular fluids. Indeed evidence of such peripheral cytoplasmic changes may be encountered even in the vascular channels as illustrated in figure 36 from a 9 mm. pig embryo in which one of the erythrocytes shown contains only a relatively narrow rim of cytoplasm peripheral to the nuclear ring, whereas it is entirely absent in the remaining two cells. In figure 34*a* the peripheral cytoplasm of the degenerating erythrocyte is much paler than that of the adjacent normal corpuscle. Instances in the circulating corpuscles of nuclear rings without any evident peripheral cytoplasm is demonstrated in figure 29 from the heart blood of a 7 mm. pig embryo. It appears evident, therefore, that in pig, rabbit and mouse embryos all transitional stages can be found between degenerating nucleated erythrocytes and the eosin staining bodies in the embryonic tissue spaces.

A further possible source of origin of many of these bodies which may be noted, especially in older embryos in which non-nucleated erythrocytes are beginning to appear, is in connection with the formation of non-nucleated erythrocytes. In a previous study of the pig embryo (Emmel '14) evidence was advanced indicating the origin of non-nucleated red blood corpuscles by a process of cytoplasmic constriction resulting in the separation of the original erythroblast into a non-nucleated remainder consisting of the erythrocytic nucleus together, not infrequently, with a small amount of cytoplasm remaining from the parent cell. This nucleated remainder may present an appearance practically identical with that of many of the eosinophilic bodies under discussion.

On the basis therefore, of their distribution, structure and close correspondence cytologically to both disintegrating erythrocytes as observed in the circulating blood, inter-cellular tissue spaces and phagocytic inclusions and to erythrocytic nuclei persisting after the formation of non-nucleated erythrocytes, the conclusion is drawn that the small eosin staining bodies in the embryonic mesenchyma are correctly interpreted as consisting primarily of disintegrating, and in many cases phagocytically ingested erythroblasts which have escaped into the embryonic tissue spaces and second, especially in older embryos, of nucleated erythrocytic bodies resulting from the cytogenetic processes involved in the formation of erythro-plastids, rather than the products of secretory or other cytological activities in mesenchymal cells.

b. Cabot's rings. The second phase of the present subject is concerned with the question of Cabot's rings. The erythrocytic ring-like structures, first observed by Cabot ('03) in anemic blood and which are now known to also occur under other abnormal conditions such as obtain in leukaemia and lead poisoning, are usually described as staining red or reddish violet with Giesma. Both Cabot (p. 455) and Naegeli ('12, p. 152), however, also record the occurrence of blue stained rings. These rings which have been interpreted as nuclear elements, possibly in part nuclear membranes, (Schliep, '07, p. 455) are regarded as occurring only in the pathological blood of the adult organism and never in either the human or mammalian embryo (Naegeli, p. 152, Grüner, '13, p. 83). But in view of the present data it may be questioned whether analogous structures are not, however, also encountered in the embryo as well as in the adult. The nuclear rings already described in the ingested erythroblasts of the coelomic macrophags, the basophilic periphery of some of the eosin bodies in the coelomic cavity (figs. 21 to 26), the nuclear ring-like structures arising in the degenerating erythroblasts in the mesenchymal (figs. 27 and 31*e*) and other tissue spaces (figs. 29, 30, 34 to 36), appear closely related if not identical with the ring bodies of Cabot. In the embryo these nuclear rings, especially in the mesenchyma are typically blue

with Giesma's stain in contrast to the reddish tone of the rings in pathological blood of the adult, although as already noted blue rings are not entirely lacking even in the latter case. This may be in part due to the different conditions under which they are formed for it is to be observed that in certain regions such as that of the brain wall and nerve ganglia many of the ring bodies stain a reddish rather than a blue tone (figs. 28 and 33*e*). In the embryo these rings are more frequently found without any surrounding peripheral cytoplasm, that they may, however, also occur within the still intact erythrocyte is well illustrated in figures 34, 35 and 36 (also p. 96). This may be due in part to an earlier disintegration of the cytoplasm in relation to the nucleus in the case of the embryo as contrasted with the adult. Although it is not to be overlooked that Gabriel ('08, p. 604) records the observation even in the adult of ring bodies a parently lacking a peripheral rim of cytoplasm. As to the normal or abnormal character of these structures there appears no doubt but that in the embryo just as in the adult (Naegeli) their production is a phenomena of abnormal nuclear disintegration and not a normal mode of erythrocytic cytomorphosis. Concerning the conditions under which the degenerating erythrocytic nucleus will present the form of a ring or that of small compact spherules, the possibility is suggested that this may be associated in part at least with the stage of cytomorphosis at which the degenerative processes are initiated. In the younger erythroblasts, as is well known, the nucleus is both relatively larger and the chromatin granules are more loosely distributed throughout the nucleoplasm, whereas in later stages of differentiation the nuclei become not only smaller but also much denser and more compact in chromatin structure. Degenerative changes initiated at these different stages may consequently be expected to manifest correlated differences in nuclear disintegration. It is possible that a longer persistence of the thickened reticulum described by Cupp ('15) at the periphery of the erythrocytic nucleus may also be associated in part with the formation of nuclear rings.

2. Degenerating erythroblasts and the so-called eosinophilic leucocytes in embryonic mesenchyma

Attention has already been directed to the fact that upon first impression certain characteristics of the eosin staining coelomic cells were suggestive of leucocytic elements but that the results of subsequent investigation indicated the nature of these cells to be that of degenerating erythrocytes. In the case of the rabbit eosinophilic leucocytes are also absent in the serous cavities of the adult (Weidenreich, '12, p. 127). In connection with this conclusion it is to be taken into account, however, that cells which appear practically identical in both nuclear and cytoplasmic structure with these degenerating erythrocytes in the coelomic cavities are also found in the intercellular spaces of the mesenchyma of the same embryos (figs. 38 to 40), concerning the nature of which Maximow ('09) in his description of the 7 mm. cat embryo reaches the theoretically important conclusion that they are eosinophilic leucocytes differentiating in situ from mesenchymal cells.

The apparent identity of these cellular elements of the mesenchyma, both as described by Maximow and as observed in the present material, with the cells interpreted as degenerating erythrocytes in the coelomic cavities has rendered it necessary to reexamine the evidence concerning the nature of the cellular structures in the mesenchyma. In presenting the results of such a study it may be stated that the following considerations have led to a negative conclusion as to the leucocytic character of the cellular elements in question in the mesenchyma. In the first instance it is to be observed that no special eosinophilic granulation can be demonstrated in these cells (figs. 38 to 40). Indeed Maximow himself, although maintaining that in the mesenchymal wandering cell the nucleus becomes lobulated into a number of subdivisions held together in some cases by only fine connecting strands, is nevertheless obliged to admit that no leucocytic granules can be recognized in the cytoplasm of the cells here in question (p. 525). In explanation of this, Maximow points out that it is likewise also very difficult to demon-

strate such granules in the granular leucocytes of even the adult cat and that consequently failure to demonstrate these granules in the embryonic cells does not constitute evidence of a necessarily negative character. With reference to this point, however, it may be noted that the eosinophilic, non-granular cells in question are not limited to the embryo of the cat for apparently the same cells can be also demonstrated in the embryonic mesenchyma of the rabbit, mouse and pig, mammals in the adults of which a corresponding difficulty in staining the granules in the granular leucocytes cannot be said to be encountered. Second it can be demonstrated that degenerating erythroblasts, occasionally found in the embryonic circulation may undergo cytological changes apparently identical with the structural characteristics to be found in the mesenchymal cells in question. Erythrocytic nuclei as is well known, may become very irregular in shape: bilobed or even constricted into several subdivisions. Early stages in such nuclear changes are indicated in figure 37 and reference has already been made to Weidenreich's account on this subject (p. 595). In man lobulation of erythrocytic nuclei has also been described under pathological conditions in the circulation of the adult (Jünger, '00, p. 109). Maximow ('09, p. 478) also recognizes the occurrence of such nuclear lobulation even in the embryonic circulation but described the nuclei in such instances as becoming smaller, more compact and taking a darker stain. It may be questioned, however, whether this is necessarily always the case. For even in the circulating blood, degenerating erythroblasts may be observed with lobulated nuclei which cannot be said to present an especially more compact structure (fig. 37). A similar comparison can be made in figure 33 of Maximow's work in which lobulated erythrocytic nuclei are shown which do not appear either essentially darker in stain nor more compact in structure than the unchanged nuclei of the adjacent erythroblasts or the nuclei of the co-called leucocytes in figure 27 of his monograph. As for the relative size of the cells no conclusive distinction can be clearly drawn on this basis between the cells in question in the mesenchyma

and the erythroblasts in the circulating blood (cf. figs. 37 and 38, drawn with the same magnification).

That the cytoplasm of the degenerating erythrocyte may become vacuolated and assume a lighter stain is illustrated in figures 37*d* and 34*a*. Similar degenerative erythrocytic changes have also been described by Minot ('12) and may be observed in tissue cultures (Emmel '14). Finally, attention has already been directed to the fact that erythrocytes frequently escape from the vascular channels into the adjacent embryonic tissue spaces where they may eventually disintegrate, be ingested by phagocytes, or possibly in some cases eliminated through the lymphatic vessels as suggested by Minot ('12) for mammals and as observed by Clark ('09) in the living tadpole. Cells which can be unquestionably identified as such degenerating erythrocytes can be readily observed in the mesenchyma. The nuclei may vary from round to highly irregular and lobulated forms and the cytoplasm may be paler in stain reaction, vaculoated and under certain conditions may even disappear. Among these cells are such forms as are illustrated in figure 40 the nucleus of which may still be identified as erythrocytic as seems clearly indicated by the persistence of a thickened peripheral accumulation of chromatin in a manner comparable to that of the circulatory erythrocytes shown in figures 37 and 34. Such a nuclear structure is in decided contrast to that of the adjacent mesenchymal cells as may be seen in the same figures. Practically all transitional stages can be found between such cells and those shown in figure 38 representative of the so-called eosinophilic leucocytes under discussion. It appears evident that such partial hemolysis, cytoplasmic vacuolation and consequent peripheral disintegration in the erythrocytes which have escaped into the mesenchyma may result in the production of cytoplasmic processes of such a character as not to be readily distinguishable from cellular processes of the adjacent mesenchymal cells with which they appear to fuse. Such cells may present the deceptive appearance of differentiation in situ from mesenchymal cells as indicated in figures 39 and 40.

In conclusion, therefore it may be stated that in view of the fact that the cellular elements under consideration in the mesenchyma are not only deficient in any definite leucocytic granules but that nucleated erythrocytes degenerating in the embryonic tissue spaces may assume cytological characteristics apparently identical with those of the non-granular eosin staining cells with lobulated nuclei in the mesenchyma, it appears difficult to escape the conclusion that many if not the majority of the latter just as in the case of the coelomic elements, are degenerating erythroblasts rather than granular leucocytes developing in situ from mesenchymal cells.

Concerning the distribution of such degenerating erythroblasts in the embryonic tissues, it is of interest to note that the corpuscles in which the nuclei assume the lobulated condition without becoming noticeably more compact in structure or darker in staining reaction occur typically in the looser tissues with larger intercellular spaces. On the other hand degenerating erythroblasts assuming the more compact forms containing either nuclear rings or dark homogenous nuclear spherules, as previously described, and presenting a great reduction if not entire absence of peripheral cytoplasm occur typically in the denser tissue regions (cf. figs. 39 and 40 with 31–33). Presumably the structural characteristics which may be presented by the degenerating erythroblasts are in part determined by the nature of the environment in which such degeneration takes place (cf. however also p. 98).

In concluding the present subject it may be stated with reference to Maximow's most interesting and stimulating work concerning the participation of the mesenchyma in the formation of blood cells, that while the present results do not in themselves necessarily constitute a conclusive argument against such a possible role of the mesenchyma, they are presented as contributing toward an evaluation of some of the evidence which has been advanced toward the establishment of such a conclusion.

VI. CONCERNING THE PRESENT STATUS OF THE QUESTION AS TO THE ORIGIN OF MACROPHAGS FROM THE COELOMIC MESOTHELIUM

1. Embryological and comparative

As already intimated, a study of the cellular elements in the embryonic coelom of mammals has not been previously made. At the same time it may be observed that the present conclusion that the coelomic epithelium may give rise to free functional elements in the coelomic cavities is indirectly supported in an interesting manner by the results of a number of recent investigations. Reference may be made to Bremer's ('14) work in which he finds anlages of the earliest blood vessels in man to arise from the surface mesothelium. His "observations point to the ingrowths of the mesothelial layer covering the yolk-sac and body-sac as the anlages of the blood vessel endothelium and of a lesser extent of the blood corpuscles" (p. 459), and the conclusion is drawn that "True blood islands may occasionally arise by the multiplication of the cells of the mesothelial ingrowths, or scattered blood corpuscles may arise singly within these ingrowths" (p. 464). For the cat embryo Schulte ('14) records the occurrence of "funnel-like diverticuli of the coelom, the walls of which are intimately united to the blood vessels," and which he suggests may be of morphological significance with reference to the development of the embryonic blood vessels (p. 80). Haff ('14, pp. 346 and 333) states the conclusion that the peritoneum covering the embryonic liver of the chick may give rise to cells within the liver differentiating into erythrocytes. Scammon ('15), in the histogenesis of the Selachian liver, records the occurrence of mesothelial tubules "the walls of which are continuous with the splanchnic mesothelium and the lumen with the coelomic cavity" (p. 276). Although unable to find that the lumen of these tubules connected with that of the blood spaces, it is stated that the tubules break up into mesenchymal strands and that "the mesenchymal and endothelial cells form free anastomosis" (p. 280). Phylogenetically reference may also be made to primitive vascular conditions in

some of the lower invertebrates. Lang ('04, p. 152) states that in the body cavities of annelids there occur not only sex cells but also amoebocytes (lymphocytes) and coelomocytes, some of which as the result of formation of hemoglobin are designated haemocytes, and that these elements arise from the coelomic epithelium. Abbot ('13, p. 6) describes the observation of hemoglobin containing cells or 'haematids' in the body cavity of the Echiurid worm, Thallasema mellita, arising from the "living membranes of the general body cavity which buds off masses of cells, usually eight to twenty-four in number, which ultimately break up into individual haematids" (p. 6). Data of a similar character could be greatly extended not only among annelids, but also in the Echinoderms and Coelenterates. The views of Bütschli ('83), His ('00) and Arnold ('04) concerning the phylogenetic origin of the circulatory system from the body cavities are well known.

2. In adult mammals

In connection with these embryological results the question arises as to whether these mesothelial activities are confined to the embryo, or whether such a potentiality may be retained even in the adult animal. Without entering into a detailed account of the extensive hematological literature bearing upon the much debated question as to the nature and origin of the free cells in the adult serous cavities (for a discussion of which cf. Weidenreich ('11, pp. 126–138), certain aspects of the problem may briefly be considered in the light of the more recent investigations.

Schott ('09), from cytological and experimental studies reaches the important conclusion that the surface lining cells of the adult body cavities in the guinea pig and rabbit are not highly specialized and fixed passive structures, without potentiality for further differentiation, but that on the contrary they may assume phagocytic activities, become detached from the serous membrane and be liberated as free, active, living cells identical with the lymphocytes and macrophags of these cavities. He states:

"Wir müssen aber andrerseits auf Grund unserer Flächenpräparate vom Netz . . . . unbedingt an der Fähigkeit der Deckzellen sowohl zur aktiven Phagozytose wie zur Isolierung und Loslösung aus dem Zellverbande festhalten" (201), and in conclusion finds: "dass die grossen ungranulierten Exudatzellen und nicht nur diese, sondern auch die grossen Elemente des normalen Transudates, Abkömmlinge fixer oder sessiler Gewebsbestandteile sind. Ursprünglich fixe Gewebselemente lösen sich aus dem Zellverbande, runden sich ab und werden zur freien Zellen der serösen Höhlen" (p. 208). Among subsequent investigators confirming this conclusion may be noted Szecsi ('12) who states: "Aus der Endothelzellen wird zuerst durch Wachstum im Plasmaabrunding der Lymphoidozyte und Macrophage" (p. 18). This is again reaffirmed in the later work of Szecsi and Ewald ('13, p. 182). Lippman and Plesch ('13) in a summary of their Thorium experiments, relative to the exudate cells of the serous cavities further support this conclusion: "Somit können die 'kleinen lymphozyten' weder Hämatogen sein (aleukozytäres Blut.'), noch von den Adventitialzellen, noch von den taches laiteuses stammen—sie sind Abkömmlinge des serosaendothels" (p. 1396). Weidenreich ('11) writes with no uncertainty concerning the origin of the large phagocytic cells of the serous cavities: "sie sind losgelöste Netzelemente, und somit sowohl Abkömmlinge von Deckzellen als auch Bindegewebszellen, ... die nicht degenerieren, sondern in Gegenteil sehr lebenskräftig und mitotischer Teilung fähig sind" (p. 133). In opposition to the above conclusion Pappenheim-Fukushi ('13) maintain that the exudate cells of the serous cavities are not mesothelial derivatives: "Sie sind Abkömmlinge nicht der Deckzellen" (p. 305). . . . . Allerdings leiten Lippman und Plesch die entzündungezellen ebenso wie Wiedenreich-Schott, auch von den serosen noch nicht angenommen werden darf" (p. 289). Recently through the employment of vital staining methods new evidence has been introduced into the discussion of the problem. Thus Goldman ('12), on the basis of his studies with vital stains, draws a distinction between the macrophags and serous mesothelium on the ground that the

latter in contrast to the former does not stain with pyrolblue (p. 45, 49) and the inference consequently arises that the macrophags of the serous cavities are not derived from the mesothelium. Tschaschin ('13, p. 350) not having succeeded in obtaining a vital stain for the peritoneal endothelium, also draws a similar conclusion. In experiments with celloidin plates inserted into the peritoneal cavity, Tschaschin ('13) furthermore failed to find that the mesothelial cells manifested any special reaction or potentiality for transformation, but that on the contrary they quickly disquamated and under the conditions of the experiments took no part in the formation of macrophags (pp. 271, 285, 289).

Unanimity cannot, therefore, be said to have as yet been attained in the solution of the problem. As already indicated with reference to negative evidence, perhaps the strongest data which has been more recently advanced is that derived from such results as that of Goldmann and Tschaschin with vital stains. It remains to be seen what is to be the final evaluation of the data derived from this method. At the present stage of such investigations it may not be without value to note the following points which do not appear to render some of the results so far attained as of a necessarily conclusive character with reference to the question in hand.

It may be observed that if the reaction of the given tissue (whether endothelial or mesothelial) to the vital stain is negative and that of the macrophags positive, it appears, in some cases at least, that this is taken as evidence that the macrophags could not be derivatives of the tissue in question. But with reference to such a conclusion the question arises as to whether it has been clearly demonstrated, first, that there is such a sharp difference in the reaction of the mesothelium and macrophags and second, that even a material difference in vital stain reaction in itself establishes an entire absence of any genetic relalationship between the tissues under consideration. In the first place it is not to be overlooked that in some instances at least both mesothelium and macrophags may react alike, for Schulemann ('12) after injection of Trypanblue in rabbits did not ob-

tain a stain reaction for either the macrophags of the peritoneal cavity or the peritoneal epithelium, a result which, as he himself notes (p. 239) consequently substantiates Schott's conclusion. On the other hand it has been shown that in cases where the macrophags do manifest a typical vital stain reaction the reaction of the mesothelium is by no means necessarily entirely negative, for Evans ('14) records the observation that whenever certain cells such as the clasmatocytes (resting wandering cells) of the connective tissues and macrophags of the great serous cavities "react in a typical intense manner to the vital stain" other cells "are normally found with much smaller often very minute granules of the stain" in which latter class, it is important to observe, is included the mesothelium "lining the peritoneum and covering its organs" (p. 100). Again it does not appear that the fact that the reaction of a tissue to the vital stain in a given case is negative or stains only slightly in contrast to the macrophags necessarily leads to the conclusion that such a tissue cannot give rise to the macrophag elements. A case in point is that of the endothelium of some of the larger blood vessels in the liver. It has been shown for example by Taschaschin ('13) that whereas after 'Kallargol' injection black silver granules are specifically deposited in the large mononuclear elements or macrophags of the blood, they are not found in the endothelial cells of the portal vessels (p. 353). On the other hand Batchelor ('14) ,while he finds that injections of trypan blue just as in the case of 'Kallorgol' ,do not normally stain the endothelium of the larger portal vessel of the liver in contrast to the positive reaction of some of the phagocytic endothelial giant cells in the same organ, nevertheless endothelial proliferations experimentally produced in the same vessels by means of albumen emboli do react to the stain: thus newly formed endothelial tissue at the site of the embolus and in its immediate neighborhood it is stated, "is stained vitally, a phenomenon never seen with the normal endothelial cells of larger vessels, and showing that the vital stain is adequate for the detection of endothelial growths although the parent tissue does not show this property" (p. 139). MacCurdy and Evans ('12, p. 1695— and

also Tschaschin ('13, p. 370), record the observation that in the case of blood vessels in the vicinity of wounds or other irritants, the vascular endothelium which normally does not stain, may under these changed conditions now react to vital stain. Finally it may be noted that the macrophags themselves do not all react alike. Thus Tschaschin ('13*b*) recognizes the occurrence of distinct variations in the intensity of the vital stain in the macrophags: "Es muss jedoch hervorgehoben werden, dass die freien Macrophagen der Bauchböhle sich vital bei weitem nicht immer gleich intensiv färben" (p. 351). Tschaschin associates this with variations in different types of stain and methods of injection, rather than as furnishing any ground for identifying these lighter stained cells with detached cells from the peritoneal endothelium, but in view of the above referred to results by MacCurdy, Evans and Batchlor, it does not appear that the latter possibility can as yet be said to have been successfully eliminated. Variations in the vital stain of such detached cells may well be correlated with different degrees of differentiation as has indeed been emphasized even by Tschaschin (p. 382) in connection with difference in the vital stain reaction of blood cells so that undifferentiated cells reacting negatively with a given vital stain may with further differentiation give a positive reaction with the same stain. Consequently on the basis of the data so far at hand, the ground does not appear clear on which it can be stated with entire assurance that the mesothelial cells which are able to take up a small number of the granules of the vital stain, may not under given conditions just as in the case of the vascular endothelium, come to manifest an increased expression of the same function such that as detached cells would identify them with true macrophags.[2]

In conclusion, therefore, it appears that a convincing case can hardly as yet be said to have been made against the possible per-

[2] In connection with the question as to the degree to which mesothelial and endothelial tissue may manifest common morphological and functional potentailities it is of interest to note the results of Hooper and Whipple ('15) indicating that mesothelium as well as endothelium may participate in the formation of bile pigments.

sistence in the adult mammal of the potentiality on the part of the mesothelium of contributing, under certain stimulating environmental conditions, liberated cellular elements to the body cavities in a manner comparable to the processes taking place in the embryonic coelom.

VII. RÉSUMÉ

1. A considerable number of free cellular elements were found to be more or less constantly present in the coelomic cavities of pig, rabbit and mouse embryos.

2. These coelomic elements may be described as falling into two groups, the one consisting of basophilic staining and usually phagocytically active cells and the other of cellular elements characterized by their eosinophilic staining qualities and non-phagocytic activity.

3. The coelomic macrophags

a. The basophilic cells may be conveniently further subdivided into the following types: 1) cells relatively smaller and more spherical in form, containing an occasional small cytoplasmic vacuole and a rather dark staining, round or kidney shaped nucleus; 2) cells usually larger in size, more oval or irregular in form, containing one or more phagocytic inclusions, having a round or kidney shaped and more or less eccentrically situated nucleus and the cytoplasm and nucleoplasm of which take a considerably lighter basophilic stain; 3) cells characterized by the vaculoated condition of the cytoplasm and the not infrequent occurrence of cytoplasmic processes or buds projecting from the surface of the cell.

b. The transitional stages which may be found between these different cells are of such a character as to justify correlating their size, form, nuclear, and cytoplasmic differences with variations in differentiation and function. Consequently practically all of these cells are here regarded as belonging to a common group which in view of their evident phagocytic functions may be designated as coelomic macrophags.

c. As to the origin of these coelomic macrophags, some of them may no doubt have entered the coelomic cavities from

extra coelomic regions, but the present results do not indicate this to be the only source of origin of these cells. On the contrary the observation that at the surface of the coelomic walls, in the free mesothelial cell masses, and the pleuro-pericardial and pleuro-peritoneal membranes, mesothelial cells are found which are rounded in form, manifest phagocytic characteristics apparently identical with that of the typical macrophags, and the evidence advanced that these cells may become detached as free cells, support the conclusion that the coelomic mesothelium is an important source of origin for the phagocytic cells found in the embryonic coelom.

4. Erythrocytic elements in the coelomic cavities.

a. The second of the two groups of coelomic cellular elements above indicated again fall into two sub-groups, the one consisting of small non-nucleated bodies and the other of larger nucleated cells.

b. The present data indicate these structures to be erythrocytic in nature; the larger nucleated cells representing degenerating nucleated red blood corpuscles and the smaller eosin staining bodies consisting chiefly of degenerating erythrocytic nuclei.

5. Erythrocytic disintegration in the mesenchyma.

a. In the embryonic mesenchyma there occur small eosin staining bodies which have been interpreted as hemoglobin containing secretion products of mesenchymal cells (Maximow). But on the basis of the present evidence concerning their distribution, structure and close cytological correspondence to disintegrating erythrocytes as observed in the circulating blood, intercellular tissue spaces and phagocytic inclusions and to erythrocytic nuclei persisting after the formation of non-nucleated erythrocytes, the conclusion is drawn that the bodies in question represent chiefly erythrocytic elements consisting of degenerating and in many cases phagocytically ingested erythroblasts and, in older embryos, including nucleated erythrocytic bodies arising in connection with the formation of erythro-plastids or non-nucleated erythrocytes, rather than the products of mesenchymal secretory activities.

b. The ring-like configuration presented by many of these degenerating erythrocytic nuclei appear identical in many respects with the nuclear structures occurring in the erythrocytes of pathological blood known as Cabot's rings and they consequently furnish evidence that the latter are not limited to the blood of the adult animal as has been previously assumed (Naegeli).

c. Certain cellular elements in the mesenchyma present in some respects the appearance of eosinophilic leucocytes and are suggestive of a possible mesenchymal origin as maintained by Maximow. In view of the fact, however, that all of these are deficient in any definite leucocytic granules and that nucleated red blood corpuscles escape into the embryonic tissue spaces where they may present degenerative nuclear and cytoplasmic characteristics apparently identical with those of the non-granular eosin staining cells in the mesenchyma, it appears difficult to escape the conclusion that the majority of the latter, just as in the case of the corresponding coelomic elements, are degenerating erythrocytes rather than granular leucocytes developing in situ from mesenchymal cells.

6. Concerning the problem of mesothelial origin of macrophags.

a. In connection with present conclusion that the coelomic epithelium may give rise to free functional elements in the coelomic cavities, it appears not without significance on embryological and comparative grounds to note the recent work of Bremer, Haff and Schulte concerning the participation of coelomic epithelium in vasculogenesis and the formation of blood corpuscles in vertebrate embryos and the conditions in certain invertebrates where the coelomic epithelium gives rise to cellular elements functioning as respiratory and phagocytic cells in the body cavities (Lang, His, Arnold).

b. Regarding the origin of the macrophags in the serous cavities of adult mammals unanimity still remains to be attained in the solution of the problem. On the basis of the data so far at hand, it does not appear, however, that a convincing case has as yet been made against the possible persistence of a potential-

ity in the mesothelium of the adult organism of contributing under certain conditions free cell elements to the body cavities in a manner comparable to the process here described as taking place in the embryonic coelom.

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PLATE 1

EXPLANATION OF FIGURES

The following figures are from camera lucida drawings in all of which, with the exception of the low power drawing in 41 and in 1 to 7, 11 to 13, which were outlined with a No. 8 ocular, the details were drawn from observations with a Zeiss appochromatic immersion objective and No. 4 and 6 compensation oculars. Fifteen of the drawings were made by Herr Kretz, artist at the Anatomical Institute of the University of Strassburg and twenty-one by Mr. Jarrett and Miss Ehinger at the Anatomical Laboratory of the Washington University Medical School. In the reproduction plates 2 and 4 were reduced by one-fifth from the original drawings.

1, 2 Cells belonging to the first of the three types described under coelomic macrophags. The cytoplasm is decidedly basophilic, contains a number of small cytoplasmic vacuoles and the nuclei are either round or kidney shaped. What appears to be a centrosphere is seen at the left of the nucleus in figure 1. These cells are interpreted as coelomic macrophags in a stage of inactivity with reference to phagocytic functions. From the pericardial cavity of a 9 mm. rabbit embryo (compare with fig. 9).

3. Mitosis in the type of cells shown in figures 1 and 2. From the pericardial cavity of the same embryo as above.

4, 6. Macrophags at a stage of active phagocytosis. The inclusions appear to consist chiefly of erythrocytic elements. The lighter cytoplasmic and nucleoplasmic stain as compared with 1 and 2 appears correlated with an advanced stage of phagocytic activities. *n*, is nuclear inclusion with a lighter stained central area. From the pericardial cavity of a 9 mm. rabbit embryo.

7 Mitosis in a coelomic macrophag containing two large cytoplasmic vacuoles (cf. fig. 3). Pericardial cavity 9 mm. rabbit embryo.

8 Two coelomic macrophags lying side by side and consequently subject to identically the same technique. They demonstrate the lighter nuclear and cytoplasmic stain in the cells at stage of greater phagocytic activity *b* as compared with the less active cells *a*. In cell *b* one of the inclusions still retains an unmodified remnant, in the form of a crescent, of the original basophilic material of the ingested nucleus. Pericardial cavity, 9 mm. pig embryo.

9, 10 Furnishes a striking contrast in the cytological characteristics of a phagocytically active (10) and a phagocytically inactive cell (9). From the same source as figure 8.

11 Coelomic cell (macrophag?) showing a highly vacuolated condition of the cytoplasm. Peritoneal cavity, 9 mm. rabbit embryo.

12 Cells occasionally found in the coelomic cavities of rabbit embryos, showing a peripheral border of eosin staining material. Similar structures are also found in the mesenchyma where they undoubtedly represent degenerating hemoglobin containing elements. Pleural cavity, 9 mm. rabbit embryo.

13 A macrophag undergoing mitosis in which the large digestive vacuole still contains a visible undigested remnant of the phagocytic inclusion. Pericardial cavity, 9 mm. pig embryo.

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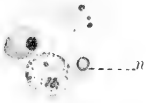

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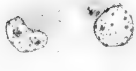

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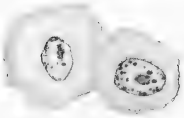

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PLATE 2

EXPLANATION OF FIGURES

14 One of fourteen sections through the pleuro-pericardial membrane of a 7 mm. pig embryo. *c*, represents a central issue core surrounded by mesothelial cells (*mes*). Many of these cells have assumed a rounded form and are partially if not entirely detached from the main mass. These modified cells are phagocytically active (*pcm*), contain cytoplasmic vacuoles (*vcm*) and present structural and tinctorial characters practically identical with that of typical macrophags. For general relations see figure 43. *e*, small eosin staining bodies (cf. figs. 20 to 26).

15 Free mesothelial cell masses in the pericardial cavity of a 9 mm. rabbit embryo. The visceral and parietal pericardial walls, respectively, are at the right and left sides of the figure (*mes*).

16 Free mesothelial cell mass in the ventral region of the pericardial cavity of a 7 mm. pig embryo. It extends through five sections, but in both this case as well as in figure 15 these cellular masses are at no point attached to the coelomic wall. Some of the mesothelial cells are partially detached, present a rounded form and a slightly more basophilic stain reaction (*m*).

17 A region of the mesothelial surface showing a rounded, partially detached basophilic cell (*m*) which appears to be a modified mesothelial cell. Visceral pericardium of a 9 mm. pig embryo (cf. figs. 41, 43, 44).

18 A group of free cells in the peritoneal cavity of a 9 mm. rabbit embryo, interpreted in the text as degenerating erythrocytes. In some of the cells indications of hemoglobin are still evident (*a*), in others the cytoplasm is paler in color show cytoplasmic vacuolation and degenerative nuclear changes (*b*, *c*).

19 Other instances of degenerative nuclear and cytoplasmic changes in erythrocytes (cf. fig. 18). Peritoneal cavity, 9 mm. rabbit embryo.

ABBREVIATIONS

cm, coelomic macrophags
d, mitotic figures
icm, coelomic macrophags, apparently in a stage of inactivity with reference to phagocytic functions
in, phagocytic inclusions
m, rounded cells interpreted as mesothelial cells in the process of becoming detached as free cells in the coelomic activities
mc, mesenchymal cells
mes, mesothelium
mm, mesothelial cell masses found free in the coelomic cavities
pcv, pericardial cavity
pcm, coelomic macrophags in a stage of active phagocytosis
vcm, vacuolated coelomic macrophags
w, vessel wall

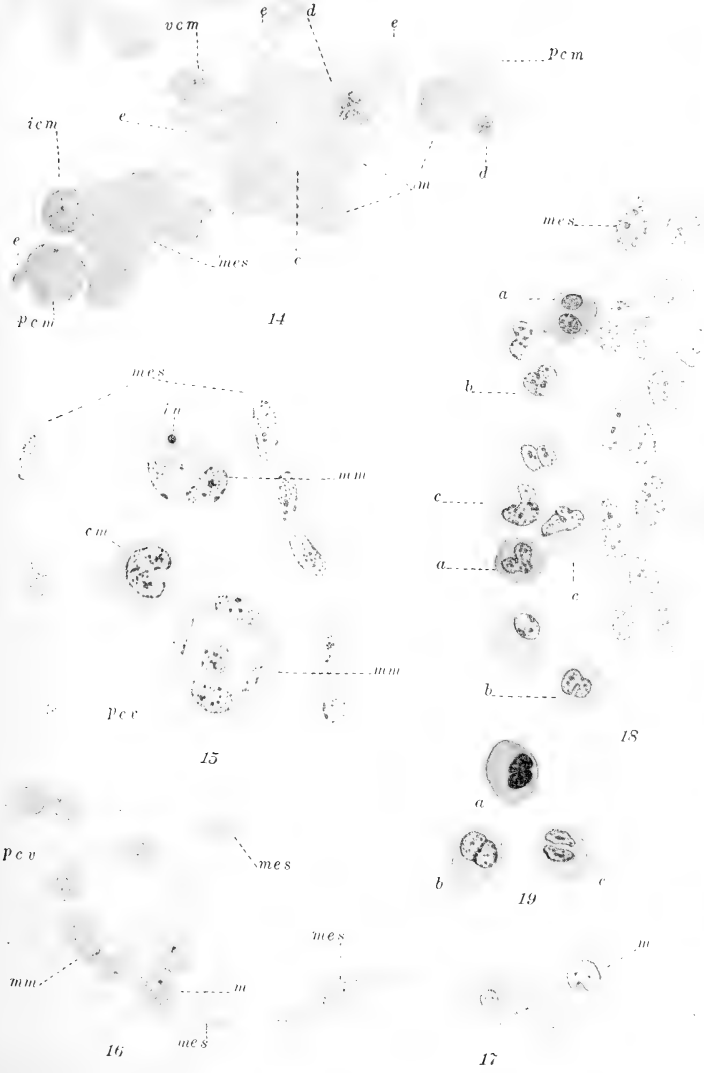
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PLATE 3

EXPLANATION OF FIGURES

20 to 26 Small eosin staining bodies (degenerating erythrocytic nuclei) in the coelomic cavities, some of which contain variable quantities of basophilic material in the form of small spherules and peripheral rings. 20, 22, 24, 26 are from the pleural cavities of 7 mm. pig embryos; 21, 23 from the pericardial cavity of a 13 day mouse embryo, and 25 from the pleural cavity of a 9 mm. pig embryo.

27, 28 Ring form structures (degenerating erythrocytic nuclei) in the tissue spaces. 27 is from the mesenchyma of the ventral thoracic wall of 9 mm. rabbit embryo and 28 from the gasserian ganglion of a 13 day mouse embryo.

29, 30 Degenerating erythrocytic nuclei in the heart cavity of a 7 mm. pig embryo (29) and of a 13 day mouse embryo.

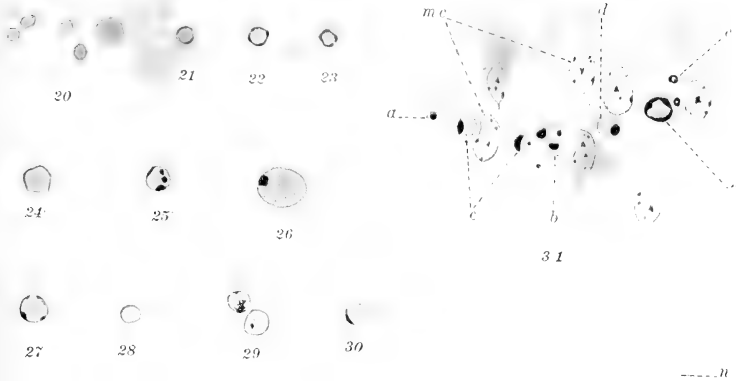
31, 32, 33 Groups of small bodies, *e* (degenerating erythrocytic elements consisting chiefly of nuclear material) in the embryonic tissues. 31 is from the mesenchyma of the ventral wall of a 9 mm. rabbit; 32 from the mesenchyma the septum transversum of a 7 mm. pig embryo; and 33 from the ventral wall of the fore-brain of a 13 day mouse embryo. Shows their variation in size, structure, stain reaction and intercellular relations.

34, 35, 36 Ring form nuclear structures (Cabot's rings) observed in degenerating erythrocytes found in the embryonic circulation. 34 and 35 respectively, are from a small blood vessel in the mesenchyma and a sinusoid in the liver of a 13 day mouse embryo; and 36 from a blood vessel (or posssibly lymphatic) in the mesenchyma of a 9 mm. pig embyro.

37 Erythrocytes in the hepatic sinusoid of a 13 day rabbit embryo showing earlier stages in degenerative nuclear and cytoplasmic changes including lobulation of nuclei, hemolysis and cytoplasmic vacuolation.

38 Cells found in the intercellular tissue spaces of the mesenchyma of the same embryo as for figure 37, concerning which grounds were advanced in the text indicative of their degenerating erythrocytic nature rather than leucocytic elements differentiating in situ from mesenchymal cells.

39, 40 Cells from the mesenchyma which appear in nuclear and cytoplasmic structure clearly intermediate between the cells shown in figure 38 and the degenerating erythrocytes in figure 37. From the same source as figure 38.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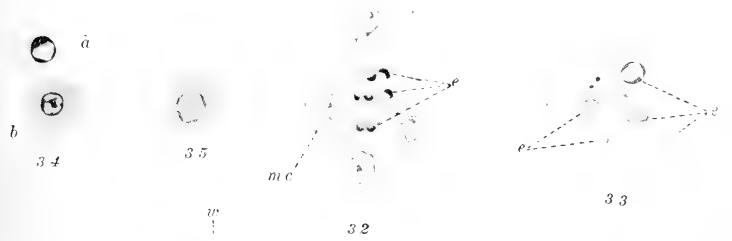
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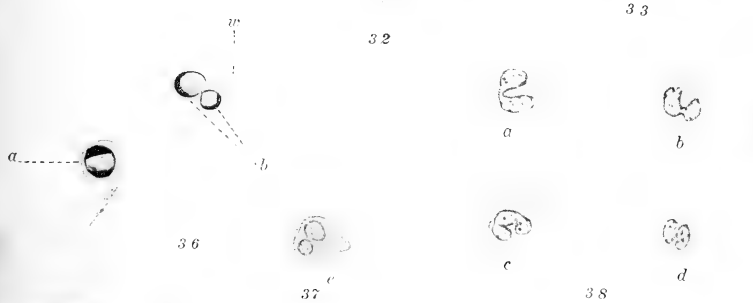
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PLATE 4

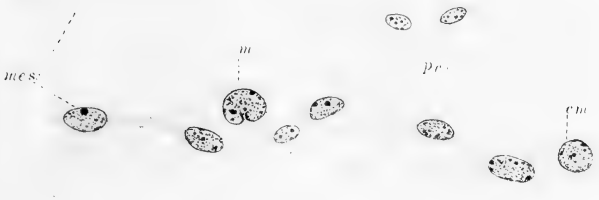
EXPLANATION OF FIGURES

41 Showing a rounded cell (*m*) projecting into the pericardial cavity from the visceral pericardium, but still attached by a slender cytoplasmic pedicle to the mesothelial surface. 7 mm. pig embryo (cf. fig. 17, 42, 44).

42 Demonstrating the potentiality for phagocytic activity on the part of the mesothelial cell. The mesothelial character of the cell can hardly be questioned, at the same time it is partially raised above the level of the mesothelial surface and the nucleus approximates a kidney-shaped form. From the parietal pericardium of a 9 mm. pig embryo.

43 Showing the position and general relations of the section of the pleuro-pericardial membrane (*pp*) drawn at a larger magnification in figure 14. It may be observed that the cellular mass in question lies in the pleural cavity (*pcv*) and that the latter is still in communication with the pericardial cavity through the pleuro-pericardial canal (*pplc*). The membrane continues through fourteen sections and is found to connect with the parietal wall at the junction of parietal-pleural (*ppl*) and parietal-pericardial (*ppc*) walls where its cells become continuous with the mesothelium lining the coelomic walls.

44 Showing a region of the visceral pericardium in which the mesothelial cells present the appearance of transformation into free cellular elements. This region continues through several successive sections. Many of the cells assume a more basophilic stain than that of typical mesothelial cells, and phagocytic activities, cytoplasmic vacuolation and peripheral processes or buds (*b*) apparently identical with that of the coelomic macrophags. 7 mm. pig embryo.



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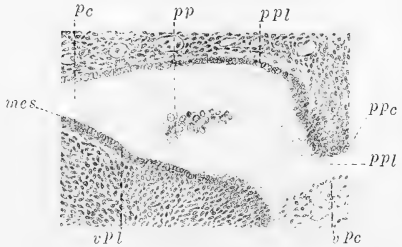

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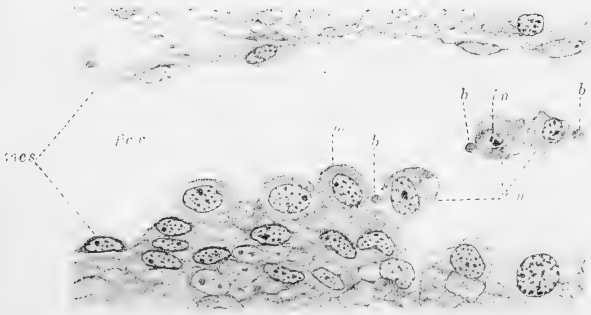

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THE GENESIS, DEVELOPMENT, AND ADULT ANATOMY OF THE NASOFRONTAL REGION IN MAN

J. PARSONS SCHAEFFER

Daniel Baugh Institute of Anatomy and Biology of the Jefferson Medical College of Philadelphia

THIRTEEN FIGURES

Owing to the contradictory and often but general statements extant in the literature on the nasofrontal connections, the writer deemed it important to make a more detailed study of the embryology and adult anatomy of this region. The present communication will in a sense supplement previous studies on the embryology of the nose by the writer. Special attention is here given to an analysis of the adult anatomy, and an effort is made at an intelligent interpretation of the complicated region by referring to the genesis and development of the parts involved. The anatomy of the nasofrontal connections is of considerable importance clinically, since the sinus frontalis is now frequently approached from the nasal cavity in operative procedures. With the latter thought in mind, important anatomic types of the region commonly encountered are illustrated by drawings from actual personal dissections. The embryology is dealt with but briefly; the reader is referred to previous papers by the author for more detailed discussions.

EMBRYOLOGY

The nasofrontal region is genetically an outgrowth from the ventral and cephalic end of the meatus nasi medius, operculated by the concha nasalis media (middle turbinated bone). The mucosa of this part of the meatus nasi medius is, therefore, the proton of what subsequently becomes the recessus frontalis of the meatus nasi medius (early in evidence) and derivatives there-

from. The recessus frontalis in turn is the anlage of the sinus frontalis and certain of the anterior group of cellulae ethmoidales (also called cellulae frontales by Killian, Onodi and others).

As early as the end of the third or beginning of the fourth month of embryologic life, one sees evidence of a beginning extension of the meatus nasi medius in a ventrocephalic direction. This early extension is the anlage of the recessus frontalis and is, strictly speaking, the first step in the formation of the sinus frontalis and certain of the anterior group of cellulae ethmoidales. For some time the lateral wall of the recessus frontalis is

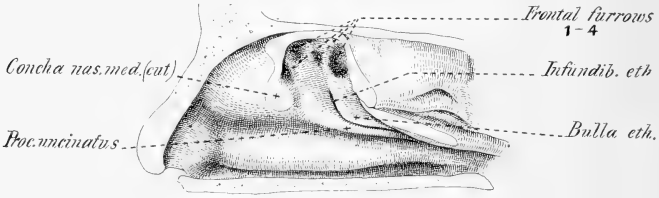

Fig. 1 From a term fetus. The recessus frontalis is exposed for study by the removal of the operculating concha nasalis media. Note the frontal furrows and the relations of the infundibulum ethmoidale. Compare with figure 6. The most ventral of the frontal furrows or pits are referred to throughout this paper as the first (1), the next in order as the second (2), etc.

even and unbroken and gives no evidence of the later configuration and complexity which characterizes the region in the adult nose. Coronal sections and transections of the recessus frontalis of a 4-month fetus show the lateral nasal plate of cartilage thickened at certain points. These thickened cartilaginous areas—the forerunners of the folds or accessory conchae which later configure the lateral wall of the recessus frontalis—vary in number and are for a period low and inconspicuous and do not throw the nasal mucosa into relief.

Upon examining the recessus frontalis in the late fetus, one finds a variable number of low accessory conchae on its lateral wall (figs. 1 to 4). The folds with the cartilaginous skeleton,

now partly ossified, are at this time sufficiently developed to throw the nasal mucosa into relief. Between the folds are found pits or furrows, the positive growth or outpouching of which aids materially in making more prominent the folds. It is appropriate to speak of the latter as accessory or hidden frontal folds or conchae and the pits as frontal furrows, of the meatus nasi medius. As mentioned above, there is no constancy in the degree of differentiation and development of the frontal folds and furrows. The number varies from a complete absence to four or five. In some instances, therefore, the recessus frontalis remains a simple blind outgrowth from the meatus nasi medius without configuration of its lateral wall (fig. 5).

The processus uncinatus and the folds composing the bulla ethmoidalis likewise should be considered as accessory conchae of the meatus nasi medius (analogues and homologues of the frontal conchae), and the infundibulum ethmoidale and the suprabullar furrow as accessory meatuses or furrows of the meatus nasi medius (analogues and homologues of the frontal furrows).

The accessory furrows of the meatus nasi medius are forerunners of certain of the sinus paranasales, i.e., the sinus frontalis, the sinus maxillaris, and the anterior group of cellulae ethmoidales (by anterior group is meant all those ethmoidal cells which communicate with the nasal fossa caudal to the attached border of the concha nasalis media, including both the anterior and middle group according to another classification).

The frontal furrows or pits early evaginate and form certain of the anterior group of cellulae ethmoidales or cellulae frontales. Semi-coronal sections through the recessus frontalis show these early cells. When these cells are followed in serial sections toward the recessus frontalis, they are shown to be extensions or outpouchings of the frontal furrows and in communication with the recessus frontalis. Some of the cellulae ethmoidales having their genesis in frontal pits remained diminutive and ethmoidal in topography, while others grow to considerable size and often develop beyond the confines of the ethmoidal bone.

It is a well established fact that the sinus frontalis develops variously by a direct extension of the whole recessus frontalis; from one or other of the anterior group of cellulae ethmoidales which have their point of origin in frontal furrows; and occasionally from the ventral extremity of the infundibulum ethmoidale, either by direct extension or from one of its cellular outgrowths. Indeed, the sinus frontalis may be unilaterally or bilaterally present in duplicate or triplicate, indicating a genesis from more than one of the aforementioned areas. The sinus frontalis is in many instances, embryologically speaking, a

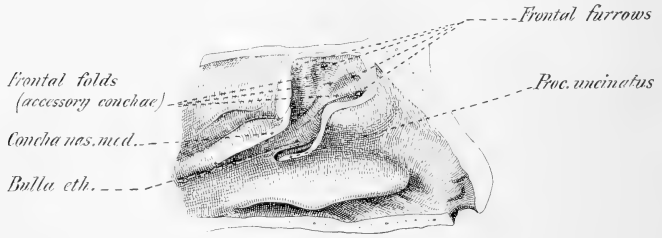

Fig. 2 From a term fetus. Recessus frontalis exposed. Note frontal furrows and folds. Especially note the continuity of the suprabullar furrow and the fourth (most dorsal) frontal furrow (see reference in text). The infundibulum ethmoidale is in line with the first frontal furrow, but not directly continuous with it. After Schaeffer.

cellula ethmoidalis anterior which has grown sufficiently far into the frontal region to be topographically a sinus frontalis.

The first evidence of the sinus frontalis must not be sought in the frontal bone, but in the recessus frontalis of the meatus nasi medius. Lack of observance of this rule has led to such statements as: "in the newborn infant no trace of a frontal sinus is visible," "the earliest sign of a frontal sinus is seen about the end of the first year in the form of a shallow depression," "the frontal sinus is completely absent in the newborn infant." Poirier states that the frontal sinus is first seen about the end of the second year. Tillaux puts it as late as the twelfth year. Onodi, Schaeffer, Davis and others recognize the sinus frontalis as such

in some instances early in extrauterine life. Killian operated upon a diseased sinus frontalis in a child fifteen months old. As stated before, the recessus frontalis of the meatus nasi medius is demonstrable as early as the fourth fetal month. During late fetal life the recessus frontalis becomes complex by the formation of frontal furrows or pits, etc. One is not justified at this time to hazard an opinion as to the specific point in the recessus frontalis from which the sinus frontalis will ultimately develop.

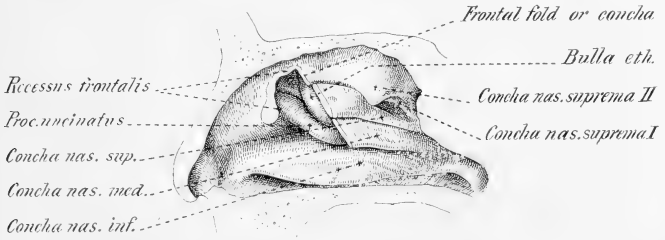

Fig. 3 From a term fetus. Here a single frontal fold or concha presents, bordered by a dorsal and a ventral frontal furrow. The infundibulum ethmoidale and the frontal furrows are continuous channels. Whether this is a secondary condition due to growth, i.e., whether the frontal furrows and the infundibulum ethmoidale were discontinuous anlagen, cannot be said. According to my series the condition is not common.

There are exceptions to this rule. Occasionally at birth the genetic point for the sinus frontalis is obvious. Again, one cannot be certain until the second or the third year.

From the suprabullar furrow develop most of those cellulae ethmoidales anterior which in time honeycomb the bulla ethmoidalis. Rarely the suprabullar furrow seems to be the genetic point for the sinus frontalis. This may be apparent only and not the actual condition. The most dorsal of the frontal pits and the suprabullar furrow are at times continuous channels (fig. 2). This might lead to the interpretation that the sinus frontalis developed from the suprabullar furrow, when in reality it developed from a frontal pit (early anterior ethmoidal cell). At times some of the bullar cells develop from occasional

furrows on the medial surface of the bulla ethmoidalis. The infundibulum ethmoidale dorsally and caudally gives rise to the sinus maxillaris and ventrally it usually ends blindly by forming a cellula ethmoidalis anterior of variable size, lateral to the recessus frontalis. Various aberrant cellulae ethmoidales anterior (agger, conchal, infundibular, etc.) also frequently develop from the aforementioned points. The posterior or dorsal group of the cellulae ethmoidales do not concern us here.

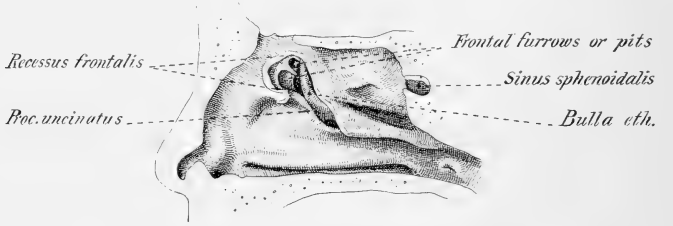

Fig. 4 From a term fetus. Recessus frontalis exposed by partial removal of concha nasalis media. Note intimate relationship between ventral extremity of the infundibulum ethmoidale and the second or most dorsal frontal furrow or pit. Should the sinus frontalis develop from the second frontal furrow, the adult relationship between the former and the infundibulum ethmoidale would be very intimate. Indeed, there might be direct continuity, allowing for further changes in development. The conclusion would be wrongly drawn, however, to say that the sinus frontalis is a derivative of the infundibulum ethmoidale.

The processus uncinatus and the folds composing the bulla ethmoidalis are often in direct continuity with one or more of the frontal folds or conchae (fig. 1). Again, the bulla ethmoidalis and the processus uncinatus are fused across the ventral extremity of the infundibulum ethmoidale (figs. 1 and 5). Likewise in many instances the dorsal extremity of the processus uncinatus divides, in a sense, into two roots, one of which turns cephalad and fuses with the bulla ethmoidalis, thus causing the infundibulum ethmoidale to end in a blind pouch dorsally (fig. 8).

The infundibulum ethmoidale and one or other of the frontal furrows or pits are in the same axis in the fetus and are at times contiguous (fig. 1). It must, however, here be pointed out that

it is unusual for the infundibulum ethmoidale to be directly continuous with a frontal furrow or pit (fig 3). The latter embryological fact is significant when one recalls the careless statement frequently made without qualification, that in the adult the "infundibulum ethmoidale is continued upwards as the nasofrontal duct into the sinus frontalis."

Because of the intimate relations, in the adult, of the infundibulum ethmoidale and the ductus nasofrontalis or the sinus frontalis directly, the infundibulum ethmoidale serves, in many instances, as a channel to convey secretion from the sinus frontalis to the sinus maxillaris. This is enhanced in those cases in which the infundibulum ethmoidale is deep and ends dorsally in a blind pouch, thus directing drainage through the ostium maxillare into the sinus maxillaris (figs. 8 and 12). In other words the sinus maxillaris is often a cesspool for infectious material from the sinus frontalis and certain of the anterior group of cellulae ethmoidales

The above well known clinical fact has doubtless led to the erroneous belief that the infundibulum ethmoidale is, in the majority of instances, directly continuous anatomically with the nasofrontal duct or, in the absence of the latter directly with the sinus frontalis. The inference is also wrongly drawn that in many cases the sinus frontalis is embryologically a direct outgrowth of the ventral and cephalic end of the infundibulum ethmoidale. From what has been said previously on the embryology, it is needless to enter further into a discussion here. Suffice it to say that it is not a common adult anatomic condition to find the infundibulum ethmoidale directly continuous with the ductus nasofrontalis, or in the absence of the latter with the ostium frontale. There are in many instances close relationships established, even a contiguity, but a direct continuity is an occasional occurrence only. According to the series worked it is, likewise, not common for the sinus frontalis to have its genesis in the infundibulum ethmoidale.

It should, however, be pointed out that in probably as many as 50 per cent of adult cases the relationship is so intimate between the infundibulum ethmoidale and the sinus frontalis or

its duct (ductus nasofrontalis) and certain of the cellulae ethmoidales anterior that drainage from these paranasal chambers finds its way in whole or in part into the infundibulum ethmoidale, thence via the latter into the sinus maxillaris.

ADULT ANATOMY

In order to properly interpret points in adult anatomy it is frequently necessary to resort to the embryology of the part or parts involved. This, indeed, is true of the nasofrontal region. Doubtless many of the erroneous statements extant in the

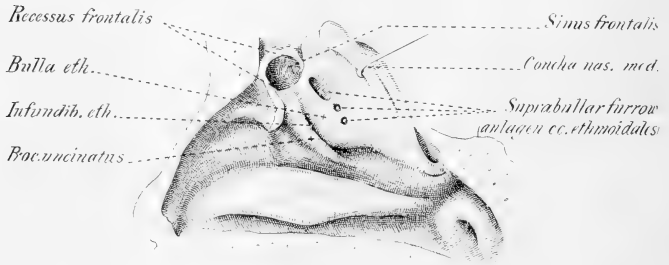

Fig. 5 From a child aged fourteen months. Note the apparent absence of frontal furrows and folds. The whole recessus frontalis is expanding or growing frontalward in the establishment of the sinus frontalis. After Schaeffer.

literature on the nasofrontal connections are the result of drawing conclusions from a study of too few specimens, of studying adult material alone, and of errors in interpretation due to the fact that embryologic and adult studies were not carried on simultaneously.

The adult nasofrontal region presents a varied anatomy, a fact in accord with the varied genesis of the parts involved. In the adult one usually finds evidence of the previous embryologic condition that must have obtained in the particular case. Careful analysis of the nasofrontal region reveals, as a rule, the derivatives of the frontal furrows or pits and of the frontal folds or

conchae; provided, of course, these structures were differentiated. As stated before, there are instances in which the lateral wall of the recessus frontalis does not become configured by pits and folds (fig. 5). In some specimens the adult anatomy is so altered that interpretation is very difficult, even impossible.

It may be well here to refer to specific dissections of the region for study and analysis. In figure 6, for example, we have represented an adult nasofrontal region exposed for study by the removal of the operculating concha nasalis media. There

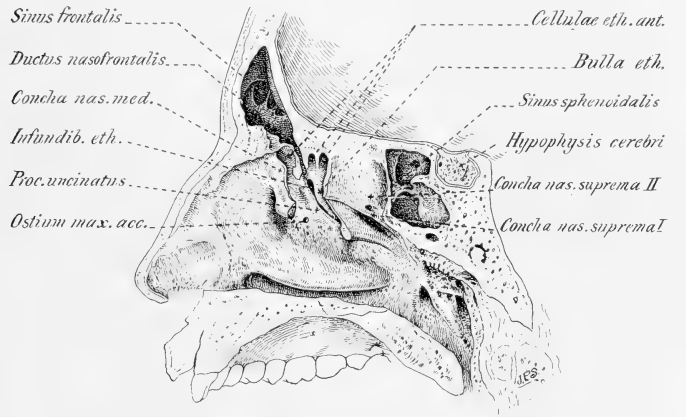

Fig. 6 From an adult. Recessus frontalis and nasofrontal connections exposed for study by the removal of part of the concha nasalis media. See text for a discussion of this dissection.

is positive evidence of four embryological frontal furrows or pits. The first or most ventral of the latter differentiated into a cellula ethmoidalis anterior of small dimensions communicating directly with the meatus nasi medius, medial to the processus uncinatus. The third and fourth frontal furrows or pits likewise developed into cellulae ethmoidales anterior both of which communicate with the meatus nasi medius cephalic to the hiatus semilunaris of the infundibulum ethmoidale.

The second frontal furrow or pit after first developing into a cellula ethmoidalis anterior continued to extend its boundaries until it became topographically the sinus frontalis. It should be noted that the duct of the sinus frontalis (ductus nasofrontalis) is in the position of the embryonic second frontal furrow or pit and that it is in the same axis as the infundibulum ethmoidale and the hiatus semilunaris,[1] but not in direct continuity with them. The sinus frontalis in this instance (fig. 6) communicates, therefore, with the recessus frontalis directly via the ductus nasofrontalis. On the other hand, the infundibulum ethmoidale ends blindly as a cellula ethmoidalis anterior (infundibular cell) lateral to the recessus frontalis and the ductus nasofrontalis.

The anatomy represented in figure 6 is that found in a certain number of adult specimens, and is illustrative of one of the anatomic types of the region. It should be noted that the infundibulum ethmoidale is not directly continuous with the ductus nasofrontalis, but that it bears an intimate and important relation to it. The relation is, in a sense, a contiguous and not a continuous one. Drainage from the sinus frontalis would find its way partly into the meatus nasi medius directly. An exploratory probe passed towards the frontal region via the infundibulum ethmoidale would, of course, find its way into the ventral, blind end of the latter and not into the sinus frontalis. To probe the sinus frontalis in this case it would be necessary to pass through the proximal ostium of the ductus nasofrontalis located in the recessus frontalis.

It is interesting and instructive to compare the embryologic anatomy of the recessus frontalis illustrated in figure 1 with the adult anatomy illustrated in figure 6. In the former the third frontal furrow and the infundibulum ethmoidale are in the same axis; in the latter, the second frontal furrow (now the nasofrontal duct) is in the same axis as the infundibulum eth-

[1] The term hiatus semilunaris should be applied to the lunate cleft which establishes a communication between the infundibulum ethmoidale and the meatus nasi medius, i.e., the slit between the free border of the processus uncinatus and the bulla ethmoidalis.

moidale. If in figure 1 the sinus frontalis had developed from the same frontal furrow as in figure 6, the relation between the ductus nasofrontalis and the infundibulum ethmoidale would have been less intimate.

The dissection of the adult nasofrontal region illustrated in figure 9 gives evidence of the early embryologic frontal furrows or pits. The adult derivatives of the latter are readily identi-

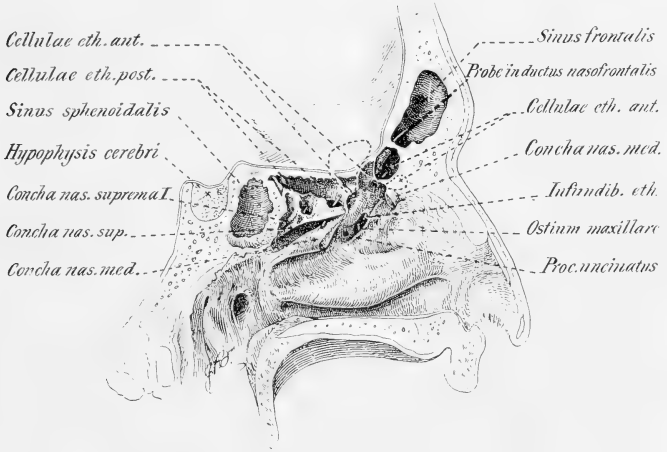

Fig. 7 From an adult. Recessus frontalis and nasofrontal connections exposed for study. Especially note the derivatives of the frontal pits, the tortuous and narrow ductus nasofrontalis, and the termination of the ventral extremity of the infundibulum ethmoidale. See text.

fied. The first frontal pit developed into a small cellula ethmoidalis anterior which is in direct communication with the recessus frontalis by means of its ostium. The second and the third frontal pits developed into sinus frontales (sinus frontalis in duplicate). Both of the latter communicate directly by means of independent ostia with the recessus frontalis—no ductus nasofrontalis being present. A study of the dissection shown in figure 9 clearly points out that the infundibulum ethmoidale ter-

minates blindly (indicated by a probe) as a cellula ethmoidalis anterior (infundibular cell) lateral to the recessus frontalis. Loose interpretation of the anatomy of this region in this instance, might lead to the erroneous statement that the sinus frontalis developed as an extension of the infundibulum ethmoidale. One sees even a channel-like depression on the lateral wall of the recessus frontalis connecting in a sense the sinus

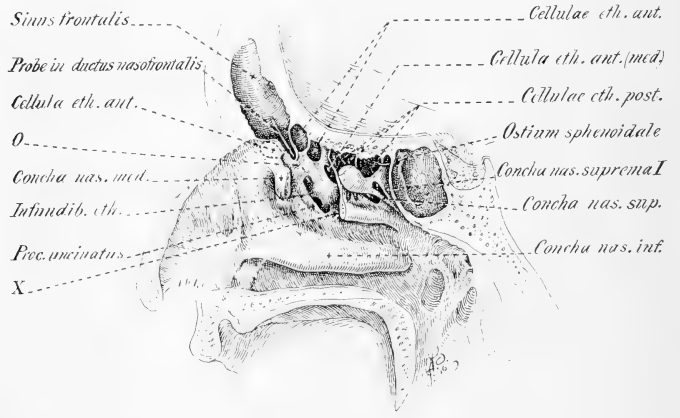

Fig. 8 From an adult. Dissection shows the nasofrontal connections and the ethmoidal labyrinth exposed. Especially note the derivatives of the frontal pits and the direct continuity of the sinus frontalis with the infundibulum ethmoidale. The dorsal blind end of the infundibulum ethmoidale, due to a mucosal fold (*X*) passing from the free border of the processus uncinatus to the bulla ethmoidalis, should also be noted. In this specimen practically all secretion from the sinus frontalis would find its way into the sinus maxillaris. See text.

frontales with the infundibulum ethmoidale. It is obvious that drainage from the sinus frontales would in part find its way into the infundibulum ethmoidale, thence via the latter to the ostium maxillare and into the sinus maxillaris (antrum of Highmore).

In figure 7 we have evidence of four embryologic frontal pits. The derivatives of these pits are two cellulae ethmoidales anterior and two sinus frontales, all in communication with the recessus

frontalis of the meatus nasi medius. The first (most ventral) and fourth (most dorsal) frontal pits developed into two small cells. The second frontal pit developed sufficiently far to be topographically a sinus frontalis (indicated in drawing as an anterior ethmoidal cell). The sinus frontalis proper took its origin from the cellula ethmoidalis anterior which had its genetic point in the third frontal pit. The result of the encroachment of the cell from the second frontal pit is a narrow channel (ductus nasofrontalis) communicating between the sinus frontalis and the recessus frontalis. As in figures 6 and 9, in figure 7 the infundibulum ethmoidale ends blindly lateral to the recessus frontalis.

As stated in previous paragraphs, fewer frontal pits and folds are at times differentiated in the fetus. This changes the picture of the adult anatomy of the recessus frontalis. In figure 12 there is evidence of but two embryologic frontal pits. The first or most ventral of the latter developed into the sinus frontalis. It should be noted that the duct of the sinus frontalis is in the same axis as the hiatus semilunaris and the infundibulum ethmoidale. The latter terminates lateral to the recessus frontalis as a cellula ethmoidalis anterior (infundibular cell). The second or dorsal pit (fig. 12) developed into a small cellula ethmoidalis anterior in line with the suprabullar furrow (now cellula ethmoidalis anterior, honeycombing the bulla ethmoidalis). This same dissection shows a well developed dorsal limb of the processus uncinatus (x). This causes the infundibulum ethmoidale to end in a deep, blind pocket just over the ostium maxillare.

In an earlier paragraph mention was made of occasional adult specimens in which the ductus nasofrontalis is in direct continuity with the infundibulum ethmoidale. In figure 8 is represented a dissection of an adult nasofrontal region in which the ventral extremity of the infundibulum ethmoidale is directly continuous with the ductus nasofrontalis and secondarily with the sinus frontalis. In this dissection one notes a plate of tissue intervening between the free border of the processus uncinatus and the bulla ethmoidalis, thus bridging over the ventral extremity of the infundibulum ethmoidale and, in a sense, replacing the hiatus semilunaris in this position. One encounters

difficulty in interpreting the anatomy of the nasofrontal connections in this specimen. Did the sinus frontalis develop from the infundibulum ethmoidale (by a direct extension or from an infundibular cell) or from the second frontal pit (early cellula ethmoidalis anterior)?

The infundibulum by its ventral and cephalic extension usually comes into topographic relationship with some of the cellulae

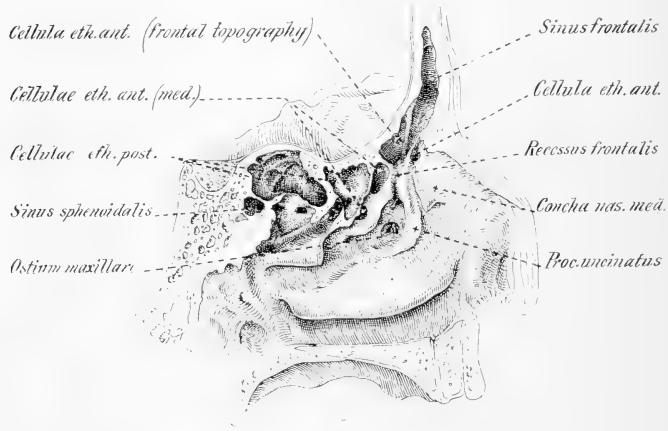

Fig. 9 Dissection from an adult. Note the two sinus frontales, the absence of ductus nasofrontalis, and the ventral termination of the infundibulum ethmoidale. See text.

ethmoidales anterior which arise from the frontal pits. In this instance (fig. 8), a relationship may early have been established with the second frontal pit (there is evidence in support of this belief).

Resorption of the intervening barrier would, of course, bring the infundibulum ethmoidale in direct continuity with the cellula ethmoidalis anterior arising from the second frontal pit, likewise with the sinus frontalis. The dissection gives positive evidence of three frontal pits (now cellulae ethmoidales anterior). Whether

an additional frontal pit which gave rise to the sinus frontalis was present in the position of the ductus nasofrontalis is, of course, impossible to say. Two of the cellulae ethmoidales anterior are separated by a considerable interval. This space may have been the second frontal pit. Again, the two frontal pits in question (cellulae ethmoidales anterior) may have been crowded apart by a

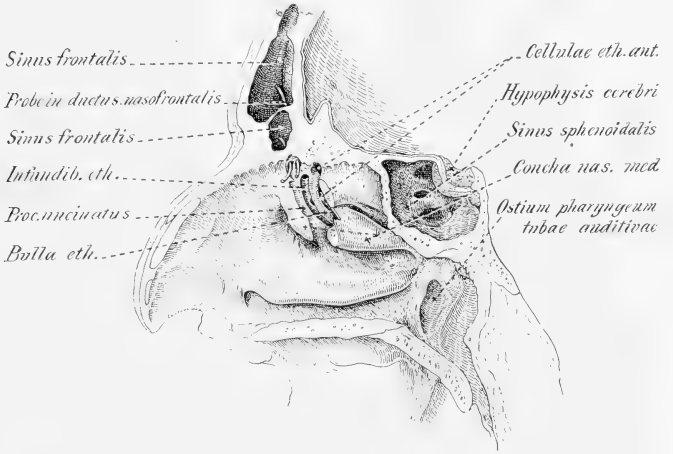

Fig. 10 Dissection from an adult. Note the sinus frontalis present in duplicate, the proximal ostia frontales in relation to the recessus frontalis, and the ventral termination of the infundibulum ethmoidale. See text.

bullous-like ventral and cephalic growth of the infundibulum ethmoidale in the establishment of the sinus frontalis. My experience has been that it is unusual for the sinus frontalis to arise from the infundibulum ethmoidale.

Drainage from the sinus frontalis in such instances (fig. 8) would almost wholly pass into the infundibulum ethmoidale, and via the latter to the ostium maxillare, thence into the sinus maxillaris. Should the floor of the infundibulum ethmoidale in such cases be largely replaced by an elongated ostium maxillare (a

rather common occurrence), the sinus frontalis and the sinus maxillaris would from a practical viewpoint be in direct communication. It should be recalled that the sinus maxillaris is genetically an outgrowth from the floor of the infundibulum ethmoidale. The initial area of the outgrowth varies considerably in extent, thus accounting for the varied size of the adult ostium maxillare.[2]

The sinus frontalis is occasionally present unilaterally or bilaterally in duplicate or in triplicate. In these cases each sinus frontalis is absolutely independent of others and possesses an individual ostium frontale. The condition of duplicity or triplicity of the sinus frontalis is readily explained when one recalls the potentiality of development referred to in previous paragraphs (figs. 1 to 5). In figures 9 and 10 are represented dissections of adult nasofrontal regions in which two frontal pits (early ventral or anterior cellulae ethmoidales) developed sufficiently far to be topographically sinus frontales. Duplicate sinus frontales are either side by side in the sagittal plane (fig. 10) or are ventral and dorsal in relation, in the coronal plane (fig. 9). Intermediate relations are, of course, encountered. In figure 11 the first and second frontal pits developed into sinus frontales; in figure 10 the second and third. In both instances the sinuses communicate independently with the recessus frontalis of the meatus nasi medius. At times when the sinus frontalis exists in duplicate (or triplicate) one sinus may encroach bullous-like on the other. The name bulla frontalis was, however, applied by Turner to infundibular cells which encroach upon the dorso-caudal boundary of the sinus frontalis.

The ductus nasofrontalis is a very variable channel. One encounters very many specimens in which no true duct is present.

[2] "In my series of 90 cases it (the ostium maxillare) has a great range of dimensions; varying from 1 to 20 mm. in length and from 1 to 6 mm. in width. In some instances where the ostium has reached considerable size, it almost entirely replaces the caudo-lateral wall of the infundibulum ethmoidale, thus forming a long, slit-like communication between the sinus maxillaris and the infundibulum ethmoidale." J. P. Schaeffer, Am. Jour. Anat., vol. 10, 1910, p. 351.

Witness, for example, the dissection represented in figure 11. Here the sinus frontalis is very small. In fact, partly ethmoidal in topography. The interesting thing about this case is that the sinus frontalis was bilaterally very diminutive in size. Its communication (fig. 11) with the recessus frontalis of the meatus nasi medius is established by means of a large ostium frontale in the same axis as the infundibulum ethmoidale.

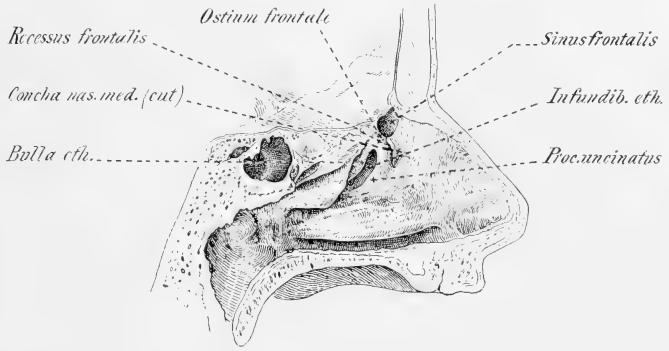

Fig. 11 Dissection from an adult. Recessus frontalis exposed. Note the diminutive sinus frontalis and the absence of a ductus nasofrontalis. "The views held on the presence and absence of the sinus frontalis are, doubtless, largely due to differences of opinion as to what should be called a sinus frontalis, and how far the development must have progressed into the frontal region before the cell has reached the dignity of a sinus frontalis." (J. P. Schaeffer).

The dissection shown in figure 9 likewise presents the sinus frontalis in duplicate in which no ductus nasofrontalis is present. Each sinus communicates with the recessus frontalis by means of a large ostium frontale. On the other hand, one encounters specimens with true ductus nasofrontales. Some of these ducts are straight and short (fig. 6), others straight and long (figs. 12 and 13). Again, the ductus nasofrontalis may be long and more or less serpentine. Witness, for example, the specimen shown in figure 7. Here is a sinus frontalis with a long,

narrow and curved ductus nasofrontalis. The duct communicates with the recessus frontalis. There are very definitely two ostia frontalia to the duct, one proximal and the other distal in position. The duct is encroached upon by a cellula ethmoidalis anterior (really a second sinus frontalis) which developed from the second frontal furrow. The slightest swelling of the mucosa of such narrow and tortuous ductus nasofrontales (figs. 7 and 10) would, of course, occlude its lumen. In some instances the

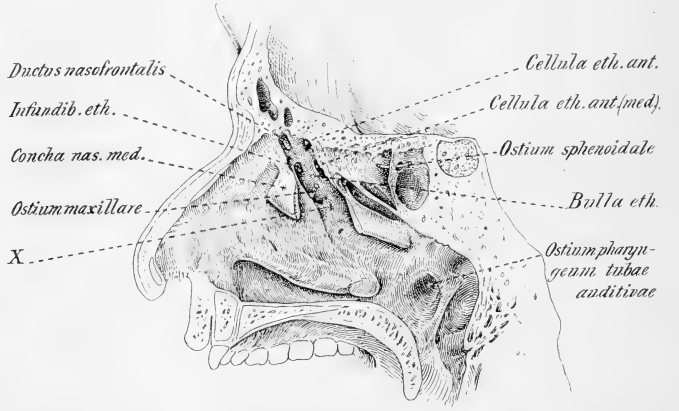

Fig. 12 Dissection from an adult. Recessus frontalis exposed. Note the discontinuous channels, i.e., the ductus nasofrontalis and the infundibulum ethmoidale.

ductus nasofrontalis is roomy, possessing large proximal and distal ostia, thus affording a better drainage channel for the sinus frontalis (fig. 13).

The ductus nasofrontalis with its proximal ostium or in the absence of a true duct, the proximal ostium frontale (in the latter the distal ostium frontale is wanting), bears a varied relation to the ventral extremity of the infundibulum ethmoidale. The latter usually ends blindly lateral to the terminal portion of the ductus nasofrontalis. The infundibulum ethmoidale and the duc-

tus nasofrontalis are at times in the same axis (figs. 6 and 12). Again the ductus nasofrontalis with its proximal ostium is not in line with the infundibulum ethmoidale. Witness, for example, figure 10: Here the proximal ostium frontale is located medial to the cephalic extremity of the processus uncinatus. Drainage in such cases would in a large measure be diverted directly into

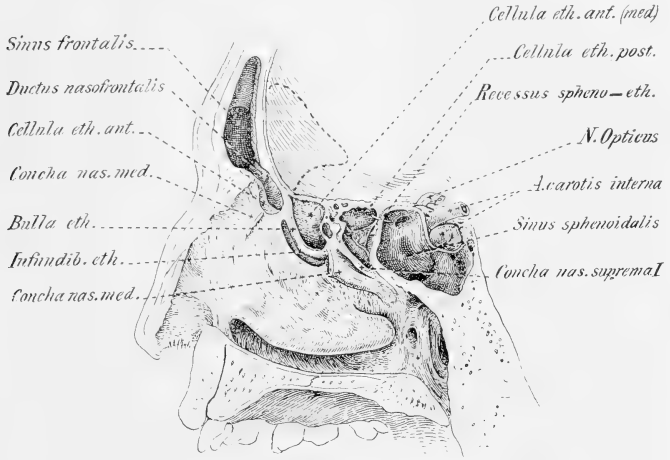

Fig. 13 From an adult. Note the roomy ductus nasofrontalis, discontinuous with the infundibulum ethmoidale. The intimate relation between the sinus sphenoidalis and the A. carotis interna is also indicated in the dissection. The section is to the right of the mid-sagittal plane, hence the absence of the hypophysis cerebri.

the meatus nasi medius. In figure 7 the relation is not one of alignment. In figure 9, passing from the proximal ostia frontalia, on the lateral wall of the recessus frontalis towards the infundibulum ethmoidale, is a shallow, gutter-like channel. Drainage from the sinus frontales here would largely find its way into the cephalic end of the infundibulum ethmoidale.

In the specimen shown in figure 8 in which the infundibulum ethmoidale and the ductus nasofrontalis are in direct continuity,

drainage from the sinus frontalis would, of course, readily find its way into the sinus maxillaris. The infundibulum in these cases acts in every sense as a gutter of communication between the sinus frontalis and the sinus maxillaris. The same is true in a large number of cases in which the relations are *intimate but not continuous* between the ventral extremity of the infundibulum ethmoidale and the derivatives of the recessus frontalis. The sinus maxillaris in turn becomes a reservoir for drainage from the sinus frontalis and certain cellulae ethmoidales anterior (some infundibular and others frontal in position). If in those cases in which the sinus frontalis or its duct is directly continuous with the ventral extremity of the infundibulum ethmoidale (fig. 8) the ostium maxillare should occupy a goodly or greater portion of the floor of the infundibulum ethmoidale (a condition encountered), the sinus frontalis and the cellulae infundibulares would, from a practical viewpoint, be in direct communication with the sinus maxillaris. This close relationship is, however, secondary and one must not infer that the frontal and maxillary sinuses and the infundibular cells arise from the same point.

CONCLUSIONS

The materials studied for this paper seem to justify the following conclusions:

1. The sinus frontalis is in the vast majority of cases a derivative (a) of the recessus frontalis directly, (b) of one or more of the cellulae ethmoidales anterior which have their genesis in frontal pits, or (c) of both, when present in duplicate or triplicate.

2. The sinus frontalis appears occasionally to arise from the ventral extremity of the infundibulum ethmoidale. This relationship, however, is in some instances secondary owing to development. It is questionable whether the sinus frontalis ever develops from the suprabullar pit or furrow.

3. The ductus nasofrontalis (or the sinus frontalis directly in the absence of a ductus) and the infundibulum ethmoidale are in the vast majority of instances, discontinuous channels in the

adult. The topographic relationships may, however, be very intimate, i.e., they may be contiguous.

4. The ductus nasofrontalis (or the sinus frontalis) and the infundibulum ethmoidale are occasionally directly continuous in the adult.

5. The infundibulum ethmoidale in approximately as many as 50 per cent of adult bodies acts as a channel for the carriage of secretion or infection from the sinus frontalis and certain of the cellulae ethmoidales anterior to the sinus maxillaris: Included in this group are (a) those cases in which the sinus frontalis and the infundibulum ethmoidale are continuous, and (b) those cases in which the sinus frontalis and the infundibulum ethmoidale are discontinuous, but intimately and vitally related topographically.

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THE LACHRYMAL GLAND

JOHN SUNDWALL

From the Hull Anatomical Laboratory, University of Chicago and the Department of Anatomy, the University of Kansas

TWENTY FIGURES

CONTENTS

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I. INTRODUCTION

The investigations on which this paper is based began several years ago ('05) when it was first learned that lachrymal glands as well as those of the nictitating membrane, plica semilunaris, of many animals stain with certain dyes which are generally regarded as specific to mucous secreting cells. It was found that these glands of the ox, pig, sheep, goat, and horse stained readily with either mucicarmin or muchaematein, while those of the cat, dog, rabbit, and guinea pig did not. Preparations of the lachrymal glands of these animals were shown at the twenty-second session of the Association of American Anatomists held at the University of Wisconsin, and a preliminary report on the staining reactions of the lachrymal gland tissue found in the nictitating membrane of the ox was published in 1906.

It was the intention at that time to publish in detail the results of the investigations on the staining characteristics of these glands. There was a delay, however, and in the meantime a paper by Hornickel ('06) on this particular subject appeared. In this he claimed that by the use of mucicarmin a positive stain was obtained on the lachrymal glands of the pig, sheep, goat, and dog, while similar glands of the horse, ox, ass, and cat were negative. His results in some instances were opposite to mine; namely, I obtained a positive stain in these glands of the ox and horse while the glands of the dog were negative. The difference in the results obtained in the two laboratories is interesting. Hornickel's paper served as added impetus to further

work on my part, and I decided that a detailed study of the structure of this gland in one species might well precede a general comparative study.

In view of the work already begun on the Harderian gland in Bos and the availability of the lachrymal gland in this species for both structural and chemical study, it was chosen for this purpose.

Numerous contributions have appeared dealing with various phases of the lachrymal gland of different animals—such as its phylogeny, embryology, innervation, gross and microscopic structures, etc. Chief efforts, however, have been directed toward the study of the structure of the cells and the secretory changes they undergo during the stages of rest and activity.

The origin and distribution of the gland in vertebrates have been described by Wiedersheim ('76) and Sardemann ('87). Wiedersheim ('07) states:

> The first attempt of a vertebrate to exchange an aquatic for an aerial existence necessitated the development of a secretory apparatus in connection with the eye. Thus in Urodeles a glandular organ is developed from the conjunctival epithelium along the whole length of the lower eyelid; in Anurans and Reptiles this becomes more developed in the region of the anterior, and in many Reptiles also of the posterior angle of the eye, the original connecting bridge gradually disappearing: thus two glands are developed from the primitively single one, each of which becomes further differentiated both histologically and physiologically. From one is formed the Harderian gland—anterior angle of the eye—while the other, posterior angle, gives rise to the Lachrymal gland. In Crocodiles, Snakes, and Hatteria, the lachrymal gland is wanting while in the Chelone it is extremely large.

Further contributions regarding the comparative anatomy of the lachrymal gland are given by Van Trotzenburg ('01) who studied it in both the old and new world monkeys and its phylogenetic relation to man.

Contributions to the embryology of this gland and its accesory structures have appeared from time to time. Falchi ('05) studied the development of the gland in the rabbit, guinea pig, sheep, and human. Monesi ('03), Matys ('05), ('06), Küsel ('06), and Lang ('11), also, have contributed to this phase of the subject. Their efforts, however, have been concerned chiefly with

the lachrymal sac and canal. Speciale-Cirincione ('08) states that in man—"Die Anlage der Tränendrüse beginnt mit dem 2. Monat, zu einer Zeit, wo die Lidspalte noch weit offen steht. Die Entwicklung geht alsdann sehr rasch vor sich, so dass von einem Tage zum anderen eine erhebliche Aenderung zu bemerken ist."

The innervation of the lachrymal gland has been studied by Dogiel ('93), Jendrássik ('94), Tepliachine ('94), Laffaye ('97), W. Klapp ('97), P. Klapp ('97), Landolt ('00), Puglisi-Allegra ('03) ('04), and Schirmer ('04). The works of Dogiel and Puglisi-Allegra are of particular interest here as the others are chiefly contributions from a clinical standpoint.

Dogiel used the methylene-blue and Golgi's methods on the lachrymal glands of the guinea pig and rabbit. According to him "Die Thränendrüse empfängt fast ausschlieslich marklose Nervenfasern, solche, die Blutgefässe und die Ausführungsgänge umflechtend, . . . und bilden ein Geflecht in dem auf der memb. Propria derselben sich lagern." A peritubular and intercellular net work of these fibers is described.

Puglisi-Allegra ('03), using Golgi's method and the vital staining with methylene-blue, describes peritubular, intrapericellular, intracellular fibres, and sympathetic ganglia cells situated on the course of the sympathetic fibres.

Schirmer describes both medulated and nonmedulated nerve fibres in this gland.

Clinical observations seem to favor the view that the secretory fibres are derived from the facial nerve through the Nervus petrosus superficialis major by way of the Ganglion Sphenopalatinum (Klapp).

The vascular supply and lymphatics have been studied by Puglisi-Allegra ('04).

The position and gross characteristics of the gland have been described by Ellenberger ('06) and by Ellenberger and Baum ('08).

Regarding the finer structures of the gland there have been many contributions. Not all are agreed, however, as to the form of the secreting elements (whether tubules or alveoli).

Leydig ('57), Frey ('59), Henle ('73), Ellenberger ('88), Toldt ('01), V. Ebner ('02), Böhm and Davidoff ('03), and others have described the lachrymal gland of man and domestic animal as being in general tubulo-acinous in form. Flemming ('88), Stöhr ('91), Zimmermann ('98), Sobotta ('02), and Schirmer, among others, describe it as a compound tubular gland, while Franck ('83), Langer ('90), and Leiserung-Mueller-Ellenberger ('90) describe it as being of the acinous type.

Boll's ('68) research on the lachrymal glands of the pig, sheep, calf, and dog was one of the first of importance. His work was confined to the star-shaped supporting cells surrounding the alveoli. Later ('71) he compares the structure of this gland to that of the salivary gland.

Schwalbe ('87) divides the gland into "eine grössere compacte obere, die obere Thränendrüse (Glandula lachrymalis superior s. innominata Galeni; Portio superior s. orbitalis [Sappey] und eine kleinere aus locker geordneten Läppchen gebildete untere, die untere Thränendrüse (Glandula lachrymalis inferior s. glandulae congregatae Monroi; Portio inferior s. palpebralis [Sappey])."

Much of the later investigation has been confined to the study of secretion granules and the secretory changes in the lachrymal gland during rest and activity. Among the most important of these are the contributions of Langley ('79), Reichel ('80), Nicolas ('92), Solger ('96), Kolossow ('98), Lor ('98), Noll ('01), and Puglisi-Allegra ('04).

Maziarski ('02), using the method of Born, constructed a model of the human lachrymal gland. "Es ist eine deutlich tubulöse Drüse; es wäre also ganz unrichtig, sie mit den Speicheldrüsen zu vergleichen."

Fleischer ('04) follows with an extensive article on the structure of the lachrymal gland of the ox.

Numerous other contributions have appeared dealing with special phases of the lachrymal gland, among which may be named Merkel ('83), Kirchstein ('94), Stanculéanu and Théorhari ('98), Garnier ('00), Axenfeld ('00), Alt ('00), Dubreuil ('07), Gotz ('08), and Riquier ('11).

II. GROSS CHARACTERISTICS

The following description of the lachrymal gland in the ox is made from careful dissections after embalming heads and injecting the arteries.

Gross structure and relations

The gland in general is a flattened oval or almond shaped structure situated on the superior contour of the bulb, with an auricular appendage which descends on the outer or posterior contour of the bulb. The gland is more or less moulded to conform with the bulb and the bony orbit so that the superior surface of the oval mass as well as the outer surface of the appendage is convex in contour while the surface directed towards the bulb is concave. Both surfaces possess the characteristic glandular lobulations.

The anterior or outer margin of the gland including the appendage measures on an average from 5 to 5.5 cm. in length. The long axis of the superior mass is from 3 to 4 cm. in length, the average width of the mass being 3 cm. and its average thickness 1 cm. The superior mass is generally termed the Pars superior while the appendage is known as the Pars inferior or accessorius. The Pars inferior or appendage generally measures about 2 cm. in length, 1 cm. in width, and 4 mm. in thickness, but this portion of the gland is subject to much variation.

The weight of the entire gland mass averages in the adult ox from 6 to 7 grams. The weight of the appendage (Pars inferior) is from 1 to 1.5 grams. It will be seen from a comparison of these weights that the larger mass of the gland is confined to the Pars superior.

The Pars superior is situated on the superior and posterior or temporal half of the Bulbus oculi while the long axis of this oval shaped mass is directed obliquely posteriorly and medianward. The anterior pole is located immediately above the insertion of the M. levator palp. sup. and M. obliquus sup. This corresponds to a point immediately behind the center of the Margo supra orbitalis. From this point, the lateral margin of the Pars super-

ior extends to the posterior or temporal canthus just under and slightly within the Margo supra orbitalis. At this point the Pars inferior or appendage takes origin and extends downward and behind the bulb, just within the bony orbital wall. It terminates at the level equal to the insertion of the M. obliquus inf. and the inferior margin of the insertion of the M. rectus lateralis.

The gland is completely enclosed within the orbit by the periorbital fascia, a thick fascia which surrounds and encloses all the structures of the orbit and lines the bony orbital wall. The gland itself is embedded and surrounded by a condensed and tough mass of areolar tissue and fat which is a part of this general tissue which fills the interstices between the muscles, nerves, and bulb. Bands of dense tissue derived from the inner surface of the periorbital fascia are fused with the capsule of the gland.

Vascular supply

The gland derives its blood supply from the A. lacrimalis which is a branch of the A. ophthalmica. The artery enters the gland from the interior or bulbar surface by numerous branches. A branch of this artery continues and enters the superior palpebral fascia. Corresponding veins leave the gland and are collected into the V. lacrimalis which empties into the V. ophthalmica.

Nerves

The innervation is from the N. lacrimalis. Two distinct lachrymal nerves take origin from the N. ophthalmicus near its origin. These nerves follow the general course of the blood vessels (fig. 1.)

Ductuli excretorii

From six to eight ducts carry away the secretion of the gland. These ducts leave the lateral margin of the gland, enter the palpebra superior and terminate by piercing the superior conjunctiva about 1.5 cm. internal to the free margin of the superior

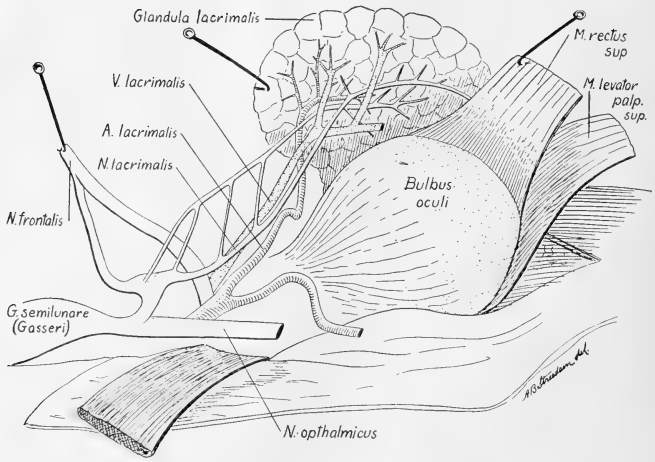


Fig. 1 Gross structure of gland showing relations. From dissection, Tech. I. Concave surface with vessels and nerves are shown.

lid. These terminals are arranged in a straight line on the conjunctiva extending from its center to the external or posterior canthus. The distance between these terminals averages about 0.5 cm. The openings readily admit a porcupine quill (fig. 2).

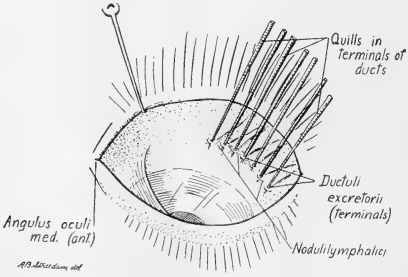


Fig. 2 Terminals of Ductuli excretorii with quills inserted. From tissue prepared by Tech. I.

The ducts range from 1.5 cm. to 2 cm. in length. They are more or less tortuous in their course as it is impossible to obtain a duct in its entirety even in thick sections. There are seen on the conjunctiva surrounding these terminals aggregations of lymph nodes. From four to six ducts leave the Pars superior while from one to two leave the Pars inferior (fig. 3).

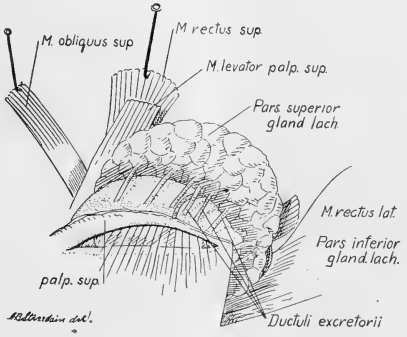


Fig. 3 Pars superior; Pars inferior; Ductuli excretorii. Specimen prepared by Tech. I.

III. SUPPORTING TISSUE

For the study of the capsule and connective tissue framework of the gland the methods and stains used were: Flint and Spalteholz, Mall's method and a modification of his method for the demonstration of reticulum, Mallory's, Van Gieson's, Weigert's, and Herxheimer's. (See Technique.)

Capsule

The capsule is fused with the general areolar and adipose tissue of the orbit. Strands of tough connective tissue fibres derived from the periorbital fascia are also intimately fused with the capsule. It is bound down to the gland by numerous interlobular septa which take origin from the capsule and ramify throughout the gland thus separating it into numerous lobules. It varies in thickness. In general that portion covering the

convex surface is thinnest, averaging 0.5 m. Where the septa take origin in this area it is much denser. The thickest portion is found on the concave surface.

It is composed of areolar tissue in general. Both collagenic and elastic fibres are present in abundance. Numerous globules of fat are seen singly and in groups irregularly distributed through out, being more abundant around the large vessels. Small lobules of gland tissue are also included within the capsule. These are more numerous in the neighborhood of the exits of the main ducts. Smooth muscle fibres are also present irregularly distributed. On the concave surface the capsule contains the large blood vessels and nerves while on the outer margin of the gland the large ducts are surrounded by the capsular tissue. The elastic fibres are long and fine and are arranged parallel to the surface of the capsule, as described by Fumagalli ('97), Riquier, and Schirmer. They are most numerous on the concave surface where they surround the larger vessels. In the region of the exits of ducts the elastic fibres are abundant also and interlace more than elsewhere, forming a network around the ducts.

The interlobular septa

The interlobular septa, which are derived from the capsule and divide the gland into numerous irregularly shaped and sized lobules, are composed of practically the same tissues described in the capsule. Secondary septa take origin from two or three large primary septa and enter the various lobules, forming the supporting structures of the lobules and surrounding the various vessels, nerves, and ducts, which are in close proximity to each other.

Observations in this laboratory regarding the capsule and septa agree with those of Schirmer. However, I have failed so far to see in the connective tissue framework of the gland—septa or intralobular tissue—of either young or old Bovidae a constant accumulation of lymphoid cells such as described by Schirmer for man and Fleischer for the lachrymal gland of the ox. Axenfeld and Gotz have also referred to lymphoid infiltrations in

man which increase from childhood to old age. According to Bensley these are probably plasma cells.

The supporting elements finally ramify and surround each acinus, or tubule, forming a network or reticulum. The larger septa contain fat and small glandular lobules in addition to the vessels, nerves, and ducts. The septa are composed chiefly of collagenic fibres (Van Gieson's). Elastic fibres are present to some extent in all the interlobular framework, especially around the ducts and vessels. Cells suggesting smooth muscle cells are sometimes seen, in addition to those seen in the vessel walls, occurring very sparsely and as a rule singly in the neighborhood of the ducts.

The intralobular septa

The intralobular connective tissue septa are practically composed of collagenic fibres and reticulum. In the larger of these, only, are elastic fibres seen independent of the vascular walls. In the smaller septa well within the lobules elastic fibres are seen only in connection with the blood vessels. No elastic fibrils are seen surrounding the alveoli of the gland.

Elastic fibres

My observations regarding the final distribution of elastic fibres do not entirely agree with Boll ('71), Schirmer, and Fumagalli, who claim that elastic fibres surround the alveoli, or tubules. Regarding these fibres Riquier remarks—"nei sepimenti connettivali attorno ai vasi ed ai condotti escretori, sono invece difficilimente dimostrabili intorno ai tubuli secernenti."

My investigations indicate that elastic fibres do not enter the lobules and surround each tubule. Repeated examinations of tissues fixed in various solutions and stained both by Weigert's and the Unna-Taenzer method—the latter was used by the Italian investigators—did not show the presence of elastic fibres surrounding the acini.

In using these stains, it is most essential to fully decolorize the section. Otherwise the usual collagenic fibres and reticulum

may assume the staining characteristics of elastic fibres. This is especially true of the Unna-Taenzer orcein method which stains elastic fibres brown. It was my practice to place the sections from the stain in 70 per cent alcohol and then decolorize under the microscope with acid alcohol until the section became for the most part colorless. The characteristic elastic fibres of the blood vessels remain deeply stained and serve as controls for the degree of decolorization. In the use of Weigert's it is also necessary to decolorize in alcohol until the section becomes yellowish or light gray. The elastic fibres alone should be stained black.

With the proper degree of differentiation, using the elastic fibres of arterial wall as the criterion, it will be found that elastic fibres are present, as described, in the capsule; to a less degree in the interlobular septa surrounding the blood vessels; and in the basement membrane of the main, primary, interlobular, and larger intralobular ducts. Only occasionally are they seen in the walls of the small intralobular ducts. They are not present as forming the walls of the acini except in rare instances, and then when lobules are in contact with capsule.

Peritubular connective tissue, 'Korbzellen', basement membrane

The finer structural tissue surrounding the terminal tubules or acini of glands in general is composed of several elements such as fixed connective tissue cells, the so called 'Korbzellen' or basket cells, and the basement membrane. Much confusion exists in the literature respecting the terms used to designate these various tissues. Some refer to all these elements as the basement membrane. Shafer ('12) states:

> In most glands the secreting cells of the alveoli and also the cells which line the ducts are bounded. . . by a thin membrane, which is sometimes continuous, sometimes interrupted, and which has nuclei here and there scattered upon it. This is the basement membrane and as the presence of nuclei indicates, it is composed of more or less fused flattened cells of connective tissue nature which are sometimes united edge to edge, sometimes connected only by branch processes so as to form a sort of flattened basket-work around the alveoli. Even

in this case the meshes of the basket-work are not quite empty, being occupied by a delicate filmy membrane which is a condensation of the reticular connective tissue.

Flint and others refer to this latter alone as the basement membrane.

Boll ('68) was the first to describe 'Korbzellen' in the lachrymal glands of the pig, sheep, calf, and dog. Owing to the resemblance of these cells, in transverse sections, to demilune cells, he regarded them as similar in nature to those cells described by Gianuzzi in salivary and mucous glands. Cells similar in structure had been described in other glands by Krause; Henle, who regarded them as nerve cells; Pflueger; and von Kölliker. Others held the same view as Boll regarding the function of these cells. Noll ('01) regarded these 'Korbzellen' as having completed a stage of secretion and having been pushed back against the wall by neighboring cells filled with secretion. In a later communication Boll ('71) refers to these cells as the basement membrane.

Kollosow refers to the cells described by Boll as muscular epithelium, as did Zimmermann ('98). Schirmer and Puglisi-Allegra described similar cells in lachrymal glands, to which they ascribe the function of contractility.

These 'Korbzellen' are readily seen in my Zenker-Van Gieson preparations. They are more prominent in the lachrymal glands of younger animals. Frequently these irregular anastomosing cells are seen between the deep red staining reticular membrane and the epithelial cells of the tubules, often obscuring the latter. I am inclined to regard them as of connective tissue origin rather than contractil musculo-epithelium. The anastomosing processes of the cells stain deeply red in Van Gieson's. Further work, especially of an embryological nature, will be essential to determine the positive nature of these cells.

Other connective tissue cells are seen in addition to these large cells—Körbe. These manifest themselves as elongated nuclei and lie alongside of the basement membrane. Occasionally nuclei appear at the intersection of the fibrils of the reticulum and as a consequence appear as a part of it. Nuclei of endothelial cells, lymph cells, and plasma cells are also seen.

The basement membrane proper has been described by Zimmermann and Fleischer in the lachrymal gland.

According to my observations, the final ramification of the supporting tissue is in the form of a reticulum enclosing within its meshes the acini, intercalary and intralobular ducts, and vessels. This reticulum forms the membranae propriae of the acini or tubules and consists of a delicate network of interlacing fibrils which intimately surround the epithelial cells of the acini and small ducts. From this basement membrane secondary

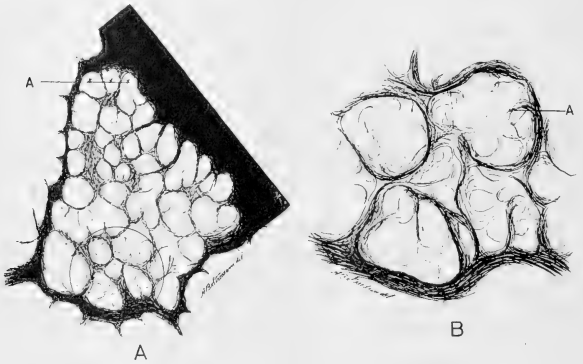


Fig. 4a Reticulum, Tech. III, 2, 3, Oc. 6, obj. 8, X 300. Parenchymatous and other tissue digested away in pancreatin. Reticulum is readily seen showing the outlines of the original tubules.

Fig. 4b Reticulum, Oc. 2, obj. 2 mm. oil, X770. Portion in figure 4a enclosed within the circle is shown here enlarged.

fibres apparently take origin and pass radially into the acini between the individual gland cells. Others may appear to enter the base of the cell, thus simulating Holmgren's Trophospongium. However, in reality this probably does not occur.

The basement membrane and its relation to the tubules, as described, can readily be seen in sections stained by Mallory's method, whereby it is stained dark blue. As a rule it is closely apposed to the epithelium, and the interepithelial processes are seen with ease. In Van Gieson's stain it is red. This network

can be considered as reticular connective tissue in the sense of Mall, and is almost identical in appearance to the reticular framework of the submaxillary gland as shown by Flint. According to the Flint, Spalteholz method (Technique II, 1) and a modification of Mall's method (Technique III, 2) it was shown that this basement membrane is not digested in pancreatin while the parenchyma entirely disappears. Satisfactory specimens for study may be made by these methods and then staining by either Mall's or Mallory's method (figs. 4 a and 4 b). Van Gieson's stain also may be used. The presence of this basement membrane after prolonged digestion shows that elastic fibres do not compose it.

Plasma cells

Numerous plasma cells are seen in both the interlobular and intralobular tissue, principally around the ducts and between the acini. These cells are typical in appearance—round, more or less eccentric nuclei with peripheral arrangement of the chromatin, and fine basophilic granules in the protoplasm. These cells are differently shaped—some spheroidal, others elongated and irregular in outline. Sections stained in neutral safranin show these cells very clearly. They may be found singly or in clusters (fig. 5).

Since Waldeyer ('75) first designated certain cells as plasma cells and Unna ('92) described these cells as purely pathological in the skin, followed by other observers who stated that they are present in normal tissues, there have been numerous contributions regarding plasma cells and their distribution in glands. Klein ('79, '82) and Cajal ('96) describe them as being present in the submaxillary gland of man. Zimmermann shows in the lachrymal gland of man "Granulirte Zellen (Plasmazellen?) mit je zwei stäpchenförmigen Centralkörpern innerhalb eines granula freien Hofes." Plasma cells have been described also by Ioannovices ('99) in glands of the tongue, and by Maximow ('01) in submaxillary and retrolingual of the dog. According to the latter they are comparatively few in the submaxillary and numerous in the retrolingual. Krause ('98) calls attention to

the relation of plasma cells to the secretory activity of the retrolingual gland of the hedge hog—they being fewer in number in the stimulated gland than in the resting. Dantchakoff ('05) observed the relation of plasma cells to the secretory activity of the submaxillary gland of the rabbit, and Hannes ('11) describes them in the lachrymal gland of man.

Fig. 5 Interstitial plasma cells. Oc. 4, obj. 2 mm. oil.

IV. DUCT SYSTEM AND TUBULES

For purposes of description I have adopted the following classification of the duct system; (1) main ducts, Ductuli excretorii; (2) primary ducts; (3) interlobular ducts; (4) intralobular ducts; (5) intercalary ducts; and (6) acini, alveoli, or tubules. I see no reason for the inclusion of separate sublobular ducts in the lachrymal gland.

The methods used for the study of the duct system have been as follows: (1) injection methods, (2) vital staining with pyronin, and (3) histological study of sections.

The injection-corrosion specimens of the duct system as prepared in this laboratory (Technique II, 2)

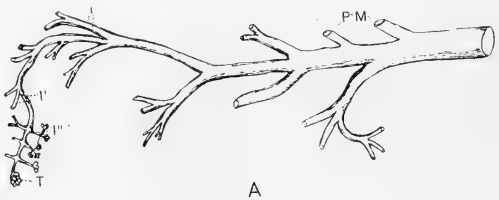
The main duct follows a more or less tortuous course through the palpebral fascia and enters into the gland substance at the outer margin. At this point it is seen to branch. The branching is somewhat variable—generally two different types are seen: (a) a dichotomous branching wherein the main duct divides into two ducts, which by the same method immediately form four ducts, etc. (This method of branching is described for the submaxillary glands by Flint) and (b) the main duct may continue for some distance into the gland substance somewhat similar to the trunk of a poplar tree, while the primary ducts take origin from this main trunk at various levels. In both instances, however, before the primary ducts take origin, several small branches are seen to leave the main duct at right angles. These are the ducts of small accessory lobules located on the outer or lateral margin of the gland.

The primary ducts vary in length, depending upon their distribution. They divide, as a rule, dichotomously into either equal or unequal branches. In the former case, where the branches are equal, these immediately undergo dichotomous branching again to form the interlobular ducts. Where the branches are unequal, the smaller one may directly form an interlobular duct, while the larger one may continue as a primary duct for some distance farther and then branch to form interlobular ducts. Frequently very small branches take origin directly from the primary ducts at right angles as in the case of the main ducts. These small branches are intralobular ducts which empty directly into the primary ducts from neighboring lobules.

An extensive ramification of the interlobular ducts is seen. The branching is much similar to that described for the primary

ducts—(a) dichotomously or (b) the main interlobular ducts continuing as such for some distance with smaller intralobular ducts taking origin at various levels.

The intralobular ducts have an extensive ramification as well. The branching is chiefly of the dichotomous type although trichotomous branching is seen. The branches are as a rule very unequal in calibre. Each lobule as a rule contains many of these ducts of unequal sizes. At various levels in the course of these ducts, nodular enlargements are seen. Frequently the ducts are seen to terminate in nodular enlargements. It is somewhat difficult to force the injection mass beyond these nodules. However, after careful and repeated trials it is possible to force it (celloidin is preferable) to the alveoli or tubules and



A

Fig. 6a Corrosion cast of main duct with branches. Drawing, binocular, somewhat diagrammatic. M, main duct; *P*, primary duct; *I*, Interlobular duct; *I'*, intralobular duct; *I''*, intercalary duct; *T*, tubules.

when this is accomplished it is seen that these nodules mark the exits of the intercalary or junctional duct.

The lumina of the intercalary ducts are exceedingly fine and threadlike as represented by the celloidin or celluloid cast. As a rule two or three of these ducts leave each nodule at right angles to the intralobular ducts and terminate in the tubules. Frequently they are seen to undergo dichotomous and in some instances trichotomous branching before so terminating. From two to three tubules mark the termination of the intercalary duct.

The entire duct system belonging to one main duct forms an elongated gland (fig. 6 a). Generally the primary duct leaves the main duct at an acute angle. The branching of the secondary

duct system—interlobular and intralobular ducts—is similar. The intercalary ducts, on the other hand, as a rule branch off at almost right angles or even greater angles from the intralobular ducts from which they have origin (fig. 6 b). Of course there are numerous exceptions to this generalization, but it is not by any means imaginative to compare these corrosion casts of the lachrymal gland to six or eight trees varying in length and placed in a line so closely that the smaller branches and leaves intermingle. The main trunks are then comparable to the Ductuli excretorii; and the primary, secondary, and tertiary, etc. branches to the primary, interlobular, and intralobular ducts. The leaves represent the acini or tubules and their stems the intercalary ducts.

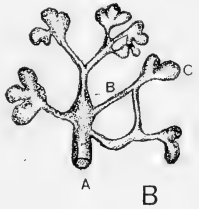


Fig. 6b Corrosion cast. High power drawing, projection apparatus. *A*, intralobular duct; *B*, intercalary duct; *C*, tubule.

In preparations where the mass has not passed beyond the nodular enlargements of the intralobular ducts the branching is readily observed through the binocular microscope. If the mass has passed into the tubules the duct system is greatly obscured. For the study of the intercalary ducts and tubules, small pieces were teased off and mounted on slides, then studied by high power.

It must be appreciated that in the study of corrosion casts, it is impossible to classify with certainty the various ducts; i.e., one cannot differentiate between the larger intralobular and the smaller interlobular ducts. As an auxiliary to this study carmin gelatin was injected into the ducts of other glands and sections of these tissues were prepared. In the latter preparations the

diameters of various ducts were ascertained and then compared with the former. Even with this aid it was impossible (and not essential) to classify all ducts.

Vital staining

Vital staining with pyronin (Technique VI, 1) greatly facilitates the study of the smaller ducts and their distribution. Bensley found that the entire duct system of the pancreas was stained

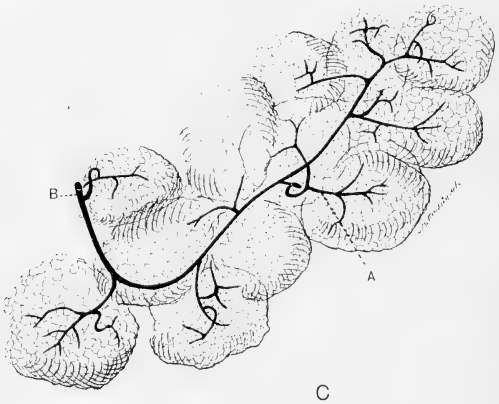


Fig. 6c Lobule of gland, fresh, after vital staining with pyronin. Drawing, binocular. The lumina of ducts are deeply stained red. *A*, intralobular duct; *B*, interlobular duct.

by this method. In application of his methods to the lachrymal gland it was found that its duct system stains a deep red while the tubules are only faintly stained (fig. 6 c). In the larger ducts the stain is limited to the periphery of the lumen, while in the smaller ducts the entire lumen stains. The smaller intralobular and intercalary ducts are especially prominent, and when fresh sections 0.5 mm. thick are examined through the binocular microscope the ramifications are clearly seen. The deep red ducts can be readily traced, in thin sections, to the acini. Very

thin sections of this tissue previously fixed in ammonium molybdate (Technique VI, 1) show that all the intercellular secretion capillaries are stained. See Secretion capillaries.

Histological structure of ducts and tubules

Main ducts. The excretory ducts in the superior lid are so tortuous in their course that it is impossible to obtain a continuous duct in longitudinal sections—only portions of one duct can be obtained. The ducts lie close to the conjunctival surface. As the terminal of the duct is approached there is seen a gradual increase of lymphoid tissue which surrounds the terminal openings as true lymph nodes.

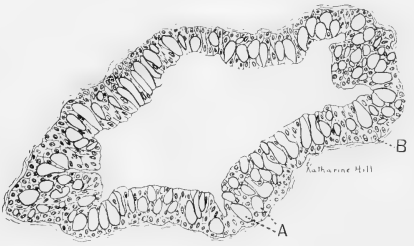


Fig. 7 Epithelium of main duct. Drawing, Zeiss, oc. 4, obj. 8, as seen in cross section of duct near its terminal in lid. *A*, goblet cells; *B*, epithelial cells. The large clear goblet cells are readily seen surrounded by smaller epithelial cells.

A cross section of the duct near its terminal shows the following characteristics: The lumen is either slit-like or very much corrugated, the epithelium being thrown up in folds with crypts between. Two types of cells line the duct—goblet cells and more or less irregularly elongated or cuboidal epithelial cells arranged in layers (fig. 7). The former cells occur in great numbers. They average as a rule 40μ by 20μ although there is much variation. Sections stained in iron haematoxylin and counterstained with mucicarmin show the surface epithelium to the best advantage. Here the goblet cells are stained red and can readily be differentiated from the epithelial cells in general. Many of

these goblet cells reach the lumen and from the ends secretion masses can be seen projecting into the lumen. Others again do not reach the surface but are entirely surrounded by the general epithelium. As a rule a flattened nucleus is seen in the base of these cells and a definite network is seen in the cytoplasm. (fig. 8).

The epithelial cells show great irregularity as to form and size. They occur in several layers. In the crypts usually two layers are seen while in the folds three or even six layers of cells are seen arranged irregularly. Those bordering on the surface (superficial cells) are usually elongated and the base is drawn out into a wedge shaped process which projects downward between the

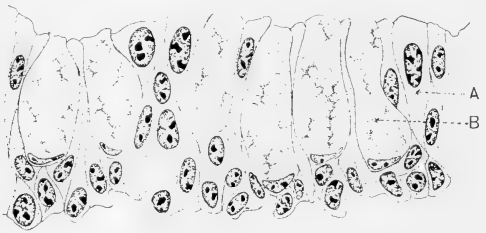


Fig. 8 Epithelium of main duct. Zeiss, oc. 4, obj. 2 mm. oil. *A*, epithelium; B, goblet cells.

deeper layers of cells. In the crypts these wedge-like projections may reach the basement membrane. The deeper layers of cells are smaller as a rule than the superficial cells and are irregularly cuboidal or polygonal in shape. The deepest layer of cells borders on a basement membrane which is readily seen in sections stained by Mallory's method. The epithelial cells in general stain deeply and homogeneously in the usual stains. Reference is made to mitochondria later on. No secretory granules can be made out in any of these cells. The nuclei are prominent, oval or spheroidal, and show irregular clumps of chromatin. As a rule no definite nucleolus is seen.

The basement membrane is directly surrounded by a circular coat of connective tissue which averages from 100 to 150μ in

thickness. The inner portion is very cellular containing numerous lymphoid cells, while the outer boundary is more or less fibrous. This outer boundary is immediately surrounded by the general connective tissue of the palpebral fascia. Sections stained by Van Gieson's method show the presence of numerous small vessels accompanying the duct. Smooth muscle cells are also seen in this connective tissue coat. They occur as a rule singly and do not constitute a coat. Collagenous fibres greatly predominate.

Weigert's stain shows the presence of numerous interlacing elastic tissue fibres which form a more or less indefinite layer outside of the basement membrane.

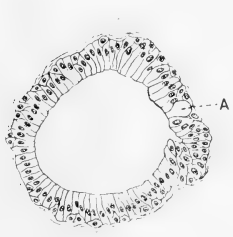
Primary ducts. These ducts represent the first branches of the main duct and are very irregular in size. As a rule the lumina are round or oval and have an average diameter of 0.2 mm. The primary ducts are embedded and surrounded by a large amount of connective tissue which is derived from the septa. The epithelial wall averages 20μ in thickness and is as a rule made up of several layers of cells—varying from two to three layers, the former predominating. In many instances, however, a single layer of long columnar cells forms the entire epithelial wall. Where two layers are present there is an interdigitation seen between the long columnar cells and the basal more or less irregularly cuboidal cells.

The cytoplasm of the surface layer in the usual fixatives stains homogeneously. The nuclei are either oval or round and have an average diameter of from 7 to 10μ. Clumps of chromatin irregularly distributed are readily made out within the nuclei. In many stains a definite cell boundary cannot be made out. However, it is plainly seen in thin sections and especially after the use of iron haematoxylin.

The cells of the outer layer, or layers, are irregularly cuboidal in form. The nuclei and cytoplasm show the same staining characteristics as the inner layer. The nuclei of the outer layer are frequently so arranged that their long axes are parallel to the circumference of the duct, while those of the inner layer are radially arranged to the duct. This condition, however, is much more pronounced in the smaller ducts.

Goblet cells are also seen in the primary ducts. They are not so numerous by any means as in the main ducts. Many transverse sections of the former may show none of these cells while others again may show from one to three. Their staining characteristics are similar to those described for these cells in the main ducts (figs. 9 and 10).

The epithelial cells of primary ducts border on a more or less indefinite basal membrane, which stains a deep blue in Mallory's stain, and is derived from and is a condensation of the circular layer of collagenic fibres which surround the epithelium and form the duct wall. The average thickness of this connective



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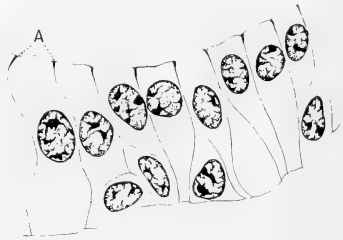

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Fig. 9 Epithelium of primary duct, cross section. Zeiss, oc. 4, obj. 8 mm. *A*, goblet cell.

Fig. 10 Epithelium of primary duct. Enlarged drawing, Zeiss, oc. 4, obj. 2 mm. oil. *A*, cement lines.

tissue wall is 50 to 100μ. Its outer borders are gradually lost in the general connective tissue septa of the gland, which are always more abundant where these ducts are found. Long fine interlacing elastic fibres are present throughout the entire thickness of this circular connective tissue coat. Immediately surrounding the basement membrane these fibres form a closely interlacing layer similar to that described for the large main ducts. As the ducts become smaller, through ramification, there is a gradual decrease of these fibres and in some of the smaller primary ducts they are difficult to make out.

The chief component of the circular connective tissue wall is the collagenic or white fibrous tissue. The collagenic fibres

stain deeply red in Van Gieson's. Within the interstices of these fibres many nuclear elements are seen which are (a) the nuclei of the endothelium, a rich plexus of arterioles, venules, and capillaries which are found in the walls; (b) lymphoid cells, which in very rare instances are so numerous that they obscure the collagenic fibres (I do not consider these lymph cell accumulations normal as they occur so infrequently); (c) occasionally solitary nuclei are seen in a cytoplasm which stains yellow in Van Gieson's. The structure of these cells, together with the staining characteristics, suggests that they are smooth muscle cells; (d) plasma cells are also seen.

In addition to the very small and numerous vessels which accompany and supply the ducts, large vessels and nerves are seen in close proximity to the ducts and following their ramifications to the gland substance. There is no regular distribution of these vessels, so far as numbers are concerned, in relation to the primary ducts. Sometimes only one of these larger arteries is seen in close proximity to a group of primary ducts. Frequently two are seen. Generally one large vein is seen to accompany these ducts. This is true also of the non-medullated nerves. However, much variation is the case with these as well.

Interlobular ducts. The primary ducts ramify to form the interlobular ducts. These further subdivide to form the intralobular ducts. It must be borne in mind, however, that intralobular ducts may originate directly from the main or primary duct. Again practically all of the primary ducts and even the main ducts for some distance are interlobular. Consequently the artificial subdivision of the duct system into primary, interlobular, and intralobular ducts must be accepted in a general sense only. In the following description of the interlobular ducts reference is made to those ducts which as a rule result from the ramification of the primary ducts and lie between the lobules.

The lumina of these interlobular ducts are wide and average as a rule about one-half to one-fourth the diameter of those of the primary ducts. Here again an explanation is necessary, for the diameters of the former are much larger at their origin than

later on after ramification. The epithelial wall, which averages from 12 to 18μ in thickness, is composed of from one to two layers of cells similar in arrangement to those described for the primary ducts. The larger interlobular ducts generally possess two complete layers while in the smaller ones there is a noticeable reduction of the cells which form the outer layer. Consequently in these smaller ducts many of the epithelial cells extend down to the basement membrane.

The single layer cells are cylindrical in form. The cytoplasm stains deeply and evenly in the various cytoplasmic stains. Study of these cells by means of the oil immersion shows the cytoplasm to be very finely granular. No basal filaments or secretory granules can be seen in the general stains. I did not see in my preparations the central bodies described by Zimmermann. Tissues fixed in acetic osmic bichromate and stained in acid fuchsin-methyl green show numerous mitochondria. These are described under 'Mitochondria.' The cell boundaries are indefinite and only in the thinnest sections can they be made out clearly. As in the other ducts where a second layer of cells is present the inner layer sends basal processes between them which reach the basal membrane. The nuclei, which average from 6 to 8μ, are round or oval and show the same staining characteristics as those of the primary ducts.

The cells in the outer layer, which, as already stated, are fewer in number, are more or less elongated—this elongation being parallel to the circumference of the duct. Here also the cell boundaries are indefinite. The cytoplasm stains similarly to that of the cells of the inner layer. In very thin sections both stain with the same degree of intensity. In thick sections, however, the inner layer appears more densely stained. This observation was made by Fleischer, who states, as a consequence, that the cytoplasm of the cells of the inner layer stains more intensely than does that of the outer layer. In my opinion this apparently deeper stain is due to the fact that these inner cells are more numerous and more compact, while those of the outer layer are fewer in number and more loosely surrounded by other cells. The nuclei of these cells are either oval or elongated, the

long axes being parallel to the circumference of the tubules. Here is seen a more pronounced example of the statement that the long axes of the nuclei of the outer layer are at right angles to the long axes of the nuclei of the inner layer cells. The cells of the outer layer are frequently so elongated that they resemble connective tissue cells. The nuclei of both layers are similar in staining characteristics.

The connective tissue wall surrounding the epithelium varies in amount depending upon the thickness of the septa in which the ducts are situated. A well defined basal membrane upon which the epithelial cells rest is plainly seen with Mallory's stain. Practically all the elements described for this wall under the caption of the primary ducts are seen in these walls—with a reduction in quantity, however. There is a marked decrease of elastic fibres. It is only necessary to contrast the smaller interlobular ducts with the larger primary ducts to appreciate this statement. In the wall of the primary ducts numerous elastic fibres are seen throughout the connective tissue wall while in the smaller interlobular ducts the elastic fibres occur sparsely or may not be seen. The accompanying arteries and veins, however, show these deep staining fibres in their walls and may be used for comparison. The decrease and disappearance of the elastic fibres take place between the origin of the primary ducts and the smaller interlobular ducts. The quantity of elastic fibres present is proportional to the thickness of the walls and septa in which the ducts are embedded. Where the connective tissue wall is much reduced and the septum is thin elastic fibres are only sparsely present or are not seen at all. The walls are composed almost entirely of collagenic fibres as revealed by Van Gieson's stain. Numerous cellular elements are present which may be classed similar to those of the primary ducts.

Intralobular ducts. Intralobular ducts vary much in the size of the lumina and the quantity of the surrounding connective tissue. Some ducts appear greatly dilated with lumina 70μ in diameter. These dilated ducts are not frequently found and are doubtless abnormal. Many again are seen which correspond

in size and structure to the interlobular ducts. The intralobular ducts present as a rule the following characteristics. There is a reduction in the cells of the outer layer so that the smaller ducts appear to possess but a single layer of cells which show the same characteristics as those described for the interlobular ducts. The nuclei are elliptical. Frequently, however, one sees ducts with two layers of cells wherein the cells of the outer layer are as prominent as are the cells of the inner layer. This is true even in some of the smallest of the intralobular ducts. The connective tissue surrounding these ducts is much less abundant than that of the interlobular structures sometimes forming only a thin layer or again it may be considerable in quantity. It is derived from the intralobular connective tissue. Mallory's connective tissue stain shows a definite basal membrane. The wall is made up almost entirely of collagenic fibres. Only occasionally are elastic fibres seen. Capillaries are seen in these walls.

No secretory granules or secretion capillaries are present in these ducts. I did not observe by using the ordinary stains the basal filaments described by Hornikel in the cells of these ducts in the lachrymal gland of the ass and the more or less indefinite pencil like structures described by Fleischer in the ox. However these are seen in special preparations (see Basal striations). Merkel saw none in the lachrymal gland of the dog.

Cement lines. These are seen in connection with the surface epithelium of all the collecting ducts—primary, interlobular, intralobular. On the surface they outline the polygonal margins of the cells and at the various angles appear as dots. In longitudinal sections this cement substance forms a well defined point which projects some distance basalward between the cells sometimes half the length of the cell. As they near the base these intercellular cement structures become finer and are finally lost in the cell membrane (fig. 20). Fleischer failed to observe this characteristic of the cement lines in the lachrymal gland of the ox. Kolossow saw no cement lines in his studies on glands. Zimmermann, on the other hand, noted this peculiar arrangement of the cement lines in the human lachrymal gland. He

describes these cement structures as bands projecting down between the cell boundaries completely surrounding the proximal end of the cell, and states that this structure of the cement is peculiar to the lachrymal gland. I am inclined to think that the basal projecting intercellular cement lines are more or less limited to the angles of the hexagonal cell margins and that they do not form bands or caps surrounding each cell. They are always seen as intercellular continuations of the fine black dots seen at these angles. Cross sections of these ducts show their complete absence between many cells. This would not be the case if they formed true bands. The entire proximal half of the cells would appear in stained sections much darker than the basal half, which is not the case.

Intercalary ducts. These have been described by many histologists among whom may be named Schwalbe, Böhn and Davidoff, Merkel, and Henle. This duct has been compared to the Speichelröhren (Schaltstücke) of the salivary glands, especially the parotid. V. Ebner describes these ducts in the submaxillary gland as short blue tubes. Their deep staining characteristics have also been described by Merkel in the lachrymal gland of the dog; and by Nussbaum and Langley in the submaxillary of the rabbit. The latter also refers to the presence in them of 'large copious granules.' Zimmermann compares this duct in the lachrymal gland in man with the parotid gland. However, he saw no basal striations in the ducts. According to him the intercalary duct in man is not so well defined as that in the ox but the transition from the intralobular duct to the tubules is much more gradual. Merkel, in his observations on the lachrymal gland of the dog, also describes intercalary ducts but "fand hier keine Gänge mit Stäbchenepithlien." According to him these ducts stain more deeply than the other structures. Regarding the intercalary duct or schaltstücke, Fleischer states—"Die Form der Zellen, ihre einschichtigkeit, das enge Lumen, ihr häufiger plötzlicher Uebergang in die Ausführungsgänge entspricht ganz dem, was zuerst V. Ebner bei Speicheldrüsen als Schaltstücke bezeichnet hat. Sehr charakteristich. . . . ist auch ihre diffuse intensive Färbung."

Secretion granules in the cells of the intercalary duct are described by him. The chief characteristics then of the intercalary duct according to these observers are the more intense staining reaction and the presence of granules.

The deeper staining of these ducts is readily seen in sections of tissue fixed in the ordinary solutions and stained with iron haematoxylin and neutral stains.

For the study of the intercalary duct I found that the most interesting results were obtained from tissues fixed in Zenker's, embedded in celloidin, and stained in muchaematein. This stain was prepared and used according to Bensley's method

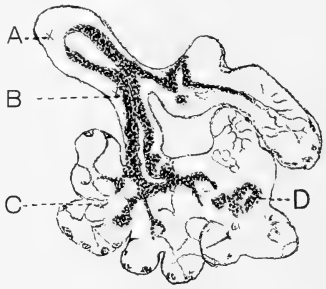

Fig. 11 Intercalary duct and tubules. Stipple board drawing projection. The granules of the intercalary duct are stained specifically with muchaematein, Tech. IV, 1. *A*, unstained intralobular duct; *B*, intercalary duct with granules; *C*, tubules; *D*, transitional cells.

(Technique IV, 1.) The sections were cut from 10–20μ thick. It was found that by following this technique the granules of the intercalary duct are stained specifically and definitely blue while the remaining tissue is unstained (fig. 11). This method therefore is of great value in the study of the intercalary duct and its relations to the intralobular duct on the one hand and the alveoli or tubules on the other. In the preparation of these sections it was found that after fixation in Zenker's the tissue should be thoroughly washed in order to remove as much of the salts as possible, for if iodine is used for this purpose the distinct granular stain cannot be obtained. Furthermore,

if in thick sections the stain affects the tubules this can be removed by the use of acid alcohol. The time required to obtain the intense blue stain is brief, as less than one minute was necessary. Sections of the salivary gland and of the mucous membrane of the intestinal tract were used for controls. I found that the muchaematein stained the granules of the intercalary ducts of the lachrymal gland in the same time that was required to obtain a corresponding stain in the control sections. The similarity of this gland in its staining characteristics to the mucous glands and cells is interesting as it suggests the possibility of the presence of mucous secreting cells in the lachrymal gland. I shall discuss this phase later on. These muchaematein stained sections show the interlobular and the intralobular ducts unstained. The tubules generally take a light diffuse stain, and connecting the tubules with the intralobular ducts are the deep blue granular stained intercalary ducts—the stain is confined to the granules only.

The intercalary ducts vary greatly in length. The longest are from 200 to 250μ in length and the shortest are about 70 to 80μ. The lumen of the intercalary duct is very narrow when contrasted with that of the other ducts. It averages about 6μ in diameter although much variation exists not only in its width in different ducts but also in a single duct. As a rule the lumen becomes wider as it approaches the intralobular duct. Primary intercalary ducts frequently give rise to a number of secondary branches which in turn terminate in one or more tubules. The cells of these ducts have an average height of 10μ. The nuclei are not stained in muchaematein, they are seen, however, as large oval structures in the basal end of the cell. They never appear angular or compressed against the base. The granules are confined to that portion of the cell forming the lumen (the proximal end) and occupy about one half of the cell mass (fig 12). This arrangement of granules is rather constant, rarely is a cell seen where the granules extend to the base. They are proximal to the nuclei and in many cases obscure their rounded proximal surface.

In cross sections of the intercalary duct the cells are pyramidal in form, the apices being in contact with the lumina which are seen to be very narrow when contrasted with the wide lumina of the intralobular and interlobular ducts. The lumina are formed as a rule by an average of seven triangular shaped cells. (These same cells in longitudinal sections appear cuboidal in form.) The granules are grouped in the apices of the cells and consequently these masses of granules are triangular on outline. A narrow

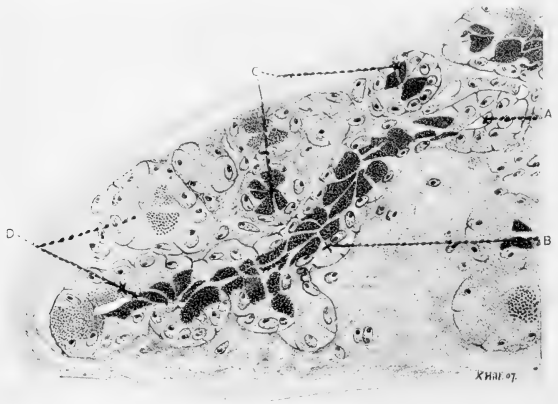

Fig. 12 Secretion granules in intercalary duct, Zenker's muchaematein. Zeiss, oc. 4, obj. 8. Granules are specifically stained. *A*, intralobula duct; *B*, intercalary duct with granules; *C*, cross section of intercalary duct; *D*, tubules.

margin of unstained non-granular cell substance is seen between these granular masses and the cell membrane.

In longitudinal sections these cells appear cuboidal in form and rest on a well defined basal membrane which represents practically all of the supporting tissue.

Not all of the cells of the intercalary duct are stained by the muchaematein. Many cells are present which possess no granules and consequently are not stained. Occasionally ducts are seen in which in longitudinal sections the majority of cells of one side show no granular stain while the cells of the other side possess the

characteristic granular stain. The presence of nongranular cells in the intercalary duct is plainly seen in cross sections of those ducts, the number of unstained cells varying in different sections. In some ducts only one cell may be present which does not possess the muchaematein stained granules, again such ducts are seen where the majority of the cells in cross sections are not stained. As a rule, however, most of these cells possess specifically stained granules.

At the origin of the intercalary ducts from the intralobular ducts the granules as a rule make their appearance in the first cells as a narrow layer in the lumen surface of the cell, having the appearance of a theca or cuticula. As the distance from the intralobular duct increases this stratum of granules widens until it occupies the entire proximal half of the cell. The complete transition from the normal amount to the complete disappearance of granules takes place in from four to seven cells. Frequently one sees an abrupt transition at the junction of the intercalary and intralobular ducts. In this type the granules in the cells of the intercalary duct remain normal and constant in quantity until the intralobular duct is reached when they disappear completely. Thus two neighboring cells may be seen one belonging to the intercalary duct and having the characteristic granules occupying the proximal half of the cell while the bordering cell belonging to the intralobular duct possesses no granules whatsoever. The granules in the intercalary duct are seen to extend to the tubules. They are readily seen in sections prepared by Weigert's method (Zenker's fixation) for the demonstration of elastic fibres. In the alcohol osmic bichromate, and methyl green anilin fuchsin preparation, the granules are well preserved and stain green.

Tubules. The cells of terminal tubules vary in size and form depending upon the stage of secretion they are in. Thus they may be columnar, cuboidal, oval, polyhedral, pyramidal, spherical, or flattened. When the cells composing these elements are in the height of the granular stage the cells are large and bulging and the secreting end may then appear as an alveolus or acinus. Again when the cells are observed in a partial granular stage

they are smaller and more cuboidal and the secreting end has a tubular form. This doubtless explains in a large measure the controversy regarding the structure of the secreting terminals—whether tubules or acini. The finer structure of the cells of tubules is considered in the Section on granules.

V. SECRETION GRANULES

Serous cells[1]

For some time there was much discussion as to whether the granules seen in fixed preparations of glands were natural or the product of fixation. A. Fischer ('99) regarded them as artefacts—Fällungsprodukte. E. Müller, ('96) on the other hand, found that certain granules seen in fresh cells became more distinct when fixed with sublimate solution. The process of fixation was directly observed through the microscope. Held ('99) and many others have substantiated Müller's observations, and now secretion granules are no longer regarded as artefacts. Milawsky and Smirnow ('93) studied, in the parotid and submaxillary glands of dogs, the secretory changes resulting from electrical stimulation of the cerebral and sympathetic nerves supplying these glands.

Bensley ('96) among others (see his various papers for bibliography) has contributed much to our knowlege of the antecedent substance which goes to make up the granules of serous cells. He has observed that

> During digestion a substance similar in chemical properties to the chromatin of the nucleus makes its appearance in the outer clear zone of the chief cells of the fundus glands. This substance, which may be called prozymogen, stains deeply and readily in haematoxylin, and presents a characteristic fibrillated appearance. (He suggests that in some cells this is dissolved in the nuclear substance and that sometimes it is collected in masses—plasmosomata.) During rest this proxymogen is used in some way giving rise to zymogen granules.

Speaking of the chief cells he states that the fibrillae in the outer zones of the cells are not so regular or distinct as in the

[1] Metzner ('07) in Nagel's Handbuch der Physiologie des Menschen gives an excellent discussion of secretion granules in general.

salivary ducts and that they remind one of the nebenkerne in the pancreatic cells of the amphibians as described by Macallum, Erberth, and Müller. In a later contribution ('08) he suggests the probability of serous cells being made up of various groups. By using the following technique (1) examination of fresh material in blood serum, teased preparations or sections cut with Valentine knife, (2) experimental fixation, by which a fixative medium was obtained which would preserve the secretion antecedents in a form which is present in living cell, (3) differential microchemical staining, Bensley has obtained results "which seem to justify the subdivision of the serous class of cells into a number of subordinate groups" such as (1) 'tropochrome cells' which stain metachromatically under certain fixations and stains, and (2) 'homochrome cells' which do not stain metachromatically and in all liklihood form a heterogeneous group. In a still later contribution ('11) he speaks of prozymogen granules "zymogen granules in process of formation. . . . These must not be confused with the basophile substance of the cell which has elsewhere been called 'prozymogen' by Macallum and myself." These prozymogen granules stain deeply in neutral red when used as a vital stain.

Mucous cells

Regarding the main differences between mucous and serous cells as revealed by more perfect fixations and staining processes, Bensley's ('03) conclusions may well be inserted here. The following reasons are given for his conclusion that the cells of the glands of Brunner, in eighteen out of nineteen genera examined, are of the pure mucous type—(1) granules (droplets?) have a low refractile index which corresponds closely to the mounting media; (2) no basal filaments, 'prozymogen,' in fixed preparations (the microchemical test for organic iron shows only relatively small amount of cytoplasmic nucleoproteid); (3) the granules do not stain in iron haematoxylin or neutral gentian; (4) mucous cell stain in mucicarmin and muchaematein; (5) granules are soluble in weak alkaline solutions, insoluble in 5 per cent solution of hydrochloric acid and in artificial gastric

juice containing 0.2 per cent of hydrochloric acid; (6) structure of cells. However "It is obvious that no absolute proof of the mucous character of the glands of Brunner can be brought forward until a positive microchemical test for the various mucins is devised." He suggests that mucous glands may contain small amounts of ferment. See also Bensley's ('02) summary of results in the histology of cardiac glands.

Similarities of mucous and serous cells

Regarding serous and mucous cells investigators agree that in both types the process of secretion is very similar and that the secretory substance is present in the form of very small globules generally termed granules. These granules are seen in fresh tissues with a few exceptions. They vary in size and in degree of light refractibility. The small granules have a greater refractive index than do the larger ones. In the case of the latter this index may be very nearly the same as that of the mounting medium so that they appear very dim, 'matte,' or can not be seen at all—this is true also of the granules of certain cells throughout their secretory activity. In the resting cell before secretion has begun the cell is full of granules, and the nucleus which may appear flattened or angular lies at the base of the cell. After stimulation the granules are greatly decreased or disappear, the nucleus becomes round or oval and approaches the center of the cell. In most instances these granules can be fixed and stained. Usually the smaller ones stain more intensely than do the larger. In many glands the larger granules disappear in the process of fixation and an intergranular protoplasm remains.

Granules in lachrymal gland

According to Langley (lachrymal gland of the rabbit)—

> In the resting gland the alveoli are throughout unevenly stained. The nucleus is irregular and lies in the peripheral portion of the cell. During activity the outer portion of each alveolus begins to stain evenly at first without much alteration in the nuclei or in the inner portions of the cells, later the nuclei become larger and travel towards

the center of the cells, they are then much less distinct, at the same time the lumen becomes more obvious.

The observations of Reichel, Kolossow, and Lor were also confined to the secretory changes. Puglisi-Allegra ('04) contributes an extensive article.

> Studio della glandola lagrimale. . . . In una glandola normalmente funzionante si possona distinguere due specie de cellule. . . . Dopo una eccitazione prolungata è facile notare numerose cellule piuttosta piccole con protoplasma oscura è fortemente granuloso, totalmente prive di secrezione.

Nicolas saw numerous granules in the lachrymal gland of man after fixation in sublimate or Flemming's solution and staining with aniline-safranin, Biondi-Ehrlich and Altmann's methods.

Noll saw in the nonstimulated fresh lachrymal gland of the cat "die meisten Zellen deutlich granulirt. Die Cellgrenzen treten nicht immer gut hervor." The nucleus—"der Basis nahe gelegen, als rund oder ovalen." According to him these granules had remarkable differences of refractibility; they disappeared when water was drawn under the cover-glass and reappeared when 2 per cent NaCl was added; other cells were seen with no apparent granules except numerous 'Protoplasmakörnchen (these cells he termed 'matte Zellen'); the tissue when fixed and stained by Altmann's method showed faintly and deeply stained cells—"hellere und dunklere Zellen." Transitional cells were also seen which showed both the characteristics of faintly stained and deeply stained cells. Noll fixed fresh tissue with Altmann's fluid and observed the process by means of the microscope. The strongly refracting granules in fresh tissue became the deeply stained granules in prepared sections while the less refracting ('matteren') granules became the faintly staining cells with the network. In the latter case the granules were not conserved but only meshes remained. The deeply and faintly staining cells then represent different stages in the secretory activities of the gland, the latter representing the higher stage of granule formation. After electrical stimulation fresh tissue showed "das die Alveolen bei Weiten nicht so viel Granula-Zellen enthalten. In der Hauptsache sieht man Zellen mit

matter Grundsubstanz." The nuclei were round, granules were present in the neighborhood of the nuclei.

Fleischer, in the lachrymal gland of the ox, describes peculiar granular forms—ring, crescent, or demilune granules—which had previously been observed by M. Heidenhain ('90) (See other references to him in Bibliography), Nicolas, and Held. Held, in the submaxillary gland of the rabbit, saw this crescent or ringlike type in addition to the highly and slightly refracting granules. Nicolas failed to observe them in the parotid gland. Heidenhain describes these Halbmondkörperchen in the accessory sex glands of the Triton. The tissue was taken during the heat period. According to him these demilune granules represent a phase in the secretion of the cell; they are formed from small structureless primary granules, and in turn pass into large round secondary granules which are excreted from the cell. . Fleischer found these types of granules in the lachrymal gland of the ox. In sections fixed in 10 per cent trichloracetic acid or picric acid with brillantschwarz-toluidin-blausafranin, he describes (1) round deeply stained small granules, (2) granules of the same size and form which take on a much lighter stain (3) granules with two zones, a dark crescent shaped cap (kapuze) which partly surrounds a lighter stained trager, (5) oval forms of the type just named, (6) granules in the form of crescents or demilunes without any indications of a trager or lighter stained area, (7) granules with ringlike borders surrounding a lighter stained center. These various types were seen without any special arrangement or distribution. Some cells possessed only the deeply stained round forms, others contained only the demilunes, and others again were seen in which all the various forms were present. Fleischer verified his observations by at least four different kinds of fixations in order to escape the possibilities of artifacts. He saw also these variously formed granules in the fresh gland. Hence he was positive of their presence. No Halbmondformen granules were seen in the parotid or submaxillary gland of the calf.

Granules in Fresh Tissue. My observations show that granules are readily seen in all fresh lachrymal gland tissue when

mounted in either serum or physiological salt solution. The refractive index of these granules is much greater than that of the mounting media and consequently the granules stand out clearly. In the great majority of tissues examined, practically all cells of both the tubules and the intercalary ducts appear to possess granules. Only rarely are any of these cells seen free from granules. This condition is due to the fact that the few granule-free cells are masked by granular cells which may be above or below them as it is impossible to procure fresh tissue sections one cell layer thick. Fresh lachrymal gland tissue consequently appears as a rule to be in an extremely granular stage. It is possible, however, after examinations of numerous glands to make out several phases in the secretory activities of glands although this is much better seen in permanent preparations. These various phases observed in fresh tissue mounted in ox serum may be generally classified into three groups or types. However, every gradation exists between these groups.

A. The first type may be termed the granular stage—in the height of granule formation. All cells of both tubules and intercalary ducts are filled with granules. The alveolus or tubule is easily recognized surrounded by the basal membrane and interstitial tissue. The cell boundaries are readily seen. The majority of cells forming the tubules are completely filled with secretory granules. Consequently no clear basal zone is present as is the case in the pancreas. These granules extend from the base of the cell to the summit. So numerous are they that most cells appear to bulge and as a consequence no lumina are seen. These granules obscure for the most part the nucleus of the cell. In many cells, however, the distal half of the nucleus can be seen lying on the base of the cell. The granules vary in size from almost imperceptibly fine structures to granules several micra in diameter. They vary also in their powers of light refractivity—the smaller ones refract light to a much greater degree than do the larger. There is no regularity in the distribution of large and small granules within the cell. They are scattered throughout the cell, appearing at both the base and the summit. Frequently large granules are seen which have

dark ring-like contours. This phenomenon is undoubtedly due to variations in the refraction of light as it passes through the granules and does not represent any peculiar constituent of the granules as held by Fleischer.

The granules in the intercalary duct show the same characteristics as do those of the tubules so far as size and the powers of refraction are concerned.

B. In the second group or type of fresh glands examined, one sees considerable variation in the form and size of the cells of the tubules as well as in the quantity of granules—large bulging cells described under A and smaller cells which have various forms (irregularly cylindrical, pyramidal, or appearing as demilunes wedged in between the bulging cells). These smaller cells may be filled with granules or may possess but few. Their nuclei are always round or oval and are separated from the base of the cell by a narrow zone of cytoplasm.

C. Glands are occasionally seen in which the tubules for the most part are made up of cells which are regular in form—low cylindrical, pyramidal or cuboidal in outline. The granules are confined to the proximal half of the cells. The nuclei are round and oval and do not touch the bases. The lumina of the tubules are wide and open. This condition represents a stage of partial exhaustion of granules.

At the margin of these fresh tissue preparations, cells are seen which have been ruptured by teasing, cutting, etc. Here the granules are seen passing from these cells into the serum and still retaining the same continuity as they possessed within the cell. In fact the entire margin of the tissue is characterized by the presence of these free granules. In order to exclude the possibility of the more finely formed elements in the blood being responsible for this loose granular mass the serum was carefully filtered before using as a mounting medium. Microscopic examination of the serum, after filtering, revealed no such formed elements.

When sections of fresh tissue are mounted in physiological salt solution, the cells show the same characteristics as those described for the serum mounted tissue. The loose granules

do not preserve their continuity as long, however, in the salt solution as in the serum.

Fresh tissues mounted in distilled water soon lose (in five minutes) their granules. As a consequence the nuclei which are round or oval become very prominent. They are seen for the most part in the basal portion of the cells and are opaque and homogeneous. No nuclear granules are seen. In no instance are the nuclei seen to be angular and flattened as in many fixed and stained preparations. They have in all likelihood taken up enough water to be swollen when observed under these conditions. The water is absorbed before the granules have sufficiently disappeared for the process to be observed. If 2 per cent salt solution is drawn under the slide after the granules have disappeared in distilled water they reappear again. This phenomenon was observed by Noll in the lachrymal gland of the cat and by Langley and others in other serous glands.

When fresh tissues are mounted in five per cent solution of hydrochloric acid or sodium bicarbonate the granules become indistinct—disappearing much more rapidly in the former solution than in the latter. An intracellular network remains after treatment with these solutions, the meshes of which were formerly occupied by the granules. This net work should be interpreted in the sense of Bensley "optical sections of the continuous cytoplasmic partitions which separate the granule holding spaces from one another."

Thin sections of fresh lachrymal gland were mounted in the following vital stains in physiological salt solution with these results:

1) naphthol blue—granules stained a purplish blue.

2) neutral red—granules were very faintly stained. I did not observe here prozymogen granules more deeply stained as observed by Bensley in the pancreas of the guinea pig.

3) janus green—the mitochondrial elements were stained. This will be discussed under that caption.

4) trypan blue—a diffuse light blue stain, granules only faintly stained.

5) isamin blue—a very faint blue stain limited chiefly to the interstitial tissue. Certain cells in the alveolus also appeared more deeply stained than did others.

6) trypan red—a diffuse light red stain.

7) sulforhodamin—a diffuse faint light red stain.

8) pyronin—intercellular canals and lumena of ducts stained.

Granules in fixed and stained preparations. Zenker's. Attention has already been called to the fact that the granules of the intercalary duct when fixed in Zenker's solution stain deeply in muchaematein and mucucarmin while the other granule stains do not affect them. The tubules, on the other hand, show no deeply stained granules but appear under low power only diffusely and faintly stained. Careful examination of these tubules with the oil emersion, however, shows various intensities of staining reactions on the part of the cells—which may be generally classed as follows: a) Large rounded bulging cells as seen in the fresh sections. Here a faintly stained cytoplasmic network is seen throughout the cell. The meshes of this network are round and appear to hold large unstained granules. The nucleus is angular or flattened and lies against the base of the cell. In fresh tissues the granules of these cells are distinctly seen and the intergranular network is not seen, while in Zenker's muchaematein preparations the granules are not stained but the intergranular network is distinctly seen. b) Cells more cylindrical in form frequently showing indented sides as if they had been pushed in by the round bulging cells (also seen in fresh preparations). They possess very faintly stained granules. The nuclei as a rule are round or oval. c) So called transitional cells. In the use of the term transitional cells reference is made to those cells that mark as a rule the junction of the intercalary duct and tubule. They possess granules similar in structure and staining reaction to those of the intercalary duct and are always preserved in Zenker's solution (figs. 11 and 12).

Bensley's solutions. For the fixation of granules in the tubules, it was necessary to use other solutions than Zenker's. The granules in tubules are best preserved by Bensley's sublimate

alcohol bichromate solution (Technique IV, 2 a) and by formalin bichromate sublimate solution (Technique IV, 2 c). Tissues must be small as the penetration of these solutions is not great. In these solutions the granules of both the tubules and the intercalary ducts are preserved. These are readily stained with muchaematein (Technique V, 1), mucicarmin (Technique V, 2) iron haematoxylin, iron haematoxylin counterstained with mucicarmin, copper chrome haematoxylin (Technique V, 5) neutral gentian (Technique V, 6) and safranin-acid violet (Technique V, 7).

Mucous stains. Sections fixed in Bensley's sublimate alcohol bichromate solution or in formalin bichromate sublimate solution when stained with muchaematein or mucicarmin show that the selective staining is confined entirely to the granules of the tubules and ducts. The various types of cells as seen in these sections depend upon the secretory state of the gland at the time of fixation. The various stages described in the tubules of fresh tissue are seen in these preparations and may be grouped as follows:

(1) Large rounded or bulging cells completely filled with intensely blue stained granules—type 1. All other structures within these cells are obscured as a rule. Frequently, however, flattened nuclei are seen at the base. These cells correspond to those described in both fresh tissues and in Zenker's muchaematein sections. In the latter case the granules were not stained but the intergranular network was prominent as a consequence of a light blue stain.

(2) and (3) Cells more or less cylindrical or pyramidal in outline, or appearing as demilunes in the periphery of the tubules completely shut off from the lumen by the large bulging cells—frequently the cylindrical cells are constricted in their vertical axis by the pressure of the bulging cells and thus simulate in outline hour glasses. The nucleus is generally round or oval and does not lie directly on the base of the cell but is separated from it by a narrow zone of cytoplasm. These cells can be divided into two classes. The one contains relatively few or, rarely, no granules. The granules are seen in the proximal end of the

cell or throughout the cytoplasm and stain with different degrees of intensity—many are but faintly stained. These will be referred to as type 2. The cells of the other group (type 3) are similar to those of type 2 in form but are full of deeply stained granules.

Generally there is no regularity of distribution of these various types. Tubules are seen wherein all the cells composing them are of the first type. Others are seen made up of all three types.

Occasionally glands are obtained wherein practically all the cells within the tubules are cuboidal in form and show the granules proximal to the nuclei as described under fresh tissue (Fresh tissue C). Here the cells are not large or bulging and the lumina are open.

In other sections, which are rare, one sees numerous granules with deeply stained rings around them. These were described by Fleischer who considered them as a stage in lachrymal secretion. I do not think such is the case, however (see Discussion of granules.)

The cells of the intercalary duct show the same characteristics as those described in Zenker's celloidin preparations.

Iron haematoxylin. In iron haematoxylin stained sections one sees the same types of cells described under the mucous stains. The large bulging type 1 cells are filled with black granules of various sizes, the nuclei angular or flattened against the bases of the cells. The type 2 cells have grayish granules and their round or oval nuclei show as a rule one or two large chromatin masses. The type 3 cells are filled with deeply stained granules or a mixture of both black and gray granules.

The different intensities of the staining reaction on the part of granules is a striking feature of the tubules. Large and small granules may be seen side by side in the cell some staining deeply black while others are gray or faintly stained. The granules of the intercalary duct vary also in the intensities of staining reactions but not to such a degree as those of the tubules.

Iron haematoxylin and mucicarmin. Sections stained with iron haematoxylin and counterstained with mucicarmin show some very interesting features. All the granules of certain

cells stain red in mucicarmin and all the granules in other cells stain black in iron haematoxylin while in still others both red and black granules are seen in the same cell (fig. 13.)

Copper chrome haematoxylin. Sections stained in copper chrome haematoxylin show the granules to be deeply stained. The cells are readily classified into the three types already described. The large bulging cells are filled with black stained granules of various sizes. The granules in the type 2 cells are very lightly stained—in many they are so faintly stained that

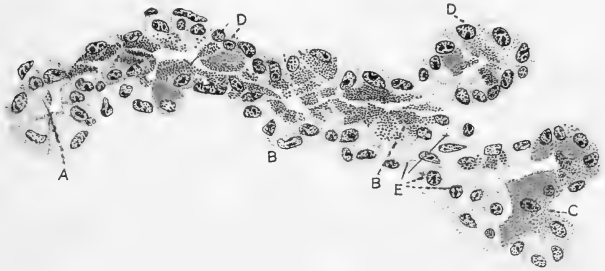

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Fig. 13 Secretion cells with granules. Tech. IV, 2, a; V, 4. Leitz. oc. 2, obj. $\frac{1}{12}$ oil. *A*, intralobular duct; *B*, cells possessing granules all of which stain black with iron haematoxylin; *C*, cells with the entire granular content stained red with mucicarmin *D*, cell with granules some of which are stained with iron haematoxylin and others with mucicarmin; *E*, secretory cells with no granules.

it is difficult to make out any granules whatsoever. The type 3 cells are filled with black granules.

Sections fixed in the sublimate alcohol bichromate solution and stained in copper chrome haematoxylin show, in the larger ducts, mitochondria either as irregularly distributed granules or as filaments (see Mitochondria.)

Neutral gentian and safranin-acid violet. These proved to be excellent stains for granules in the lachrymal gland. Tissues fixed in either the sublimate alcohol bichromate or the formalin bichromate sublimate solution show the characteristics already

described. In fresh safranin-acid violet the nuclei stain red while the granules stain a dark greenish blue. The advantage of this stain lies in this contrast. In the large bulging cells the red flattened basal nuclei are generally seen while in the other stained cells they are made out with difficulty since the colors of nuclei and granules are similar.

The granules in the cells of the intercalary duct stain similarly to those of the tubules. Lumina are always seen but vary in diameter. The cells are generally uniform in size and shape. No bulging cells are seen. In each cell there is a narrow zone of non-granular cytoplasm surrounding the granules. No variations are seen in the size, shape, and position of the nuclei whether the cell contains the maximum of granules, only a narrow zone at the proximal end, or no granules whatsoever. They are similar in structure and staining characteristics to the round and oval nuclei seen in the tubules.

In thin sections stained with neutral gentian the outer layer of cells surrounding the secretory cells of the intercalary duct are readily made out. Near the origin of the duct these cells are still more or less cuboidal but towards the tubular end the cells with their nuclei become more and more elongated. These cells stain more faintly than do those of the inner layer. They surround the tubule as elongated cells between the secreting elements and the basal membrane (see Connective Tissue.)

As in the case of the iron haematoxylin stained sections one is especially struck by the various intensities which these granules stain with neutral gentian and safranin-acid violet. In well fixed tissues the majority of granules stain deeply in both of these dyes. However, one frequently sees in the same cell, in both the tubules and the intercalary ducts, granules lying in close proximity to each other some of which are stained deeply blue, others only faintly yellow or brown, and still others barely stained at all. This variation in intensity of staining is in no way related to the position of the granule or its size. Cells are frequently seen bordering each other, one possessing deeply stained blue granules throughout, the other possessing yellowish brown or faintly stained granules. The probable expla-

nation of this phenomenon is, as suggested by Bensley—"the granule substance has a primary difference of density due to difference in water content or a change in dispersion grade of the colloid." Certainly some granules retain the stains much more tenaciously than do others. I have not observed these extreme variations in the staining reaction of granules in other glands such as the pancreas and submaxillary.

The secretion granules of the lachrymal gland are not stained in neutral red or in any other of the vital stains employed.

I saw no free granules in the lumina of either the tubules or the ducts. Frequently such appears to be the case however, for one sees, especially in the intercalary duct, elongated groups of granules which seem to be in the lumen. Careful study, however, reveals the fact that these granules mark the proximal end of the other cells which contribute to the formation of the lumen.

No cuticle, striated or otherwise, was observed in the epithelium.

Discussion of granules

Secretory changes in cells. Unfortunately the lachrymal gland of the ox does not lend itself for experimental study of a stage of rest, a stage of activity, and a stage of exhaustion. Studies based upon microscopic changes in the cells as induced by rest and stimulation must be accomplished in the more accessible laboratory animals. My study of granules was based chiefly on the methods of their conservation and their staining characteristics. However, a sufficient number of glands was examined to gain some light upon the changes that do occur in the normal secretory activity of the gland. According to the tissues fixed in Bensley's sublimate alcohol bichromate solution and in formalin bichromate solution supplemented by a study of fresh tissues the secretory phases of the gland may be classified as follows:

A. A maximum granular stage in which most of the cells of the tubules and intercalary ducts are filled with granules. The majority of cells in the tubules belong to those described as type

1 under the various stains—completely filled with granules and bulging, lumina of the tubules not visible.

B. A medium granular stage in which the tubule possesses for the most part cells of the types described as types 2 and 3. Rarely does one find an entire tubule made up of these types alone, as bulging cells of type 1 are generally present. It will be recalled that type 3 cells are those described as cylindrical, pyramidal, or crescent cells filled with deeply stained granules. The nuclei of these cells are still round or oval and separated from the base of the cell. This type doubtless represents an earlier phase to that of type 1 in the stage of granule formation. Granules have formed in sufficient quantities to fill the cells but not to such an extent as to enlarge them—cause them to bulge.

Cells described as type 2 in all likelihood represent two phases in secretion. a) Those which possess few granules at their proximal ends suggest the terminal phase of secretion wherein the granules for the most part have passed out of the cells. b) Those in which comparatively few granules are seen scattered throughout the cells suggest cells in the beginning phase of granule formation. These granules stain with varying intensities. Some are large and pale, others small. There is no regularity to their distribution. The few nongranular cells may be interpreted as occupying a position between the terminal of granule expulsion and the beginning of the formation of new granules. The very few nongranule cells seen and the fact that the type 2 cells usually possess granules both at the lumen end of the cell (terminal secretion) and also a few granules throughout the cytoplasm (beginning granule formation) strongly suggest that the extrusion of granules and the new formation of granules go hand in hand in the same cell during the normal secretory activity of the gland. We have then in this gland a continual secretion as indicated by the cells showing all phases of secretory activity.

C. In the third group of glands the secreting cells were much reduced in size, the lumina wide and open, the few granules present confined to the proximal end of the cell, and the nuclei round and oval. This condition was uniform throughout the gland and simulated preparations obtained by other observers

after stimulation with pilocarpin. In my opinion these glands represent a condition of intense secretion before the animals were killed. Many factors could be responsible for this excessive activity.

The results of my study of the various phases of secretion agree in a general way with those of other observers. Detailed study of the cellular changes during secretory activity, must be, as stated, on the laboratory animals. Preliminary work on certain species of Anura and Urodela gives much promise, and I trust that a paper will soon appear on this subject. In the lachrymal glands of the ox I have failed to observe the secretory elements of the cell divided into two zones by a strand of reticular protoplasm stretching across the cells as noted by Bensley ('02) in the cardiac glands of mammals and in the glands of Brunner (Bensley '03). Nor was an intermediate stage present as is seen in the Pancreas (Bensley, (11) which contained small granules (prozymogen granules) that stain with neutral red, intra vitam.

I have failed to observe the paranucleus described for secreting cells, including the lachrymal gland, by Gaule, Ogata, Nussbaum, Garnier, and others. As stated elsewhere, the three zones described by Zimmermann were not observed. No definite light has been thrown as a consequence of this study upon the origin of these granules. They seem to make their appearance anywhere within the cytoplasm, independent of position or of any marked demonstrable antecedents such as a basophil substance (toludin blue), iron possessing substance (Macallum reaction), or prozymogen (Bensley's vital neutral red). Exception to this statement should be made if we are to consider mitochondria as an intermediate substance. This will be discussed under mitochondria. Perhaps the presence of such antecedents should not be anticipated, as the lachrymal gland possesses so far as I know no demonstrable specific enzyme. Whether the nucleus plays any part in the formation of granules, I am unable to say. It is true that in the tubules when in a stage of maximum granule formation (cell type 1), the nucleus is more or less flattened and compressed against the base while

in cell types 2 and 3 it is round or oval and separated from the base of the cell by a narrow zone of cytoplasm. However, no distinct changes are seen in the chromatin. In both instances one or frequently two large chromatin masses are surrounded by numerous finer granules of chromatin. The change of position and form of the nucleus may be due entirely to the quantity of granules. The nucleus of the intercalary duct, on the other hand, shows no appreciable change in form or position whether the cell is at its maximum stage of granule formation or its minimum.

The fixation and staining of granules. When the tissues are properly fixed in either Bensley's solution or formalin bichloride bichromate solution all granules are conserved. Such is not the case when alcohol, Zenker's or numerous other fixatives are used. Zenker's and Altmann's fix the granules in the intercalary duct and frequently in some few cells in the tubules. The granules in the large bulging cells are not preserved in alcohol, Altmann's, or Zenker's but, instead, the intergranular network remains.

Neutral gentian, safranin acid violet, copper chrome haematoxylin, iron haematoxylin, muchaematein, and mucicarmin proved to be the most satisfactory stains for the granules.

Are secretory cells of tubules similar in function? Notwithstanding the variable staining reactions on the part of granules and the various sizes and shapes of cells, cells of the tubules are doubtless similar in function. We have no evidence that more than one functional type of cell is present. Proof of this is seen in the granular stage when all the cells of the tubules stain similarly. Furthermore other structures in these cells are alike so far as I have been able to make out. The changes in position and form of the nuclei only represent different secretory phases.

Staining reaction of granules, selective action. In order to prove that the cells in both the tubules and the intercalary ducts are capable of taking various granular stains, serial sections three micra thick were cut and fastened to different slides—one section for each slide. The first section was stained with neutral gentian, the second with muchaematein, the third with

iron haematoxylin, the fourth with mucicarmin, and so on until all the granule stains were used. It was found that all granules in these sections were stained with the various dyes. This demonstrated that the granules of the same tubule and intercalary duct are capable of taking various stains. Furthermore the same granule is apparently capable of taking any one of these stains. This is shown in the counter staining. When sections stained in either iron haematoxylin or copper chrome haematoxylin are differentiated to such an extent that the granules are pale, then stained with mucicarmin they appear red. This condition is further substantiated by sections from those rare tissues which contain the peculiar ring-like or crescent granules. In many of these granules the caps or crescents and the rings stain black in iron haematoxylin while the bodies of the granules stain red after counterstaining in mucicarmin. That many granules, however, show an affinity for certain stains seems very probable, as cells are present in the tubules and intercalary ducts the entire granular contents of which stain black with the iron haematoxylin while all the granules of the neighboring cells stain red after counterstaining with mucicarmin, and again a mixed condition of red and black granules is seen in other cells. However, the distribution of these red and black granules within one cell as well as the distribution of cells containing either all black or all red granules in the tubules and intercalary ducts is very irregular and the ratio of these cells to each other is very inconstant.

Various explanations for this selective action on the part of some granules for iron haematoxylin, others for mucicarmin may be advanced as follows:

1) The granules at different stages of their development have different selective action for various stains. Against this view, however, is the fact that this selective action on the part of granules bears no relation to their size or distribution in the cell. Large and small granules, either at base or summit, may take one or the other stain. The large bulging granular cells of the tubules seem to have a greater affinity for mucicarmin, however, many exceptions to this observation were noted.

2) Even the granules within one cell may be subdivided into a number of groups, each group representing a specific chemical structure and contributing some element to the sum total of secretion. If such be the case one may readily assume a selective action on the part of each group for specific stains. Of course we have no proof for such an assertion.

3) A possible explanation lies in the fact that in the differentiation of sections stained in either iron haematoxylin or copper chrome haematoxylin some granules hold these stains much more tenaciously than do others. This may be due to varying degrees of fixation of the granules. It is the faintly stained granules, those differentiated most, that are stained again in mucicarmin. This observation had been made.

However, as Bensley has pointed out:

> The difficulty of all these discussions of the different staining properties of granules in the same cell arises from our ignorance of the changes which would be produced in the absorptive properties of these colloids with variations in their water content and so in their dispersion grade. The dyes which we use for this staining are all dyes which may be absorbed. I think under the circumstances it would be rash to assume a difference in the fundamental composition of the granules on the basis of the different staining.

Are the cells of the tubules and intercalary ducts of the same functional type? Attention has been called to the fact that the intercalary duct stains more deeply in the ordinary dyes than do the tubules (v. Ebner, Nussbaum, and Langley, submaxillary gland; Merkel and Fleischer, lachrymal gland). Fleischer concludes that this duct has a specific secretion differing from that of the tubules. He gives as his reason the presence of such large granules.

I too have observed that the intercalary duct as a rule stains more deeply in the ordinary dyes. This can be readily explained by the fact that the cells are smaller and more compact and that the granules are conserved in such general fixatives as Zenker's solution, while in the majority of cells of the tubules the granules are not fixed but an intergranular cytoplasmic network remains.

Fleischer holds that a specific secretion is produced in the intercalary duct:

> Das Vorhandensein derartiger grosser Granula in den Schaltstückzellen, wie ich sie auch in der Thränendrüse gefunden habe, spricht mit grosser Wahrscheinlichkeit für eine besondere sekretorische Bedeutung dieser Zellen und zwar muss es sich um eine andere Art von Sekret handeln, als dasjenige, das die Zellen der End-abschnitte ausscheidet.

While I found that the granules in the cells of the tubules—cell type (1)—in well fixed preparations are as large or even larger than those in the intercalary ducts, still certain marked differences do exist between the cells of the intercalary duct and the tubules. The granules in the intercalary ducts are fixed in Zenker's solution while those of the tubules are for the most part not preserved in this solution. When fixed in this solution the granules of the intercalary ducts stain deeply and readily in mucicarmin and muchaematein while the other granule dyes do not affect them. In tissues fixed in Bensley's acetic, bichromate osmic solution and stained with anilin fuchsin-methyl-green these granules are stained green while they are not preserved in the bulging cells of the tubules.

A narrow band of non-granular cytoplasm is always seen between the cell membrane and the granules. The granules never reach the base of the cells. The cells do not undergo any marked variation in size or shape during their secretory activity. The nuclei remain practically constant in form and position whether the cells are empty or possess numerous granules. No secretory capillaries are present. Frequently, however, intercellular indentations are seen. These are some of the features that characterize the cells of the intercalary duct.

In the tubules, on the other hand, the granules in the majority of the cells are not fixed in Zenker's solution, only those that mark the transition from the duct to the tubule are fixed. The granules when fixed in the height of the secretory stage fill the entire cell and cause it to bulge out. Marked changes consequently appear in the form and position of the cell which is true also in the case of the nucleus. Secretion capillaries are also present.

Nature of granules. Ellenberger states that the lachrymal gland of the pig is a mucous secreting gland, while Boll states—regarding pig, sheep, calf, and dog—"Es ist hieraus mit Sicherheit zu erschliessen dass das Secret der Thränendrüse nie mucin enthält." Which is true in the case of the lachrymal gland of the ox? Although the specific reaction of these granules to the mucous stains might indicate that this gland may be mucous in character, in my opinion it cannot be classed as mucous, since:

1) If one takes fresh pieces of the pancreas, submaxillary, and lachrymal glands and compares the secretions which can be pressed out one is impressed with the similarity of certain physical properties of the secretions of the pancreas and lachrymal glands. The fluids of both are thin and watery with no adhesive qualities. The secretion from the submaxillary gland, on the other hand, is viscid, thick and sticky to such an extent that pieces of tissue are readily supported by it. 2) A careful analysis of numerous lachrymal glands from the standpoint of their physiological chemical structure showed that they are in no wise similar to mucous glands. 3) The structure of the cells of the tubules and intercalary ducts does not simulate those of the submaxillary gland and other mucous glands. 4) One finds that the lachrymal gland has the following characteristics which are true of serous glands in general: a) The granules have a high refractive index. b) The granules are insoluble in weak alkaline solution. Following Bensley's procedure I found that sections fixed in his bichromate alcohol sublimate solution showed no change after having stood for twelve, twenty-four and forty-eight hours in 5 per cent potassium carbonate solution at a temperature of 38°, while in similarly treated sections of the submaxillary gland the granules of the mucous cells had completely disappeared in twenty-four hours. The granule stain in the lachrymal gland is as definite and as intense as is seen in the stained normal sections. Similarly treated sections in five per cent hydrochloric acid for the same length of time did not affect the granule stain in the least. c) The granules stain readily in all serous granule stains. d) The presence of secretory capillaries. On the other hand certain characteristics

are present also which are generally true of mucous secreting cells: a) The flattened nucleus seen compressed against the base of the cell in the large bulging cells. b) The absence of basal striations as seen in many serous cells. c) The presence of but minute traces of prozymogen (toludin blue stain) and organic iron—the latter demonstrated by Macallum's method d) The specific reaction to the mucous stains.

5) Examination of lachrymal secretion in the human shows only a small amount of mucin present which is readily accounted for by the presence of goblet cells both in the ducts and conjunctiva.

Analyses of human lachrymal secretions show:

	FRERICHS (46)		ARLT LERCH[2]	MAGAARD (82)
Water	99.06	98.70	98.233	98.1200
Epithelium	0.14	0.32		
Albumin	0.08	0.10	0.504	1.4638
Mucus and Fat	0.03	0.34	Trace	
Salt NaCl				
Phosphate	0.43	0.54	1.257	0.4160
Other salts			0.016	

No positive proof can be advanced that these cells are either serous or mucous in character as we have no specific methods for determining this. The lachrymal gland may be considered as not highly specialized in function when compared with such glands as the pancreas. It appears to occupy an intermediate position between the more highly specialized serous and mucous glands and possesses many characteristics of both. My results plainly show that great care must be taken in determining the nature and function of glands from the standpoint of microchemical staining.

Rings and demilune granules. Attention has been called to the fact that these peculiarly shaped granules have been described by Held, Nicholas, and Heidenhain in other glands, and especially by Fleischer in the lachrymal gland of the ox, who described

[2] Nagel's handbuch.

them as being present in all lachrymal glands and stated that they represent constant and distinct phases in the evolution of the granule. My observations do not agree with those of Fleischer. While these peculiar forms of granules were seen occasionally in the numerous preparations of lachrymal gland which I made, their occurences were relatively so rare that they must be considered as exceptional. I have also seen these peculiar granules in the accessory lachrymal gland tissue of the third eyelid of the ox as well as in the orbital glands of frogs. Frequently ring granules are seen in fresh preparations of these glands, but the explanation lies in the effect that a large granule may have on light passing through it. Light may pass through the center of the granule with but little or no refraction while it may be greatly refracted at the contour. I think that these peculiar granule structures in fixed preparations can be explained from the standpoint of fixation. It is the large granules which show these peculiarities when present at all. It can be readily conceived that the surfaces of these granules may be acted upon more thoroughly by the fixing fluids than the centers and consequently stain more deeply. Small tissues well fixed in Bensley's solution or formalin bichromate sublimate solution seldom show these peculiar granule structures. It is difficult to conceive of these granules of fluid or semifluid consistency taking the form of rings, demilunes or crescents. Our knowledge of the physical properties of matter in such condition will not permit of such a conclusion.

VI. MITOCHONDRIA

I found that lachrymal gland tissues prepared according to Bensley's method for the demonstration of mitochondria—acetic osmic bichromate, anilin fuchsin methyl green—show these elements very clearly.

Tubules. In the tubules two chief cell types are made out somewhat similar to those described by Noll in the lachrymal gland of the cat after fixing with Altmann's solution—light and dark cells.

Light cells: The light cells correspond to the large bulging cells (type 1) heretofore described. The secretory granules are not preserved in this solution but an intergranular cytoplasmic network remains. The meshes, which originally possessed granules, now appear empty. This condition is seen also in tissues fixed in Zenker's solution. Irregularly distributed throughout this dark stained cytoplasmic network are seen these minute fuchsinophil granules or mitochondria. The majority are slightly elongated simulating somewhat bacilli. Others again are round, cocci like. They are seen in every portion of the cell from the base to the summit. (fig. 14). In no instance

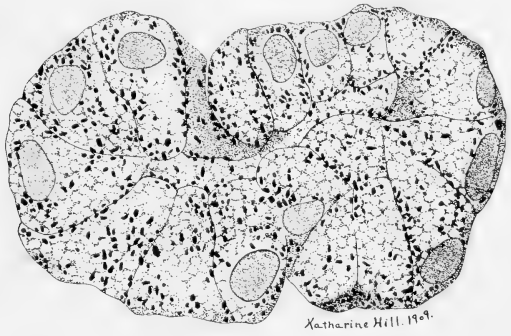

Fig. 14 Mitochondria in cells of tubule, Tech. IV, 2, e; V. S. Zeiss, oc. 6, obj. 2 mm. oil. Mitochondria are readily seen irregularly distributed throughout the cells.

have I seen in these large bulging cells mitochondria arranged in the form of filaments at the base of the cell. Nor is this to be anticipated, for these cells in other fixations (formalin Zenker and alcohol bichromate sublimate solution) are filled with granules from the base to the summit. Occasionally larger fuchsinophil granules are seen which resemble the secretion granules in size and shape, and are seen surrounded by the darker cytoplasmic network. These secretion granules when present show as much affinity for fuchsin as do the mitochondria. I have

frequently observed these fuchsinophil secretion? granules in the orbital glands of frogs. Just what the significance of these granules is, I am unable to state. The explanation that they represent an intermediate stage between the secretion granules and mitochondria is unwarranted in view of the fact that so few are seen.

In some cells, though rarely, fuchsinophil globules are seen which are much larger than the secretion granules. It will be well to add here that red blood cells possess a marked affinity for fuchsin, although I was unable to make out mitochondria within them, confirming Cowdry ('14 c).

Other structures are seen in the cell. These are stained black with the osmic acid. The larger spherical ones undoubtedly are neutral fats. These can be demonstrated with Herxheimer's stain. The finer structures are liposomes. (See discussion under Fat.)

Dark cells: These cells are so termed because the darkly stained cytoplasm shows no network with empty meshes but instead is either filled with deep green stained secretion granules or is continuous. These cells correspond to types 2 and 3 heretofore described. In form they are irregularly cylindrical, pyramidal, elongated, or hourglass shaped due to indentation caused by the bulging type 1 cells. Many appear as demilunes. The position and form of the nucleus are similar here to that already described for these cells. The transitional cells of Noll are seen also wherein the central half of the cell possesses the characteristics of the light cells while the basal half shows the structures described in the dark cells. Throughout the cytoplasm mitochondria are readily observed. In the cells filled with secretion granules they are very plainly seen between the granules. The contrast is marked. In those cells which possess few or no granules, the red stained mitochondria are readily observed in the dark stained cytoplasm.

There is no regularity of distribution of mitochondria in the cells of the tubules in any of the types. Frequently one sees them in clumps at the central end, frequently at the basal end.

More often, however, they are irregularly distributed throughout the cell. In all the cells of the tubules the mitochondria appear as very short irregular rods or as minute spheroids which may be slightly irregular in outline. In no instance have I seen long rods, threads, or loops as described especially for growing cells. Further, in the tubule the mitochondria do not form rows in the base of the cell and perpendicular to its base (filaments) such as found in the larger duct cells. Careful study failed to reveal any general irregularity in the quantity of these fuchsinophil granules. While some cells appear to possess more than do others yet the light cells (type 1) which represent the maximum of the granular stage apparently possess approximately the same number as do the dark cells (types 2 and 3) which represent either earlier or later stages of secretion granule formation. The mitochondria in these cells are not numerous when contrasted with those seen in the cells of the intralobular ducts (Cf. figs. 14 and 15). Consequently the granules do not appear to be used up in the formation of secretion granules.

Intercalary duct. The secretion granules in the intercalary duct are well preserved in Bensley's acetic osmic bichromate solution. In fact, so far as the preservation of secretion granules is concerned the effect of this solution is similar to Zenker's. The granules here have a marked affinity for the methyl-green. Between these green granules red stained mitochondria are readily observed. They simulate those in tubules so far as size, shape and distribution are concerned.

Larger ducts. In the intralobular and interlobular ducts the mitochondria are especially abundant. In the smaller intralobular ducts they may be so numerous that the entire cell, with the exception of the nucleus, appears to be composed entirely of these fuchsinophil granules. In these cells there exist marked irregularities so far as their distribution and arrangement are concerned. Some cells may be full of these granules while others again possess but relatively few. Generally the granules are arranged in rows—these rows being parallel to each other, perpendicular to the base of the cell, and extending through-

out the entire length of the cell (fig. 15.) Cells are frequently seen, however, in which the mitochondria appear in heaps with no definite arrangement whatsoever.

The mitochondria of the ducts are readily seen when preserved in Bensley's sublimate alcohol bichromate solution (Technique IV, 2) and stained in copper chrome haematoxylin (Technique V, 5). Here they have an arrangement similar to that already described. Whether the mitochondria in the tubules and inter-

Fig. 15 Mitochondria in interlobular duct, Tech. same as figure 14. Here they are arranged as filaments.

calary duct are preserved I can not say. It will be recalled that this fixative preserves the secretion granules and these stain deeply black in this copper stain. Consequently the mitochondria if present are masked. I did not see them in tissues of this fixation when stained with iron haematoxylin or the neutral stains—neutral gentian, and neutral safranin. In all likelihood

mitochondria are affected by these latter stains but in the differentiation do not hold them so tenaciously as do the secretion granules. The presence of demonstrable mitochondria in tissues fixed in alcohol sublimate bichromate solution is interesting in view of the observations of others that mercuric chloride and alcohol fixatives do not preserve them.

The mitochondria were also stained by the intra vitam method of Michaelis ('00) (Technique VI, 1, 2). Here they appear as deep blue structures being the only stained constituents of the cell. In size, form and distribution they appear as already described.

Function of mitochondria in gland cells. Regarding the nature and function of mitochondria in general many hypotheses have been advanced which may be briefly summarized as follows: 1) the mitochondrial theory—that these elements are specific elements of the cytoplasm just as the chromosomes are fixed elements of the nucleus and like the latter arise from preformed elements in sex cells and are carried over in all mitosis—this view was championed by Benda and Meves. It has many supporters and is gaining ground. 2) Others do not accept the mitochondrial theory but claim that mitochondria arise from nuclear material, from ferment products of the centriole, or that they represent other phases in the metabolic activity of the cell. Many of those who accept the mitochondrial theory hold that these structures later develop into the fixed specific structures of cells; i.e., neurofibrils (Meves and Hoven '10), muscle fibrils, etc. Cowdry ('14 a) has demonstrated that in the case of nerve cells this is not true.

Cowdry (14 b) assumes that they have to do with the metabolism of the cell since they are almost coexistent with all active protoplasm. The fact that they do not occur in red blood cells (Cowdry), in superficial layers of epithelial cells (Firket), and in the terminal stages of the cycles of development of certain grains and legumes (Guillermond), according to Cowdry, partially substantiates this view because these cells are in the terminal stages of metamorphosis. Mitochondria, according to him,

is in all liklihood a lipoid albumen complex. In nerve cells if they are numerous the lipoid granules are few and vice versa. In my opinion Cowdry's conclusions are correct.

Regarding their function in secretory cells, Altmann held the view that they were capable of forming secretion granules and also related to the formation and absorption of fat. Noll held views similar to Altmann regarding their relation to secretion. Schirmer could not say. Bensley ('11) states that certain types may possibly represent secretion antecedents. Champy ('11) makes the bold assertion that secretion granules originate from mitochondria. Hoven ('11, '12) also hold that the different products of the mammary gland originate from these granules and according to him this is true of the secretion granules for all glands. Arnold ('11) likewise holds the same view.

My studies on the lachrymal gland have not revealed any positive evidence that secretion granules have their origin from mitochondria. Certain facts may suggest that such a hypothesis is tenable. These are, 1) The absence of demonstrable antecedent substances such as prozymogen—basophile substance (toluidin blue), prozymogen granules (intra vitam, neutral red), nuclear material in cytoplasm (Macallum reaction). 2) The secretion granules seem to make their appearance in any part of the cell independent of the nucleus. 3) The small amount of mitochondria in the secreting cells as contrasted with that in the cells of the ducts suggests that it may be used up in the formation of secretion granules.

On the other hand as valid objections can be advanced against this theory—1) Demonstrable antecedents for secretion granules are not found in many other serous and mucous cells. 2) The secretion may arise directly from other cytoplasmic structures independent of mitochondria. 3) If secretion granules originate from mitochondria one would expect to find variations in quantity depending upon the secretory stages of the cells. 4) The universal distribution of mitochondria in all cells speaks against a specificity in gland cells. Much light on this subject would result no doubt from both embryological and comparative study of glands aided by certain pharmacodynamic reactions.

VII. BASAL STRIATIONS

Whether such structures occur in the lachrymal gland as were early described by R. Heidenhain in fresh gland tissue and by J. Müller and Pflüger especially in the salivary ducts, has occasioned much discussion. Boll described 'Tränenröhren' in the lachrymal glands studied by him. Maziarski found none in man. Merkel found none in the lachrymal gland of the dog. Garnier, on the other hand, describes them in this gland in the dog and cat while Zimmermann claims that basal striations in the cells of the human lachrymal gland correspond to a lamellar structure. Fleischer, Hornickel, and Puglisi-Allegra have also recorded indications of the presence of such striatious or lamellar structure.

Thus we find much difference of opinion regarding the nature and presence of these structures. Even investigators working on the same gland have disagreed regarding them. In some contributions it is often difficult to determine just what particular cells are said to possess them. To Bensley ('11) we are especially indebted for clearing up the situation. He has shown that the so termed striations are due to two distinctly different substances —a) mitochondrial filaments of Altmann and Michaelis, and b) basal filaments of Solger and others. The former are seen in fresh tissue, are stained vitally by janus green, can be demonstrated by fixing tissues in acetic osmic bichromate solution and staining by Bensley's acid fuchsin methyl green method in which these filaments are fuchsinophilic in reaction, and are readily destroyed in solutions containing much acetic acid. The basal filaments, on the other hand, are due "to the fact that there are in the cell (speaking of his pancreatic acini fixed in chrome sublimate and stained in toluidin blue) unstained areas shaped like the filaments observed in the fresh cell after staining with janus green. These are the spaces originally occupied by the mitochondrial filaments." The basal filaments, then, are intermitochondrial basophile substance. In preparations fixed in solutions containing sufficient amounts of acetic acid to destroy the mitochondria this basophile substance is broken up into a feltwork of fine filaments. "These are the familiar basal fila-

ments of Solger or the ergastoplasmic filaments of Prenant, Garnier, Bouin." Bensley is inclined to the opinion that these basophile filaments are fixation artifacts. At least he did not see them in the living cells.

Unfortunately many investigators have failed to take cognizance of this valuable contribution of Bensley's. Since its publication many still are laboring under the old confusion. Even Hoven ('12) speaks of "vegetativen Ergastidien und Chondriomen" as the same structures. Champy likewise fails to appreciate their difference—"Mitochondria und Ergastoplasma scheinen eine einzige und die gleiche Formation darzustellen."

Basal striations then are due to (a) Mitochondria which are either rod- or thread-like structures or granules so arranged as to form rows (Chondriomiten). The long axis of the former and the rows of the latter are generally arranged parallel to each other and perpendicular to the base of the cells. (b) An intermitochondrial cytoplasm which may be basophile in reaction and depends upon the existence of the former (a). When the mitochdonria are not preserved or remain unstained the latter (b) is prominent.

The presence, shape, and arrangement of mitochondria, then, should determine to a great extent whether basal striations are present. It will be recalled that in the discussion of mitochondria these structures in the cells of the secreting tubules and intercalary ducts are more or less spherical or very short rods and irregularly distributed throughout the cytoplasm. No parallel rods or rows were seen. Further, no deeply staining basophil substance was observed. Again the secretion granules filled the entire cell. These conditions exclude the possibility of basal striations being present. I have not observed any semblance of such in the tubules or intercalary ducts in any of my preparations fixed in at least ten different fixing solutions.

The intralobular and interlobular ducts on the other hand possess an arrangement of mitochondria necessary to form basal filaments. However, no intermitochondrial basophil substance is present. It was not until I had availed myself of Bensley's method that I was convinced that basal striations of any kind

are present in these cells. In most fixations the basal half of the cell appears in no wise different from the proximal half. I could not account then for the claims of Fleischer who states that he saw 'pinselartige Aufaserung' in the cells of these ducts, ox—5 per cent ammonium chromate solution; Hornickel, beim Esel, and others. There is no doubt in my mind now but that the structures seen by these observers were imperfectly fixed mitochondria, which are readily seen in the acetic osmic bichromate-anilin fuchsin methyl green, and alcohol sublimate chromate-copper chrome haematoxylin preparations.

VIII. FAT

Axenfeld states that the presence of fat in the secreting elements of the human lachrymal gland is a normal condition and that apparently it is related to the secretory processes. Many globules of fat are found in the small dark cells. Kirschstein speaks of the large amount of fat in the interstitial connective tissue of the human lachrymal gland of old people. Ellenberger states that globules of fat are normal constituents of the lachrymal glands of all domestic animals except the pig, as does Lutz ('99). Stanculéanu and Théorhari describe much fat in the human lachrymal gland after epiphora. This condition probably was one of fatty degeneration. Schirmer—"Jedenfalls ist es von Wichtigkeit zu betonnen, dass in ganz normalen Drüsen grossere mengen Fett sich finden können." Hornickel—"Das Fett tritt als konstanter Zelleinschluss bei allen Tieren auf." According to him, in the horse and pig, the droplets of fat are small and in the periphery of the cell. In ruminants the distribution is regular, in the dog there is much variation, and in the cat the least amount.

More recent studies regarding the distribution of fat and lipoid substances in other tissues have been made by Bell ('10) and Kingsbury ('11). The former shows that in fresh tissues Herxheimer's stain affects all fat globules as well as the liposomes. According to him tissues fixed in formalin have a tendency to lose the liposomes while the neutral fat globules are unaffected. He shows that the granules obtained by Albrecht ('02) in the

kidney after staining with neutral red are not lipoids. Kingsbury used osmic acid and Weigert myelin sheath method.

For my purposes I found that Herxheimer's stain (Technique III, 5) served best notwithstanding that it stains lecithin, cholesterin, and myelin. This however did not materially affect the results. Osmic acid was also used and proved satisfactory but with this fixation it is very difficult to differentiate between the finer lipoids and other structures within the cell. Numerous glands were examined and it was found that the fat content of the cells is subject to much variation. As was pointed out by Bell, fresh glands show more of the finer liposomes than do those after preservation for some time in formalin.

In some glands numerous droplets of fat were present in all the epithelial elements. The sizes of these varied from some as large as the nucleus to others so fine that they could barely be made out. In these glands the larger fat goblets were generally seen in the base of the cell while the finer ones were located more in the body and proximal portions. There was, however, much irregularity in the distribution. In other glands again the amount of fat present within the epithelial cells was limited to very fine globules irregularly distributed throughout the epithelial elements. An abundance of interstitial fat is always seen both in the capsule and the trabeculae.

In comparing these preparations with similar ones of other glands (pancreas and submaxillary gland of the same animals) the lachrymal gland in the majority of those examined showed very little more fat, if any, in the epithelial cells than did the others. My conclusions then are that frequently the lachrymal gland cells show considerable fat globules but that as a rule the cells in this gland do not possess more than are ordinarily seen in other secreting glands, and that where numerous globules are seen it should be considered in the nature of pathological degeneration.

Frequently the technique of preparing tissues by frozen method involves, by many, the infiltration with gum arabic. The latter stains red in Herxheimer's solution and unless it is completely removed it may simulate fat globules. I was misled by this at first.

IX. SECRETION CAPILLARIES AND CEMENT SUBSTANCE

Injection of various masses into the duct system of glands was an early method employed for the demonstration of secretion capillaries. Langerhans, Saviotti, Gianiozzi, Pflüger, Ewald, and Boll, among others, utilized this means. Objections, however, to this method were advanced by some observers in that the injection mass under pressure produced these capillaries. It was found that Golgi's silver impregnation method demonstrated the secretion capillaries very clearly, as a black deposit is formed within them. Cajal, Retzius, E. Müller, Langendorf, Laserstein ('94), employed this means.

While intercellular secretion capillaries for serous glands in general are readily demonstrated by these methods as well as with certain stains much controversy has existed regarding their terminations. Some claim that they enter the cell, intracellular, as in the case of bile canaliculi, while others maintain that they remain intercellular throughout their course.

Noll described intercellular secretion capillaries in the lachrymal gland of the cat and Fleischer describes them in the tubules of the lachrymal gland of the ox, of varying lengths—some mere depressions, others almost touching the basal membrane, branching. Hornickel agrees with Fleischer regarding the presence and relations of these capillaries in the lachrymal glands of domestic animals. He further states that none are present in this gland in the pig and dog and explains this condition as being due to the fact that these glands are mucous in character as they react to mucous stains. Puglisi-Allegra demonstrated these structures by Golgi's method.

For the study of secretion capillaries I used the following: Kopsch-Golgi method (Technique II, 4); vital staining with pyronin (VI, 1); and tissues fixed in sublimate alcohol bichromate solution (IV, 2, a) or formalin bichromate sublimate solution (IV, 2, c) stained with iron haematoxylin, copper chrome haematoxylin, neutral gentian, and neutral safranin (V, 3, 5, 6, 7). My results agree with those of Fleischer and Hornickel.

Kopsch-Golgi

In Kopsch-Golgi preparations the lumina of the intercalary ducts and tubules and the secretion capillaries are definitely marked by the heavy black deposit, which gives an exact cast of them (fig. 16.)

In the tubules numerous secretory capillaries are seen radiating from the central irregular lumina. These vary in length, some forming only slight indentations between the cells while others almost touch the basement membrane. The ends of

Fig. 16 Kopsch-Golgi silver impregnation, Tech. II, 4. Zeiss comp. 18, obj. AA—showing secretion capillaries, lumina of tubules and ducts.

these capillaries are rounded and in many instances terminate in slight bulb-like swellings or knobs. Many branch at irregular distances from their origin. Some branch immediately upon leaving the lumina—others, after coursing some distance between two cells, branch to straddle the small demilune cells. In many instances the terminal branches are so numerous that they form irregular rosettes with many short club-like rays projecting in every direction. These rays frequently appear to be

intracellular although it is impossible to demonstrate by this method that such is the case.

The lumina of the intercalary ducts appear either smooth and even or many short knot-like projections are seen on either side showing that the surface cells which form the lumina are either in close apposition to each other or have indentations between them. That both conditions may be present has been considered in "Duct System and Tubules."

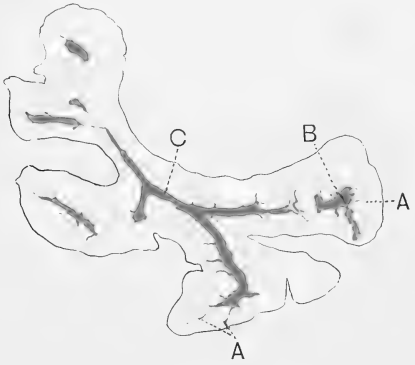

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Fig. 17 Tubule and intercalary duct after vital staining with pyronin. Tech. VI, 1. *A*, secretion capillaries; *B*, lumen of tubule; *C*, lumen of intercalary duct.

Pyronin

The most satisfactory method for the study of secretion capillaries is the vital staining with pyronin. The capillaries as well as the lumina of the tubules and ducts stain a deep red while the cells are unstained (fig. 17). The advantage of this method lies in the fact that very thin sections from 2 to 5μ can be obtained and these can be further stained with either iron haematoxylin or copper chrome haematoxylin. Thereby the relation of the capillaries to the other structures of the cells can be readily made out.

In sections thus prepared the red stained secretion capillaries are seen between the cells. Frequently they become finer and finer and gradually disappear as they approach the basal ends

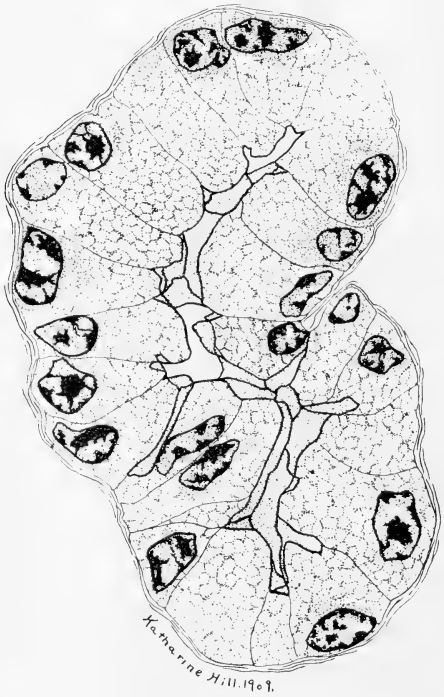

Fig. 18 Tubule showing secretion capillaries and cement lines. Secretion granules are not preserved. The intergranular cytoplasmic network is seen.

of the cells. Others terminate in bulb-like swellings. In no instance was I convinced that they had an intracellular termination.

Fixed and stained preparations

Secretion capillaries are readily seen in tissues stained with the serous granule stains already named (figs. 18 and 19). Here they are seen as intercellular structures and are outlined by a thin deeply stained cement substance. These sections plainly show that the secretion capillaries are much modified by the stages of granule formation within the cells. If bordering cells are in a maximum stage the capillaries are not seen, only the cement line projects downward between these cells. On the other hand, if the cells are in a medium or minimum granular stage the capillaries are wide and open and outlined by the cement substance.

In none of my preparations have I observed true intracellular secretion capillaries, notwithstanding they have been described by others. It might be well to add here that the literature on this particular subject is very confusing. Under the caption of intracellular canaliculi, one finds at least three separate and distinct types of these canals described without any particular discrimination as to their nature—(a) intracellular secretion canaliculi as described by E. Müller and R. Krause; (b) intracellular blood capillaries, in liver cells, as shown by Schäfer; and (c) the canalicular apparatus (reticular apparatus) (Holmgren's canals) which is found in most animal and vegetable cells. The adoption of a uniform nomenclature is certainly desirable in this instance. It is interesting to observe that similar methods (silver impregnation) have been used for the demonstration of all of these and is it not possible that in many instances intracellular secretion capillaries have been described when the canalicular apparatus of Holmgren alone was present? The latter is discussed under X.

The cement substance of glands was first described by Heidenhain and later by Zimmermann, Bonnet, Cohn, Solger, Carlier, Meyer, Oppel, Bensley, and others. Kolossow, on the other hand, doubted its existence.

I have already described the cement structures in the larger ducts (VI. The Duct System and Tubules.) Cement lines are

readily demonstrated with iron haematoxylin, copper chrome haematoxylin, neutral gentian, and neutral safranin. In the tubules and intercalary ducts they appear either outlining the lumina and the secretion capillaries or as lines varying in length projecting basalwards between the cells (fig. 19). As already

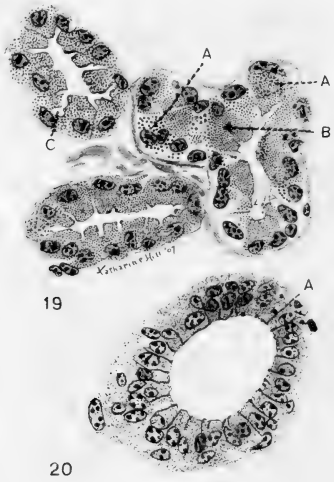

Fig. 19 Tubules, Tech. IV, 2, a; V, 6. Zeiss, oc. 2, Leitz. obj. $\frac{1}{12}$ oil—showing secretion capillaries and cement substance. Note varying intensities of staining of the granules. *A*, deeply stained granules; *B*, faintly stained granules; *C*, secretion capillaries.

Fig. 20 Intralobular duct, cross section, tech. same as figure 19. Cement lines projecting towards the base of the cells and between them—*A*.

stated, the cement substance is much modified in appearance by the secretory stage of the cells. If the cells are in a maximum stage of granule formation the secretion capillaries may be completely obliterated, in which case the cement substance likewise may be completely obscured or may be seen as fine lines extending between the cells towards their basal membrane.

As Carlier ('99) and Bensley ('02) have shown in gastric epithelium, the cement substance frequently appears on the free surface of the cell as a fine irregular network.

X. CANALICULAR APPARATUS

It has been shown that many types of cells possess within their cytoplasm very fine anastomosing canaliculi. These were first described by Golgi ('98) in the nerve cells. Later his students, Negri ('00) among others, demonstrated them in many gland cells. Golgi's silver impregnation method was used for the demonstration of these minute canals.

Kopsch ('02) showed that they could be demonstrated by long immersion of the tissue in 2 per cent osmic acid. By this method von Bergen ('04) demonstrated the canaliculi in a great many animal cells.

Holmgren ('02), in a number of papers beginning with 1899, has contributed much to this particular subject, using picric acid-sublimate, toluidin blue and erythrosin method, and later trichloracetic acid and fresh Weigert's resorcin fuchsin. At first Holmgren thought that the canals were lymphatic in nature as he had demonstrated to his own satisfaction that they communicated with the exterior. Later, mainly as a result of his second technique, he held that these canals (in nerve cells) possessed a network of fibres which had their origin from the nerve capsule. To this network of anastomosing fibres he applied the term spongioplasma and from this developed the well known 'Trophospongium theory.'

Bensley ('10 b) has shown the analogy of these canaliculi to the vacuoles of plant cells. In the latter (root tip of onion) using formalin bichromate sublimate solution and Kopsch solution as fixatives, he has demonstrated that in the youngest cells the well known vacuoles appear as a canalicular system similar to that in the characteristic animal cell. As the plant cell becomes older the canals enlarge and finally form vacuoles. These were seen by him in the living plant cells, as well. In no instance did he find these canals communicating with the exterior. Bensley ('11) describes the canaliculi in both the acinus

cells and islet cells of the pancreas. In the former, after stimulation in order to rid the cell of the secretion granules which hide the canaliculi, they are seen—"the apparatus is located in the portion of the cell between the nucleus and the lumen but branches of the canal may appear basalwards along the sides of the nucleus." In the islet cells they resemble those in the acinus both in topography and relation. In neither were communications with the exterior seen.

Cowdry ('12 b) describes them in the spinal ganglion cells of the pigeon.

That these canaliculi communicate with the exterior of the cell has been claimed by Holmgren and Retzius, among others; that they do not, by Golgi, Kopsch, Misch, Studnicka, and Bensley.

The canalicular apparatus in the cells of the tubules of the lachrymal gland of the ox can not be easily studied owing to the general presence of secretion granules. However, in many glands cells with few or no granules were seen in which this apparatus could be made out readily. It was seen for the most part in that part of the cytoplasm proximal to the nucleus. It appears as a network of open spaces or canals with branches which frequently terminate in slight nodular enlargements. A layer of cytoplasm was always seen between these terminations and the cell membrane. The apparatus at one end comes into close proximity to the nucleus, and processes are frequently seen extending around the nucleus towards the base of the cell.

This apparatus is seen best in sections preserved in formalin Zenker's solution, where it is seen as clear spaces in the cytoplasm. In the tissue stained by the vital pyronin method, which demonstrates the intercellular secretion capillaries, the canalicular apparatus of Homlgren can be made out as well. Here also it appears unstained, in contrast to the red secretion capillaries, and is seen as clear spaces in the cell. Consequently it is readily differentiated from the intercellular structures. Since the pyronin stains the secretion substance, one can readily conclude that the apparatus is in no manner concerned with the secretion substance of the cell and is independent of the secretion capillaries.

XI. TECHNIQUE

I. For the study of the gross characteristics of the gland, calves' heads were embalmed by injecting, under pressure, into the carotid arteries equal parts of glycerine, 95 per cent alcohol, and carbolic acid full strength. A suspension of red lead, starch and hot water was then injected, shortly after which dissection of the orbit was begun.

II. For the study of the ducts and their ramifications the following methods were used:

1. Flint ('02), Spalteholz ('97).

2. Injection method, see Hüber's technique given in American Journal of Anatomy, vol. 6, 1907, The Arteriolae Rectae of the Mammalian Kidney. The celluloid mass withstood the corrosion much better than the celloidin. Total peptic digestion required on the average two weeks.

3. Vital staining method with pyronin (See VI, 1).

4. Kopsch ('10)—Golgi chrome silver method (somewhat modified) for the demonstration of lumina of tubules and secretion capillaries.

III. For the study of the connective tissue framework.

1. Flint, Spalteholz—After complete digestion, tissues were imbedded in paraffin and thin sections made.

2. Mall's method ('96) for the demonstration of reticulum with frozen sections (25μ).

3. Tissues fixed in 70 per cent alcohol, imbedded in celloidin, sections 10 to 20μ thick, fastened on slides, and digested and stained by Mall's method were especially useful for the study of the framework. The method (Jeffrey's) was as follows: Sections were placed in a mixture of equal parts of glycerine and 95 per cent alcohol for some time before mounting. A thin coating of Mayer's albumin was applied to clean slide, to which section was transferred. A piece of smooth writing paper was placed on the section and over this several layers of blotting paper. A second slide was prepared in the same manner and the two were bound tightly together with the blotting paper in the middle. This was placed in a thermostat 60° C. for one-half hour after which the slides freed from the paper. The slides

(to which the sections adhered) were then placed in alcohol and ether until the celloidin was dissolved (15 to 20 minutes); transferred to absolute alcohol, 95 per cent alcohol, 70 per cent alcohol, and finally to water; and digested in pancreatin, which required from one to three days.

Tissues fixed in any of the chrome salts were not affected by pancreatin in one week's time.

4. For further study of the connective tissue, tissues were fixed in Zenker's solution, embedded in celloidin and stained as follows: (a) for collagenic fibres and smooth muscle—Van Gieson's, Mallory's and haematoxylin and eosin; (b) for elastic fibres—Weigert's and Unna-Taenzer ('10).

5. For the study of fat in connective tissue, pieces of gland were fixed in 10 per cent formalin twenty-four hours, washed, cut by frozen method, and stained by Herxheimer's ('10) method —absolute alcohol 70 cc., sodium hydroxide (10 per cent solution) 20 cc., water 10 cc., Sharlach R. to saturation. After staining for from five to ten minutes sections were washed in 70 per cent alcohol, washed in water, and mounted in glycerin.

IV. For the study of secretion granules and the finer histological characteristics of the cell.

1. Granules in the intercalary ducts—Fixed in Zenker's, embedded in celloidin, fastened to slides (see III, 3); celloidin dissolved off; and section stained in muchaematein or mucicarmin prepared according to Bensley's method (see Stains).

2. Granules in the tubules as well as those in the intercalary ducts and the minute cell structures—Small pieces of tissues were fixed in the following solutions, embedded in paraffin, and cut from 2 to 5 μ thick.

(a) Bensley's ('96) sublimate alcohol bichromate solution—equal parts of saturated solution $HgCl_2$ in 95 per cent alcohol and $2\frac{1}{2}$ per cent aqueous solution of $K_2Cr_2O_7$. Small pieces of the tissue were placed in this fixative for about three hours. The solutions were not mixed until time of using. Fresh mixtures were used every thirty minutes during fixation.

(b) Modification of Kopsch's formalin bichromate solution—

Formalin 40 per cent, 1 part...
$K_2Cr_2O_7$ 3 per cent Aq. sol., 3 parts.....one part
Sat. sol. of $HgCl_2$ in 95 per cent alcohol.....one part
Distilled water.....two parts

Fixative was made at time of using. Small pieces of tissue were placed in it for about three hours to insure proper fixation.

(c) Formalin bichromate sublimate method—Fixed for twenty-four hours in the following solution: neutral formalin 10 cc. and Zenker's solution without the acetic acid 90 cc.

(d) 70 per cent alcohol.

Tissues fixed in (a), (b), (c), and (d) were stained in muchaematein, mucicarmin, iron haematoxylin counterstained with mucicarmin, Bensley's ('11) neutral gentian, Bensley's ('11) safranin-acid violet, and copper chrome haematoxylin used singly or with mucicarmin (see Stains).

(e) Acetic osmic bichromate method (Bensley '11, p. 308).

V. Staining methods.

1. Muchaematein (Bensley '03)—haematein 1 gram, aluminum chloride 0.5 gram, 70 per cent alcohol 100 cc. Haematein and chloride rubbed together, dissolved in alcohol and allowed to stand for a week to insure ripeness. (If alcohol is made by diluting absolute alcohol with tap water stain can be used immediately.) Sections were flooded with stain, placed on stage under microscope and watched until deep color, then rapidly washed in 95 per cent alcohol, dehydrated, cleared and mounted in xylol balsam. The granules in the intercalary duct stained definitely and intensely blue.

2. Mucicarmin—1 gram of carmin and 0.5 gram aluminum chloride ground in porcelain evaporating dish after which small amount of water was added; heated over a Bunsen burner, grinding process continued during heating, till mass became very dark red (almost black) then dissolved in absolute alcohol and filtered. Stain must be used while fresh. Technique same as V, 1.

3. Iron haematoxylin.

4. Iron haematoxylin counterstained with mucicarmin.

5. Copper chrome haematoxylin (Bensley '11, p. 310).

6. Neutral gentian—solution of gentian-violet (crystal violet) precipitated by its equivalent of orange-G solution (Bensley '11, p. 308).

7. Safranin-acid violet—precipitate of a saturated solution of safranin O with solution of acid violet (Bensley's 11, p. 309).

8. Acid fuchsin methyl green—(a) Altmann's acid fuchsin anilin solution: acid fuchsin 20 grams, anilin water 100 cc. and (b) 1 per cent solution methyl green (Bensley '11, p. 309).

9. Macallum's ('95) iron reaction.

VI. Vital staining methods.

1. Pyronin. (Bensley '11, p. 305.) About eight liters of 1–1000 solution in isotonic salt solution injected into the carotid arteries of heads of freshly killed calves; cut arteries in neck clamped off to prevent leakage. Lachrymal gland was deeply stained. By means of the Valentine knife sections 0.5 to 1 mm. were cut and studied with binocular. The acini or tubules stained only lightly while the ducts, as well as the lumina of the acini including the intercellular secretion capillaries, were deeply stained. The capillaries were best studied by fixing small pieces of this pyronin stained gland in 8 per cent solution of ice cold ammonium molybdate for twelve to twenty-four hours, after which they were placed in ice cold 95 per cent alcohol one hour, absolute alcohol one hour, toluol one hour, and paraffin one-half hour. Sections were made and capillaries studied with the microscope.

2. Janus green—one gram in 15,000 cc. of isotonic salt solution. (Bensley '11, p. 305.) Technique same as VI, 1.

3. Methylene blue—one gram in 10,000 cc. of isotonic salt solution. Technique same as VI, 1.

4. Neutral red—one gram in 15,000 cc. of isotonic salt solution. Technique same as VI, 1. This stain cannot be fixed.

For detailed consideration of these stains see Bensley ('11).

XII. MISCELLANEOUS-OTHER FIXATIONS AND STAINS

In addition to the technique outlined and referred to in the general discussion other methods of fixation and staining were employed, the results of which may be briefly summarized as follows:

1) Fixations with alcohol failed to preserve the secretory granules and the mitochondria. The cells of the tubules show only the intergranular cytoplasm which appears as a network.

2) When 5 per cent glacial acetic acid was added to the 70 per cent alcohol it was found that the granules were partially preserved. In these preparations many of the ring and crescent granules of Fleischer were seen.

3) The same results outlined in (1) are obtained when Carnoy's fixation is used.

4) The secretion granules are fairly well preserved in picric acid. When stained in the neutral stains of Bensley, one is impressed with the varying intensities to which granules stain. One cell may be filled with the characteristic dark blue granules while the neighboring cell may be full of lightly stained yellowish granules. The granules in cell type 1 generally show in this fixation greater affinity for the neutral stains than do those in other cells.

5) Tissues fixed in trichloracetic acid preserved the secretion granules fairly well. However, they did not show great affinity for the neutral stains, for after slight differentiation they are but faintly stained.

It will be recalled that fixations (4) and (5) were used by Fleischer in the demonstration of ring and crescent granules. While these were frequently seen in these fixations they were by no means constant.

6) Using Kolossow's method, I was unable to demonstrate the epithelial intercellular bridges as described by him. Much shrinkage of tissue was in evidence.

My experiments with neutral red, vital staining, were not very satisfactory. Four different calves heads were stained by this method and in each instance the gland remained either uncolored or appeared slightly pink. This was found to be in marked contrast to the results obtained in other animals where the gland stained deeply red (monkey). Microscopic examinations show that all cells are diffusely but faintly stained. The granules of the tubules and intercalary ducts also appear very faintly stained. As elsewhere stated, careful examination

failed to reveal prozymogen granules as described by Bensley for the pancreas.

Vital staining with methylin blue showed the sympathetic nerve fibres of the gland, as demonstrated by Dogiel. The granules are not stained.

XIII. SUMMARY

1. The lachrymal gland in Bovidae is made up of two parts—a Pars superior which comprises by far the greater bulk of the gland and an auricular appendage which extends downwards between the Bulbus oculi and the outer bony orbital wall—the Pars inferior.

2. The gland is composed of a series of from six to eight compound tubular glands serially arranged and in close apposition to each other. Each gland may be subdivided, beginning with the terminal opening and proceeding to the secretory elements, into the following structures: main duct, primary duct, interlobular duct, intralobular duct, intercalary duct, tubule or alveolus.

3. Elastic fibres are abundant in the capsule and in the larger interlobular septa. They appear to some extent in the more prominent intralobular septa but in the finer they are not seen. These fibres do not surround the individual secreting tubules as claimed by Boll, Schirmer, Fumagalli, and others, in various animals. Lymph cell infiltrations of the septa are not normal conditions though so held by Schirmer for this gland in man and by Fleischer in the ox. The basement membrane is formed by a reticular connective tissue similar to that seen in the salivary gland. In addition to this membrane, irregular, anastomosing connective tissue cells are seen between and surrounding the tubules together with plasma cells, endothelial cells, and lymph cells. While smooth muscle cells are frequently seen occurring singly in the capsule and in the largest interlobular septa, they are not found within the lobule surrounding the individual tubules as held by Kollosow, Zimmermann and others.

4. The epithelial cells of the main duct are irregular in outline and arranged in several layers. Numerous goblet cells are seen.

In the primary ducts a gradual reduction of both goblet cells and the layers of epithelial cells takes place. The interlobular ducts possess no goblet cells, and two layers of epithelial cells form the lumina. In the intralobular ducts one or two layers of epithelial cells are seen. The outer layer gradually disappears in the smaller intralobular ducts and in the intercalary ducts.

5. Secretion granules are present in both the intercalary duct and in the tubules. They are not found in the other ducts. The granules are readily seen in all fresh glands when examined in serum or isotonic salt solution. They disappear when the fresh cells are placed in distilled water but reappear upon the addition of 2 per cent sodium chloride solution.

6. The granules in the intercalary ducts are preserved when the tissue is fixed in Zenker's solution and stain specifically in muchaematein and mucicarmin. They are not stained—when fixed in Zenker's —with the serous granule stains. The granules in the tubules are not preserved as a rule in Zenker's solution. This phenomenon suggests that the cells or the tubules may differ in function from those of the intercalary duct. I am not prepared, however, to make this claim.

7. The granules of both the intercalary duct and the tubules are fixed in Bensley's alcohol sublimate bichromate solution and in formalin bichromate sublimate solution. When tissues are fixed in these solutions the granules in both the tubules and intercalary ducts stain in the well known mucous stain—muchaematein and mucicarmin as well as in the serious granule stains iron haematoxylin, copper chrome haematoxylin, neutral gentian, neutral safranin. After staining tissues fixed in the former solution with iron haematoxylin and then counterstaining with mucicarmin, all the granules in certain cells stain black with the former stain and all the granules in other cells stain red in the latter, while in other cells both black and red granules are seen—thus showing that even within the same cell some of the secretion granules are affected by serous stains while others are affected by mucous stains. Notwithstanding this double staining reaction, there is not sufficient evidence to claim that the cells forming the tubules are heterogeneous in character.

8. The cells constituting the tubules or acini present different pictures depending upon the secretory stage they are in. In the maximum granular stage the cells are large spherical or oval (bulging), the granules fill the entire cell and the nucleus is flattened against the base. When the secreting cavity is made up of these cells it may have the form of an alveolus or acinus. Cells in a medium or minimum granular stage are cylindrical, pyramidal, hour-glass shaped, or may appear as crescents. As a rule they are seen compressed between the large bulging cells in each tubule. They are frequently seen, however, making up the entire secreting cavity. These cells are much reduced in size when compared with the former. Granules may fill the entire cell or only few may be present. Occasionally these cells show no granules. The nucleus is round or oval and is always separated from the base of the cell by a zone of cytoplasm.

9. The cells of the intercalary duct do not show such disturbances. Whether these cells are in a maximum or minimum granular stage, the size and shape remain practically the same. No changes are seen in the form or position of the nucleus.

10. The lachrymal gland of the ox is not a mucous secreting gland notwithstanding that the granules stain specifically in the mucous stains. I do not agree with other observers, namely Fleischer, that the secretion granules in the process of formation regularly assume various peculiar forms such as rings and demilunes. Rings and demilunes are sometimes seen in fixed preparations but these peculiar forms are no doubt due to the action of the fixation fluid.

11. No light has been gained as a result of my studies on the origin of secretion granules. The absence or at least the presence of very small amounts of demonstrable secretion granule antecedent substances—prozymogen, nuclear derivatives, etc.—does not admit of the hypothesis that the nucleus is the sole originator of secretion granules. On the other hand the abundance of granules, their variation in size without regard to position in the cell (the largest and smallest granules are seen side by side in any portion of the cell), their apparent origin from the cytoplasm—all strongly suggest that the cytoplasm plays a

very important rôle in the formation of granules. I have seen no evidence that mitochondria are diretly concerned in the production of secretion granules.

12. Mitochondria are abundantly present in the cells of the larger ducts—intralobular, interlobular, etc.—where they are either arranged as filaments or appear irregularly distributed without any special arrangement whatsoever. In the cells of the intercalary duct and secreting tubules they are much less numerous and are distributed irregularly throughout the cytoplasm. The number of mitochondria for these cells appear approximately equal—whether the cells are a maximum granular stage or contain the minimum of granules.

13. I did not find that an abundance of fat globules in the secreting cells is characteristic of the lachrymal gland as claimed by other investigators. While glands were studied in which numerous fat globules were observed, in the vast majority the quantity of fat globules was found to be no more than that observed in the submaxillary gland and the pancreas.

14. Intercellular secretion capillaries varying in width and length—depending upon the secretion stages of the cells—and bounded by cement substance are prominently seen in the tubules. In the intercalary duct these capillaries may not be seen or they may appear as slight indentations between the cells. Cement substance is found throughout the secretion passages. Pyronin when used intra vitam selectively stains the secretion capillaries.

15. The canalicular apparatus is readily seen in the cells of the lachrymal glands. In the cells of the tubules they are only seen when few or no granules are present.

16. I have failed to observe the constant presence of centrosomes described by Zimmermann and Fleischer. Likewise I have failed to find the constant presence of the paranucleus described by other investigators.

I am especially indebted to Professor R. R. Bensley of the University of Chicago for numerous suggestions. This work was begun in his laboratory under his inspiring direction. Since

then I have taken advantage of his numerous contributions to the knowledge of gland structures, and the new methods of technique devised by him have been of greatest value in my work.

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All drawings are made from preparations of the lachrymal gland obtained from Bovidae.

EXPERIMENTAL MITOCHONDRIAL CHANGES IN THE PANCREAS IN PHOSPHORUS POISONING

W. J. M. SCOTT

Anatomical Laboratory, Johns Hopkins University

SEVEN FIGURES (ONE PLATE)

The interest which has been aroused in mitochondria in recent years and their possible importance in the cell economy is evident when Champy ('11, p. 154) is willing to make the statement that "I would not regard as living a cytoplasm which does not contain mitochondria" and when Cowdry ('16 b) remarks that "mitochondria are as characteristic of the cytoplasm as chromatin is of the nucleus."

There is one cardinal fact which must be emphasized and given its true value in any discussion of the literature on mitochondria in pathological conditions. This important point, so frequently ignored, is that these structures were brought into prominence over a quarter of a century ago by the brilliant investigations of Altmann, but they were seen and described by many investigators before Altmann, notably by Flemming. It is indisputable that many of his granules, particularly those styled vegetative filaments, are identical with mitochondria. His investigations had a very stimulating effect upon pathologists with the result that several important and truly classical papers on Altmann's granules in various pathological conditions appeared in rapid succession. Reference need only be made to the early numbers of Lubarsch and Ostertag's Ergebnisse and to the extensive bibliography given by Galeotti ('95, p. 544).

Now Altmann's work fell into disrepute and was actually scoffed at on account of the bizarre theoretical interpretations in which he indulged. He thought, for instance, that the granules were elementary organisms which existed in the form of colonies in all cells. This very naturally prejudiced investi-

gators against him and his work and they speedily lost sight of his very valuable objective findings. Soon his researches lost all their novelty and were forgotten together with the pathological studies based on them.

Very recently interest has been revived in these granules of Altmann, now called mitochondria, but unfortunately we are rather inclined to regard their study as something new and novel. We are altogether too apt to think that anything relating to them in pathology is new. On the other hand, there is a danger in accepting Altmann's work unreservedly because the technique which he used was far from specific and brought to light many granules which are certainly not mitochondria. So that we have to deal with two mitochondrial literatures in pathology, separated by a gap of twenty years or more, the first was stimulated by Altmann, and the second is to be regarded as one of the manifestations of the recent revival of interest in protoplasm, as contrasted with the nucleus.

So far as I have been able to ascertain there are no observations on mitochondrial changes in phosphorus poisoning in the older literature but several contributions of importance have been made in the last few years. The first of these is a paper by Ciaccio ('13, p. 725) who has made use of phosphorus, along with other poisons, to bring about experimental changes in mitochondria. His one brief reference to the pancreas is the only observation which I have been able to find concerning mitochondrial changes in that tissue as the result of phosphorus poisoning. He has two figures illustrating the phenomena which he observed. They show simply a diffuse knotty swelling of the mitochondria to which he applies the term 'Praeplastorhexis.' Indeed he does not mention these changes in the pancreas in the text at all, apart from the general discussion of the changes in other organs.

We owe by far the most detailed information regarding the mitochondrial changes in phosphorus poisoning, as well as in a large variety of other intoxications, to Mayer, Rathery and Schaeffer ('14, p. 607) who worked on the liver. They found that the mitochondria respond by a change in their form and staining reactions. On the basis of their observations they divide

the reactions of liver cells into two types. The first is characterized by cytolysis and chondriolysis. The cells increase in size, clear spaces appear in their cytoplasm, the mitochondria decrease in number and the nuclei change in appearance. In the second there is a "homogéneisation protoplasmique" and a "chondriomegalie," the protoplasm staining diffusely with fuchsin, the mitochondria increasing in size, and the cells themselves decreasing in volume.

These investigators also showed that, when they were able to increase the amount of mitochondrial substance in the liver experimentally, the same liver, on chemical analysis, showed an increase in the content of phospholipin. In this way they connected up their histological with their chemical findings, a result which might be expected in view of the evidence that the mitochondria are themselves, at least in part, composed of phospholipin. Three investigators, Regaud, Fauré-Fremiet, and Löwschin, working independently on mitochondria in mammals, invertebrates and plants, each came to the conclusion that they were made up of a combination of lipoid and albumin.

The possible significance of mitochondria in this connection is brought home to us when we reflect upon their lipoid nature and upon the increasing importance which investigators are now inclined to attach to the rôle of lipoids in cell processes. This point of view is aptly stated by Mathews ('16, p. 88) who regards phospholipins as the most importance substances in living matter:

> For they are found in all cells, and it is undoubtedly their function to produce, with cholesterol, the peculiar semifluid, semisolid state of protoplasm. This physical substratum of phospholipin differs in different cells and probably in the same type of cells in different animals, but everywhere, from the lowest plants to the highly differentiated brain cells of mammals and of man himself, it possesses certain fundamental chemical and physical properties.

Workers in this field, however, have been slow to realize the importance of the relation which may exist between the histological study of mitochondria and the new analyses of the lipoidal content of cells.

The idea which underlies most of the modern work on mitochondria in pathological conditions is that they constitute a

new and delicate cytoplasmic criterion of cell activity which is indeed supported by recent observations. Thus Dubreuil ('13, p. 138) found that they increased definitely in inflammatory processes just as Romeis ('13, p. 12) observed an increase in regenerating tissues. Homans ('15, p. 12) was able to relate them to an increased demand upon the activity of islet cells of the pancreas in experimetal diabetes; and Goetsch ('16) discovered that an increase in their numbers was correlated with an increase in the activity of the thyroid epithelium and with the severity of the clinical symptoms of hyperthyroidism, in man.

MATERIAL AND METHODS

White mice were used. The phosphorus was administered by subcutaneous injections of an olive oil solution. In the first experiments the concentration of phosphorus in the solution was 0.0125 per cent which was later increased to 0.05 per cent. Several injections of various amounts of 0.1 to 0.2 cc. were given to each mouse at intervals of a day or more—for a longer or a shorter time depending upon whether it was desired to bring about a severe or a slight reaction. The animals usually showed pronounced symptoms within a few days, so that the intoxications must be regarded as rather acute.

Animals in the desired stage of poisoning were killed with chloroform. Pieces of the pancreas were first removed to physiological salt solution. A part of it was then examined in the fresh condition. Other portions were vitally stained by immersion in solutions of janus green as well as with the other dyes in general use. Pieces were also fixed in a variety of mixtures, chief among which may be mentioned formalin and bichromate (Regaud) and acetic osmic bichromate (Bensley) for mitochondria; osmic acid, commercial formalin, neutral and alkaline formalin, among others, for the demonstration of fat and other substances. Many different methods of staining were employed; the acid fuchsin methyl green and the iron hematoxylin methods for mitochondria; Sudan III, Scharlach R, Mallory's stain, hematoxylin and eosin and a variety of others were used.

The best preparations of mitochondria were obtained in pancreases fixed by injection through the blood vessels with a mixture of neutral formalin and potassium bichromate in accordance with instructions given by Cowdry ('16 a). Fixation by injection in this way obviates the factor of poor penetration as well as certain other undesirable accompaniments of the more crude method of fixation by immersion only. This is even more necessary in investigations on the central nervous system. I venture to quote this method of technique in detail:

Fixation. Chloroform the animal. Inject warmed 0.85 per cent NaCl solution into the aorta through the ventricle. Clamp the vessels to the part which it is not desired to fix. Continue the injection until the salt solution is returned uncolored through the veins. Gravity pressure of not more than 6 feet may be employed.

Follow the salt solution with the formalin and bichromate mixture: 3 per cent potassium bichromate, 4 parts; neutral formalin, 1 part. The potassium bichromate acts best when freshly prepared. Neutral formalin is made from the commercial variety by the addition of magnesium carbonate, a deposit of which should always remain at the bottom of the formalin bottle. It is important that the pressure should be at the maximum when the mixture is first injected, so that the blood vessels may be fixed in a state of dilation. If the pressure is low when the fixative comes in contact with the vessel walls they will be fixed in a condition of collapse. It will then be difficult, or even impossible, to obtain a complete injection. The injection should be continued for about an hour.

The organ is then dissected out and immersed in the fluid. It should be cut into pieces not more than 1 cm. cube. The fixative must be changed every day for 4 to 5 days, otherwise it undergoes a change evidenced by a darkening in color. This change is accelerated by light and by heat, so that the tissue should be kept in the dark and in a cool place. Fixation may also be effected by simple immersion of the tissue in the fixative, instead of by injection, but this procedure is not recommended.

After this prolonged fixation the tissue is mordanted in a fresh 3 per cent solution of potassium bichromate in which it remains for 8 or 9 days, changing every second day.

Wash in running water for 24 hours. The object of this careful washing is to remove most of the formalin and bichromate, for otherwise the tissue will be extremely brittle and hard to cut.

Dehydration and embedding. 50 per cent alcohol 12 hours; 70 per cent and 95 per cent alcohol 24 hours each; absolute alcohol 6 to 12 hours; half absolute and xylol 6 hours; xylol 3 hours; paraffin 60°C. 3 hours; cut in 4 serial sections.

Staining. 1) Pass the sections, mounted on slides, down through toluol, absolute, 95, 70 and 50 per cent alcohol to distilled water.

2) 1 per cent aqueous solution of potassium permanganate 30 seconds; but this time must be determined experimentally.

3) 5 per cent aqueous solution of oxalic acid also about 30 seconds.

4) Rinse in several changes of distilled water about a minute. Incomplete washing prevents the staining with fuchsin.

5) Stain in Altmann's anilin fuchsin, which is to be made up as follows: Make a saturated solution of anilin oil in distilled water by shaking the two together (anilin oil goes into solution in water in about 1 per cent). Filter and add 20 grams of acid fuchsin to 100 cc. of the filtrate. The stain should be ready to use in about 24 hours. It goes bad in about a month. To stain, dry the slide with a towel, except the small area to which the sections are attached. Cover the sections on the slide with a small amount of the stain and heat over a spirit lamp until fumes, smelling strongly of anilin oil, come off. Allow to cool. Let the stain remain on the sections for about 6 minutes. Return the stain to the bottle.

6) Dry off most of the stain with a towel and rinse in distilled water, so that the only stain remaining is in the sections. If a large amount of the free stain remains it will form a troublesome precipitate with the methyl green; on the other hand, if too much stain is removed the coloration of the mitochondria will be impaired.

7) Again dry the slide with a towel, except for the area covered by sections. Allow a little 1 per cent methyl green, added with a pipette, to flow over the sections, holding the slide over a piece of white paper so that the colors may be seen. Apply the methyl green for about 5 seconds at first and then modify the time to suit the needs of the tissue.

8) Drain off excess of stain and plunge the slide into 95 per cent alcohol for a second or two, then rinse in absolute for the same time, clear in toluol, and mount in balsam.

Several difficulties may be met with: 1) The methyl green may remove all the fuchsin, even when it is only applied for a short time. This is due to incomplete mordanting of the mitochondria by the chrome salts in the fixative. It may often be avoided, either by omitting the treatment with permanganate and oxalic acid, or by treating the sections with a 2 per cent solution of potassium bichromate for a few minutes immediately before staining (as advised by Benseley). The action of the permanganate and oxalic is to remove the excess of bichromate. 2) Or the fuchsin may stain so intensely that the methyl green removes it very slowly or not at all. This, on the other hand, is due to too much mordanting. It may be corrected by prolonging the action of the permanganate and osalic. 3) Sometimes, after obtaining a good differentiation, the methyl green is washed out before the slide is placed in toluol. This may be avoided by omitting the 95 per cent alcohol, by passing from the methyl green to the absolute direct. 4) Unfortunately the stain is not very permanent. Under favorable conditions it will last for 3 or 4 years. The fading in color is hastened by light and by heat, and it proceeds very rapidly in a damp atmosphere.

OBSERVATIONS

The mitochondria in the normal pancreas of the white mouse differ in no noteworthy particular from the mitochondria in the pancreas of other animals, which, indeed, have been described again and again (for reference see Bensley '11, p. 361). Attention, however, may be called to the fact that the mitochondria are filamentous, that they occupy the basal zone of the cell and that they possess bleb-like swellings all of which can easily be distinguished in the living cells. This may be seen by reference to figure 1.

The first change resulting from a very mild degree of phosphorus poisoning consists of the loss of the swellings on the mitochondrial filaments (fig. 2). At the same time the mitochondria become shorter and thicker. Some of them appear spherical, others ovoid. These altered mitochondria can be clearly distinguished from the zymogen granules by their staining reactions. The zymogen granules stain a purple or an olive brown color depending upon the degree of differentiation, and the mitochondria bright crimson with the fuchsin methyl green stain. This alteration in the mitochondria precedes any noticeable change in the other cell constituents. The nucleus still stands out sharply, the nuclear membrane being quite distinct, the cytoplasm stains a homogeneous bright green color, and the zymogen granules occupy the distal zone of the cell.

The second stage of the process is represented in figure 3. It can readily be seen that the mitochondria exhibit a remarkable tendency to clump together like agglutinating bacilli. The clumps occur most frequently in the basal portions of the cells. They are of variable size. Some of them are composed of only three or four mitochondria. In others, however, it is possible to count eighty or even more individual granules. The clumps vary in consistency, some of them being loose and others quite compact. The mitochondria are more closely crowded in the center. But even here the individual granules may be distinguished from one another by the reflection from their curved surfaces. The peripheral part of such a clump contains mitochondria less densely packed together. Strands, three or four

mitochondria thick, often radiate from such a clump. These usually extend parallel to the nearest margin of the cell, and the mitochondria in them are oriented so that their axes correspond in a general way with the direction of the strand. The cell on the right shows two such strands running out from the large mass of agglutinated mitochondria. In some cells almost all the mitochondria are agglutinated in a single large mass, but in others many scattered clumps of small size are formed. Where there are large clumps of mitochondria those of them which are usually distributed throughout the cytoplasm are greatly diminished in number. There seems to be a complete transition between the condition of unclumped mitochondria (shown in fig. 2) and these masses of agglutinated ones. Parallel with agglutination of mitochondria there are other evidences of cellular damage: the nuclei, instead of being sharp and clear, are hard to define; frequently the nuclear membrane cannot be distinguished, the nucleoli are apparently absent; and the cytoplasm stains intensely as well as unevenly.

In the next stage, that shown in figure 4, a noticeable change is observed in the clumps above described. The agglutinated mitochondria fuse together and lose their individuality. Figure 4 is the representation of a single cell which shows the transition from agglutination to fusion. The triangular shaped mass just above the nucleus is made up of closely packed, yet discrete granules. It is a typical clump of agglutinated mitochondria. The second large mass in the cell stains crimson with the acid fuchsin just as the other does. Its outline, however, is roughly spherical, but still somewhat irregular in shape, and it is impossible to resolve it into separate mitochondria. It is not perfectly homogeneous, though, for it shows evidence of its original granular nature. These two masses represent practically the entire mitochondrial content of the cell although occasional scattered mitochondria remain. It is possible to see in one section many cells which illustrate well the agglutination as well as others which show complete fusion.

Figure 5 shows the results of a more severe intoxication with phosphorus. The cells contain globular masses which bear a

remarkable resemblance to those formed in the preceding stage through the fusion of the agglutinated mitochondria. They differ, however, from these in that they are more spherical and show not the least evidence of a granular structure. Some are quite small, but the largest may actually exceed the nucleus in size. There is a variable number of them in each cell. Their properties, so far as I have observed, are as follows: 1) They are drop-like in form; 2) They stain faintly bluish with hematoxylin and eosin; 3) They do not stain specifically with Sudan III or Scharlach R; 4) They are insoluble in absolute alcohol and toluol after chromatization (3 per cent solution $K_2Cr_2O_7$ eleven days, 8°C.); 5) They stain with acid fuchsin, in fuchsin methyl green, and with Orange G in Mallory's triple stain. I assume that they are lipoid.

Within these droplets are to be observed little spherical vacuoles which do not stain at all. These clear spaces are sometimes quite numerous especially in the largest droplets. Some of the droplets, indeed, appear as only a rim of stainable substance about such a vacuole. That this phenomenon is not due to the imperfect penetration of the chromatizing solution is proven by the fact that the vacuoles may be arranged along the periphery as is seen in figure 5 in the largest droplet. It must be due to a difference in the solubility, to a loss of the staining capacity, or to an alteration in the composition of this part of the lipoid droplet. The protoplasm surrounding these droplets stains very intensely with the methyl green. Further away this dark color shades gradually off into the light green which the cytoplasm usually stains. In this more deeply staining zone mitochondria are seldom present; but in that part of the cytoplasm possessing the more normal staining reaction they are to be observed. It is to be especially noted that they show no tendency to agglutinate and possess no bleb-like swellings. There are many nuclei which seem to be quite normal in appearance but there are others which clearly bear evidence of excessive cell damage.

In the same specimen which shows these large lipoid droplets cells are to be found riddled with clear vacuoles (fig. 6). Such

cells are relatively scarce. The vacuoles range in size from 0.1μ to 3μ in diameter. They have every appearance of being the empty spaces left after neutral fat has been dissolved out. It seems quite probable that this is the case for the chromatization (eleven days at about 8°C.) would be quite insufficient to render neutral fats insoluble. It is to be particularly noted that such a cell contains the lipoidal droplets, and that its cytoplasm stains deeply. A few mitochondria are still present in cells of this sort visible between the vacuoles. This cell is of interest since within it are shown the results of two processes, fatty infiltration, and the formation of the lipoid droplets from mitochondria. This great accumulation of neutral fat within the cell is not associated in any demonstrable way with the formation of the lipoid droplets, and is not a part of any stage of this process, as is evidenced by the fact that only a few scattered cells show it and that it is not a regular accompaniment of any stage in the agglutination or fusion of mitochondria.

A very interesting condition, quite unlike any described above, is observed in a number of the cells in the same sections from which figures 5 and 6 were drawn. The whole cell is densely packed with zymogen granules of varying sizes, which are usually confined in the normal acinus cell, to the distal zone (fig. 7). It is possible to distinguish clearly mitochondria as brightly stained rods between the zymogen granules. These mitochondria are short and thick and have no blebs. They certainly do not seem to be increased in number; and, while it is impossible to estimate their number exactly, because the zymogen granules are so densely packed, the impression is gained that they are somewhat diminished in number. The mitochondria and secretion granules can be clearly distinguished here also by their difference in morphology and staining reaction: the zymogen granules being always perfectly spherical and staining an olive-brown to a purplish red (depending on the differentiation), while the mitochondria are scarcely ever perfectly spherical and stain a brilliant crimson. Indeed it is possible in all our preparations to distinguish between mitochondria and zymogen granules by their staining reaction in the

acid fuchsin-methyl green preparations after neutral formalin-bichromate fixation. In the living cell the distinction is easy, too, by reason of the relatively high refractive index of the zymogen. These secretion granules are in no discoverable way different from those in the normal cell. They stain specifically with neutral gentian, and their size and form is unchanged. There are no droplets of lipoid and no agglutinated masses of mitochondria in these cells. The only possible interpretation of this condition is that it be either a retention of secretion or else an excessive formation of it. Curiously enough such cells packed with secretion and exhibiting mitochondrial changes of only the first stage are found near cells containing the large lipoid droplets and very few, if any, zymogen granules. Cells of this variety are really quite numerous. They frequently occur together in acini but they may also be seen in acini with other cells which show the typical lipoid droplets, and scarcely any zymogen granules. The reason for changes seemingly so opposite in nature occurring in neighboring cells is not at all apparent. It is true, however, that the reaction of individual cells to the poisoning in the other types of change varied greatly in degree. It may be that this heaping up of secretion in the cell is merely an evidence of an altered metabolism, possibly an abnormal stimulation, in these cells showing the least evidence of damage. This type of cell, whatever be its cause, emphasizes the fact that whenever the poisoning has affected a change in another constituent of the cell the mitochondria are found to be altered.

Among the large number of pancreases examined certain of them showed intracellular infiltration of hyaline substance in both acinus cells and islet cells, while others contained small areas of necrosis in which the mitochondria had practically disappeared. Even some of the control animals presented minute foci of fatty infiltration in the pancreas.

Other tissues of the animals poisoned with phosphorus were examined as a check upon the changes in the pancreas. The alterations are of course most pronounced in the liver and my findings in this organ are in a measure confirmatory of the observations of Mayer, Rathery and Schaeffer ('14, p. 608), but I

have not seen their stage of "homogenéisation protoplasmique" in which the mitochondria are supposed to go into solution and the whole cell to stain intensely with acid dyes. Briefly I find that the mitochondria lose their filamentous or rodlike outlines, become spherical and progressively lose their staining reactions so that they finally are not seen at all or merely as shadowy structures. There is also, as is to be expected, a very pronounced fatty infiltration. The alterations in the kidney are those of a typical cloudy swelling in which the mitochondrial changes have been carefully described, under the heading of Altmann's granules by several authors since Lubarsch's original publication. The cells of the central nervous system show no outspoken changes in their mitochondrial content. Finally it must be noted that the pancreas alone showed agglutination and fusion of mitochondria and that no tendency of this sort was observed in any other tissue.

DISCUSSION

The points of chief interest in this work are (1) the immediate loss of the bleb-like swellings on the mitochondria as the first evidence of pathological change, (2) the phenomenon of agglutination as a type of mitochondrial reaction and (3) the source of the lipoid droplets.

The swellings in the course of the mitochondrial filaments are considered by many investigators to be the precursors of secretion. The crucial point is the similarity or the dissimilarity between the mitochondria, these blebs and the zymogen granules. Key ('16, p. 216) is opposed to this view since he finds that, on increasing the secretory activity of the cell, through the administration of secretion and pilocarpin, the mitochondria show no change indicative of participation in the formation of zymogen. Moreover Cowdry ('16 b) has been able to stain the blebs in quite a different way from the zymogen granules as well as from the remainder of the mitochondrial filaments. He fixed mouse pancreas by injection of neutral formalin-bichromate through the blood vessels. The tissue was then mordanted in bichromate and embedded in the usual way. Sections stained with iron

hematoxylin and counterstained with safranin and light green showed the mitochondria and the zymogen granules blue-black, the nuclei bright red and the blebs light red against a green background.

My observations seem to bear upon this problem in two ways. In the first place I have found that the blebs in the normal pancreas, with the method of technique employed, always stain differently from the zymogen granules. In the second place the changes which I have observed in phosphorus poisoning seem to be still more suggestive. The fact that the blebs are the very first structures to disappear with a very mild degree of phosphorus poisoning (fig. 2) and that zymogen granules are heaped up in enormous numbers in some cells of animals more severely affected (fig. 7) would seem to indicate that the production of zymogen granules does not necessarily cease with the disappearance of the blebs, which is rather at variance with the hypothesis that the mitochondria participate, through their bleb like swellings, in the formation of zymogen granules.

The agglutination observed during the course of phosphorus poisoning is a new reaction on the part of mitochondria. It is interesting to compare this clumping of mitochondria (which are thought to be lipoidal in nature) with that of bacilli and red blood cells. Jobling and Peterson ('14, p. 453) make the statement that "with or without a morphologically distinct limiting membrane we can reasonably assume that the external surface of the bacterial cell is potentially lipoidal" and the lipoid nature of red blood cells is well recognized. In each of these three cases the fact that this phenomenon of agglutination is a reaction to pathological conditions should not be lost sight of. Moreover agglutination is a phenomenon which always occurs in a fluid medium which fact is not without significance from the point of view of cell structure because it indicates the fluid nature of the protoplasm of the pancreas cell, and militates against the doctrine of a cytoplasmic reticulum.

It has long been known that there is a deposition, or perhaps more correctly speaking a formation, of fatty lipoid droplets in the cells of the pancreas as the result of phosphorus poisoning,

but the source of the material has been much debated. There are several possible, though unlikely, sources of these lipoid droplets which must be considered. Normal pancreases often contain, in their acinus cells, small droplets of neutral fat and of lipoid in variable quantities. I have studied their distribution by staining with Sudan III and Scharlach R, after formalin fixation, and by vitally staining with dilute solutions of nile blue B extra, Meldola's blue (Sandoz, same as naphthol blue) and brilliant cresyl blue. Fat of this sort does not stain with janus green. It is also well known (Bensley '11, p. 363) that the mitochondria, themselves, occasionally contain droplets of fat, though I have searched diligently, but without success for traces of it in the mitochondria of acinus cells of white mice. No relation could be found between the formation of the lipoid droplets in the poisoned pancreas and this neutral fat occurring free in the cell or embedded in the filaments.

Pieces of the pancreas, fixed in neutral formalin and bichromate and stained in the routine manner with hematoxylin and eosin show these droplets but do not indicate their source. The whole process is made clear, however, when sections prepared in this way are stained by the fuchsin methyl green technique which is here advocated. The mitochondria are brought to light and the changes of agglutination (figs. 3 and 4) and of fusion (figs. 4 and 5) to form the lipoid droplets, which they undergo, are at once revealed. It seems that the mitochondria are the actual source of the droplets.

I wish to take this opportunity to express my appreciation for the continuous advice and interest of Dr. E. V. Cowdry in this research.

CONCLUSIONS

Mitochondria are the first constituents of the acinus cell of the pancreas to show pathological change in phosphorus poisoning. They lose their filamentous form, become shorter and thicker, and their bleb-like swellings which are so characteristic of the normal pancreas completely disappear (figs. 1 and 2). Then

follows the stage of agglutination in which the mitochondria collect in large compact clumps (figs. 3 and 4). The mitochondria in these agglutinated masses fuse to form droplets possessing the characteristic properties of lipoid (figs. 4 and 5).

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DESCRIPTION OF FIGURES

All the illustrations have been drawn from pancreas cells of white mice, all except the control showing varying degrees of poisoning with phosphorus, fixed by injection of a mixture of neutral formalin and potassium bichromate through the blood vessels, according to the instructions given by Cowdry ('16 a). Sections, 4μ thick, were stained with fuchsin and methyl green. In the original preparations the mitochondria are bright crimson, the zymogen granules purple and the ground substance green. Zeiss apochromatic objective 1.5 mm., compensating ocular 6 and camera lucida were used in making the drawings. They were not reduced in reproduction so that they represent a magnification of 1640 diameters as they now appear on the plate.

PLATE 1.

EXPLANATION OF FIGURES

1 A portion of an acinus of the pancreas of a normal female white mouse weighing 36 grams illustrated for control. Note particularly the long filamentous mitochondria with their bleb-like swellings stretching from the basement membrane toward the lumen. The zymogen granules are present, in moderate amount, in the distal parts of the cells.

2. A group of acinus cells of a female white mouse, weighing 17 grams, which was injected subcutaneously with 0.2 cc. of an 0.0125 per cent solution of phosphorus in olive oil. Five days later the animal became comatose, and was killed and examined. The cells show very nicely the first stage in phosphorus poisoning in which a change in the mitochondria is alone noticeable. They have lost their bleb-like swellings and have become shorter and more rounded. Compare with figure 1.

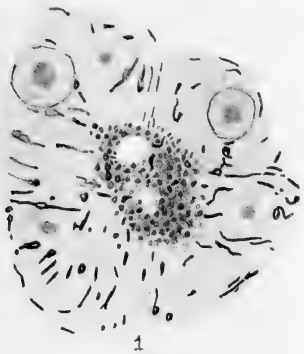
3 Two acinus cells showing a rather more pronounced change. The mitochondria are seen in clumps in the basal parts of the cells, the zymogen granules are decreased in amount and the nuclei are hard to define.

4 A single acinus cell, also from the same pancreas, which is of interest because it shows on the left hand side a triangular agglutinated mass of mitochondria and, on the right, such a clump which has undergone partial fusion in the process of formation of a lipoid droplet.

5 Portion of an acinus of a female white mouse weighing 16 grams which was more severely poisoned with phosphorus. It received 0.2 cc. of a 0.05 per cent solutions subcutaneously. On the third day the mouse was comatose. It was killed and examined in the usual way. All the agglutinating mitochondria have, in this stage, fused, and have formed numerous spherical lipoid droplets. These droplets contain clear vacuoles and the cytoplasm surrounding them stains intensely.

6 A cell from the same pancreas showing a pronounced fatty infiltration as well as the formation of lipoid droplets.

7 Another cell from the same pancreas showing a great increase in the number of zymogen granules. One or two mitochondria may be seen scattered among them.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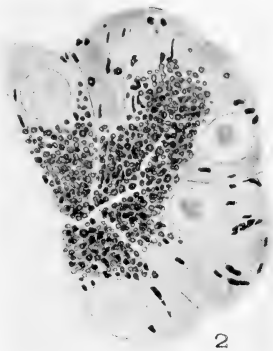
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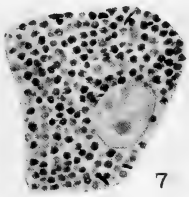
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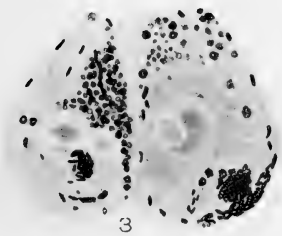
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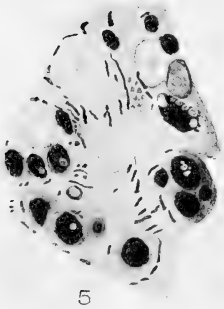
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EQUIVALENCE OF DIFFERENT HEMATOPOIETIC ANLAGES. (BY METHOD OF STIMULATION OF THEIR STEM CELLS)

I. SPLEEN

VERA DANCHAKOFF

The Wistar Institute of Anatomy and Biology

TWO TEXT FIGURES AND NINE PLATES

1. INTRODUCTION

Statement of the problems of hematology. Different methods of attacking the problems. Myeloid metaplasis

The special hematological literature has been rapidly and constantly growing during the last decade, and there is enough ground to anticipate a further specialization in this partial domain of biology. Surveying the voluminous hematological literature, one is astonished by the discrepancy between the simplicity of the fundamental problems of hematology and the entanglement which they have undergone during the last

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decade. One is naturally inclined to doubt, whether the small variations in the innumerable schemes are as important, as they are represented in the deductions of different workers.

The fundamental problems of hematology have a close bearing upon practical medicine as well as on the general problems of biology. Wilson and Conklin (6) especially, and many other biologists established as a fact the gradual segregation of different chemical materials collected in the egg. This is accomplished by a range of cleavages beginning in certain eggs at the first cleavage, in others delayed to later stages. As a result there appear groups of cells, different in their constitution and therefore possessing different potencies for differentiation. These cell groups proliferate and give tissues, which in their own differentiation may exhibit a great complexity. The diversity in the products of differentiation may be due either to differences in the physico-chemical constitution of the cells, or to differences in environmental conditions. One of the important questions of biology consists in determining the limit to which each of the agents cited has an active role. Does the segregation lead to a production of a definite number of uninterchangeable blood stem cells, of which the differences in the chemical structure imply a definite metabolism and their further specific development? Or does the process of segregation lead to a production of one group of numerous homogeneous primitive blood cells, which under different conditions in the embryo, as well as in the adult organism, split off variously differentiated cells? This problem was differently conceived by the monophyletic and polyphyletic schools.

The importance of a true conception of this fundamental problem of hematology for practical medicine is obvious. The uninterrupted, everyday destruction of the blood elements is too often accompanied by a failure of regeneration in the organism. A whole range of stimulating agents for blood regeneration was found empirically. Still the success of interventions in similar cases as well as of interventions in various other deviations from the normal course of hematopoiesis (leukaemias) depends greatly upon a clear understanding of the action of the

agent on the processes underlying a definite symptom complex. An expenditure implies a complete knowledge of the reserve stock, the reserve being represented in the particular case by the group of cells, which by their uninterrupted reproduction and differentiation may supply the deficiencies.

The reserve stock is differently appraised by the monophyletic and polyphyletic schools. According to the polyphyletic school these reserves are formed by a number of specifically differentiated cell groups, which may multiply and ripen into still more specialized cells. The monophyletic school, on the contrary, finds in the organism along the various intermediate stages of differentiation also the young, undifferentiated polyvalent cells, which in the embryo have been the source of the various lines of blood cell differentiation. The monophyletic school admits a greater amplitude in the regeneration, and hence a wider sphere of influence upon the blood tissue. These common stem cells were identified by Pappenheim (31) as the large lymphocytes, by Dominici (10) as the small lymphocytes, by Weidenreich (44) and Downey (11, 12) as reticular cells.

However great the differences between the mono- and polyphyletic schools may seem, both groups of hematologists admit in the adult organism the existence of younger and riper cells and of their gradual differentiation products. The difference between them consists chiefly in the value of the amplitude of the differentiation process in the adult organism, in other words in the admission of different degrees of segregation, which may preserve or dissolve the common stem cells for various blood elements.

Various methods were applied for the elucidation of the fundamental problems of hematology, as well as for the understanding of the mutual relationship of the various blood-cells and of the structure of the hematopoietic organs. As expected, the histological study of normal preparations of hematopoietic organs in adult organism was unable to solve the problem. The mere statement of coexisting cells in the hematopoietic organs offers a wide field for personal interpretation. Therefore the most

contradictory conclusions regarding the structure of the blood tissue were made on the basis of this method.

The embryo-genetic method may give more definite results if used exhaustively. The gradual appearance of the different blood cells becomes a reliable criterion for the judgment of the mutual relationship of the blood cells. The discovery of younger cells, which first develop in the embryo and the study of their gradual differentiation may be of great help in the identification of the blood cells in the more complex structure of the hematopoietic organs in the adult organism. However, the demand for strict exhaustiveness is often disregarded and leads to gaps, which are filled by a number of more or less keen interpretations of the investigator. The omission of the study of the first stages in the development of blood cells led investigators to an admission of specific stem cells for various differentiation products—Denys, (8), Bizzezero (3), and others. However, the recent histogenetic studies made by different investigators on various animals gave similar results. Bryce (2), Danchakoff (9), Maximow (25), Mollier (28), Haff (18), all admitted that the different blood cells are derived from mesenchyme of mesodermal origin, and that the various blood-forming organs developed autochthonously at the expense of mesenchymal cells of which the various differentiation may depend upon external physico-chemical agents.

Studies of pathological changes in hematopoietic organs did not contribute greatly to the solution of the problems of hematology. The complexity in the mutual relationship of the blood cells was recognized and led many pathologists to take recourse to embryo-genetic studies, (Fischer (16,) Schridde (38), Schmidt (39) and others). The conclusions drawn from studies made by these methods were based upon the existence of morphological similarities and differences between various blood cells. They lead to more or less plausible probabilities but do not determine irrefutably the functional dynamics of the various blood cells in different hematopoietic organs.

The recourse to the experimental method may contribute more definite information about mutual relationship of blood-cells as well as about the source of their regeneration and the function of different blood-forming organs. The study of intense general destruction of blood tissue through bleedings and of its partial destruction by X-ray application, followed by the study of its regeneration, have already contributed undeniable data. The use of a method known as lympho and myelotoxic intoxication, (I would better say stimulation) may give in this respect most conclusive results. This method was indicated in 1901 by Dr. Flexner (17). Specific leucolytic, spleno- lympho-marrow-lytic sera are injected into animals. The consecutive circulation of antibodies present in these sera find adequate receptors in certain cell groups and a proliferation follows as a result of molecular changes in these cells.

One of the most striking results obtained in experimental pathology of the hematopoietic organs is the myeloid metaplasis of lymphoid tissue. First described by Fraenkel it was experimentally obtained by Dominici (10) by producing traumatic anaemias and typhoid infection. Later the myeloid metaplasis of the lymphoid tissue was repeatedly observed during various pathological processes, (anaemia, leukemia, intoxications, infections, tumors). According to the statements of various investigators, the myeloid metaplasis invariably consists in a simultaneous development of erythro- and leucopoietic or granuloblastic tissues. This was accounted for by most of the pathologists as being a proof of a close relationship between these tissues. The common origin of leucocytes and erythrocytes was therefore admitted by many pathologists who formed the so-called dualistic school, which distinctly separated the myeloblastic (erythro-granuloblastic) tissue from the lymphatic tissue.

The present paper has for its main subject the study of a very extensive chiefly granuloblastic (or myeloid) metaplasis of the embryonic mesenchyme, therefore a survey of different opinions concerning the subject may be permitted.

The myeloid metaplasis was observed chiefly in the spleen, and occasionally mentioned in the lymph-glands and other

organs of the adult organism. Dominici (10) admitted in his paper ('01) that cells with the structure of small lymphocytes give rise to the myeloid tissue. "Les petits mononucléaires en question (in another part of his paper he uses the term of small lymphocytes) ont des dimensions égales ou inférieures à celles des hématies: un noyau rond, une bordure protoplasmique mince. Un élément figuré, offrant de tels attributs, n'est ce pas ce que l'on a dénommé communément une cellule embryonnaire?" A closer study of the embryogenesis of the blood tissue would certainly not have allowed Dominici to consider the small lymphocytes as being young embryonic cells, for the cells bearing the structure of small lymphocytes appear in the organism considerably later than other lymphatic cells, at least in mammals, birds and reptiles. Though the stem cells of the myeloid tissue, according to Dominici, bear a morphological structure identical with cells, which differentiate into lymphatic tissue, he still attributes to the myeloid stem cells specific potencies of differentiation, which they preserve from early embryonic period. Later, in 1909, Dominici (10) changed his opinion and attributed to the lymphatic tissue itself the faculty of myeloid transformation. Dominici was thus first to stand for the conception of autochthonous development of the myeloid tissue in the lymphatic organs.

According to Ehrlich (13) the myeloid tissue, which appears under certain pathological conditions in different parts of the adult organism, derived from the central myeloid organ, namely from the bone-marrow. This opinion was expressed by Ehrlich at a time when the differentiation of every blood-cell seemed to be predestined generations back by the specific constitution of their ancestral cells. Ziegler (47) and Helly (19) still adhere to this view. They base their opinion upon the occurrence of bone-marrow Ausschwemmungen, which follow venous injections of parenchymal mash, also after bone-marrow traumas. Helly finds support to his view also in the fact that after

Beeinflussung des Knochen-marks mittels Bakterien schon nach kurzer Zeit in der Milz des Kaninchens, welche unter normalen Verhältnissen so gut wie gar keine specifischen Markelemente jüngerer

Generation wie etwa Myelozyten, enthält, eine derart hochgradige und herdweise auftretende Einlagerung solcher Zellen vorkommt, welche sich nicht nur im Blute sondern auch in den Kapillaren anderer Organe reichlich finden, dass die Erklärung mit Hülfe der gedachten Verschleppung bei weitem die nahe liegendste ist.

However, the existence of myeloid metaplasis without any changes in the circulating blood, and further the discovery of diffuse autochthonous development of granuloblastic tissue in early stages of embryonic development, led most of the investigators to oppose strongly Ehrlich's conception of the myeloid metaplasis as being a metastasis from the central myeloid organ. If most of the investigators are inclined to consider the myeloid metaplasis as a local autochthonous process, their opinions concerning the source of the myeloid tissue, which develops in lymphatic organs, differ widely.

The fact that the myeloid metaplasis is chiefly localized in the pulpa of the spleen, became one of the main arguments of the dualists for denying any relationship between the lymphatic and the myeloid tissue in the adult organism. According to the dualists, cells from remote embryonic periods remained in the regions of the spleen-pulpa. Though their differentiation has not fully been accomplished, yet it reached stages at which these young cells respond to a stimulation by development of only myeloid tissue, (containing erythroblastic, leucoblastic tissue and megakaryocytes). According to them, the myeloid tissue, which in embryonic life is more diffusely spread out, does not disappear completely, but persists in a sort of latent stage. Bezançon et Labbey (1), Fischer (16), Heinecke (26), Lobenhofer (24), Meyer (27), Nägeli (31), Schridde (38), Schmidt (39), Sternberg (40), Türck (42), and others uphold this view. Most of them attribute the development of myeloid tissue under pathological conditions to a "sudden wakening" of embryonic potencies in various cell-groups. These cell-groups are differently identified by different investigators. Preexisting myeloid tissue is the source of the myeloid metaplasis for Sternberg (40), indifferent lymphocyte like pulpa-cells for Meyer and Heinecke (25, 27), Schmidt (39), Lobenhofer (24), Fischer (16), connective

tissue cells for Fischer (16) and Klein (22) and finally reticulum cells for Klein (22). An attempt was made to explain the myeloid metaplasis, not only by attributing potencies, characteristic for embryonic cells to cell-groups in adult hematopoietic organs, but also by assuming the existence of a mysterious process of dedifferentiation of already differentiated cells, Schridde (38), Fischer (16), Nägeli (31).

Among the investigators who studied the myeloid metaplasis only a few admitted the existence of a common stem-cell for various blood-cells in the adult organism. Pappenheim (32), Werzberg (46), later Dominici (10) and Blumenthal (4) became supporters of the monophyletic interpretation of the myeloid metaplasis. They pointed out that the localization of the new myeloid tissue during myeloid metaplasis is not as strict as the dualists admit. Moreover, the separate localization of the myeloid and the lymphatic tissues, where it exists, may become the differentiating factor for a common stem cell, which of course could not develop under equal conditions into different products. The monophyletic interpretation of the myeloid metaplasis was corroborated to a great extent by embryogenetic studies, Bryce (2), Danchakoff (9), Maximow (25), Mollier (28), Haff (18) and by histological studies (Weidenreich (44), Downey (11, 12), Ferrata (33).

The study of myeloid metaplasis of lymphatic tissue has been made in adult organs, in which at least a temporary embryonic or even a permanent partial granuloblastic differentiation existed. Therefore a possibility of persistence of specific granuloblastic stem cells in such organs could not be denied and a differentiation of specific cells could be explained by the specific constitution of their stem cells. Under definite stimulation, these specific stem cells might intensely proliferate. On the other hand, the hematopoietic organs could also preserve young specifically undifferentiated cells, polyvalent in their potencies. Their partial and local differentiation into myeloid tissue might have been caused by specific conditions of their environment.

It was repeatedly pointed out that the stem cells, which in the pulpa and in the follicle of the spleen give different products of development, seem to bear a perfectly similar morphological structure, Dominici (10), Hirschfeld (21), Meyer u. Heinecke (26), Weidenreich u. Downey (12), Butterfield (5), and others. However, many recent data seem to indicate that the isomorphism does not imply either isogenesis nor especially isodynamics.

The study of myeloid metaplasis in such organs, in which both directions of differentiation coexist permanently in the adult organism or temporarily in the embryo, does not evidently offer favorable opportunity of solving the problem: whether the adult organism does preserve a stock of embryonic undifferentiated cells capable of various differentiation, or not. Neither do these studies solve the question: whether the morphological structure so characteristic of and common to the young cells both of the lymphatic and myeloid tissue is a result of definite physico-chemical constitution of which further changes are implied by differences in environmental conditions; or whether a group of morphologically identical cell units may have different physico-chemical constitution which would imply their further different development.

My (9) personal studies on the normal histogenesis of the blood-cells and of the hematopoietic organs in birds and reptiles led me to a monogenetic conception of their origin. The study of regeneration of hematopoietic tissue after bleedings as well as that of changes undergone by this tissue during starvation (9) seemed to corroborate the monogenetic interpretation.

The admission of the existence of common stem cells in different hematopoietic organs implies therewith the admission of identical reaction of these stem cells to a stimulating agent as far as these stem cells are submitted to the same conditions. The structural environment in the full-grown organism is, however, highly differentiated in the various organs. The stem cells in different hematopoietic organs are of course expected to respond to stimulation by simultaneous proliferation; but their differentiation will be specific according to environmental conditions, for their differentiation depends upon conditions, usually

not experimentally controlled, upon the localization of the stem cell in the pulpa or in the follicle, in the case of the spleen. The possibility of identical reaction of the stem cells to definite stimuli is, however, not completely excluded. There may be certain kinds of stimuli, which may cause deviation from the normal differentiation of stem cells. A similarity of the reaction of stem cells in different organs may be expected also in case the different environmental conditions are made alike, or in case the environment is not fully differentiated, as for example in an embryo.

Under the influence of these premises an observation made by Dr. Murphy of the Rockefeller Institute attracted my attention. He observed 2–3 years ago an enlargement of the spleen in the host embryo after grafts of various tissues.[1] A closer study of this process led me to conclude, that the considerable enlargement of the embryonic spleen is induced by an intense proliferation of the young stem cells. This fact seemed to enable a

[1] This observation was not published by Dr. Murphy at the time. While this paper was in press a brief note by Dr. Murphy regarding the general effect of the spleen grafts on the organism of the host embryo appeared in the Journ. Exp. Med., July, 1916. It appeared after a number of my papers and communications (Meeting at New Haven, Staff Meeting at the Rockefeller Institute) and after numerous demonstrations to Dr. Murphy of my preparations. Dr. Murphy states in the above quoted note, that at his suggestion I undertook "the working out of the finer histological details of the process," discovered by him. This, in view of the above mentioned facts, I venture to consider unwarranted.

Dr. Murphy writes the brief note "*for completeness and record,*" and gives a reference of his previous work, in which "observations were made on the effects of certain organ grafts on the embryo itself. Murphy, Jas. B., Journ. Exp. Med. 1913, vol. 17, p. 482." This paper however does not contain any observation on this subject. The only passage in this paper, referring to the effect of the graft upon the embryo, reads: "Apart from the thin continuation of the chick membrane, which covers the tumor and the ingrowth of vessels with their scant accompanying stroma, *there is no histological evidence of reaction on the part of the embryo to the invasion of foreign tissue.*" Nor is it possible to find the slightest indication about the effect of the grafts in the body tissues of the host in any of Dr. Murphy's previous papers. Through personal communication from Dr. Murphy I knew about the enlargement of the embryo spleen. By deduction from my previous hematopoietic work I reached the conclusion regarding the necessary coexistence of analogous changes in other hematopoietic organs, a conclusion which the results of the experiments undertaken proved to be correct.

thorough revision of the fundamental problems of hematology. Indeed, if the various anlages of the hematopoietic organs were equivalent and contained identical stem cells, hematopoietic organs other than the spleen must have reacted also. Since the structural peculiarities in the different organs of the embryo are not fully differentiated, the reaction of the stem cells in different hematopoietic organs could be expected to be more homogenous. As shown further, the specific intervention was indeed followed by changes both in the spleen and in all the other hematopoietic organs and the reaction of these different organs was substantially similar. These facts were mentioned and demonstrated by me at the New Haven Meeting.[2]

The grafting of adult spleen on the allantois of the embryo is a complex intervention. The grafted tissue contains small and large lymphocytes, together with the so-called reticular tissue, and with the vessels and their different layers, all belonging to a full-grown organism. Which of these elements has to be regarded as the source of the stimulation could not yet be defined conclusively. However, there is no doubt the intervention applied introduces in the embryo heterogeneous substances.

It is known that the organism reacts to the introduction of heterogeneous substances by a production of antibodies and to the introduction of heterogeneous cells by production of the so-called lysins. The erythrolysins which are developed by the immunized animal have a dissolving power on the red blood corpuscles against which the animal is immunized, the leuco-

[2] Abstr. Proc. Anat. Record, Jan. 1916.

In this connection I desire to call attention to the substitution of a reference to Dr. Murphy's last communication (21) on p. 96 of No. 1, vol. 24, Journ. Exp. Med. July, 1916, in my paper on "Differentiation . . . " for a footnote, in which I stated: "I wish to express my indebtedness to Dr. J. B. Murphy, who kindly demonstrated to me the method of grafting described in this paper. Murphy, J. B. and Rous, P., 1912. The behavior of chicksarcoma . . . . Journ. Exp. Med. vol. 15." My paper, which was received for publication in February '16, and published in July '16 could certainly not contain a reference to Dr. Murphy's communication, which received for publication in May, appeared also in July. This alteration seems to me the much the more inappropriate, because it has been made to apply to the demonstrations given by me at the Anatomical Meeting, 1915.

lysins stimulate the hematopoietic organs and induce intense proliferation of their cells.

Antibodies are regarded as being specific and may influence only cells in which they find adequate receptors. Dr. Flexner (17) has shown an apparent lack of specificity both of certain kinds of leucolytic sera to different hematopoietic organs and of different leucolytic sera to one definite hematopoietic organ. The spleno- lympho and marrow-lytic toxins, each of them acted in a stimulating manner upon all the hematopoietic organs,—hence the antibodies of the leucolytic sera evidently found cells with adequate receptors in all hematopoietic organs. These cells under the influence of certain amboceptors responded by common proliferation in different hematopoietic organs.

The results of the experiments cited offer a further corroboration of the monogenetic conception of blood development. The histological studies established in all the hematopoietic organs—the presence of cells, of which the morphological structure seemed to indicate a great potency for differentiation and proliferation. The embryogenetic studies pointed out these cells, as the true stem cells, common to all the hematopoietic organs and endowed with faculty to intense polyvalent differentiation. Finally, studies on stimulation of the hematopoietic organs by agents, which were supposed to be specific, seemed to indicate in all the hematopoietic organs the presence of cells, which respond to each of these stimuli by a common proliferation.

The experiments used in the present work are closely related to the experiments referred to above. The present study, however, is connected exclusively with the stage of antibody production. The requirement of heterogenicity of tissues might be found in the differences of the tissues in the adult organism and the embryo. (Even the morphological structure of the same kind of cells, for example the hemocytoblasts, changes somewhat with age, the cells undergoing an ontogenetic development.)

The intense proliferation, exhibited after the appearance in the embryo of heterogeneous substances by all the embryonic hematopoietic organs seems to indicate that different hemato-

poietic organs in the embryo all contain elements, which react in analogous manner to the appearance of these substances. The fact that the proliferating cells exhibit a more homogeneous differentiation in the various embryonic organs than in adult organism may be accounted for by absence of specific structural environmental conditions in the embryonic organs. Though chiefly concerned in histogenesis of the blood cells, the present paper may have a close bearing on the immunity problem in establishing a connection between introduction of heterogeneous substances and reactions exhibited by hematopoietic tissue.

The grafting of an adult spleen on the allantois of the embryo produces changes similar to those described in myeloid metaplasis, and in principle are similar in different hematopoietic organs. However, these changes seem to be differently exhibited in hematopoietic organs at different stages of embryonic development. The stimulating agent may differently influence various cell groups in the hematopoietic organs at different stages of their development and leads often to the appearance of peculiar pathological processes. There appear in different organs characteristic changes depending upon their structural peculiarities which will be studied and described consecutively. As the most conspicuous phenomenon is the appearance of an enormous hypertrophy of the spleen, therefore a study of this organ will give a basis for further investigation of the changes undergone by other hematopoietic organs, including the mesenchyme of different parenchymal organs. A study of changes in the circulating blood of the embryo, as well as a study of the growth and differentiation of the grafts themselves will follow.

2. METHODS OF INVESTIGATION AND TERMINOLOGY

It has been shown in the introduction that the usual methods of investigations applied to the problem of the origin and the mutual relationship of blood cells failed in the attempt of solving them. There must be found new methods for the elucidation

of the problems so unsuccessfully discussed year after year. Such a new method for study of hematological and other biological problems may be offered by the study of transplantations of tissues on the allantois of an embryo and also in the study of the changes, which occur in the tissues of the host after grafting. The influence of identical environment upon different hematopoietic organs may be easily tested by this method. Transplantations on the allantois of embryos were used by Murphy and Rous (30) in their studies of transplantability of tissues to the embryo and by Murphy (29) in his study of the factors of resistance to heteroplastic tissue-grafting. Transplantations of adult spleen and bone-marrow seemed to supply the embryo with a refractory mechanism against heteroplastic grafting, which in a normal embryo is lacking. These transplantations as told are followed by a considerable enlargement of the host spleen.

Every theory is a deduction of a limited number of facts, but if the theory is true, it must apply to all analogous cases. The enlargement of the embryonic spleen, mentioned above, which soon was discovered to be a true hypertrophy, could not be explained from the standpoint of the monophyletic school as an isolated process. The monophyletic school, if true, had to assume that changes in the embryonic spleen were accompanied by analogous changes in other hematopoietic organs. Since the hyperthrophy of the embryonic spleen has been involved by a considerable proliferation of the stem cells, stem cells in other hematopoietic organs admitted by the monophyletic school equal for all, must have been affected also and must have proliferated. Since the structural environmental conditions in the embryo are less differentiated than in the adult organism, the reactions in the different embryonic hematopoietic organs may be expected to be more homogeneous. As will be seen later, this assumption, resulting from the premises of the monogenetic conception of the blood origin, has been fully confirmed by the results of the experiments.

In principle similar to the tissue cultures the grafting method has a great advantage over them. The allantois offers for the

transplanted tissue ideal conditions in supplying the growing tissue with nutritive material and also in withdrawing the products of the metabolism of the developing and differentiating cells. Though the tissue growth and differentiation cannot be observed right under the microscope, this is compensated by the facility of obtaining abundant experimental material in different development stages.

As Murphy and Rous (30), I used the method developed by Peebles for studies in experimental embryology. Since I had to overcome many difficulties, and failures were due sometimes to apparently minute details, I will undertake a thorough description of the culture-method on the allantois of the chick embryo, as I used it in my experiments. I am indebted to the kind efforts of Mr. Ebeling of the Rockefeller Institute for being supplied through the whole winter with fertilized eggs, which developed in the incubator in the number of 60 to 70 per cent. The cultures succeed on the allantois easily from the beginning of the 7th day of incubation. The cultures of the hematopoietic organs grow if in contact with the mesodermal surface of the allantois. The ectoderm, which overlies the area vasculosa and the yolk does not offer favorable conditions for cultures of hematopoietic tissue. From the 7th day of incubation, however, the allantois appears as a well-sized sac, flattened under the eggshell.

It is easy to determine by means of illumination, which of the incubated eggs started to develop. The localization of the embryo body as well as that of the allantois with its vessels comes thereby clearly out. Good places to choose for grafting are regions between the junction of two vessels at a distance of 1 to 2 cm. from the embryo body. The illumination and the provisional marking of the eggs must be done quickly and the eggs immediately returned to the incubator. A sawing out of small windows in the region marked on the eggshell follows. It is advisable to saw the windows in the form of a trapezium, which form allows to orientate it easily, when the window has to be closed. A great care must be observed in sawing the windows out of the eggshell. The pressure of the instrument has

to be slight, otherwise the shell cracks easily outside the region marked. Splits may be stopped by paraffine and the egg still used. As instruments for sawing, small scalpels used in ophtalmologie may be recommended, a few jaggs on their edge are often useful. An experienced hand will easily determine the time when the eggshell is passed through. Then the egg is again returned to the incubator. After all the eggs are opened instruments for aseptic extraction of organs, (a few scalpels, scissors, forceps and bone cutter), and instruments for grafting, (tissue crusher—it is a syringe with a bolt-bottom, another syringe with divisions of 0.1 gram, and a needle, a fine forceps and two pairs of scissors), are sterilized.

All the next work has to be done quickly. The organ, aseptically extracted, is cut in small pieces and passed through the tissue crusher, the tissue mash is then pulled into the syringe and is ready for grafting. Now egg after egg is taken out of the incubator, the shell window is removed, the shell membrane is lifted by the small forceps and an opening cut out with scissors. The allantois becomes visible and often in earlier stages falls off somewhat from the eggshell. Through the needle 0.1 to 0.2 of the tissue is then pushed in, introducing the tissue if possible under the eggshell at least under the shell membrane. Great care must be taken in grafting eggs in advanced stages, because the allantois then bleeds intensely, and the contact of the transplanted tissue with the allantois is removed by the extravasat. No graft usually takes, if the grafted tissue is introduced into the cavity of the allantois or deeper. In these cases the tissue is found floating in the form of a greyish mass, containing numerous small grains.

After the tissue has been implanted on the surface of the allantois, the window is closed by the piece of the eggshell withdrawn and the splits are covered by paraffine. The paraffine of a higher melting degree is preferable, in order to prevent its melting during the following incubation of the eggs. If a local graft is desired any mechanical disturbance should be avoided, otherwise a more diffuse distribution of the grafted tissue is easily obtained. The graft takes more successfully if the egg

during the first twelve hours is put in the incubator, the window down. This secures a closer contact of the tissue introduced with the surface of the allantois. Next day I usually changed the position of the egg in directing the window above. The strict observance of the directions given secures usually 80 per cent of successful cultures. Interesting results in the form of diffuse growth of transplanted tissue were obtained by introducing an emulsion of the tissue mash between the allantois and the chorion under the eggshell. The whole allantois appeared in a few days covered by innumerable small grafts. The same results were occasionally observed also after applying the usual method of grafting.

Twelve hours after transplantation, the tissue is usually in firm contact with the allantois. Later it is attached by numerous vessels growing from the allantois into the tissue. For fixation of the material, it is advisable to free and fix first the embryo after a ligature of the vasa umbilicales is done; then to cut out a large piece of the shell in the region of the culture. The allantois which covers the shell is transported in the fixing fluid together with the shell and is removed from the shell 5 to 10 minutes later. The fixation of the allantois and of the graft is completed in $\frac{1}{2}$ to 1 hour in Zenk.- formol. The celloidin was successfully substituted by the parloidin Dupont, and I may recommend this product as being in no way inferior to the celloidin Schering. The imbedding in celloidin or parloidin remains a sine qua non for hematological work, what easily was deduced by a study of a few specimens imbedded in paraffine last autumn (1915) when no more celloidin was available. Since the staining of preparations attached to the slides and freed from parloidin is more effective, it may be permitted to recall the method of Rubashkin (36), somewhat modified by Danchakoff (9, '08*d*).

The greatest part of the material was stained by eosin-azur and some of the preparations by Dominici and Pappenheim. For the staining of fibrous tissue the iron-hematoxylin and subsequently van Gieson were used. Most of the illustrations are given in black on account of the special temporary conditions,

and one colored plate is added, as example of the preparations, from which the ink drawings were made. The photographs on plates 1 and 2 were kindly made by Mr. Schmidt of the Illustration Department of the Rockefeller Institute.

Since the nomenclature in the hematology has become nowadays extremely complex and often under one name different cell units are understood, or even oftener one cell unit is termed by different names, it is useful to state in advance what terminology will be used in this paper.

The stem cells of the different blood elements, which first appear after isolation of the blood-islands, have the structure of the well-known large lymphocytes. The term of large lymphocytes was applied for these cells by Pappenheim (33), Danchakoff (9) and Maximow (25). Studies of hematopioetic tissue led to recognize everywhere cells of the structure of large lymphocytes and to identify them as stem cells for the blood tissue. Only few differentiation potencies were first assigned to the large lymphocytes. This however corresponds but little to the various differentiation potencies, exhibited under different conditions by this cell. So the name of large lymphocyte seemed to correspond little to the given cell in its new conception. Since personal studies did not give me any data, bestowing all the lymphatic cells with equal potencies, the less appropriate seemed to me the name of large lymphocyte in connection with the stem cell for different blood elements. In my last papers I called the stem cell, which in itself is a good name, lymphoid hemocytoblast—lymphoid in order to take into consideration its morphological structure, hemocytoblast on account of its potencies to differentiate into various blood cells. The same name will be used throughout the paper. The names of erythroblasts and erythrocytes do not require any explanation.

The names of myeloblast and myelocyte seem to me unfitted for the purpose used. This name is wrongly applied to cells which neither appear first in the bone-marrow, nor are cell units exclusively characteristic of this organ at any time of its existence. These terms will be substituted, as in my previous papers, by granulocytoblasts and granulocytes or leukocytes.

However, the term myeloid tissue, or myeloid metaplasis, is used throughout the paper as a collective name, under which all the characteristic cell elements of the bone-marrow are understood, it is the erythroblastic, granuloblastic tissues, and respectively the megakaryocytes. The terms promyelocytes, metamyelocytes, mikromyelocytes, which correspond to intermediate stages between a lymphoid hemocytoblast and a leukocyte are omitted. These stages are characterized by unessential features and are often overstepped in embryonic life during intensive regeneration. The use of so many terms for expressing small differences between development stages in a cell lineage seems more to confuse than to help. For this reason I did not introduce them lately in the scheme given in the Anatomical Record, and in the present paper, only terms which designate definite morphologically well defined stages will be used, and these are lymphoid hemocytoblast, granulocytoblast, and granulocyte or leucocyte.

The reciprocal relations in the lymphatic cell group are somewhat more obscure. The different lymphatic cells are looked upon by Maximow (25) and Weidenreich (44) as being merely temporary appearances of young undifferentiated cells, all characterized by the same differentiation potentialities. Though these authors admit a specific morphological structure for the large and the small lymphocytes and the histogene wander cell, yet they assume that these cells may easily change reciprocally their structure according to the environmental conditions. In birds and reptiles (Danchakoff (9)) as well as in mammals (Maximow (25)) it is easily demonstrated that small lymphocytes may both proliferate and differentiate further, but their lines and products of differentiation are not identical with those of the large lymphocytes (Pappenheim (32), Danchakoff (9)). Neither is it proved definitely that the small lymphocytes may grow into the large. Nor is a possibility of erythrocyte development at the expense of small lymphocytes shown to exist in birds and reptiles, as Freidsohn (14) admits lately for amphibians and Venzlaff (43) for birds. Therefore, under the name of small lymphocyte, cells characterized both by a definite

morphological structure and by well defined differentiation potencies will be understood.

The histogene wander cells, which term I substituted by histiotopic wander cells seem to be in close relation to the hemocytoblasts and often are merely an intermediate but morphologically well defined stage of development between a mesenchymal cell and a hemocytoblast.

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3. HISTOGENESIS IN THE SPLEEN IN RELATION TO STRUCTURAL ENVIRONMENT

A. HISTOGENESIS IN A NORMAL CHICK SPLEEN

The study of histogenesis in the spleen by the method of stimulation of the stem cells in the spleen anlage requires a thorough knowledge of the histogenetic processes, which normally take place in the spleen. The early stages of spleen development in birds were studied by Tonkoff (41). Yet no investigation of embryogenesis of the characteristic spleen elements was made by modern technique. The general lines of differentiation of the spleen elements were studied by Danchakoff ('16a) in Tropidonotus natrix, but the question—what are the conditions which determine the differentiation of the polyvalent stem cell—remained unsolved. It seems, therefore, necessary to make first a study of normal spleen development in the chick, and to attempt to determine the conditions which imply the various differentiation of the stem cells.

The appearance of the spleen anlage in the chick embryo and its first development corresponds to the description given by

Tonkoff (41) in 1900. The spleen anlage appears in an embryo nearly 4 days old—in the mesenterium dorsale duodeni in the region of pancreas dorsale. It is distinctly separated from the coelomic epithelium, which surrounds it. In the I stage of its appearance the spleen anlage is purely mesenchymal. It is distinguished from the surrounding mesenchymal tissue by the denser appearance of the tissue. At this time mesenchymal cells of the anlage present short ramifications, which soon are lost, the cells multiplying intensely and joining finally in a common syncytium (fig. 5 and 6). The spleen anlage at early stages is identified rather by its localization than by the character of its cells. Similar agglomerations of syncytium-like mesenchyme are encountered in many other places, and their further development exhibits a great analogy with that of the spleen anlage, resulting in a differentiation into lymphatic tissue. The more intense proliferation of the mesenchyme in the region of the spleen is evidently due to local favorable conditions. The development of the spleen at the expense of mesenchymal cells without any relation to the endoderm nor to the coelomic epithelium may be regarded as a well-founded fact.

The appearance in the spleen anlage of numerous ameboid cells, the lymphoid hemacytoblasts, and their differentiation into granulocytoblasts and into small lymphocytes was described by Danchakoff (9) ('16a), in Tropidonotus natrix. The fact that the small lymphocytes develop in the spleen in later stages was also noticed. However, it is improbable that the different stages in themselves should be accounted for as differentiating factors. If the small lymphocytes do appear later, the granulocytoblasts nevertheless continue to differentiate in the spleen, at least in embryonic life. Conditions for both lines of differentiation must therefore coexist in later stages. The differentiating factors should be sought rather in different structural conditions, appearing at definite stages, and determining from the time of their appearance the lines of differentiation of the polyvalent stem cells.

Since the structural peculiarities are exhibited in a more striking manner in the spleen of an adult or a young chick, it

may be advantageous to demonstrate them by a study of a fully developed spleen, and then to attempt to find out whether the gradual development of the peculiarities influence the histogenesis of the hematopoietic tissue. A distinctive feature in the spleen structure is given by its special vascularization. The regions with veinous and arterial vascularization, though they penetrate each other, remain nevertheless independent, and communicate together only in places, where the white pulpa passes into the red. The most characteristic cell element of the spleen—the small lymphocyte—belongs to the white pulpa and accumulates here in the form of follicles and follicular strings. The granulocytoblasts, though not numerous in the adult spleen, are chiefly localized in the pulpa. Other ameboid elements, the basophilic large lymphocytes (lymphoid hemacytoblasts), and mononuclear leucocytes, often in the form of macrophages, are common to all the regions of the spleen. The syncytial cell reticulum is also ubiquitous; it forms in the red pulpa wide meshes. In the white pulpa the cells of the reticulum appear denser, and the meshes formed by their ramifications are smaller.

As a further study of the spleen development will show, the chief characteristic feature, primarily, determining different regions of the spleen as red or white pulpa, consists in the type of vessels by which a region of the spleen is supplied rather than by the presence of certain kind of ameboid cells. The wide veinous capillary net together with sinuses and lacunae forms the red pulpa, the bunches of arteries resolving themselves into a net of narrower branches—belong to the white pulpa. The question, whether the specific differentiation of the ameboid elements depends upon the peculiar vascularization of the spleen may be decided after a study of the spleen development. The chick spleen is a favorable subject for elucidation of this question, for the identification of the vessels is easy in the spleen anlage from the time of their appearance.

As mentioned above, the development of the spleen in the earliest stages is characterized by its loose mesenchymal structure. The intense cell proliferation leads soon to a transformation of the loose mesenchymal anlage into a denser syncytium.

Numerous basophilic cells differentiate at the expense of the syncytium and become ameboid (figs. 5 and 6, *L. Hbl.*). Acidophylic granules appear in the cytoplasm of a part of these cells and characterize them as granulocytoblasts (fig. 8, *Grbl.*). The development of these cells (lymphoid hemocytoblasts and granulocytoblasts) is not specific for the spleen mesenchyme and is observed in other regions of the embryo body also. At the same time the spleen anlage becomes vascularized, and its vascularization is at this stage of its development exclusively veinous. In the peripheral layers of the anlage, later on through the whole organ, appear splits, which evidently are filled by a liquid, which separates the cells. These splits are at first surrounded by the irregular surface of the mesenchymal cells. Some of the cells show still their processes, projected in the lumen of the sinuses. (fig. 6, *S.*). These sinuses soon join together and form a net. From the other hand a communication with branches of the intestinal veins is established. The whole mesenchymal anlage exhibits at this second stage of its development a spongious structure. Whether the appearance of the splits in the anlage is due to a secretion of the surrounding cells, or to a transudation of a liquid through the vessels growing from outside is difficult to determine. There is, however, no doubt that the splits mentioned are of local origin. This has been shown by Laguesse (23) in fishes.

The appearance of the splits in the tissue of the spleen anlage is accompanied by more intense isolation of ameboid cells (fig. 6, *L.Hbl''*). Some of them are surrounded by a developing sinus and become situated in its lumen. These cells have invariably first the structure of lymphoid hemocytoblasts (large lymphocytes). Similar cell groups are not seldom encountered in the larger sinuses of the peripheral layers in the spleen. (fig. 5, *S*). As soon, however, as these lacunae unite with the veinous vessels, what is indicated by the sudden appearance of differentiated erythrocytes within the lacunae, the lymphoid hemocytoblasts begin here their differentiation into erythroblasts (fig. 5, *Erbl.*). The plasma of the blood must evidently contain factors for differentiation of lymphoid hemocytoblasts into ery-

throcytes. The slowness of the blood current in the large veinous capillaries and sinuses offers moreover favorable conditions for this line of differentiation.

The vascularization of a normal spleen proceeds, however, gradually. The cells, surrounding the splits, become gradually flattened and finally form an even endothelial surface (fig. 8). The sinuses of a normal spleen contain usually merely a few young cells undergoing an erythroblastic differentiation. I do not think it right therefore to consider the normal embryonic chick spleen as an active erythropoietic organ, though potentially it must be considered as such. It may be noticed that spleens of embryos at the same stage may offer in this respect well pronounced individual differences. New sinuses continue to appear with the growth of the spleen. In later stages, at the 11th day of incubation, as the fig. 7 shows, in newly formed sinuses there may be found large groups of lymphoid hemocytoblasts, which soon undergo an erythroblastic differentiation. Between the new formed vessels the mesenchyme continues to proliferate and to split off lymphoid hemocytoblasts. They multiply also and partly differentiate into granulocytoblasts, which increase also their number by mitosis and partly differentiate further into granulocytes (figs. 8 and 9).

The processes of growth and differentiation proceed slowly in a normal spleen. In an embryo of 9 days—5 days after the beginning of its development—the size of the spleen reaches 1—1.5mm the long diameter. The abundance of nuclei however may serve as index of intense proliferation processes taking place in the syncytial tissue of the embryonic spleen. The spleen during its II stage of development is characterized by a development of a net of wide veinous capillaries developed in the mesenchymal syncytium, by an intense granulopoiesis outside the vessels and by a potential erythropoiesis within the vessels. These lines of differentiation, as known, are also characteristic of the myeloid metaplasis in the spleen pulpa. The study of the normal spleen development leads to the conclusion that the first differentiation processes of the mesenchymal spleen anlage transform it into a pulpa-like organ (figs. 7 and 8). The spleen remains pulpa-like until the 12 to 13 day.

The intense development of the arterial vascularization begins at this time and the spleen enters in the III phase of its development, characterized by the appearance of follicles. A cursory glance on spleens during development of arteries brings forth a striking difference between the development of the veinous and of the arterial vascularization. The arteries and their smaller branches never appear as irregular splits limited by mesenchymal cells. These vessels on the contrary always develop as regular narrow tubes (fig. 9, *Art.c.*) I did not intend to undertake a special study of the vascularization of the spleen, therefore I did not apply special methods of investigation for this purpose. However, a thorough study of preparations may give some information upon the development of the veinous and arterial vascularization. The veinous sinuses and capillaries are local formations, the arteries and their branches seem to grow into the spleen from outside and here ramify by budding (fig. 9).

The arteries, growing into the pulpa like tissue of the spleen, divide it in regions, which are soon further subdivided by smaller arterial ramifications. The arteries lie first in the mesenchyme, where numerous granulocytoblasts are present (fig. 9). The mesenchymal cells continue to proliferate around the arteries. The process of splitting off of lymphoid hemocytoblasts persists in these regions, but their differentiation into granulocytoblasts is suspended under conditions in which the mesenchymal cells develop around the narrow arteries. These conditions do not correspond to those which prevail around the thin walled veinous sinuses. The arteries and their smaller branches finally become surrounded by clear zones of mesenchymal cells (fig. 10), which markedly contrast with the granuloblastic tissue. These zones appear in preparations in the form of islands of mesenchymal tissue, which fill up all the interstices between the arterial vessels and the pulpa like tissue of the spleen anlage. These islands are anlages of follicles.

At the time of the intense development of the mesenchyme around the arteries a new line of cell differentiation may be traced in the spleen which soon will become predominant; this is the differentiation of small lymphoctyes. The figures 12

and 13 show the development of small lymphocytes in the follicles. From right to left the figure 12 represents the tissue of the follicle from the periphery to its center. In the peripheral parts of the follicle an intense isolation and proliferation of lymphoid hemocytoblasts takes place (5 mitoses in a part of the microscopical field) which leads to the formation of dwarf hemocytoblasts (fig. 12 *S.L.Hbl.*). A process of differentiation, starting in the groups of small hemocytoblasts (fig. 12) soon transforms them into true small lymphocytes (*S.Lmc.*). The cytoplasm around the nucleus of these cells becomes smaller, the typical nucleolus of the hemocytoblast is replaced by chromatin particles which may be now permanently discovered in the nucleus (fig. 12, *S.Lmc.*). A similar development of small lymphocytes is shown in the figure 13 in the region adjacent to the red pulpa (right edge of the drawing). Both in the red and in the white pulpa numerous larger and smaller hemocytoblasts are present and offer in both regions similar morphological structures. In both regions they sometimes appear as groups of cells joined together,—probably an index of their syncytial origin. The further differentiation of the lymphoid hemocytoblasts is shown to be different, according to their localization. In the red pulpa they are in close contact with the larger veinous capillaries and differentiate into granulocytoblasts. In regions with scarce arterial vascularization they undergo a differentiation in small lymphocytes.

As in the thymus, the differentiation of small lymphocytes is preceded in the spleen also by development of generations of small-sized hemocytoblasts. Their appearance in the thymus seemed to depend upon an intense proliferation of the cells in a limited space. If the cells in the spleen are not heaped up, as they are in the thymus, yet the analogy of the conditions for their differentiation in both regions may be easily traced. Both in the thymus and in the spleen the small lymphocytes develop under conditions of poor nutrition. They appear in regions of the spleen where the swift blood current passes by the narrow arterious vessels.

The first groups of small lymphocytes differentiate between

the 15 to 17 days of incubation. They increase their number by differentiation at the expense of lymphoid hemocytoblasts and by their own proliferation. They gradually infiltrate the regions with arterial vascularization; finally they accumulate in dense masses around the arteries. One may observe amongst them a few degenerated cells, even in an embryonic spleen; they are phagocytosed by mesenchymal and endothelial cells and sometimes even by hemocytoblasts. A number of mesenchymal and endothelial cells are gradually transformed into typical macrophages (Evans) (15). Similar macrophages are observed also in the lumen of the sinuses, where they lie free, or form a part of the surrounding mesenchymal tissue (fig. 17, 18, 19). In the pulpa, however, the activity of the macrophages is directed chiefly toward the degenerated erythrocytes. Though at this time the connection between the arterial and the veinous vessels is completed, these regions continue to be very distinct.

The appearance of the follicles and of the new line of cell differentiation does not seem to influence the life of the cells in the pulpa. Both the granuloblastic and the lymphoblastic processes of differentiation coexist now in the spleen, and are displayed by lymphoid hemocytoblasts in different regions under different structural conditions. The granulo- or leukopoiesis develops around the large veinous sinuses under conditions identical to those under which they develop in the yolk sac and in the bone-marrow. The leukopoiesis in the spleen is much reduced after the embryo is hatched, and the spleen becomes chiefly a lymphopoietic and erythrolytic organ.

The structural peculiarities which determined the various lines of differentiation of the polyvalent stem cells remain in the adult spleen unchanged. Different stimulating agents may cause a proliferation of the stem cells. Their differentiation, however, will correspond to the structural environmental conditions to which they are submitted. It is therefore only natural that the myeloid metaplasis in the adult spleen should develop in the pulpa, where the structural conditions determine normally a granuloblastic differentiation of the hemocytoblast. A differentiation of small lymphocytes in the pulpa, or vice

versa, could be expected only in the case if the structural conditions of the pulpa be changed and corresponded to those in which the small lymphocytes normally develop.

The study of the normal development of the spleen in the chick allows the following conclusions:

1. The anlage of the spleen develops from the mesenchyme of mesodermal origin. It appears at the end of the 4th day of incubation. It consists first of a loose mesenchymal tissue, which gradually becomes denser and finally is transformed into a syncytium.

2. The development of a veinous vascularization transforms the spleen anlage into a homogeneous pulpa-like tissue. This stage is characterized by a well developed granulopoiesis and a potential erythropoiesis. The stem cell for both directions of differentiation is the lymphoid hemocytoblast, which develops at the expense of mesenchymal cells. The line of the stemcell differentiation depends upon the conditions to which the stemcells are submitted.

3. The development of the follicles and of the lymphoid tissue coincides with the development of the arterial vascularization. The small lymphocytes develop at the expense of the lymphoid hemocytoblasts and their differentiation is preceded by appearance of generations of small-sized hemocytoblasts. The small lymphocytes in the spleen, as well as in the thymus, appear in regions, characterized by special structural conditions of the environment.

Different kinds of cells appear, as final results of differentiation processes, observed in the spleen. Reticular tissue cells, and endothelial cells, erythrocytes, granulocytes, small lymphocytes and macrophages develop in the same organ. Can anything definite be told on the basis of the study of normal spleen development about the origin of these cells? Are all the cells of the mesenchymal spleen anlage identical and polyvalent, their further development being determined by the structural environmental conditions, or though apparently identical in morphological structure their potencies to different development are predetermined by intrinsic imperceptible differences between them?

The general outlines of the development of the spleen anlage

under normal conditions should be regarded as necessarily determined. The organ develops in a complex environment of other organs which proliferate and differentiate at the same time. Each of these factors, which necessarily ensues from the collective action of all the surrounding conditions, becomes itself one of the factors which partly impels the results of the general differentiation. The necessity and mutual dependence of the development processes result from reciprocal influence of the factors and is characterized by striking purposefulness. The general outlines of the development may therefore be changed, as will be shown later, only in the case if the regular interaction of factors has been disturbed.

Variations in the intensity of differentiation of various cell groups may be observed, however, in normal spleens. Numerous or merely scant lympho- and granulocytoblasts may develop at the expense of mesenchymal cells. The proliferation of granulocytoblasts may also be more or less intensive. But this differentiation process is extended in the spleen anlage more or less uniformly at a time when the anlage presents a homogeneous structure and is lacking special conditions of vascularization. The differences in the extent of the normal granulopoiesis are, however, not excessive and deviation in other lines of differentiation may compensate them. A new line of differentiation necessarily starts with the appearance of new structural characters, namely with the development of the arteries. After this new factor has been established, it will permanently influence the cells, which stand under its control. The existence of regular unchangeable relations between definite structural conditions and differentiation of certain kind of cells seems to explain sufficiently the necessity which appears in a group of identical stem cells to diverge in their further development. Is there a need of recurring to invisible differences between cells, lest it should be necessarily required by deduction from the results of development?

One phase in the spleen development could, however, be interpreted by the dualists in their favor—this is the apparently late ingrowth of the arteries in the pulpa-like spleen anlage. If both

arteries as well as veins developed in loco, there would remain no doubt in the common origin of the lymphatic and the myeloid tissues. The ingrowth of the arteries may be regarded as a mere stimulus for a new intense proliferation of the mesenchyme and its subsequent differentiation into small lymphocytes. On the other hand, the ingrowing vessels might have brought new cell material for a new line of differentiation, which starts at this period. It is difficult to establish with certainty whether the mesenchymal cells of the follicle anlages develop at the expense of the local mesenchyme, which remains undifferentiated, or whether they are derived from the tissue brought by the growing arteries. No matter how this question will be solved, both in the pulpa and in the follicles, the mesenchymal cells bear the same morphological structure. They present moreover in their first differentiation stages undeniable analogies. In the pulpa as well as in the follicles they partly differentiate into lymphoid hemocytoblasts and partly form the so-called reticular tissue. The special differentiation according to environmental conditions is then undergone by the lymphoid hemocytoblasts.

The dualists admit that the development of the myeloid metaplasis of the spleen, which under definite pathological conditions is localized in the pulpa is due to proliferation and differentiation of adventitial or endothelial vascular cells. Endothelial cells are present both in the pulpa and in the follicles, the adventitial cells are even more numerous in the arteries of the follicles. If the strain in the further differentiation of the lymphoid hemocytoblast has to be laid in the cell itself and not in the physico-chemical conditions, given by structural peculiarities of the environment, the myeloid metaplasis should have appeared as a diffuse process, for endothelial and adventitial cells are found in the pulpa as well as in the follicles. In order to remain consequential, the dualists ought to recognize various kinds of adventitial and endothelial cells—the ones being capable of a myeloid differentiation, the others not.

The striking dependence of the various differentiation of morphologically identical cells upon a change in the environmental conditions may greatly strengthen the monogenetic

conception of the blood origin. It cannot, however, be considered as definite proof, because various differentiation products could under different conditions derive from isomorphic but heteropotential cells. The study of the spleen development under influence of stimulation of its stem cells will give further information about causal relation between structural environmental conditions and development of the polyvalent stemcells.

B. HISTOGENESIS IN A CHICK SPLEEN AFTER STIMULATION

An intense stimulation of the mesenchymal spleen anlage is obtained by means of grafting a tissue mesh of an adult spleen on the allantois of a chick embryo. The photographs on figures 1 and 2 demonstrate the enlargement of the host spleen. Both photographs belong to an embryo of 18 days. The spleen is enlarged in both embryos. While the spleen on figure 1 as compared with a normal is enlarged approximately 4 times the spleen on figure 2 shows a much more intense enlargement. The long diameter of the spleen at this stage is about 1–2 mm. After the stimulation it may exceed 1 cm. It is remarkable that the enlargement of the spleen is usually in a visible relation to the intensity of the graft growth. The spleen *a* corresponds to the graft on figure 3, the spleen *b* to the graft on figure 4.

The macroscopical appearance of the enlarged spleen does not give any indication whether the spleen enlargement is due to a local proliferation of the embryonic tissue. This proliferation could be incited by heterogeneous products of metabolism, which from the transplanted and growing cells could penetrate in the vessels and be transported by the blood current into the embryo body. On the other hand, cells from the graft could be transported in the spleen and here under favorable conditions proliferate. Tumor like accumulations of tissue would develop in the last instance and enlarge the spleen. The surface of the spleen after stimulation is often covered by protuberancies, numerous small whitish points may be seen on its section. These facts at first suggested rather the idea that the enlargement of the spleen was caused by tumor-like metastases brought by the blood

current from the growing graft. However, the systematical study of the gradual enlargement of the spleen urged to finally admit a growth of the embryonic spleen tissue in loco. This local growth, at least in the beginning, seemed to be caused by an intense uniform stimulation of the mesenchymal cells and of the lymphoid hemocytoblasts and their further differentiation. After the hypertrophic character of the enlargement of the spleen had been established, it was proposed on the basis of this fact to test the validity of the monogenetic conception of the blood origin. If true, the monophyletic interpretation implied analogous changes in other hematopoietic organs as seen in the spleen. The chief character of the changes observed in an embryo,after grafting of an adult spleen, consists indeed in a stimulation of the whole hematopoietic tissue. The hematopoiesis, stimulated experimentally follows strictly the fundamental principles, established for birds and reptiles during embryonic and adult life. The hematopoiesis develops however in a peculiar way; first in that groups of cells, which normally would slumber indefinitely, become involved in the process; secondly, that an outburst of hematopoiesis is incited at a time when the normal hematopoiesis is conveyed in definite channels; and finally, that various directions of differentiation are displayed by hemocytoblasts in places where they are usually absent. The study of the changes in the spleen after grafting will form a basis for a comparative study of the changes, incited by the same intervention in other hematopoietic organs.

Since the stimulation leads to different changes according to the stage at which it has been applied, the results of the stimulation in early and later stages will be described separately. As mentioned, grafts of hematopoietic tissue on the chick allantois take easily, if grafted from the end of the 6th day of incubation and on. The grafts, made before the establishment of arterial vascularization in the spleen, incite in the spleen anlage uniform proliferation, which may considerably vary in intensity. The changes are different if the stimulation has been applied after the arterial vascularization has taken place.

a. Changes after stimulation in early stages

Transformation of the mesenchymal spleen anlage into a uniform granulobastic tissue. The study of the normal spleen development has shown that the spleen anlage at the stage of 6 to 8 days consists of a more or less dense mesenchymal tissue in the form of syncytium. The oblong nuclei, characterized by the presence of well pronounced nucleoli and scant chromatic particles lie closely together. At the periphery of the organ the nuclei are more scattered and the tissue looser. The limits of the cells are here also undefinable, for the cells all are united together by numerous short processes. In the peripheral layers of the organ sinuses are already developed, their connection with the veinous circulation is effected and the stimulating substances find easy access into the organ.

The first effect of the stimulation is exhibited by an intense proliferation of the mesenchymal cells and numerous mitoses. The sinuses spread swiftly over the whole organ and form a net with large meshes. The protoplasm of the mesenchymal syncytium loses soon its uniform structure and becomes less dense; numerous vacuoles appear. At the same time many of the cells become isolated and lose their connection with the plasmodial cell mass. These cells appear intensely basophylic and are very ameboid in their character.[3]

An intense development of lymphoid hemocytoblasts is seen on figure 6. The differentiation of lymphoid hemocytoblasts can assume such proportions, that the greatest part of the mesenchymal anlage may be converted into free ameboid cells and merely a few mesenchymal cells may remain between them (fig. 20). No regular development of sinuses can take place in such cases. The lymphoid hemocytoblasts multiply intensely, many of them show a beginning differentiation into granulocytoblasts and acidophylic small granules appear in their

[3] The changes which occur immediately after stimulation at early stages of embryo development merely accelerate and intensify the normal histogenetic processes in the spleen. Therefore illustrations given on plate 3, figures 5 and 6, refer at the same time to normal development as well as to development after stimulation.

cytoplasm. Numerous spleens of embryos, which were grafted in early stages were transformed 4 to 5 days after grafting into a tissue, similar to that represented on the figure 20.

The ameboid cells appear here pressed closely together, are often of polygonal form and fill up all the interstices between the few sinuses. This uniform wide-spread conversion of the spleen anlage into a hemogranuloblastic tissue offers some points of general interest. These changes show first, that the action of the stimulus applied is intense and imperative. Products of metabolism appear in the blood current from the growing graft cells. These substances incite an intense proliferation of the mesenchymal cells in the spleen anlage and swiftly transform the syncytial plasmodium of the anlage into an accumulation of ameboid cells, which proliferate and differentiate further. The results may remind one of the experiments of Rous,[4] in which connective tissue cells became spherical under the action of trypsin.

As mentioned above the transformation of the mesenchymal cells into lymphoid hemocytoblasts in the spleen anlage may be universal, if stimulation is applied in early stages and merely a few mesenchymal cells may remain undifferentiated, while in normal development numerous mesenchymal cells persist and give in course of time the reticulum tissue. Mollier (28, '11) describes this tissue as a cellular syncytium with a fibrous net adjusted to the cells. The changes in the experiments reported are so intense (fig. 20), that it may be right to assume that numerous cells, which under normal conditions would have passed into the reticulum tissue, now become hemocytoblasts and differentiate further into granulocytoblasts. This result can be taken as a corroborative proof for the polyvalency of the mesenchymal cells, which may either differentiate into granular leucocytes or become a constituent part of the reticular tissue. The general granuloblastic differentiation of the mesenchymal spleen anlage is observed in embryos to which the stimulation was applied before a development of vascularization has taken place.

[4] Personal communication.

Development of intense erythropoiesis after stimulation.—The stimulation of the lymphoid hemocytoblasts to intense proliferation may influence the development of the vessels and of their contents in the mesenchymal spleen anlage. As above stated, the stimulation in early stages leads often to defective development of veinous sinuses and large parts of the spleen anlage are transformed into granuloblastic tissue. If stimulation is applied a little later, at the ninth day, when a number of sinuses are already formed, others still appear, a development of intense erythropoiesis may be observed in the spleen anlage. The normal spleen tissue at this stage consists of a mesenchymal tissue, in which numerous hemocytoblasts continue to develop. The stimulation of the lymphoid hemocytoblasts and the simultaneous opening of splits in the mesenchymal tissue leads to the appearance of numerous hemocytoblasts within the vessels. Larger groups of lymphoid hemocytoblasts may occupy the lumina of the sinuses and here undergo an erythroblastic differentiation. Figure 15 shows a similar split developed locally in the spleen mesenchyme. It is of irregular form and surrounded by mesenchymal cells. These cells enter as a constituent part into the general spleen tissue and send some of their processes within the lumen of the sinus. A development of numerous lymphoid hemocytoblasts is observed in this tissue (fig. 15 *E.L.Hbl.*) and large groups of lymphoid hemocytoblasts are discharged in the lumen of the sinuses (fig. 15, *I.L.Hbl.*). Outside the vessels the lymphoid hemocytoblasts differentiate into granulocytoblasts (fig. 15, *Grbl.*), as elsewhere, within the vessels they develop into erythroblasts (fig. 20, *Erbl.*) and erythrocytes (*Erc*). The process of the normal differentiation of a lymphoid hemocytoblast into an erythrocyte was studied in birds by Danchakoff (9) and corresponds to what is seen under the condition of an experimental stimulation. Therefore I refer in this respect to my previous papers on the development of different hematopoietic organs in birds and reptiles.

Figure 16 shows the spleen tissue in later stages (four days after stimulation of an eight day embryo). The veinous sinuses appear surrounded by flattened mesenchymal cells. The inner

surface of the sinuses appears even and regular and is covered by cells which resemble endothelial cells. The vessels still contain a large number of young undifferentiated blood cells which continue to proliferate and to differentiate. The tissue between the vessels begins to assume the character of a reticular tissue, in the meshes of which numerous free ameboid cells are lying. They chiefly consist of lymphoid hemocytoblasts, granulocytoblasts, and granulocytes (fig. 16, *E.L.Hbl.*, *Grbl*, *Grc*) together with cells at intermediate stages of differentiation. However a few macrophages may occasionally be seen.

The intense development of erythropoiesis and the activation of granulopoiesis convert the spleen at this stage into a true myeloid organ. As above stated, an occasional differentiation of lymphoid hemocytoblasts into erythroblasts may be observed during normal development of the spleen. Under stimulation large groups of lymphoid hemocytoblasts fall into the developing sinuses. At this time the vascularization in the spleen appear as a net of large veinous capillaries annexed to the portal system and the blood current must be here very slow. Situated in the sinus lumen the lymphoid hemocytoblast is not conveyed away by the blood current, but it remains in the sinus, proliferates and differentiates into a red blood cell. The conditions of the development of the erythropoiesis in the spleen correspond to those known in the yolk sac annexes and in the bone-marrow. A development of large veinous capillaries with a slow blood current and a close connection between erythro- and granulopoiesis is seen in all these regions.

However, the erythropoiesis incited by the experimental stimulation does not persist in the spleen permanently. When the arterious vascularization develops and the connection with the sinuses is effected, the blood current becomes swifter, the younger cells are withdrawn and gradually replaced by differentiated erythrocytes. In other cases in which the arterial vascularization develops defectively and no regular food supply is established a lack of nutritive material finally may determine the suspense of erythropoiesis. Large macrophages develop in the sinuses and an intense phagocytosis of erythrocytes may be observed (figs. 17, 18, 19).

The intense development of erythropoiesis at the expense of lymphoid hemocytoblasts, which develop locally is another striking evidence for the truth of the monogenetic conception of the blood development and consequently for the polyvalency of the mesenchymal cells in the spleen anlage. The same mesenchymal cells, which normally would remain between the vessels and either become a part of the reticular tissue or differentiate into granular leucocytes now differentiate into red blood cells after they are discharged into the vessels.

Deficiencies in the development of vascularization and their effects. —The deficiencies in the vascularization of the spleen anlage occur as results of the changes in the spleen incited by stimulation of the stem cells. They can affect either the development of the veinous net or the ingrowth and the distribution of arteries.

The stimulation applied at the 7 to 8 day of incubation may convert the whole spleen anlage or considerable parts of it into granuloblastic tissue. The sinuses develop thereby defectively and considerable accumulations of granuloblastic tissue are often provided merely with scant and narrow capillaries. The scarce development of veinous sinuses is the natural sequence of the excessive differentiation of granuloblastic tissue. The study of the normal spleen development has shown, that the sinuses develop locally as splits amidst the mesenchymal tissue. Since the greatest part of mesenchyme may be transformed into accumulations of free ameboid cells (fig. 20), the net of sinuses cannot locally develop between the free cells.

Defects in the development of veinous sinuses become themselves the source of interesting alterations in the spleen tissue. The normal histogenesis leads to a gradual differentiation of granular leucocytes at the expense of granulocytoblasts. The granulocytoblasts, though ameboid, do not migrate normally in the vessels which evidently do not contain adequate chemiotactic substances. The granular leucocytes, however, penetrate easily into the veinous sinuses through thin walls (fig, 11, *Z*) and are drifted by the blood current away. Though the differentiation of granular leucocytes is in a normal embryonic spleen continuous,

they never accumulate in the tissue. The conditions develop quite differently after stimulation, and lead to an excessive granulocytspoiesis (figs. 20 and 21). In this case two factors work together in one direction—first: the granular leucocytes are formed in excessive numbers and secondly vessels develop defectively. Considerable accumulations of granular leucocytes remain therefore in the spleen tissue. They may densely infiltrate the mesenchyme, if there is mesenchyme tissue left (fig. 21).

On the other hand, they may form enormous agglomerations in the form of spherical masses of semi liquid tissue and finally perish (fig. 22, *Grc.'''*). Centers of necrotic tissue appear in the spleen as a result of the excessive production and stagnation of granular leucocytes. The development of large accumulations of granular leucocytes are interesting in so far as it seems to indicate that in the particular case cells cannot stop in their development. The reactions displayed by these living cells necessarily lead the cell to the last stage of its differentiation.

The universal and imperative conversion of the spleen mesenchyme into granuloblastic tissue also offers an example of tissue reaction, which being a response to the stimulation, seems to lack the character of purposefulness. The stimulation breaks up the reciprocal normal proportions of the development processes in the spleen. The exclusive development of granulopoiesis in the spleen-anlage leads to formation of considerable centers of completely differentiated cells which finally succumb in large masses. The embryos, in which such wide spread changes are observed, do not hatch, and usually die after 16 to 19 days of incubation.

Around the necrotic centers, if they are not too considerable in size and numbers, a characteristic reaction of mesenchymal tissue may be observed. The mesenchymal cells proliferate and form plasmodial masses in the form of giant cells around the necrotic center (fig. 22, *Gt.c*). The specific appearance of the nuclei accumulations may suggest here also the idea of occurrence of amitosis (fig. 22, *Y*). These giant cells are perfectly similar to those encountered around the foreign bodies. Though their

appearance seems to be purposeful, I do not feel right to take them for such. It seems more correct to attribute their appearance to a reaction similar to that which characterizes the development of macrophages—reaction to particulate matter, Evans (15). The considerable hypertrophy of their cytoplasm is brought by digestion of phagocytozed material. Smaller necrotic centers can undergo a complete resorption by giant cells. The formation of giant cells around necrotic centers can serve as an example of a new differentiation potency assigned to mesenchymal embryonic cells.

Besides deficiencies in the development of veinous vascularization, irregularities in the development of arterious vascularization may also be observed. A partial lack of development of arterious ramifications in districts of the spleen is a frequent occurrence under the conditions of the experiments. A partial deficiency in the veinous vascularization usually coincides and this leads to a nearly complete lack of vascularization in parts of the spleen. In such regions mesenchymal cells are still capable of proliferation, but they soon develop very differently than they do normally, namely, they become fibroblasts. Large regions of the spleen can be transformed into typical connective tissue (fig. 23). The elongated cell bodies may appear pressed closely together, in other instances they are separated by accumulations of collagenous fibers. A few mitoses are usually observed in these cells. Sometimes the presence of a few lymphoid hemocytoblasts or granular leucocytes may indicate a more intense hematopoietic differentiation which previously has taken place in such regions.

The development of fibrous tissue in the embryonic spleen completes a whole range of transformations to which the young mesenchymal cells are capable. The leucocyte, the erythrocyte and perhaps the fibroblast and the macrophage are under normal conditions final non interchangeable differentiation stages; the endothelial, the giant and reticulum cells are different morphological structures, which a mesenchymal cell may assume. All these cells derive from one stemcell, and its differentiation depends upon the various conditions to which the polyvalent cell is submitted.

b. Changes obtained by stimulation in the stage with definite spleen vascularization

Studying the myeloid metaplasis, Hertz (20) found that besides an intense development of granuloblastic tissue in the pulpa, well pronounced changes in the follicles appeared also. The tissue of the whole follicle might have undergone a differentiation into large lymphocytes (lymphoid hemocytoblasts). The changes observed in the spleen after stimulation in later stages (at 14 to 15 days of incubation) in which the structural peculiarities of the organ are developed, correspond closely to those described by Hertz. The large lymphocytes (lymphoid hemocytoblasts) develop, according to Hertz, in the follicles partly at the expense of the reticulum cells, partly at the expense of small lymphocytes. The development of lymphoid hemocytoblasts at the expense of the mesenchymal reticulum is most evident in the embryonic spleen after stimulation. However, the present experiments offer no evidence that they develop at the expense of small lymphocytes, because they appear at a time when the small lymphocytes are either still very scant or have not yet developed at all.

Figure 24 represents the tissue of a follicle 3 days after stimulation, applied to a 12 days embryo. The largest part of the cells consists of lymphoid hemocytoblasts, which multiply intensely in a hetero- and homoplastic way. The artery walls themselves are usually infiltrated by large basophylic cells, though normally they are formed at this time by a loose tissue, in which free cells are absent. The splitting off of lymphoid hemocytoblasts by the mesenchymal reticulum in such follicles is easily discernible. Though this splitting leads to the development of numerous hemocytoblasts, it however never attains the intensity observed in the pulpa-like spleen (fig. 20) after stimulation at early stages. Between the ameboid cells a distinct net of mesenchymal cells is apparent and they proliferate and continue to split off hemocytoblasts. Endothelial cells of the arteries and their smaller branches seem to be exempt from the stimulating action. Whether the lack of a reaction in the endothelial cells is caused by the final specialization of these cells, or whether the endothelial cells are merely

slow in their response, cannot be definitely determined on the basis of the available experimental material. A similar intense development of lymphoid hemocytoblasts at the expense of reticulum cells is observed in the pulpa. Thus the stimulation applied at a stage with developed spleen vascularization incites equal reactions through the whole organ. Both in the pulpa and in the follicles this reaction is manifested by an intense splitting off of lymphoid hemocytoblasts and their intense proliferation.

A study of further development of such spleens shows, however, that the differentiation of the lymphoid hemocytoblasts develops differently according to their localization in the pulpa or in the follicle. The study of the normal histogenesis has demonstrated that lymphoid hemocytoblasts, situated in the cavernous tissue of the pulpa, differentiate finally into granular leucocytes; in the reticular tissue of the follicle they develop into small lymphocytes. The same strong dependence of differentiation of lymphoid hemocytoblasts upon the environmental structural conditions is observed in a spleen after stimulation. The stimulation stirs up the less differentiated cells, which are the mesenchymal cells and the lymphoid hemocytoblasts. The further differentiation is effected in compliance with the conditions met and granular leucocytes are differentiated in the pulpa, and small lymphocytes in the follicles.[5]

[5] It is difficult to harmonize with the results of my present paper, the data given by Dr. Murphy in his recent note regarding the effect of adult spleen grafts on the organism of the hostembryo (Journ. of Exp. Med. July, 1916) in which he states that, "while the spleen of a normal embryo of this age (18 days) presents only a beginning differentiation of cells, after grafting this process is well advanced and numerous cells of both the granular and non-granular type are found."

As seen in figures 7, 8, 9, 10, 12 and 13 of the present paper taken at the 11th, 13th and 15th days of incubation a normal spleen presents a highly advanced differentiation of granular cells from the 11th day and a good start of development of small lymphocytes from nearly the 15th day of incubation.

The changes in the host spleen after grafts at the 17th and 18th day of incubation, 10–11 days after grafting (the only stages to which Dr. Murphy refers in the above quoted note) present the ultimate results of intense modifications, which are very different from the advanced stage of differentiation mentioned by Dr. Murphy.

A remarkable stimulation of granulopoiesis can be seen however in early stages,—at the 2nd, 3rd, and 4th day after grafting. A stimulation of development of small lymphocytes has not been observed as yet.

There were repeatedly described cases of myeloid metaplasis, in which the chief character was represented by a development of granuloblastic tissue and a consequent suppressing of lymphoid tissue. For the true understanding of similar conditions one may remember that granulocytoblasts only might have undergone a stimulation. Granulocytoblasts are specifically differentiated cells, the proliferation faculty of which may be aroused by specific agents, which could leave inert the other offspring of the common stem cells and the stem cells themselves.

The general proliferative reaction of the younger undifferentiated cells to the stimulation observed in the present experiments, no matter where these stem cells are localized—whether in the pulpa or in the follicle—point again to a similarity of these cells in their simultaneous reaction to the same factor. It seems, therefore, that to morphological and histogenetic data, new data of uniform biological reaction may be added as evidence for the uniformity of the hemocytoblastic cell group. This biological reaction consists in an intense heteroplastic and homoplastic multiplication as response to the stimulus. Isomorphism, isogenesis and isodynamism under equal conditions, evidently associate in the existence of the lymphoid hemocytoblasts.

C. CONCLUSIONS

On the basis of observations and experiments described in the present paper some data may be won concerning (1) the histogenesis of the blood cells in the spleen, (2) the cell differentiation, and (3) the general meaning of the myeloid metaplasis.

The data concerning the histogenesis of the spleen cells were recapitulated at the end of every section, therefore, I may here merely outline the general conclusions.

The chief results of the study of normal spleen development is the statement of a regular and unalterable relation between differentiation of certain kinds of blood cells and structural environmental conditions.

These results have been confirmed by the experimental part of the work. Moreover it has been shown experimentally that

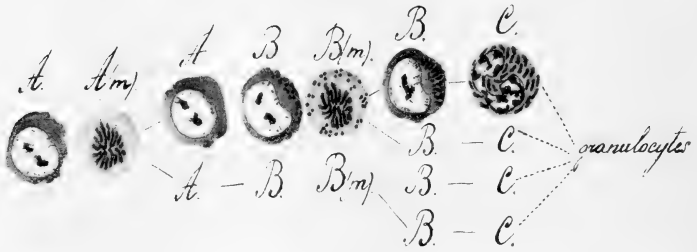
the development of mesenchyme and lymphoid hemocytoblasts can be intensely stimulated and that the respective boundaries, in which the differentiation of certain cell groups usually takes place, can be shifted (displaced), in other words, that the prospective potency (after Driesch's terminology) of the blood stemcells is greater than their prospective value.

The fact that under certain conditions nearly the whole amount of the mesenchymal cells of the spleen anlage may undergo a granuloblastic differentiation; that under other conditions they show a fibroblastic, or an erythroblastic differentiation, and so forth, is the natural sequence of the polyvalency of the mesenchymal cells in the spleen anlage. The early process of segregation must have led in the spleen anlage to a production of one group of numerous homogeneous stemcells, which under different conditions split off variously differentiated cells.

Data concerning the conception of cell differentiation

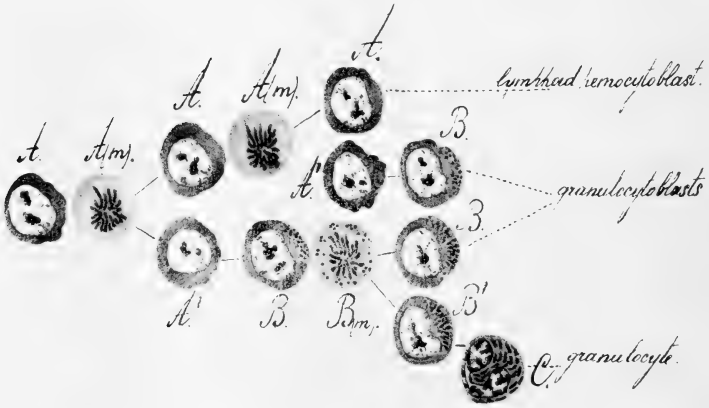
The histogenetical studies of the spleen under normal conditions and after stimulation have shown that a mesenchymal cell, which normally would contribute to the formation of reticulum tissue, can develop into a hemocytoblast or into a fibroblast or a giant cell, that the lymphoid hemocytoblasts can develop into a granular leucocyte, or into an erythrocyte or a small lymphocyte. The study of hematopoiesis in birds and reptiles shows very definite conditions for each of the lines of differentiation. Though Haff (18) has lately described the presence of extravascular erythropoiesis in the liver of hens, yet I was not able to confirm his data. My personal observations concerning the existence of small centers of erythropoiesis in the connective tissue of the hen do not seem to contradict the general conditions. These centers, scattered irregularly and finally phagocytosed may be interpreted as locally developed blood and vessel anlages, of which the connection with the general circulation has not been fully effectuated.

It is known, however, that both erythropoiesis and granulopoiesis develop in mammals extravascularly and, according to Maximow (25) ('11)—"zwei neben einander liegende ganz gleiche



Vera Danchakoff

Text fig. 1. Scheme of cell differentiation and multiplication by equal division gives as result a range of differentiated cells.



Vera Danchakoff

Text fig. 2. Scheme of cell differentiation and multiplication by unequal division gives as result a range of differentiated cells, a number of stem cells and intermediate stages of differentiation.

Lymphocyten, die sich ja sicherlich auch in gleichen äussern Existenzbedingungen befinden, doch zu verschiedenen Endprodukten entwickeln." Maximow rightly points out, that the problem of the differentiation of blood cells forms merely a partial question of the general problem of differentiation of an egg in a multicellular organism. The observation that cells begin their granuloblastic differentiation soon after completion of mitosis, led Maximow to connect differentiation with mitosis. The differentiation factor is conceived by Maximow as "eine tiefe Gleichgewichtsstörung" which occurs during cell division is not reversible, and concerns both daughter cells. It leads to specific qualitative changes,—to the development in the cytoplasm of hemoglobine or granules—"Warum aus einem sich teilenden Lymphocyt in dem einen Fall ein Paar junger Myelozyten, in einem anderen ein Paar junger Erythroblasten u. s. w. hervorgeht, hängt wahrscheinlich vom Zufall ab." It is, however, not determined how the chance can lead to definite and well pronounced differences in the development of two identical cells which seem to be submitted to equal surrounding conditions. Maximow's hypothesis attributes the differentiation to 'Gleichgewichtsstörung,' which depends upon chance. It seems to me that the factor of differentiation which so evidently appears in birds and reptiles, and consists here in definite structural conditions, cannot be essentially different in mammals. However, these conditions are more difficult to trace in mammals.

The proliferation, of the mesenchyme and lymphoid hemocytoblasts—and perhaps the development of lymphoid hemocytoblasts at the expense of mesenchymal cells—seems to depend upon stimulation by enzyme-like substances, which appear in the organism of the embryo from the growing graft cells. The circulating substances call forth an enormous production of lymphoid hemocytoblasts through the whole spleen tissue. In early stages, when the spleen tissue is homogenous and the conditions correspond to those which are required for granulopoiesis, only granular leucocytes are developed, and the differentiation of small lymphocytes does not appear until structural conditions develop in the spleen under which normally

small lymphocytes are differentiated. According to new environmental conditions the mesenchymal cells may develop into typical connective tissue. Again giant cells appear as a reaction of the mesenchymal cells around the necrotic centers. Thus the study of the spleen development in normal conditions and after stimulation adds strong evidence for the conception that at least one of the factors for the differentiation of the polyvalent stem cells consists in the physicochemical conditions to which the cell is submitted.

If the environmental conditions which determine the various differentiation of the stem cells are easily traced, it is much more difficult to understand how differentiation of the stem cells takes place simultaneously with their uninterrupted multiplication. The process of differentiation affects both the cytoplasm and the nucleus. Specific substances are developed in the cytoplasm. The nucleus during differentiation process loses gradually its typical nucleolus and accumulates chromatin, which permanently remains in the form of intensely basophylic particles. Maximow's recourse to the "Gleichgewichtsstörung" during mitosis cannot explain the persistence of the stock of the young stem cells. May the persistence of the young stem cells be explained perhaps by a higher rate of cell multiplication in comparison with their differentiation? I do not think so. If the differentiation is a result of the influence of certain conditions upon the cell, whatever rate of cell proliferation may be admitted, certain kinds of cells under definite conditions will all differentiate simultaneously. The simultaneous and permanent differentiation and multiplication of the lymphoid hemocytoblasts cannot be explained by a high rate of cell proliferation.

A group of hemocytoblasts (let us say a group of similar *A* cells) develops in the loose connective tissue. Some of these cells differentiate into *B* cells, or granulocytoblasts; others continue to multiply as such. If the environmental conditions, as they seem to appear, are similar for all these cells, how is the difference in their behaviour to be explained? Is a difference in the constitution of different cells involved and does the group of

differentiating cells, A, consist of a number of Aa, and of Ab cells? The Aa cells may be supposed to be excluded from the differentiating influence of the environmental conditions and multiply as such. The Ab cells may possess the faculty of developing acidophylic granules and become granulocytoblasts. What will then happen? The Aa cell will divide and give two Aa's, the Ab cell will differentiate into a B cell. At what time, then, does the differentiation of an Aa cell into an Ab cell take place? If between two mitosis, then it is to be expected that under definite conditions at a given time all the Aa cells will differentiate into Ab cells and further into B and C cells (text fig. 1.) Yet the stock of A cells does not show any signs of exhaustion and the hemocytoblasts preserve their existence as such.

The simultaneous differentiation and proliferation of young cells could hardly be explained otherwise than by a specific process of differentiation during mitosis (text fig. 2.) The division of a cell A must lead to the development of a cell A and a cell A^1. The cell A will continue to give rise to cells A and A^1 and will truly become the inexhaustible source of the young undifferentiated cells. The cell A^1 will undergo further differentiation and will develop finally into a B cell, or a granulocytoblast, which again will divide into B and B^1. The cell B^1 will differentiate into a C cell, or a granular leucocyte. The differentiation of the hemocytoblasts into granulocytes outside the vessels as well as their development into erythroblasts and erythrocytes within the vessels and their simultaneous inexhaustible proliferation could hardly be explained on other grounds.

I have not been able yet to trace any difference between two daughter cells. However, numerous examples of similar unequal cell division may be found in the life history of other cells. Boveri represents the differentiation of germ and somatic cells in Ascaris as due to cleavages, which result in formation of cells, of which a part conserves the whole chromatine and another loses a considerable amount of it. Again the differentiation of various cell ranges with persistence of the stem cells in Clepsines depends upon unequal division of the stem cells.[6] Conklin

[6] This example was kindly given to me by Prof. H. H. Donaldson.

sees in the cytoplasm division itself a differentiation factor, which accomplishes the segregation of different substances in the cytoplasm. Lately interesting observations were made in the laboratory of Prof. C. McClung by Dr. Wenrich and Miss Carothers, on formation of heteromorphic chromosomes in the spermatogenesis of grasshoppers. The admission of similar unequal chromosome-division in somatic cells may explain the gradual development of different mitosis figures characteristic of different tissues. The small sizes of the somatic cells do not allow to gain direct data on the inequality of the daughter cells. However, the examples cited above sufficiently explain the possibility of simultaneous persistence of young stem cells and their further differentiation.

In connection with the problem of cell differentiation, the existence of a large number of amitotic cell divisions, may be mentioned. Lately Patterson has described in the Keimblättern of the pigeon, the presence of numerous amitoses. In regions of intense proliferation the cells seemed to undergo a full amitotical division, and give rise to apparently normal daughter cells, which could themselves multiply mitotically. Maximow (25) ('08) found similar conditions in certain regions of embryos in mammals. In early stages of spleen development in chicks, when the nuclei proliferate intensely in the mesenchymal spleen anlage, numerous pictures of amitotical nucleas division may be encountered. Figure 14 illustrates four cells in a stage of amitotic nuclei division. As a result of the division of the nucleus two daughter nuclei arise, which can have both the same dimensions, or differ considerably in size. Both daughter nuclei receive always a part of the nucleolar substance. In most cases the nuclei divisions are not followed by cell divisions, for the very fact that their localization in the syncytium mass of the mesenchyme does not allow a division of the cytoplasm. However, sometimes free cells may be observed undergoing amitotic nuclei division (fig. 13, 14 d). The further destiny of such nuclei cannot be followed directly. The frequence of occurrence of amitotic nuclei division must, however, lead in the spleen mesenchyme to a production of a larger number of nuclei, derived through amitosis. These nuclei contribute in a great part to the growth

of the apparently homogeneous mesenchymal anlage, at the expense of which many different cells are developed. I cannot enter now into a detailed consideration of this problem. However, the question whether the amitosis may not be looked upon as one of the factors in the differentiation of the living substance, can find its justification.

Data concerning the general meaning of the myeloid metaplasis

The results obtained in the embryonic spleen at different stages of its development have a close bearing upon the myeloid metaplasis, well known to the pathologists. What is the general meaning of these changes, and what are their relations to the stimulus applied and to other reactions displayed at the same time by the organism? The specific conditions under which I had to work last winter did not allow me so far to obtain definite data in this respect. However, the study of the experiments made may give a few suggestions concerning the problem.

There is no doubt the intervention applied introduces in the organism heterogeneous substances. The connection between the introduction of these substances and the changes described above may be conceived in two different ways. Either these substances have general stimulating action on the mesenchyme and on the blood stem cells (as for example the thyroid substance in the experiments of Gudernatsch[7] on the development of tad-pole limbs); or the action of these substances may be similar to that of specific antigenes, which introduced in the organism incite the production of antibodies. In the latter case the proliferative reaction exhibited by the blood stem cells may be looked upon as a material basis for the phenomena of immunity. The proliferation in this case would have been brought about by the interaction between specific antigenes and their cell receptors. Only results of new series of experiments will decide which of these two conceptions has to be accepted.

The analogy between the myeloid metaplasis and the results of the experiments described is obvious. It is important to

[7] Gudernatsch, F. Feeding Experiments on Tadpoles. II, A further contribution to the knowledge of organs with internal secretion, Am. Jour. Anat., 1914, vol. 15.

notice that the myeloid metaplasis is produced by different causes. The toxines of various bacteria, the specific products of metabolism of malignant tumors, finally, inorganic chronic intoxications may incite an extensive myeloid metaplasis. It is difficult to conceive in such qualitatively different agents a specific stimulating influence on the stem cells. The response to the action of these factors is specific in so far as it is exhibited by a certain kind of tissue (even not of cells). The stimulus itself may largely vary. The cell, being understood as a complex group of receptors, may offer adequate receptors to different stimuli or antigenes. A similar example of stimulation to proliferation by different agents may be found in the phenomena of fertilization. The specific or usual stimulus in the form of the spermatozoon may be replaced by other chemical stimuli, which may find adequate receptors in the egg cell and incite therefore molecular changes, which are followed by proliferation.

If more than occasional coincidence is to be seen in the regular connection of the appearance of different antibodies and the myeloid metaplasis after infections, the specific antibodies may consist of substances derived from the proliferation and differentiation activities of the hematopoietic tissue. If so, a mere stimulation of the hematopoietic tissue would suffice for strengthening or developing immunity; the production of a large amount of antibodies would follow as a result of stimulation plus specific action of the antigene. Otherwise how could be explained the development of immunity against heteroplastic grafting by introduction in the organism of an emulsion of various tissue (Da Fano)? This intervention, similarly to the experiments described, must have also stimulated the hematopoietic tissue.

It is too early now to attempt to draw more definite conclusions concerning the specific functions of the hematopoietic tissue. Confronted with the simultaneous development of widespread changes in the mesenchyme, which occur after the appearance in the organism of antigen-like substances and usually followed by a production of specific antibodies, one may find it natural to think of the mesenchyme and its differentiation products as of an organ in close relation to the production of immune bodies.

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PLATES

EXPLANATION OF PLATES

All the figures were drawn with the camera lucida at stage level with Zeiss Apochromat 2 mm. oil immersion obj. The compensatory ocular 4 was used for the figure 10, the oc. 6 for the figures 7, 8, 9, 11, 12, 13, 23 and 24, the oc. 8 for the figures 15, 16, 17, 18, 19, 21 and 22 and the oc. 12 for the figures 5, 6, 14 and 20.

ABBREVIATIONS

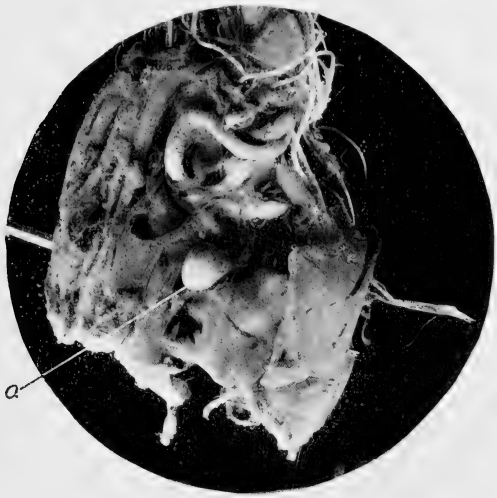
Art., artery
Art.c., arterial capillary
Erbl., erythroblast
Erc., erythrocyte
Erc.,''' degenerated erythrocyte
Fbr., fibrous tissue
Grbl., granulocytoblast (myelocyte)
Grc., granulocyte (granular leucocyte)
Grc.,''' degenerated granulocyte
Gtc., giant cell
L.Hbl., lymphoid hemocytoblast, with a denomination—mitosis of the corresponding cell
L.Hbl.,'' lymphoid hemocytoblast in the stage of its isolation from the mesenchymal syncytium
Msc., mesenchymal cells
Ms.St., mesenchymal syncytium
S., sinus
S.L.Hbl., small lymphoid hemocytoblast
S.Lmc., small lymphocyte
I.L.Hbl., intra-vascular lymphoid hemocytoblast
E.L.Hbl., extra-vascular lymphoid hemocytoblast

PLATE 1

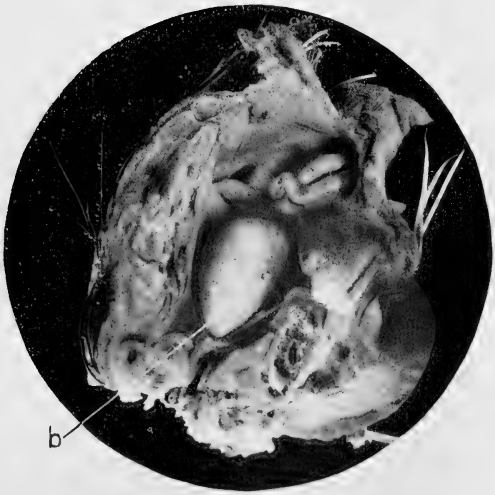
EXPLANATION OF FIGURES

1 Slight hypertrophy of the spleen, corresponding to the graft, reproduced on figure 3. Eighteenth day of incubation.

2 Enormous hypertrophy of the spleen, corresponding to the graft, reproduced on figure 4. Eighteenth day of incubation.



1



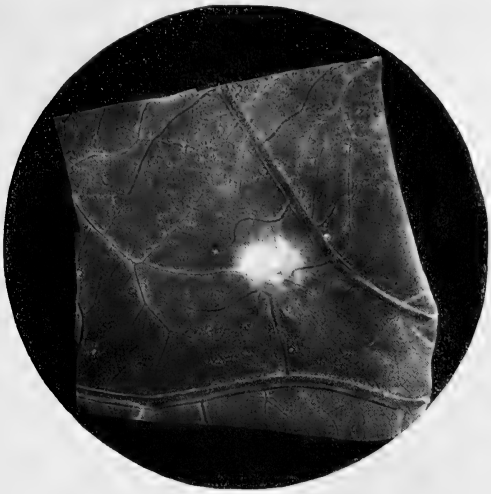
2

PLATE 2

EXPLANATION OF FIGURES

3 Spleen graft of adult tissue, growing on the surface of the allantois of a chick embryo. The culture gave merely a slight growth.

4 The same. Graft intensely growing. The tumor-like graft is well provided with vessels. The culture was made on the 7th day of incubation. The graft is fixed on the 18th day of incubation, being 9 days old.



3

4

PLATE 3

EXPLANATION OF FIGURES

5 Part of the spleen, adjacent to the surrounding loose mesenchyme. Large group of hemocytoblasts within the sinus, beginning their differentiation into erythrocytes. Eighth day of incubation.

6 Part of the spleen from the center of the organ. *S. Anl.*, sinus anlage. Ninth day of incubation.

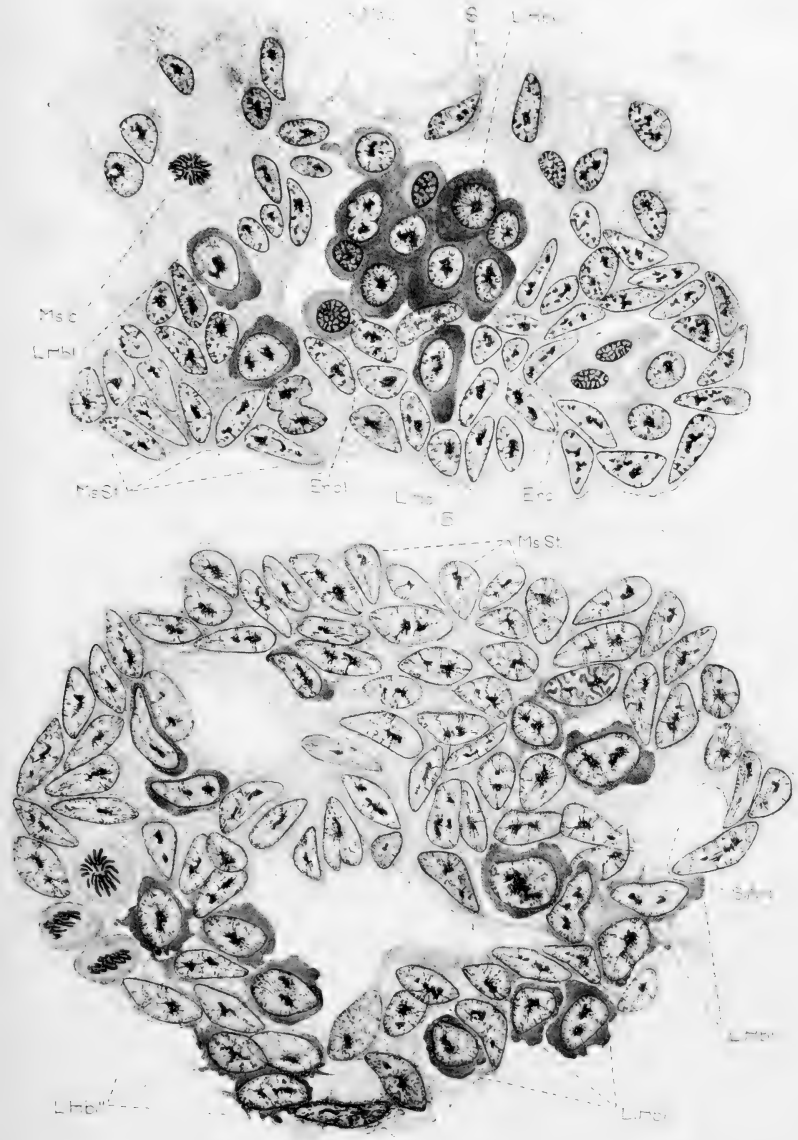
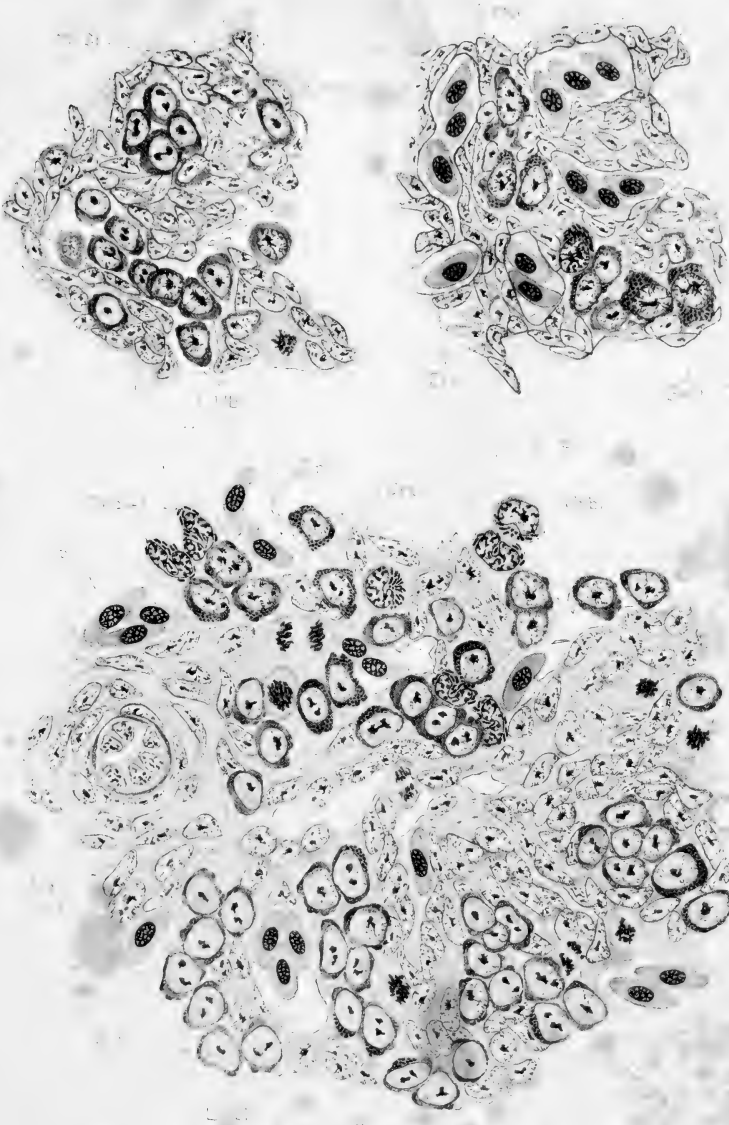

S
Ms c
Lmbl
Ms St
Ect
Lmb
Ect
B
Ms St
Lmb''
Lmb

PLATE 4

EXPLANATION OF FIGURES

7 and 8 Parts of a normal pulpa-like spleen. Eleventh day of incubation. Figure 7 shows newly formed sinuses with particularly numerous lymphoid hemocytoblasts inside. Figure 8 shows a part of the spleen with definitely formed veinous capillaries and well developed granulopoiesis between the vessels.

9 Ingrowth of arterial capillaries into the pulpa-like spleen. Thirteenth day of incubation.



Vera Danchakoff

PLATE 5

EXPLANATION OF FIGURES

10 Part of a normal spleen, in which the follicle is adjacent to the pulpa.

11 Infiltration of the spleen tissue with granulocytes and immigration of granulocytes into the vessels. *Z*, immigration of a leucocyte in the vessel. Eight days after stimulation of an 8 day embryo.

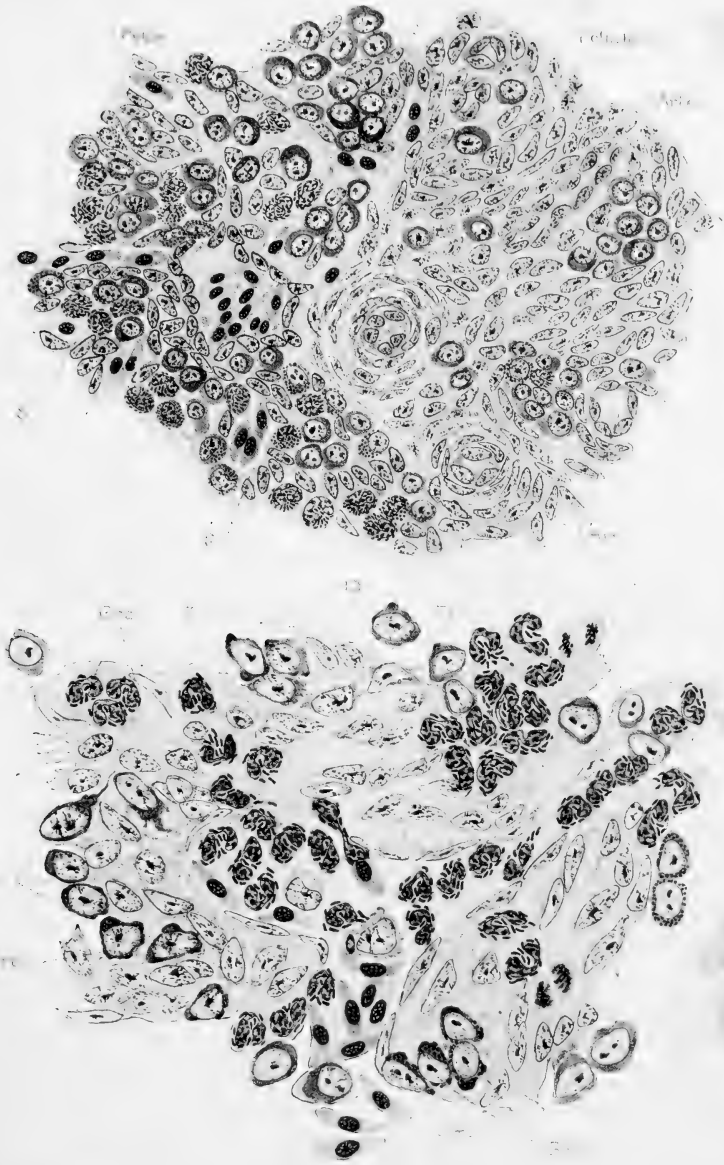


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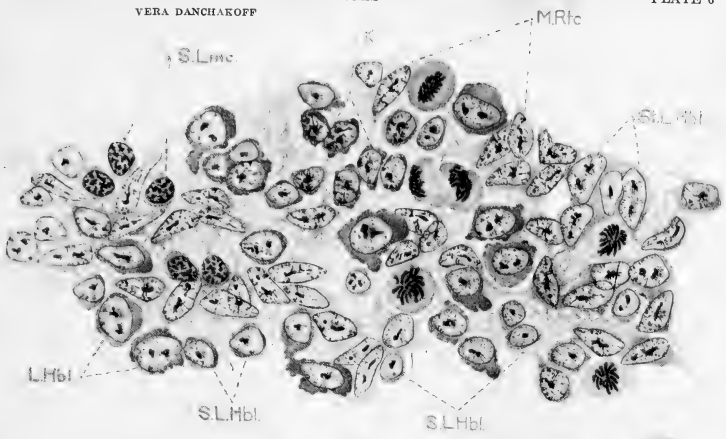
PLATE 6

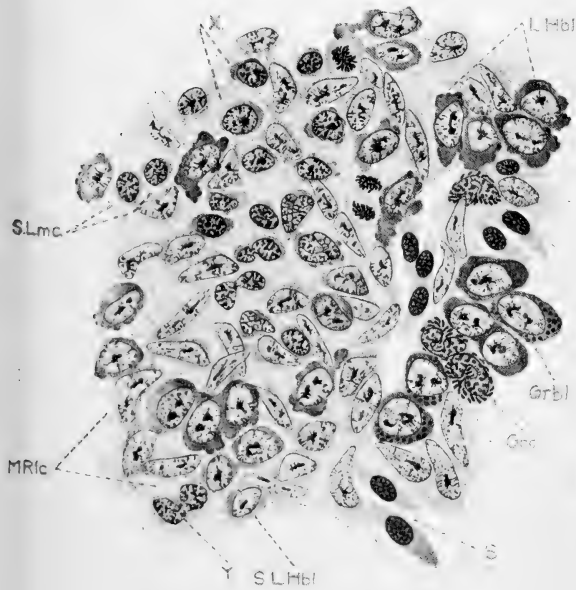
EXPLANATION OF FIGURES

12 and 13 Differentiation of small lymphocytes in the follicle anlages. Figure 12 shows the center of a follicle, figure 13 a part of a follicle adjacent to the pulpa. *M.Rtc.*, mesenchymal reticulum; *St.L.Hbl.*, group of lymphoid hemocytoblasts forming a kind of syncytium; *Y*, amitotic division of a nucleus in a reticulum cell; *X*, intermediate stage between a lymphoid hemocytoblast and a small lymphocyte. Fifteenth day of incubation.

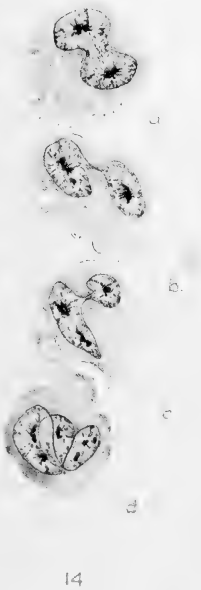
14 Different stages of amitotical division of the nucleus; *a*, *b*, *c*, in mesenchymal cells, *d* in a free cell.


M.Rlc
S.Lmc
S.L.Hbl
L.Hbl
S.L.Hbl
S.L.Hbl

12


L.Hbl
S.Lmc
Grbl
Gmc
MRlc
S.L.Hbl

13


a
b
c
d

14

PLATE 7

EXPLANATION OF FIGURES

15 Intense erythropoiesis within developing sinuses. Granulopoiesis outside the vessels. Two days after stimulation of an 8 day embryo.

16 The same. Four days after stimulation of an 8 day embryo.

17, 18 and 19 Intense phagocytosis of erythrocytes by reticulum and endoth. cells in figure 17, by a polynuclear macrophage in figure 18 and a lymphoid hemocytoblast in figure 19.

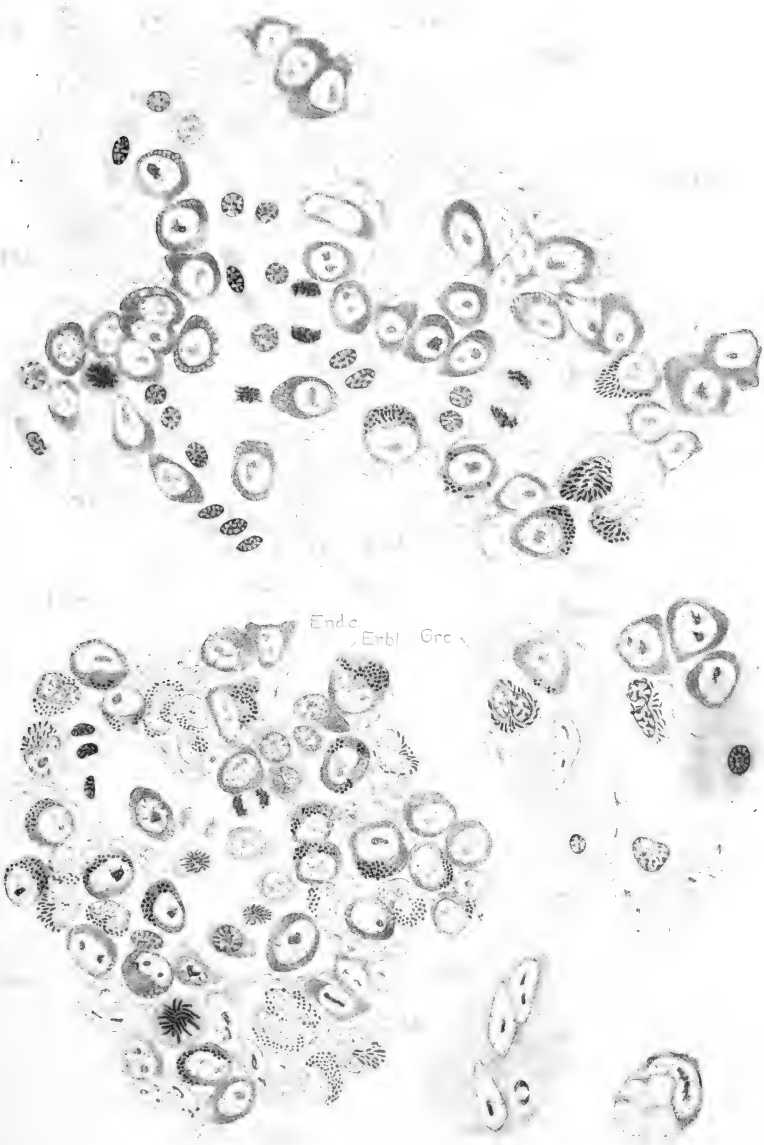

Endo
Erbl
Gro

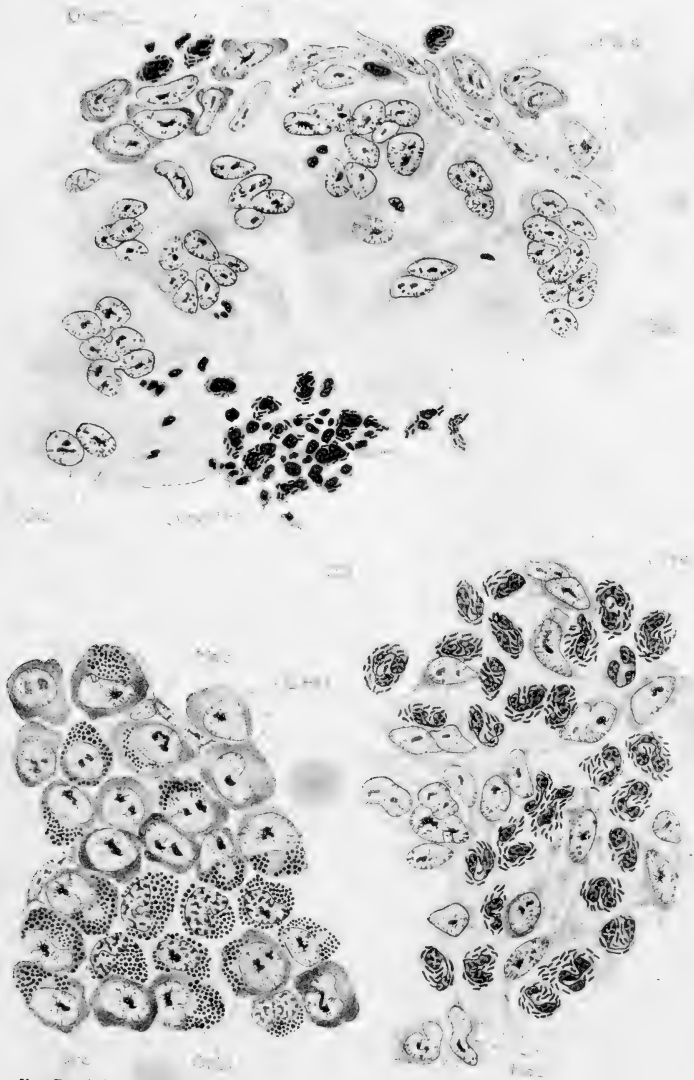
PLATE 8

EXPLANATION OF FIGURES

20 Granuloblastic transformation of the spleen anlage 5 days after stimulation of a 6 day embryo.

21 Leucocytic infiltration of large regions in the spleen 6 days after stimulation of an 8 day embryo.

22 Reaction of the spleen tissue around large necrotic accumulations of granulocytic tissue. Eight days after stimulation of an 8 day embryo.



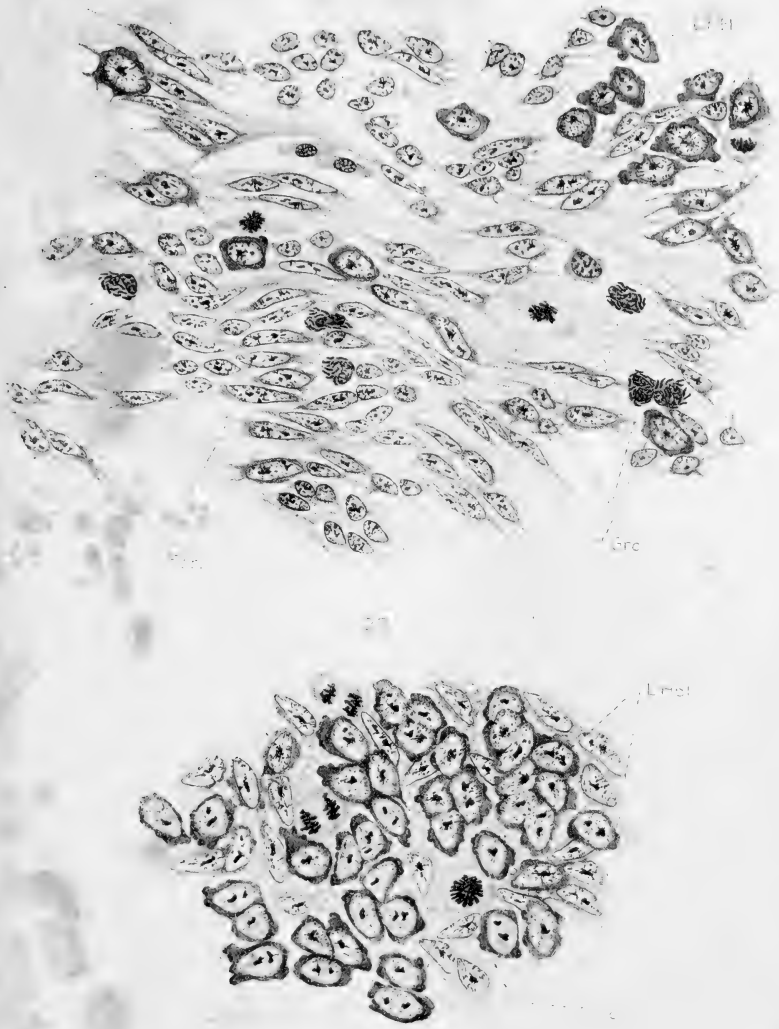
Vera Danchakoff

PLATE 9

EXPLANATION OF FIGURES

23 Transformation of the spleen tissue into a fibrous tissue. Ten days after stimulation of a 9 day embryo.

24 Lymphocytoblastic transformation of a follicle 3 days after stimulation of a 15 day embryo.



MORPHOLOGICAL AND MICROCHEMICAL VARIATIONS IN MITOCHONDRIA IN THE NERVE CELLS OF THE CENTRAL NERVOUS SYSTEM

NORMAN CLIVE NICHOLSON

Anatomical Laboratory, Johns Hopkins University

TWO PLATES

One of the landmarks in neurology is the demonstration by Nissl in 1885 that different types of nerve cells may be distinguished from one another by the arrangement of the basophilic material within them. By common consent this basophilic material came to be called the Nissl substance. This departure in neurology, new at that time, proved extremely fruitful. Investigators flocked to the study of the Nissl substance and through their investigations brought to light many facts of fundamental importance. Since the arrangement of the Nissl substance is more or less specific in different types of cells it was thought that the cells might well be functionally as well as structurally different, just as muscle cells of different structure, gland cells and blood cells of different appearance are assumed to function differently. Thus arose the doctrine of neurone specificity according to which it is supposed that the nervous impulse varies in character with different cell types.

Through the recent discovery of mitochondria in the cells of the central nervous system it has become possible to attack this old problem from a new point of view and with greatly improved methods of technique. The object of this investigation is to ascertain whether the morphology and microchemical reactions of the mitochondria vary in different types of nerve cells. It is essentially a study of qualitative mitochondrial variation, and as such it is supplementary to the investigation of Thurlow ('16, p. 253) on quantitative variations. The Nissl substance as we

see it in fixed and stained preparations is undoubtedly an artefact (Mott '15, p. 68, who unfortunately extends his artefact idea to the mitochondria also), caused by the action of the fixative upon a material present as a diffuse deposit in living cells, which fact does not, however, detract in any way from the value of the numerous detailed and careful observations on Nissl bodies in fixed tissues, because obviously, changes in the character of the coagulum (or precipitate) must be the visible manifestation of either quantitative or qualitative variations in the diffuse deposit occurring in the living cell. But it is important to note that the morphology of mitochondria in fixed preparations of nerve cells is, so far as can be ascertained, identical with that seen in the living condition, which of course gives additional value to observations on mitochondria. Then again mitochondria are bodies quite different chemically from the Nissl substance, playing in all probability an altogether different rôle in the cell economy.

It is clear then, that although this study of their morphology will be complementary and supplementary to the older work, it should enable us to push our investigations much further than is possible with studies upon the Nissl substance alone.

Comparatively little has been done thus far by other workers, who have, for the most part, confined themselves to determinations as to whether or not mitochondria do occur in adult nerve cells and to the relations of mitochondria to other constituents of nerve cells. The only paper on the mitochondria in the different kinds of nerve cells, apart from several preliminary notices of a page or more, is one by Busacca Archimede ('13, p. 322) on Testudo Graeca. No precise information has been gathered regarding qualitative variations in mitochondria in the nerve cells of the brain of any animals above the reptiles, or below them, for that matter. The following investigation was undertaken with the hope of being able to fill up this gap to a certain extent by careful study of a mammal.

MATERIAL AND METHODS

White mice of known age were used. Care was taken that they were in good condition. They were killed with chloroform, thus reducing the possible factor of fright to a minimum. They were then fixed by injection through the blood vessels of a mixture of formalin and bichromate so as to guard against the production of mechanical injury on removal of the brain as well as to insure a good penetration of the fixative. Sections were stained according to the method recommended by Cowdry ('16 b, p. 30), the essentials of which follow: (1) a fixation by injection, through the blood vessels, of a mixture of neutral formalin and potassium bichromate; (2) a mordanting in bichromate, followed by dehydration and imbedding in the usual manner; and (3) a staining of the sections, cut 4 microns in thickness, with fuchsin and methyl green. The fuchsin stains the mitochondria a bright crimson color and the methyl green colors the Nissl substance in the same cell green, thus giving a good color contrast between the two. Specimens were also stained with iron hematoxylin and by the Benda method, for control. They gave results confirmatory in every way.

The observations are based upon actual measurements of mitochondria which were made by using one of the new Spencer wheel ocular micrometers. Each measurement was made five times and the average taken so as to reduce the experimental error as far as possible.

OBSERVATIONS

Morphological variations. A general survey of the central nervous system was made and it became at once evident that the mitochondria did present considerable variation in form in different types of nerve cells. Nerve cells of the same kind, in the same nuclei, generally contained the same form of mitochondria. Somewhat more individual variation appeared in cells of the spinal cord, spinal ganglion and Gasserian ganglion, due perhaps to mechanical injury on removal. Mechanical manipulation and faulty technique will bring about great varia-

tion in the morphology of mitochondria occurring in the same type of cells, in a single center, which actually possess similar mitochondria. In fact one comes to suspect those preparations which exhibit a great dimorphism of mitochondria in cells of the same type. Poor penetration of the fixative generally results in the filamentous mitochondria breaking up into granules, or else forming spherules; a phenomenon well known in other tissues. There is no evidence that filaments are ever formed as a result of bad technique. In all my observations a careful check has been kept upon the technique.

In some nerve cells, like the anterior horn cells of the spinal cord (fig. 1), and the large cells of the reticular formation of the midbrain (fig. 11), the mitochondria are present in the form of filaments of variable length, which frequently attain a length of 4.66 microns in the former and 6.49 microns in the latter. They are much longer in the processes than they are in the cell body. They are more granular in the immediate vicinity of the nucleus where they are also more numerous. This is a striking feature of all the cells observed. It is well to remember at this point that filamentous mitochondria occur in other types of cells also; indeed, filamentous mitochondria occur more frequently than any other form in the central nervous system.

Again we find cells with mitochondria in the form of granules or short plump rods. The cells of the mesencephalic nucleus of the trigeminal nerve (fig. 5) are crowded with mitochondria of this description. The granules are about 0.29 microns in diameter and the filaments about 0.62 microns long. Mitochondria of intermediate size and shape are invariably to be seen. It is interesting to note that the same sort of mitochondria are encountered in the large cells of the Gasserian ganglion, because the nature of these mesencephalic cells has been long in dispute and this new point of similarity between them and cells known to be sensory constitutes additional evidence that they themselves are sensory, and as Thurlow[1] also has emphasized, supports the view that they are in reality neural crest cells which have been enclosed in the neural tube in the course of development. This

[1] Thurlow, personal communication.

similarity is the more striking when we bear in mind that granular mitochondria are not at all common in the cells of the central nervous system. The small cells of the Gasserian ganglion contain exceedingly minute granular mitochondria (fig. 6) which are usually clumped together in the vicinity of the nucleus leaving the peripheral cytoplasm free. This last fact considered in connection with Cowdry's ('14, p. 27, fig. 13) demonstration of mitochondria of like nature and distributed in the small cells of the spinal ganglion, which Ranson ('14, p. 123) believes to be concerned in conduction of pain and temperature sensations, is significant. One might expect similar functions to be the property of these cells, and we should be on the lookout for changes in them in cases of trifacial neuralgia.

All nerve cells, even those with otherwise granular mitochondria invariably contain filamentous mitochondria in their processes, whether they be dendrites or axones, from which it follows that there is greater variation in the mitochondria in cell bodies than in cell processes. It may be recalled that the mitochondria are all filamentous in the cells of the neural tube of the developing embryo. In other words, mitochondria retain their embryonic form in the processes and become specialized in the cell bodies. The mitochondria are usually filamentous in the axone hillock.

The cells of the nucleus of the corpus trapezoideum present as peculiar a picture as is found in any other part of the nervous system (fig. 2). Large block-like mitochondria are found in the peripheral layer of the cytoplasm. The large mitochondria are frequently oblong. Some of those illustrated in the figure are as much as 1.74 microns long by 0.63 microns in breadth. This suggests a possible relation to the unique synaptic connection of these cells (Collin '05, p. 313). The peculiarity of the connection lies in the very large pericellular fibres which arborize about the cell circumference and encompass it in a sort of cone. Nowhere else in the nervous system do we find such large fibres making connections of this kind. Further examination of the cell shows, more centrally, a distinct diminution in the size of the mitochondria which here occur as small grains or filaments,

almost beyond the limits of accurate measurement. Comparatively large areas of cytoplasm seem to be devoid of mitochondria. An abrupt change occurs in the form of mitochondria as we pass to the neighboring cells of the pontile nucleus, in which the block-like mitochondria, as well as the peculiar synapses, are absent.

It has been generally assumed (Busacca Archimede '13) that the mitochondria occur between the Nissl bodies and not within them. So far as I have been able to ascertain, with a method of staining which permits of observation of Nissl substance and mitochondria at the same time, this is not the case. Indeed one would not expect to find the mitochondria between the Nissl bodies in view of the fact which Cowdry ('14, p. 20) emphasizes, that the Nissl substance is present as a homogeneous diffuse deposit in the living cell and that the Nissl bodies as seen in the fixed preparations are produced by a process of coagulation or precipitation.

It may be mentioned in passing that cells with the typically filamentous variety of mitochondria (reticular formation cells, fig. 11), the granular or rod-like mitochondria (mesencephalic cells, fig. 5) and the blocklike mitochondria (cells of trapezoid nucleus, fig. 2) all occur in the same section, proving that the differences in form of mitochondria cannot be due to variations in technique. Furthermore the variations in morphology were found to occur constantly in all members of the species which were examined.

Another fact worthy of note is that cells of quite different type like the mitral cells of the olfactory bulb (fig. 3), the Purkinje cells of the cerebellum (fig. 9), and the cells of the septum (fig. 10) all contain mitochondria of the same kind. It is clear that variations in the form of mitochondria cannot be used to differentiate between sensory and motor cells, nor can quantitative variations be so used, according to Thurlow ('16, p. 253). This is in marked contrast to variations in the Nissl substance, which can be used for such differentiation (Malone '13, p. 129). Again, the general assumption that the morphology of the mitochondria is related to the shape of the cell containing them

does not seem to hold, for instance, the shape of the cells of the corpus trapezoideum (fig. 2) and the cells of the corpus striatum (fig. 8) is somewhat similar, yet the mitochondria in them are quite different. An inspection of the cells of the mesencephalic nucleus of the trigeminal nerve (fig. 5) and of the large and small cells of the Gasserian ganglion (figs. 6 and 7) shows that the mitochondria are rod-like or granular when present in great abundance (which would agree well with Dubreuil's hypothesis to be mentioned subsequently). I have not observed any change in the nucleus, or in the nucleoli, or in the Nissl substance, or indeed in any other cell structure which runs parallel with the above mentioned variations in mitochondria and which might offer a possible explanation of them. I have observed mitochondria in the surrounding cell processes but am unwilling to state that they actually occur between the cells themselves (that they are intercellular).

I have not found mitochondria with the bleb-like swellings, which are so common in secreting cells; or in networks. (All the net-like formations observed are illusory, being due to superposition of individual filaments, which can usually be resolved by careful focussing.) Neither have I seen them swell up to form vacuoles with clear centers, and there is no evidence of an agglutination of mitochondria as occurs in pathological conditions (Scott '16, p. 249). I should therefore feel that the occurrence of such mitochondria was evidence of pathological change.

Microchemical variations. In the inspection of a large amount of material a certain number of brains were studied, which were for some reason poorly fixed, and it was noted that in these, certain groups of cells contained mitochondria while others did not. An instance in point is that of a brain in which it was observed that, while all the cells of the mesencephalic nucleus of the trigeminal nerve contained their normal complement of mitochondria, the neighboring cells of the locus cœruleus, scattered among them were found to be devoid of mitochondria. In order to ascertain whether there were differences in the solubility of mitochondria beyond chance variations, the following experiment was carried out.

Brains of mice were fixed by injection in the regular manner, with the formalin bichromate mixture to which in one case 0.5 per cent, in another 1 per cent, in a third 2.5 per cent, in a fourth 5 per cent and finally 10 per cent of acetic acid had been added. They were carried through and stained in the usual way.

No mitochondria were found after using the mixture containing 10 per cent acetic acid except in the cells of the hypophysis. This was also true in the case of the 5 per cent mixture. With increase in concentration of acetic acid the sections became more and more difficult to stain and required longer and longer treatment with permanganate and oxalic. The fluid containing 2.5 per cent acetic acid gave apparently the same fixation as the 5 per cent mixture, but the 1 per cent acetic mixture preserved the mitochondria in the Purkinje cells of the cerebellum and destroyed the mitochondria in the nerve cells of the medulla.

With regard to variations in staining reactions it need only be said that we do observe mitochondria taking the stain more intensely in certain parts of the cell. This is often the case in the region of the axon hillock. As the staining reaction does not occur regularly and inasmuch as it may be due to differences in the degree of mordanting with the bichromate I am not inclined to attach much significance to it. Moreover when mitochondria are very abundant they sometimes stain more intensely, which may be due to the presence of the stain in greater mass and consequently washing out more slowly than where only a few mitochondria occur. Careful search has not revealed any definite difference in the staining reactions of the different forms of mitochondria, although one might expect this, if differences in morphology were assumed to be related to differences in density.

DISCUSSION

Significance of morphological variations in mitochondria. The true significance of the morphological variations in mitochondria is unknown. Yet the demand for information is very insistent as it is highly desirable that we should in some measure understand the variations which unquestionably do occur both in

normal states and in pathological conditions. Two important interpretations have been advanced.

Rubaschkin ('10, p. 428) found, in the study of guinea pig embryos, that the mitochondria were granular in the primordial germ cells and filamentous in other more specialized epithelial cells. He arrived at the general conclusion that the primitive granular form of mitochondria is peculiar to undifferentiated cells and that the process of differentiation shows itself by a change of the primitive granular type into chain-like and filamentous forms. This view has been much criticised. It is inconsistent with the investigations of Swift ('14, p. 495) who found that in the primordial germ cells of the chick the mitochondria are rodlike and do not differ from those in somatic cells. It is sufficient merely to state that my observations, that granular mitochondria occur constantly in some types of nerve cells and filamentous ones in others, are also at variance with Rubaschkin's hypothesis, because both the types of nerve cells in question (large cells of the Gasserian ganglion (fig. 7) and anterior horn cells (fig. 1)), are undoubtedly highly differentiated.

Dubreuil ('13, p. 137), on the other hand, is of the opinion that granular mitochondria are in a state of rapid multiplication by division and are characteristic of active stages in the life of the cell and that filamentous ones are indicative of rest. He bases this belief upon his study of the changes which the mitochondria undergo in the development of fat cells from fixed connective tissue cells. He found that when the cells are most active the mitochondria are most numerous and are granular; when the cells are less active the mitochondria are filamentous and less abundant. He adds to this the observation that when inflammation sets up, the mitochondria immediately increase greatly in number and are granular. The observations recorded in this paper would, at first sight, seem to support this view, for an inspection of the plates reveals at once that where the mitochondria are most abundant, that is to say in cells of the mesencephalic nucleus of the fifth nerve (fig. 5) and in the large cells of the Gasserian ganglion (fig. 7) they are also granular. The conten-

tion is, however, ruled out, by the fact that in other tissues the mitochondria may still be filamentous even though they be increased greatly in amount (Policard, '10, p. 284).

The variations in the form of mitochondria must be due to differences in themselves or in their environment or in both.

There is evidence that the chemical constitution of mitochondria is different in different cells. Regaud ('10, p. 301) has shown that there is a progressive increase in the resistance of mitochondria to acetic acid in the course of spermatogenesis. My experiments have shown that the mitochondria in the nervous system also differ in their susceptibility to acetic acid. In the nervous system this difference in chemical behavior does not seem to be related to a difference in morphology, as mitochondria of quite different form exhibit similar solubilities. So much for chemical composition. Now with regard to density, the only indicator which I have is the difference of intensity in staining with fuchsin and as I have said this is not uniform and is of uncertain meaning. That the form of mitochondria is, in a measure dependent upon their own organization is evident when we remember that if the long filamentous mitochondria in the acinus cells of the pancreas are squeezed out of the cell into the surrounding fluid they maintain their original form, unaltered, for a surprisingly long time.

As to the differences in the cytoplasm in which the mitochondria are embedded I have observed that the cells of the mesencephalic nucleus of the trigeminal nerve are more noticeably shrunken in some preparations than the other cells in the vicinity, which may be accounted for on the assumption of a higher water content. The difference in form of mitochondria and of water content of their surroundings may not be unrelated. As has been noted the mitochondria are invariably filamentous in the processes, though they may sometimes be granular in the cell bodies. This led to the belief, that there might be a difference in water content in gray and white substance of the brain, which curiously enough, was found, on looking up the literature, to be actually the case. The possible influence of the water content seems the more likely since Löwschin ('13, p. 203)

has been able to alter the form of his artificial mitochondria, made out of lecithin, by varying the physico-chemical properties of their environment. Since there is a general concensus of opinion in favor of the view that mitochondria are a combination of lipoid and albumin it is possible that alkalinity or acidity would affect their form. The acidity acting upon the protein fraction might cause it to become hygroscopic and to swell (Cowdry '16b, p. 440). The fact that the Nissl bodies, as well as the mitochondria, are often larger in the peripheral cytoplasm than they are in the immediate vicinity of the nucleus, would seem to indicate that some common environmental factor may be operating in the case of both, notwithstanding the fact that the Nissl bodies are probably a coagulum or a precipitate resulting from fixation.

In a general discussion of this kind the mechanical factors which sometimes operate, in the surrounding fluid, in shaping the morphology of mitochondria must not be lost sight of. Thus N. H. Cowdry has observed the changes in the form of mitochondria in the streaming protoplasm of living plant cells. He has seen straight filaments assume the form of loops and spirals in response to currents and eddies in the stream, indicating clearly that they are flexible and that their form is in a measure determined by their environment. Conditions of protoplasmic stress and strain, occurring especially in the course of development, probably influence the form of mitochondria also. In the outgrowing nerve fibers, for instance, the mitochondria are generally filamentous. Whether mechanical factors of this sort may play any considerable rôle in determining the form of mitochondria in adult nerve cells is unknown. It is highly probable that some combination of the two factors of variation in internal composition and of changes in the surroundings are mutually responsible for the variations in form observed.

Bearing upon doctrine of neurone specificity. These observations on variations in the morphology of mitochondria bring to light another specific difference between the internal structure of nerve cells of different categories; for it has already been pointed out that these differences in the form of mitochondria

are in all likelihood associated, in some obscure way, with changes in their environment; that is, in the cytoplasm. The intimate bearing of such differences in the cytoplasm upon the doctrine of neurone specificity is apparent, inasmuch as any difference in the specialized activity of a cell, like conduction, is in all probability related to some difference in the cytoplasm rather than in the nucleus. This is not at variance with the results which others have obtained.

Since Cowdry ('14, p. 21) has found that there is a surprising constancy in the mitochondria in the spinal ganglion cells of different vertebrates, including man, it is altogether likely that the variations in morphology which I have described in the mitochondria in different types of cells in the brain of the white mouse may hold in other mammals also. This seems to indicate that when differences in the morphology of the mitochondria occur they are not chance variations but are fundamental differences ingrained in the very organization of the cell.

Moreover Thurlow ('16, p. 253) has found, by a detailed quantitative study of mitochondria in the cells of the different cranial nerves, that the actual number of mitochondria per unit volume of cytoplasm varies considerably. The largest amount of mitochondria was found by her in the cells of the mesencephalic nucleus of the trigeminal nerve and the least in the visceral motor nucleus of the seventh. Even if we adopt the ultraconservative view that the mitochondria are purely deutoplastic elements and do not play an active rôle in cellular physiology (which I would be loath to do) it is perfectly obvious that their presence in great amount in some cells and in very small numbers in others, must indicate either a qualitative or else merely a quantitative difference in the nature of the activity of the cells in question. For it is impossible to regard the activity of a cell as being entirely uninfluenced by the heaping up in it of inert substances.

Pathological bearing. The practical value of this work lies in its possible pathological application. The standardization of material for experiment, the enumeration of the qualitative variations which normally occur in mitochondria in the differ-

ent types of nerve cells of the central nervous system of a mammal and the mention of the forms of mitochondria which should, in any experimental study of the nervous system, be regarded as pathognomonic (i.e., net-works, bleb-like swellings, agglutinations and vacuolations), are all of interest from a pathological point of view. The necessity of taking cognizance of mitochondrial changes is brought home to the clinician through the recent investigations of Goetsch ('16, p. 132) who found that an increase in the mitochondria in the thyroid epithelium was associated with an increase in the activity of the epithelial cells and with the severity of the clinical symptoms of hyperthyroidism in man.

As nothing has yet been done on the changes in the mitochondria in the nerve cells of man in pathological conditions it is quite clear that the following are merely suggestions.

If Regaud ('11, p. 20) is at all justified in his statement that "by a still unknown physico-chemical mechanism, the mitochondria retain a great variety of substances which come in contact with the protoplasm, normally as well as accidentally (medicines, poisons, toxins, etc.)" and if the conclusion is warranted that the point of action of tetanus toxin on the nerve cell is lipoidal, it is quite possible that a study of mitochondria in the nervous system in tetanus might yield interesting results. At any rate the fact that tetanus toxin is rendered innocuous when mixed with an emulsion of brain pulp (Wassermann and Takaki '98) is evidence that the toxin combines with some component of nerve tissue. Leathes ('10, p. 123) believes that it acts upon a fat which he calls 'cerebrone.'

There are up to the present no observations on mitochondria in nerve regeneration, although there is evidence from many sources that the mitochondria are delicate indicators of cell activity, and may reveal interesting facts with regard to the formation of myelin, etc.

Pathologists have frequently noted that the mitochondria are the first structures in the cell to respond to disturbances in function, which suggests the possibility that a study of them in the nervous system may serve to localize brain lesions which

heretofore have eluded the grasp of neurologists. One would naturally inquire first into those diseases in which chemical analysis has revealed a disturbance in the lipoid content, particularly general paralysis since Koch and Mann ('09) have detected a destruction of the brain phosphatides in this condition and there is a good deal of evidence that the mitochondria are, themselves, closely related to phosphatides.

Before concluding I want to express my deep appreciation to Dr. E. V. Cowdry for his friendly interest and encouragement.

SUMMARY

There are qualitative differences in the mitochondrial content of certain types of nerve cells in the brains of white mice. The variation in morphology between cells of different variety is often quite pronounced. Filamentous mitochondria are the most common form met with in the cells of the central nervous system. They are particularly apparent in large anterior horn cells (fig. 1) and in the large cells of the reticular formation (fig. 11). Rod-like and granular mitochondria are rarer. They are characteristic, however, of the cells of the mesencephalic nucleus of the fifth nerve (fig. 5) as well as of the cells of the Gasserian ganglion (figs. 6 and 7). The cells of the nucleus of the corpus trapezoideum (fig. 2) may be distinguished by their large, swollen block-like mitochondria.

There is also, in the majority of cases, a variation in the form of mitochondria in different parts of the same cell. For instance, they are usually more granular in the vicinity of the nucleus than in the peripheral parts of the cytoplasm, and in the processes. In the processes they are invariably rod-like or filamentous. This is shown in most of the drawings but it is particularly well illustrated in figures 1, 5, 9, 10, 11 and 12. The cells of the nucleus of the trapezoid body constitute a special case because in them the mitochondria always occur in the form of long blocks in the peripheral cytoplasm in sharp contrast to the minute granular and rod-like mitochondria in the immediate neighborhood of the nucleus. The mitochondria occur not

only between the Nissl bodies (as is generally believed) but also embedded in them.

They also vary in different kinds of nerve cells microchemically. The most striking instance is the greater resistance of the mitochondria in some cells to fluids containing acetic acid as compared with other cells of different types. The mitochondria in the different parts of the cytoplasm of the same cell react in the same manner to solvents.

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DESCRIPTION OF PLATES

The figures have been drawn from cells in the central nervous system of a female white mouse, thirty-five days old, weighing 9 grams, body length 6.8 cm. and tail length 6.1 cm., fixed in neutral formalin and bichromate and stained with fuchsin and methyl green. By this method the mitochondria are stained bright crimson against a green background of Nissl substance. The mitochondria are represented in black in the illustrations which were drawn with Zeiss apochromatic objective 1.5 mm., compensating ocular 6 and camera lucida. The figures are reproduced without reduction so that they represent an actual magnification of 1660 diameters as they now appear on the plates. They are intended to illustrate differences in the morphology and in the arrangement of mitochondria, which are peculiar to and which are constantly met with, in certain types of nerve cells in the brains of white mice.

PLATE 1

EXPLANATION OF FIGURES

1 Large anterior horn cell of the spinal cord in which the mitochondria are typically filamentous. They are longer in the processes than they are in the neighborhood of the nucleus. They may be seen imbedded in the flake-like Nissl substance.

2 Cells from the nucleus of the corpus trapezoideum. The striking feature is the presence of relatively enormous block-like mitochondria in the peripheral cytoplasm, leaving certain areas devoid of mitochondria. It is to be noted furthermore that the mitochondria are quite minute in the immediate vicinity of the nucleus. A large pericellular arborization is visible between the two cells.

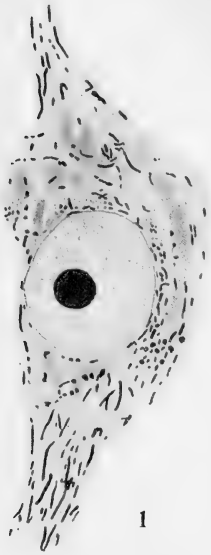
3 A mitral cell from the olfactory bulb. Filamentous mitochondria are to be seen embedded in a homogeneous background of Nissl substance.

4 Large pyramidal cell from the hippocampus showing practically the same arrangement of mitochondria as in the preceding.

5 A large cell of the mesencephalic nucleus of the fifth nerve with a cell of the locus cœruleus immediately adjacent. This large cell is among the most remarkable seen in the whole nervous system. It contains large numbers of small rod-like mitochondria and the striking resemblance which it bears to the large cells of the Gasserian ganglion (fig. 7) is at once apparent.

6 Small cell of Gasserian ganglion with minute granular mitochondria clumped about the nucleus.

7 Large cell of Gasserian ganglion containing an abundance of granular mitochondria in sharp contrast with the filamentous mitochondria which occur in the majority of other nerve cells.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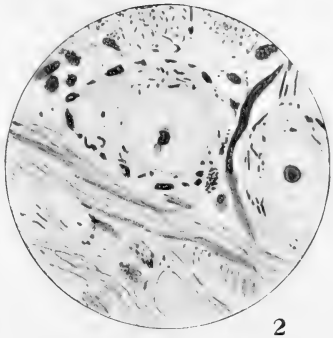
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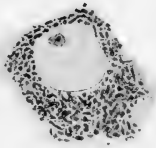
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PLATE 2

EXPLANATION OF FIGURES

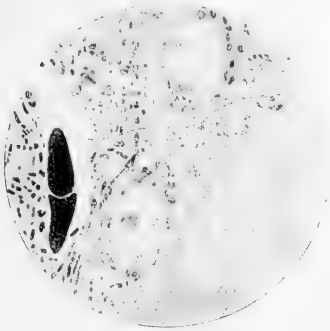
8 Two cells of the corpus striatum. They contain spherical and rod-shaped mitochondria which, in fact, resemble closely the fine medullated fibres cut in section in the neurophil about them. To the left two red blood cells are to be seen in a capillary.

9 Large pyramidal cell of the cortex cerebri with its contained mitochondria which are quite filamentous. This cell also is embedded in a mass of fibers which are cut in section and which resemble mitochondria very closely.

10 A cell from the septum.

11 Large cell from the formatio reticularis of the mid brain. The mitochondria are thread-like and remind one of those which occur constantly in anterior horn cells. They are, as is invariably the case in all the cells studied, more filamentous in the processes than in the cell body.

12 Purkinje cell of the cerebellum with neighboring granule cells just beneath it. The mitochondria look like minute bacilli near the nucleus but they become elongated as one proceeds toward the great dendrite. The granule cells also contain them. The black dots in the molecular layer are cross sections of fibres.



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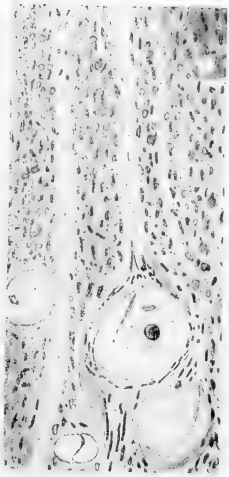

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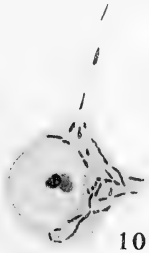

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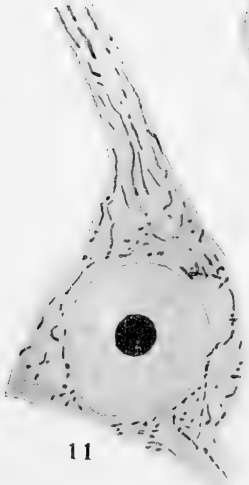

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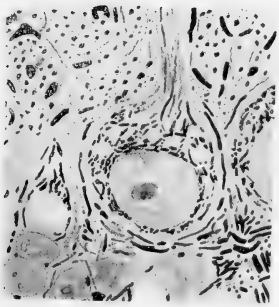

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ON THE DEVELOPMENT OF THE ATRIAL SEPTUM AND THE VALVULAR APPARATUS IN THE RIGHT ATRIUM OF THE PIG EMBRYO, WITH A NOTE ON THE FENESTRATION OF THE ANTERIOR CARDINAL VEINS

C. V. MORRILL

Department of Anatomy, Cornell University Medical College, New York City

NINE FIGURES

The method of formation of the atrial septum and nearly related parts in the mammalian heart has been known since the careful investigations of Röse ('88 and '89), Born ('89), and more recently Favaro ('13). These writers are in substantial agreement regarding the structures concerned in this process, though differing in some respect as to detail. The now classical work of Born on the rabbit has been used extensively as a basis for descriptions of cardiac development in some of the more recent text-books of embryology, notably by Hochstetter ('01–'03) and Tandler ('12 and '13). Born in his account, which included some observations on human embryos, corrected the error of His ('85) regarding the formation of the atrial septum and its relation to the foramen ovale. His description differed from the earlier one of Röse ('88) in several important respects but later Röse ('89) accepted Born's corrections. Röse and Favaro extended their observations to a number of different mammals, but not, as far as I am aware, to the pig. Retzer ('08) published a brief note on the development of the heart in which he claimed that Born's account of the atrial septum in the rabbit could not be applied to the pig. Since pig embryos are extensively used for study in American laboratories, it seemed advisable to re-examine the development of the septal

and valvular apparatus in this form in order to clear up any uncertainty which may exist regarding the processes involved.[1]

This study is based on series of pig embryos of 6.8, 7.9, 8.5, 12.3, 15.2 and 21 mm. total lengths, supplemented by dissections of the hearts of foetuses from the 45 mm. stage to birth. The hearts of the 7.9, 15.2 and 21 mm. embryos were reconstructed by the wax-plate method. In addition several human embryos from 7.6 to 22 mm. were examined for comparison.

The terminology of Born which has been largely adopted by other writers will be used, with some modifications, in this account.

In the stage represented by a 6.8 mm. embryo, septum I forms an incomplete interatrial curtain. Ostium I is still widely open and ostium II has begun to form. In the reconstruction of a 7.9 mm. embryo (fig. 5), the relation of these three structures is shown. Septum I (*S.I*) though fused for the most part with the atrial walls, still presents a short, free border facing the now very narrow ostium I (*O.I*). In the posterior superior corner of the septum, the new opening or ostium II (*O.II*) is well advanced and much larger at this time than ostium I. It is apparent from the figure that ostium II is formed by fenestration in the pig and is not at first a single opening as Born found in the rabbit. Born thought that a primitively single opening was normal for mammals generally, but Röse found a fenestrated septum in three cow embryos, a mole embryo and two rabbit embryos and states that Bruch's observations on sheep, cow and horse and Rokitansky's[2] on a human embryo support the conclusion that fenestration is the more usual condition. Favaro found numerous orifices, representing ostium II in the sheep, but only a single opening in the guinea-pig. As a rule, Röse believes, the larger openings coalesce to form a single ostium II while the smaller ones close up.

[1] This investigation was begun in the anatomical department of the University and Bellevue Hospital Medical College and completed at Cornell University Medical College, New York.

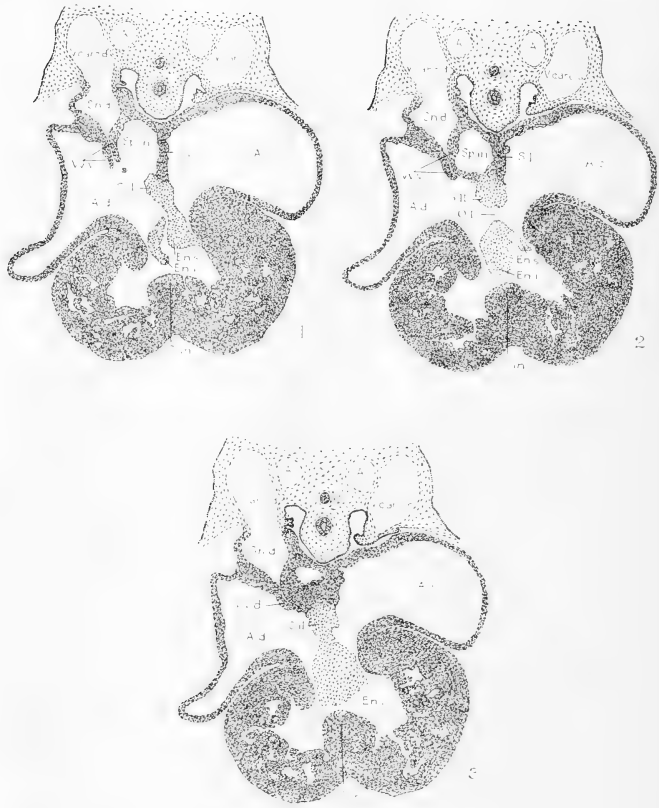
[2] The observations of Bruch and Rokitansky are known to me only through the brief mention made by Röse ('88).

Exceptionally the latter may persist until birth. In the pig later stages always show a single opening.

The closure of ostium I in the pig is effected in the same manner as described for the rabbit, guinea-pig, man and other mammals. This process is illustrated in figures 1, 2, 3, and 5. Septum I is composed for the most part of developing muscle which is continuous with the musculature of the roof, posterior wall and floor of the atrium. Its free border, however, is capped by a prominent endocardial thickening. As the septum grows downward and forward toward the atrial canal, the endocardial thickening develops two extensions or horns which fuse with the endocardial cushions of the atrial canal. The upper one blends with the upper cushion (fig. 1), the lower one with the lower cushion (fig. 3). Ostium I (fig. 2) is thus entirely surrounded by thickened endocardium except where it is continuous with the narrow slit (transverse fissure) between the endocardial cushions of the atrial canal. At a slightly later stage (8.5 mm.) the blending of the separate portions of endocardium is complete and ostium I together with the transverse fissure is closed. In the 15.2 mm. stage (fig. 6) ostium II (*O.II*) is much enlarged and forms a single large opening between the atria. Septum I (*S.I*) presents a very irregular free border which faces upward and slightly posteriorly toward ostium II. (The irregularity of the free border could not be represented in wax.) The septum as a whole bends toward the cavity of the left atrium.

The mode of formation of septum II (the later limbus Vieussenii) is first clearly indicated in the 7.9 mm. embryo though a faint trace is sometimes distinguishable in an earlier stage. Concerning the origin of this septum there has been some difference of opinion. Indeed Retzer ('08) has denied its existence in the pig, though Röse ('88 and '89), Born ('89) and Favaro ('13) have found it in all the forms they studied, Röse expressly stating that it is characteristic of all placental mammals. In the present study it will be shown that septum II is as definite and well developed in the pig as in any form so far examined.

The model of the 7.9 mm. embryo (fig. 5) shows a very distinct spur-like thickening (*S.II*) which projects into the cavity



Figs. 1 to 3 Transverse sections of the heart region of a 7.9 mm. embryo (histological structure semi-diagrammatic). Figures 1 and 3, above and below the level of ostium I respectively; figure 2, through ostium I. *A.*, dorsal aorta; *A.d.*, right atrium; *A.s.*, left atrium; *En.s.*, upper endocardial cushion; *En.i.*, lower endocardial cushion; *O.I*, ostium I; *S.in.*, interventricular septum; *S.I.*, septum I; *S.II*, septum II; *Su.d.*, right sinus horn; *Sp.in.*, spatium interseptovalvulare; *V.car.c.d.*, and *V.car.c.s.*, right and left common cardinal veins; *V.v.d.*, right sinus valve; *Vv.v.*, sinus valves. Magnified about 25 diameters.

of the right atrium in the angle between the floor and septum I, and just posterior to ostium I (*O.I*). It is continuous with the left sinus valve. Transverse sections through this region (figs. 1, 2 and 3) show that at first this spur (*S.II*) is composed of connective tissue alone and is sharply marked off, histologically, from the developing musculature of the sinus valves, septum I and the atrial floor. It appears to be nothing more than a projection, toward the right, of the endocardial thickening of septum I (*S.I*) and is continuous through the latter with the endocardial cushions of the atrial canal (*En.s.* and *En.i.*). This structure which is the anlage of septum II, is undoubtedly what His ('85) called the spina vestibuli and, taken together with the endocardial cushions, would constitute his septum intermedium. The lower end of the left sinus valve blends with it, while the corresponding end of the right valve flattens down in the atrial floor close to it (figs. 2 and 5, *Vv.v.*). At this stage, the anlage of septum II is not in relation with ostium II.

In later stages, when ostium I has completely closed, the spur lengthens out into a definite ridge. This is well shown in the model of a 15.2 mm. embryo (fig. 6, *S.II*). When traced from its point of origin in the anterior inferior corner of the atrium, this ridge extends upwards then bends backward along the roof toward the posterior wall where it flattens down and may become continuous with two or three muscular trabeculae which develop in this region, i.e., the spatium intersepto-valvulare. These trabeculae are not constant in number. The portion of the ridge in relation to the roof of the atrium[3] is broad and low contrasted with its lower anterior end or root which is sharp and well-defined. Both sinus valves (*V.v.s.* and *V.v.d.*) now blend with it, the right, however, slightly covering its lateral side.

The internal structure of septum II is shown in a transverse section taken at about the middle of its anterior vertical portion (fig. 4, *S.II*). The endocardial thickenings which entered into its formation in an earlier stage, have fused together into

[3] Owing to the position of the model this part of septum II is hidden in figure 6.

a solid quadrilateral mass. A cap of developing muscle covers its posterior border (upper in the figure) and extends anteriorly for some distance along its lateral surfaces. This muscular mass is continuous with the musculature of septum I and, at a lower level (not shown in the figure), with that of the two sinus valves which, as mentioned, blend with the root of the septum. A careful study of serial transverse sections shows that the musculature of this part of septum II is derived from that of

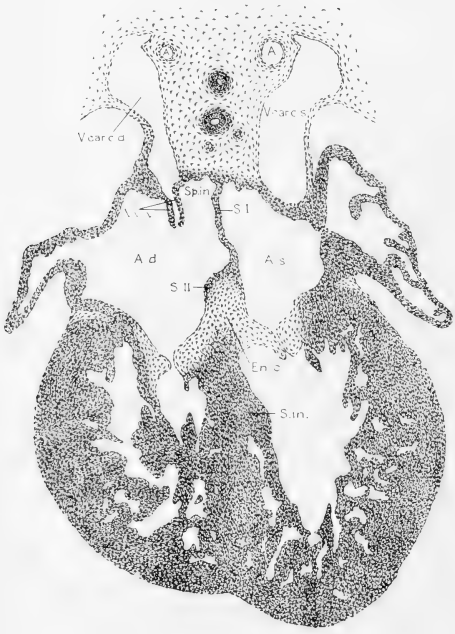


Fig. 4 Transverse section of the heart region of a 15.2 mm. embryo at the level of the root of septum II (histological structure semi-diagrammatic). *A.*, dorsal aorta; *A.d.*, right atrium; *A.s.*, left atrium; *En.c.*, fused endocardial cushions; *S.in.*, interventricular septum; *Sp.in.*, spatium intersepto-valvulare; *S.I.*, septum I; *S.II*, septum II; *V.car.c.d.*, and *V.car.c.s.* right and left common cardinal veins. Magnified 25 diameters.

the sinus valves and septum I. The developing muscle elements appear to invade the connective tissue and gradually to replace it.[4] This invasion has not yet begun in the 8.5 mm. embryo but is well advanced in the 12.3 mm. and still more so in the 15.2 mm. described above. The anterior portion of the connective tissue mass or septum intermedium[5] (fig. 4 *En.c.*) broadens out in a lateral direction between the two atrio-ventricular orifices and is joined by the interventricular septum, the line of fusion being toward the right.[6] The fusion is not complete, however, for the interventricular ostium (fig. 6, *O.in.*) is still widely open at this stage. If septum II is now traced upward and backward into the roof of the atrium, the connective tissue elements gradually disappear and it appears as a low, broad thickening of the atrial musculature which follows the external depression caused by the bulbus in this region. It is not, however, due to an infolding of the atrial wall, but to a local increase in the developing muscle elements. This upper part of septum II now comes into relation with ostium II (fig. 6, *O.II*) which meanwhile has become much larger.

Passing now to the 21 mm. embryo, the further development of septum II (fig. 7, *S.II*) is clearly shown. It appears as a crescentic ridge extending from the lower inferior corner of the atrium, upward and backward toward the posterior wall. Its lower segment or root which is thickest and most sharply defined is partly hidden by the high right sinus valve (*V.v.d.*) which blends with its lateral surface. Traced upward it curves over the roof of the atrium bordering ostium II (*O.II*) and reaching the posterior wall, bends downward somewhat in the region of the spatium intersepto-valvulare (not shown in the

[4] The invasion of the connective tissue mass (septum intermedium) by the musculature of the sinus venosus was described by Retzer ('08) as the source of the Purkinje fibres in the ventricles. The present investigation leads to the conclusion that a part of this muscle, at least, thickens to form the root of septum II.

[5] The term 'septum intermedium' originally used by His ('85), may be conveniently retained for this structure as suggested by Favaro ('13), although Born and some others discarded it.

[6] A portion of the septum intermedium thus separates the right atrium from the left ventricle (conus arteriosus of the aorta) forming the septum atrioventriculare of Hochstetter.

figure). This upper segment is broad and low where it faces ostium II but becomes narrow and sharp in the posterior wall. Septum I meanwhile has changed its position relative to septum II, so that its free border now faces upward and forward, and ostium II has become an oblique cleft, the definitive foramen ovale, between the septa.

Before considering the later changes in septum II it will be convenient to compare the conditions found in the pig with those described for other forms. According to Born septum II first appears (rabbit and man) in the upper part of the posterior wall of right atrium a little to the right of septum I. It is a crescentic spur which encroaches upon the spatium interseptale and forms the principal part of the limbus Vieussenii. In the pig irregular muscular ridges develop in the same locality but they vary greatly in form, size and number. In the model of the 15.2 mm. embryo, two such ridges can be distinguished (fig. 6). When septum II has extended backward to the posterior wall, one or more of these ridges is incorporated in it, but no one of them is sufficiently well-marked to be taken as a starting point for the developing septum. Further, at the time when the anlage of septum II is first distinguishable in the lower anterior wall (7.9 mm. embryo) the spatium intersepto-valvulare is relatively very wide and numerous small irregular ridges appear in its upper posterior wall. Along with the narrowing of the spatium, the ridges decrease in number until finally they are reduced to one or two which, as stated, are incorporated in the septum.

An examination of several human embryos seemed to indicate that much the same sort of process takes place there. In an embryo of about 12 mm., total length, at least three muscular ridges could be distinguished in the upper, posterior wall of the spatium intersepto-valvulare, while in one of about 14 mm. only one such ridge appeared which, however, was quite prominent.[7] In the lower anterior part of the right atrium of both

[7] Through the kindness of Dr. Thyng I have had the opportunity of examining the human embryo which formed the subject of his paper ('14). In this specimen (17.8 mm.), the region of the spatium intersepto-valvulare was entirely devoid of muscular ridges.

these embryos there was a thick connective tissue ridge or prominence continuous with the fused endocardial cushions and partly overlaid and invaded by developing muscle. As in the pig this musculature was continuous with that of septum I and the sinus valves. The resemblance between this structure and that described for the pig in the corresponding place is so close that I am inclined to think it represents the origin of septum II in man. Indeed Thyng ('14) in his description of a human embryo notes the presence of a "ridge or tubercle" in the same region and states that "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."

Born seems to have overlooked the early appearnace of this ridge entirely, though he states that in man the bay of the crescent (septum II) swings further downward in later stages and finally unites with the lower end of the left sinus valve. He thus includes the region in which the ridge is found in the pig. In the rabbit, however, septum II remains as a narrow short crescent in the anterior upper wall of the atrium. One may conclude from this that the ridge if present at all is poorly developed in this animal.

Röse ('89) in summarizing the results of his earlier work ('88) speaks of septum II (limbus or annulus ovalis) as follows:

> On the anterior and upper atrial wall there appears a ridge-like infolding, the septum musculare which, together with the septum intermedium . . . . . is formed into a closed ring diaphragm, the annulus ovalis . . . . . The septum intermedium arises from a connective tissue spina vestibuli overlaid, however, on its upper surface by a thin continuous muscle layer, which unites with the broad atrioventricular cushions, the latter fused together in the middle.

It is apparent that this description corresponds very closely with that given for septum II in the pig with the exception that the upper part of this septum corresponding to Röse's septum musculare is not formed by an infolding of the atrial wall but by a local thickening. In his later paper ('89) following Born's work, Röse modified his description of the atrial septa

extensively. He rejected the idea that the septum intermedium is concerned in the formation of the limbus or annulus ovalis. He also discarded the term 'septum musculare,' calling it simply the limbus (Vieussenii) and described it as arising in the anterior upper atrial wall as an infolding caused by the truncus arteriosus (bulbus) imbedding itself between the two atria. From here it spreads along the upper and then along the lower wall of the right atrium. It contains a connective tissue core which was pinched off from the surface of the truncus during the process of infolding. The presence of this core, Röse thinks, is a proof that infolding has occurred.

In the pig the upper part of septum II or limbus which corresponds to the curve of the bulbus, is at first a broad thickening of the musculature of the atrial wall which in later stages becomes more prominent. Only in its lower anterior part or root are connective tissue elements found. These are the remains of the original thickened endocardium which was shown to form the basis for the septum. Retzer ('08) in denying the presence of a septum II in the pig says: "This supposed septum which His correctly described as a 'muskulöse Leiste' is nothing but a fold in the atrial wall at that place." It is formed by the atrial growing around the conus arteriosus as a fixed point, thus causing a bulging inwards of the atrial wall.

The descriptions of Röse and Retzer thus correspond quite closely as far as the origin of the muscular fold is concerned. Retzer, however, thinks it never attains sufficient size to be called a septum. His ('85) described the same structure, calling it the anterior septum or 'sickle' which later gives rise to the limbus Vieussenii. He thought that it was formed by the septum intermedium growing up on the anterior atrial wall to meet the anterior end of the septum superius (septum I). This upgrowth of the septum intermedium, or a portion of it overlaid by muscle, is practically what takes place in the pig, but as pointed out previously, there is no evidence of an infolding of the atrial wall at a higher level.

Favaro's ('13) recent account of the septum secundum in the guinea-pig and sheep differs very little from that given above

for the pig. He finds that the myocardium which invades the connective tissue of the septum intermedium becomes more compact and raised up into a distinct prominence on the right side of the insertion of the atrial septum (septum I). This, he says, represents the site of the inferior segment of the limbus fossae ovalis. The prominence is continued upward as a thickening of the myocardium of the ventral wall of the atrium on the right of septum I. It corresponds to a broad sulcus externally but there is no infolding as described by Röse and Retzer. In the guinea-pig and sheep, however, Favaro finds that the septum spurium or tensor valvulae bends over the upper wall to join the newly-found limbus or septum II. This does not occur in the pig as reference to figures 5, 6 and 7 will show. In this form the tensor valvulae[8] (*T.vv.*) extends upward to the roof of the atrium where it is lost sight of among the developing trabeculae in that region. In the human embryos examined the course and relations of the tensor seemed to be more like those in the guinea-pig and sheep as described by Favaro.

From the first the lower end of the left sinus valve blends with septum II as mentioned previously. The right valve is at first independent of the septum (fig. 5) but later when the septum has increased in height, the valve becomes attached to its right side (figs. 6 and 7). In later stages the right valve increases rapidly in height so that when seen from the right side, it completely covers the lower end of the left valve and the root of septum II (fig. 7). When a portion of the right valve is cut away (fig. 8) the connection between the left valve and septum II can be seen. Separating the orifices of the inferior vena cava (*V.c.i*) and the coronary sinus[9] (*Sn.cor.*) is a thick ridge, the sinus septum (*S.sn.*), which extends from the root of septum II downward and to the right toward the attached border of the

[8] The term 'tensor valvulae,' first proposed by Röse ('89), seems more appropriate than the older 'septum spurium' of His. This structure probably helps to approximate the valves and render them tense during systole.

[9] When the model was sawn through, the cut opened the right extremity of the coronary sinus which forms a deep bay as it turns backward and upward to its orifice (figs. 7 and 8).

right sinus valve, where it blends with the floor of atrium. Owing to the extreme height of the right valve the inferior vena cava and coronary sinus appear to open into a common chamber flanked by the valve.

The further changes in the right atrium may now be considered. From the 21 mm. stage (figs. 7 and 8) up to about 58 mm. the relations of the parts in question change very little. Septum I or the valvula foraminis ovalis, as it may now be called, becomes thinner and somewhat folded. Its free border is fimbriated. The left sinus valve is still well developed, its lower end blending with the root of septum II. It now lies so close to the septal wall of the atrium that the spatium intersepto-valvulare is reduced to a narrow cleft. The right sinus valve remains high, guarding the orifices of the inferior cava and coronary sinus. The sinus septum between the two orifices is behind and medial to the valve but not fused with it. In two specimens (58 to 62 mm.) and possibly a third, a new opening appeared in the lower anterior part of the right sinus valve close to its attached border. The course of the blood-stream from the coronary sinus in this case was directly into the lower anterior part of the atrial cavity rather than upward, backward and medially toward the foramen ovale. The usual pathway, however, was not shut off. His ('85) described a secondary opening of the coronary sinus in human embryos and assumed that it represented the normal condition. Other writers have not found this new opening, Born expressly denying its existence. It probably occurs exceptionally in man as in the pig and may represent a premature degeneration of this part of the valve.

The tensor valvulae (septum spurium) in the older foetuses becomes flattened down considerably in its upper part, though still fairly well marked near the sinus valves. Meanwhile a new muscular ridge, the crista terminalis has appeared in the atrial wall just to the right of the right valve. Its upper part blends with the trabeculae in the roof of the atrium near the tensor valvulae. Traced downward it passes first close to the attached border of the valve and then diverges from it more

and more until it is lost among the trabeculae of the lateral wall of the atrium. The right sinus valve does not seem to take any part in its formation, though both Röse and Tandler ('13) consider that the upper part of this valve is involved.

The final disposition of the various parts of the septal and valvular apparatus is shown in figure 9 which represents a dissection of the heart of a foetus of about 85 mm. Owing to the preponderating growth of the upper part of the atria, the region of the septa appears sunken into the entrance of the inferior cava, while the orifice of the superior cava (*V.c.s.*) remains high up on the posterior wall. Septum I, now the valvula foraminis ovalis (*V.f.o.*) lies in the bottom of a slight depression or fossa whose margin is formed for the most part by the limbus fossae ovalis (*L.V.*). The free, fimbriated border of the valvula extends obliquely into the cavity of the left atrium and overlaps the limbus on that side. The oblique cleft between them is the foramen ovale. Above the fossa and extending downward on each side of it, the limbus is thick and muscular. This part is formed by septum II.

Continuous with the anterior end of septum II is the left sinus valve (*V.v.s.*) which in this place is muscular like the septum and may be said to form part of the limbus. When traced backward and upward around the fossa, the valve becomes reduced to a thin, pale streak which finally crosses the posterior part of the limbus proper and then extends along the left margin of superior caval orifice where it gradually fades out. This is practically in agreement with the descriptions given by Born, Röse and others.

The right sinus valve (*V.v.d.*) is still well marked at this stage. Its lower anterior part bounds the orifice of the inferior cava laterally and extending forward and medially partially covers the orifice of the coronary sinus. The first portion represents the valvula Eustachii, the last (*X* in fig. 9) the valvula Thebesii of other mammals. In the pig the right sinus valve never becomes divided structurally. There is always a narrow cleft between it and the sinus septum (*S.sn.*). The upper part of the right valve extends as a thin narrow membrane

along the right border of the superior caval orifice almost to its upper end. The tensor valvulae has entirely disappeared.

At this stage the crista terminalis (*Cr.ter.*) forms a thick ridge extending downward from the roof, in the angle between the posterior and lateral walls of the atrium. It was pointed out that when the crista first appears it is entirely independent of the right sinus valve, but as it becomes broader and more fully developed, the extreme upper part of the valve comes to lie right on its root. There is usually, however, a narrow cleft separating it from the remains of the valve below. For this reason, the crista terminalis of the pig cannot be said to mark exactly the boundary between the primitive sinus cavity and the atrium proper as has been maintained by His, Röse and Tandler for man.

A glance at figure 9 will show that there is a broad, rounded ridge in the angle between the orifices of the superior and inferior venae cavae (the line marked *V.v.s.* passes across it). It is formed by a thickening of the musculature of the atrial wall in this region and represents the tuberculum intervenosum (Loweri) of the human heart. There has been some doubt about the occurrence of this structure but recently Tandler ('13) came to the conclusion that it is always present in the adult human heart if hardened in situ, i.e., with the pericardium intact. He suggests the term 'torus Loweri' as more appropriate. In other mammals he finds it more strongly developed than in man, especially in the horse and still more so in the seal where it forms a 'veritable septum.' In the pig this ridge occurs constantly in older foetal stages. Owing to its position it would tend to direct the blood-stream from the superior cava toward the right atrio-ventricular orifice.

The chief results of this investigation may be summarized briefly as follows:

In the pig, the method of formation of septum I and ostia I and II is essentially the same as described in other mammals.

The present account of septum II, however, differs considerably from those of other writers with the exception of Favaro. The anlage of this septum in the pig appears in the lower anterior

corner of the right atrium. It is a spur-like thickening developed on the right side of the line of insertion of septum I and is composed, at first, of connective tissue. Later it lengthens out and becomes overlaid and invaded by developing muscle. It then extends upward and backward along the roof of the atrium as a low, broad thickening of the musculature in this region and finally reaches the posterior wall where it bends downward as a narrow, sharp ridge. For the reasons stated in the text, I am inclined to believe that septum II is formed in the same manner in the human embryo.

The right sinus valve is well-developed in the pig. Its lower part guards the orifices of the inferior vena cava and coronary sinus in later stages but does not become divided by the sinus septum into Eustachian and Thebesian valves as in other forms.

The crista terminalis develops independently of the tensor valvulae (septum spurium) and right sinus valve. It does not accurately mark off the primitive sinus cavity from the atrium proper.

A tuberculum or torus Loweri is always present in older foetal stages.

One further point which is illustrated by the models may be mentioned although it is not directly related to the subject of this paper, namely, the fenestration of the proximal part of the anterior cardinal vein. In the 7.9 mm. embryo (fig. 5, *V.car.a.*) this process is well advanced and many of the fenestrae have coalesced, thus tending to separate off a dorsal portion of the cardinal which would receive the intersegmental veins in this region. In the 15.2 mm. embryo (fig. 6, *V.car.a.*) the separation is complete up to the point where the subclavian vein (*V.scl.*) enters. On the left side (not shown in the figure) where the subclavian appears to enter one segment higher (cephalad) than on the right, the separation is carried upward correspondingly. These observations support the opinion of Thyng ('14) that the proximal part of the vertebral vein is segregated from the main venous channel (anterior cardinal) in the manner described. In an earlier paper Thyng ('11) figured this condition in a pig

embryo of 7.8 mm. Lewis ('03) mentions a splitting of the anterior cardinal vein near its entrance into the duct of Cuvier, the subclavian vein arising from its outer part. A comparison of his Plate IV and Thyng's ('11) figure 2, with figures 5 and 6 of the present paper seems to indicate that this process is normal for the pig embryo.

In conclusion I wish to express my thanks to Prof. H. D. Senior who kindly suggested a re-investigation of the atrial septum and to Prof. F. W. Thyng for advice and criticism.

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PLATE I

EXPLANATION OF FIGURES

5 Reconstruction of the heart of a 7.9 mm. embryo. The right atrium and right ventricle have been opened from the right side, the cut passing through the junction of the transverse part of the sinus venosus (*Sn.t.*) and the right sinus horn (*Sn.d.*). Magnified 25 diameters.

6 Reconstruction of the heart of a 15.2 mm. embryo, the right atrium and right ventricle opened from the right side. Magnified 25 diameters.

ABBREVIATIONS

A., Aorta dorsalis
Ao., Aorta (ventral part)
A.d., Atrium dextrum
A.s., Atrium sinistrum
Bulb., Bulbus cordis
Cr.ter., Crista terminalis
En.s., upper endocardial cushion
En.i., lower endocardial cushion
En.c., fused endocardial cushions
L.V., Limbus fossae ovalis (Vieussenii)
O.I, Ostium primum
O.II, Ostium secundum
O.in., Ostium interventriculare
Per., Pericardium
S.I, Septum primum
S.II, Septum secundum
S.in., Septum interventriculare
Sn.t., Sinus venosus, trans. part
Sn.d., Sinus venosus, right horn
S.sn., sinus septum
Sn.cor., Sinus coronarius
Sp.in., Spatium intersepto-valvulare
Sul.in., Sulcus interventricularis
T.vv., Tensor valvulae = Septum spurium
Vv.v., Valvulae venosae
V.v.d., right valve
V.v.s., left valve
V.f.o., Valvula foram. ovalis
V.car.c.d., right com. card. vein
V.car.c.s., left com. card. vein
V.car.a.d., right ant. card. vein
V.car.p.d., right post. card. vein
V.c.s., Vena cava superior
V.c.i., Vena cava inferior
V.az., Vena azygos
V.scl., Vena subclavia
V.h., Vena hepatica
X, (fig. 9)= part of right sinus valve guarding coronary orifice

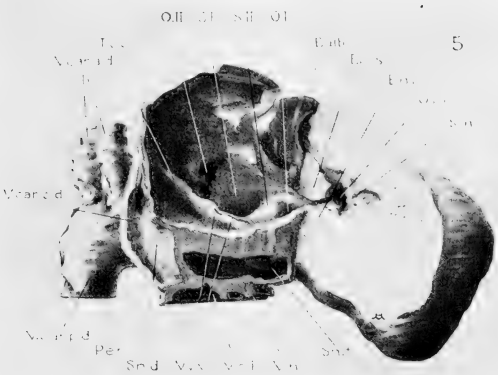

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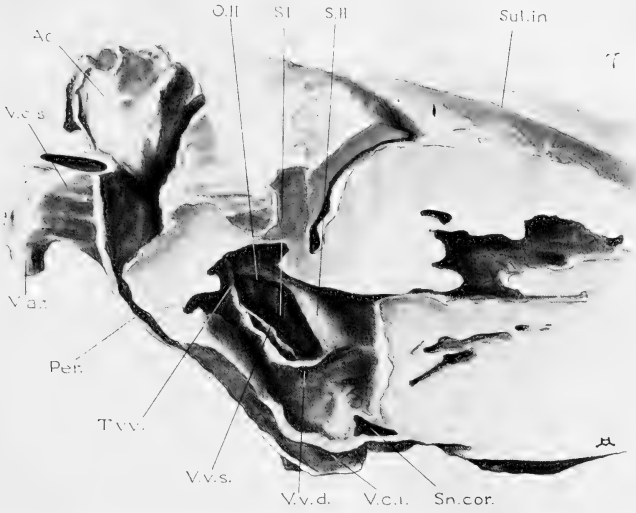
PLATE 2

EXPLANATION OF FIGURES

7 Reconstruction of the heart of a 21.0 mm. embryo. The right atrium and right ventricle have been opened from the right side, the cut passing through a part of the coronary sinus (*Sn.cor.*). Magnified about 25 diameters.

8 Same as figure 7 but in slightly different position. The right sinus valve (*V.v.d.*) has been partly cut away, exposing the orifices of the coronary sinus and inferior vena cava. Probes have been inserted in the superior and inferior cavae and the coronary sinus. The probe in the coronary sinus passes across the sinus septum (*S.sn.*) under the right valve. Magnified about 25 diameters.



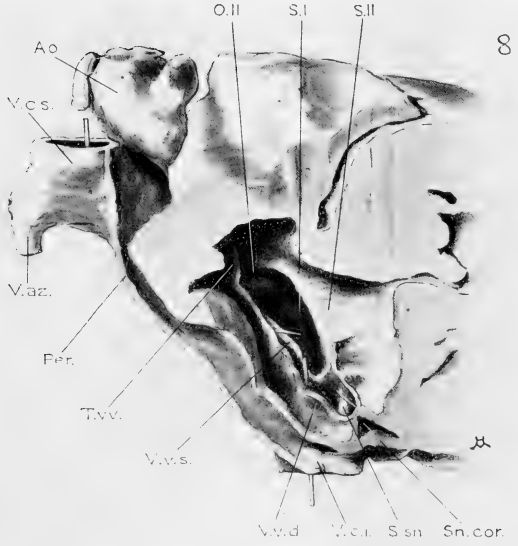


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PLATE 3

EXPLANATION OF FIGURES

9 Dissection of the heart of a foetus of about 85 mm., the right atrium and right ventricle opened from the right side. The lateral wall of the inferior vena cava (*V.c.i.*) and the right sinus valve (*V.v.d.*) have been divided by a vertical cut and the parts somewhat separated. Magnified about 10 diameters.

H. Murayama del.

ORIGIN OF THE SEX-CORDS AND DEFINITIVE SPERMATOGONIA IN THE MALE CHICK

CHARLES H. SWIFT

From the Hull Laboratory of Anatomy, University of Chicago

SIX FIGURES

CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

The year 1870, in which appeared Waldeyer's famous article, Eierstock und Ei, really marks the beginning of modern research relative to the sex glands, their origin and products.

According to a majority of the investigators who have studied the question since 1870, there are two possible sources of origin of the definitive male cells—either from cells of the germinal epithelium or from primordial germ-cells.

These terms require some elucidation and will be explained before taking up the more important articles which bear on the subject.

In the early stages of all vertebrate embryos, the epithelium, or mesothelium, lining the coelomic cavity is flat and a single layer of cells in thickness. After the appearance of the Wolffian body the coelomic epithelium on its ventro-medial surface begins to change. The cells begin to elongate and become columnar or cylindrical and they may finally be several layers in thickness. This change is strictly limited; viewed in section

the elongated cells protrude out from the general level of coelomic epithelium like a small narrow hillock and if looked at from above, the area has the appearance of a light narrow streak or stria on the medial and anterior face of the pink mesonephros. This region of differentiated coelomic epithelium is the germinal streak of Kölliker ('61) and the germinal epithelium of Waldeyer ('70).

The primordial germ-cells (ureier, keimzellen, gonocytes, ovules mâles, ovules primordiaux, urgeschlechtszellen, and germ-cells of the various authors) are found among the cells of the germinal epithelium from its very commencement. They are easily recognized, for they are quite different from the surrounding cylindrical cells. They are large, oval or round, have a large excentric nucleus, which appears vesicular and does not have much chromatin. The cytoplasm also stains faintly. These cells were discerned by Bornhaupt ('67) and were clearly described by Waldeyer ('70), both investigators noticing them in the germinal epithelium of the chick. Since Bornhaupt many investigators in every vertebrate phylum have seen the cells, and yet there is great uncertainty, even at the present time, in regard to their origin and fate.

The earlier students, as a rule, believed that they arose in situ, in the germinal epithelium from the epithelial cells by a process of differentiation (Waldeyer, '70; Balfour, '78; and Semon, '87). Of late, however, there is an increasing belief that they antedate the germinal epithelium; that they originate in some region of the embryo at a distance from the site of the future gonads, and only migrate into the Wolffian region at about the time of the appearance of the germinal epithelium (Nüssbaum, '80; Hoffman, '92; Eigenmann, '92; Beard, '04; Rubaschkin, '07; and Swift, '14).

Having given these short explanations of terms which will be frequently employed we are now in a position to review the more important papers which relate to the origin of the definite sex-cells in the male.

Waldeyer ('70), although he described so exactly the germinal epithelium and primordial germ-cells, did not believe that either

played any rôle in the histogenesis of the male sex gland. He believed that the sex cords arose in the middle portion of the Wolffian body and that they grew into the stroma under the germinal epithelium. The primordial germ-cells degenerated and the definitive sex elements arose from the Wolffian tissue.

Braun ('77), like Waldeyer ('70) and Bornhaupt ('67), described two kinds of cells in the germinal epithelium, the cylindrical, differentiated, peritoneal or coelomic cells and the large primordial germ-cells. Braun found in reptiles (Lacerta) that the walls of the Wolffian tubules produced cell buds, which grew in height, anastomosed one with the other, and ended by sending up cord-like, cellular processes which fused with the germinal epithelium. He called these processes segmental cords, and believed that they gave rise to the seminiferous tubules of the testis.

In reality, Braun did not describe the origin of the true sexual cords, but rete cords, or cords of uro-genital union. Up to the time of Mihalkowics ('85) the true sex cords were constantly confused, both as to origin and function with the rete cords. The rete cords, or cords of uro-genital union, arise prior to the sex cords in the male and female. The rete cords, according to Mihalkowics ('85) and Sainmont ('05), are derived from the Wolffian tubules and the epithelium of Bowman's capsule. According to Allen ('04, '05 and '06), they appear in reptiles and mammals as ingrowths of the germinal epithelium. Bouin ('00), in the frog, Firket ('14), and Swift ('15), in the chick, believed them to arise as condensations of the mesenchyme between the germinal epithelium and the Wolffian body. Whatever their origin they do not produce the definitive sex-cells, but are developed into the tubuli recti and rete testis; interposed between the tubuli contorti, which are of true sex cord origin, which produce the definitive sex-cells, and the vasa efferentia, which are modified Wolffian tubules.

Mihalkowics ('85) described the origin of the true sex cords from the germinal epithelium and replaced Braun's name segmental cord with sex cord. He did not get the details exactly correct—that was left for Janosik—but his idea was correct.

He believed that cells of the germinal epithelium migrated into the subjacent mesenchyme and grouped themselves into cords which later united with the overlying epithelium, while Janosik described the sex cords arising as invaginations or processes of the germinal epithelium. For a very complete account of the origin of rete cords and the two series of sex cords in the female and the single series in the male, the reader is referred to Allen ('04) and Firket ('14).

Nüssbaum ('80) in Rana and in Teleosts, agreed with most of investigators of the period prior to Mihalkowics, that the segmental or medullary cords arose as outgrowths of the Malphigian capsules. However, he disagreed with them as to the origin of the large clear, primordial germ-cells which were present in the cords and germinal epithelium. He did not believe them to be differentiated coelmic cells because he found them away from the site of the gonads, in the mesentery, because there were no transition forms and because they contained a great deal of vitellus, which was not present in the neighboring cells of the germinal epithelium. He believed them to be primitive cells and the direct ancestors of the adult sexual cells.

In passing it should be said that Nüssbaum was the first to hint at a distant site of origin and migration for the primordial germ-cells. He also formulated the hypothesis that "the sexual cells do not come from any cells that have given up their embryonic character or have gone into building any part of the body, nor do sexual cells ever go into body formation." Here, then, is a suggestion of the germ-plasm idea.

Laulanié ('86), in the chick, thought that the primordial sexual gland was bi-sexual; that the germinal epithelium produced the female elements and that the male elements or cells were present in the network of cords, which were developed from the stroma in the midst of the gonad. The cortical ovules, in the germinal epithelium were female and the medullary ovules were male. According to his idea, the gonad was not indifferent but hermaphroditic. Later on in development one of the sexual elements degenerated and the gland became male or female as

the case might be. This idea is of interest only because of its novelty and historic interest.

We now come to two important papers, which will always stand out as landmarks, and which, together with those of Waldeyer, Nüssbaum and Mihalkowics, will always be studied in connection with any work on the origin of the sexual cells. I refer to the work of Semon ('87) and Hoffman ('92).

Semon studied the indifferent gonad and the testis of the chick. Like Balfour ('78), Braun ('77), and Nüssbaum ('80), he believed the sex cords to be outgrowths of the capsules of the Wolffian bodies. He saw, as Waldeyer did, two kinds of cells in the germinal epithelium, the columnar epithelial coelomic cells, and the large clear primordial germ-cells. He described the various stages which the columnar cells passed through in becoming the primordial germ-cells. In fact, his opinion in regard to the primordial germ-cells is exactly that of Waldeyer. He found that the sexual cords, growing out from the Malphigian bodies, reached the germinal epithelium about the sixth day and that the primordial germ-cells passed into them. The cords became separated from the epithelium by a connective tissue layer, differentiated from the stroma, called the albuginea. The cords anastomosed freely with one another, became tubular and in this way developed the tubuli contorti seminiferi. The cavity in the cords began to appear during the third week of incubation and at hatching had reached a great size. These tubes contained two kinds of cells, like the germinal epithelium, the small peritoneal cells and the large clear cells which resembled and were descendants of the primordial germ-cells. In regard to the products of these two varieties of cells it is better to quote Semon: "Es kann natürlich keinen Zweifel unterliegen, das die kleinen Zellen der Segmentalstränge die sogenannten Stützzellen der Samenkanälchen, die Ureier aber die grossen, runden Hodenzellen repräsentiernen."

Semon and Nüssbaum ('80) were the first to show a continuity between the primordial germ-cells and the male sexual cells of the adult. It must be remembered, however, that Semon obtained his ureier from the cells of the germinal epithelium by

a process of differentiation, while Nüssbaum noticed the primordial germ-cells at a distance from the sexual primordium.

Hoffman ('92) employed in his researches about a dozen species of birds, mainly taken from the group of waders, and in three of them, Haematopus ostralegus, Sterna paradisea, and Gallinula chloropus, there was sufficient evidence brought out to show that some, if not all the primordial germ-cells, did not arise in the modified coelomic epithelium. In the three species mentioned above, he found at the proper time, numbers of the primordial ova in the germinal epithelium, but, in addition, he found cells, supposedly primordial ova, because of their resemblance to those found later in the germinal epithelium in embryos of 23 somites. An embryo of 23 somites does not possess the so-called germinal epithelium, the coelomic epithelium over the Wolffian body not having been modified at this age, yet in these he found primordial germ-cells far removed from the site of the future sex gland, in the splanchnic plate of mesoderm, in the region between splanchnic mesoderm and entoderm and in the entoderm itself.

To quote Hoffman:

> Maintenant qu'il est évident que les ovules primitifs ne dérivent pas des cellules péritonéales prévilégiées, mais qu'ils se rencontrent déjà dans très jeunes périodes de développement, bien que leur première ébauche chez les Vertébrés, soit encore entièrement inconnue il sera nécessaire de laisser tomber le mot "épithélium germinatif." Voila pourquoi j'appellerai desormais, cette partie de l'épithélium péritonéal, qui se transforme en un assise, dont les cellules sont disposées en pousieurs rangées et entre lesquelles sont placées les ovules primitifs, couche des ovules primitifs.

This "couche des ovules primitifs" gave origin to the true sexual cords in the male, and the medullary cords and cortical cords in the female.

The true sexual cords in the male and the medullary cords in the female are homologous—the cords of first proliferation according to Firket ('14). The cortical cords—cords of second proliferation—occur only in the female and appear later. The true sexual cords in the male produce the sexual elements of the male. The medullary cords in the female—their equivalents—go partly into the formation of ovarian stroma, but in greater part disappear, while the second crop in the female—the cor-

tical cords—give rise to the sexual elements in the female and the follicular epithelium (Swift, '15).

Hoffman found in Totanus calidrus, Vanellus cristatus, and Limosa ergocephala that the true sexual cords, just after hatching, developed a lumen and became the seminiferous tubule.

To quote Hoffman again:

> Les tubes seminifères renferment deux espèces de cellules; des cellules grandes et rondes et des cellules plus petites d'une forme conique ou cylindrique qui les première enveloppent. Les grandes cellules ont un noyau arrondi et volumineux, qui ne contient que peu de chromatine et qui ne se colore que très faiblement par les reactifs; son diamètre est de 9–11 μ. Le corps protoplasmatique est également très pâle, les contours sont ordinairement très indistincts. Le noyau des petites cellules est ovale, il a une longueur de 6–7 μ, une largeur de 4–5 μ et est tienté très fortement. Les deux espèces de cellules se trouvent mêlées sans aucun ordre, ne formant ordinairement qu'une seule assise, comme cela se voit dans les sections, qui ont coupées le tube ou exactement dans son axe longitudinale ou transversale. Entre les grandes cellules des tubes seminiferès et les ovules primordiaux de la couche des ovules primitifs, il n'y a pas de moindre différence. Il n'est pas douteux que les grandes cellules soient des ovules primordiaux émigrés et qu'elles représentent les propres cellules du testicule—les spermatogonies—tandis que les petites cellules forment les cellules de soutient (Stützzellen des auteurs allemands).

Hoffman's work is of extreme importance because of the continuity which he established. The primordial germ-cell was first seen away from the germinal epithelium; it migrated into that structure, then passed into the sexual cord, which the germinal epithelium produced, became a spermatogonium, and later developed into the mature sexual cell.

According to Semon the peritoneal mesoblastic cell is the ancestor of the sperm; according to Hoffman the primordial germ-cell of whose ancestry he is ignorant, is the direct forebear of the sperm.

Allen ('04) made a very thorough study of the development of the testis and ovary of the pig and rabbit and found the sex- and rete-cords to be invaginations of the peritoneum.

In regard to the primordial germ-cells he said:

> Primitive sex cells are being formed in the rete cords from the syncitial cells that have retained the primitive character exhibited by the

peritoneal cells, from which these cords arise. This development of undifferentiated peritoneal derivatives to form primitive sex cells is probably homologous with the process by which germinative cells (descendants of the peritoneal cells of the germinal epithelium) of the seminiferous tubules of the testis and the cells of the cords of Pflüger of the ovary are being transformed into primitive sex-cells.

And again:

Clear transition forms are found to connect the primitive sex-cells with the germinative cells of the seminiferous tubules. Transition forms connecting the germinative cells with the sex cells occur in the pig embryos between 2.5 cm. and 13 cm. length; later states show no transition forms.

As regards the formation of the definitive sexual cells he said:

It has been shown in the pig and rabbit, however, that these sex cells, appearing in the indifferent stages, do not contribute to the formation of functional sex products in the ovary. The same is probably true of the testis, it being at least certain that the great bulk of the spermatogonia are formed from the germinative cells—cells derived originally from the peritoneum and maintaining, first, their indifferent character, at least so far as our technique is able to show.

Dustin ('07, '10) studied the question of germ-cells origin, migration and evolution in Amphibia—Triton and Rana—and in Reptilia—Chrysemys.

In the first paper, dealing with the Amphibia, he reported that the primordial germ-cells were of mesoblastic origin; from a part of the primitive coelom which he called gonocle. He called the germ cells gonocytes of the first line and found that they reached the sites of the definitive sex glands by active migration. He found that a second line of gonocytes was produced by transformation of small germinative cells, which were in their turn the result of peritoneal epithelium proliferation. Both lines of gonocytes were able to produce definitive sexual cells.

In his second paper relating to Chrysemys, he found that there were also two lines of gonocytes, with this exception, that in the reptile the primary gonocyte came from the entoderm.

Deux espèces de cellules sexuelles se succèdent donc au cours de l'ontogenèse; l'une privient de l'éndoderme; elle est extraordinaire-

ment précoce et subit une série de migrations qui lui assurent trois localisations successives (gl. paires primaries, glande impaire, gl. paires définitives); l'autre est d'origine mésodermique et se développe in situ, au niveau des glandes définitives.

Chez Chrysemys marginata, les gonocytes de la première espèce ne présentent de karyokinesès qu'au début de leur différenciation; ultérieurement ils ne se multiplient plus; beaucoup d'entre eux dégénèrent même.

He believed that more of the primary gonocytes functioned, in producing definitive sexual cells, in Amphibia than in Reptilia.

Rubaschkin ('12), working with the guinea-pig, stated that the definitive sex cells in both testis and ovary are descendants of the primordial germ-cells. Rubaschkin used as his criterion for distinguishing the primordial germ-cells the granular type of mitochondria. He believed that the primitive cells, or blastomeres, possessed this type of mitochondria, and that as the somatic tissues were differentiated from them, the cells of the tissues acquired the rod-shaped mitochondria. In this way he was able to recognize the germ-cell at any stage in the young embryo and differentiate it from the ordinary somatic cell. By this means he came to the conclusion that the definitive sex cells were descended from the primordial germ-cells.

For a more detailed account of the literature on the subject of the origin of the definitive sexual cells, the reader is referred to the ample bibliographies of Bouin ('00), Allen ('04), and Firket ('14).

MATERIALS AND METHOD OF STUDY

In the course of this investigation two fixatives were employed-namely, Meves' modification of Flemming's fluid and the acetic, osmic-bichromate mixture. In a previous investigation on the primordial germ-cells of the chick ('14) the osmic acid content of these fluids was found to be a serious disadvantage owing to their staining of the numerous vitellus granules, which, when blackened, obscured the cytological details. However, that drawback was not present in the embryos and young chicks used in this research for the germ-cells had lost their vitellus

content, and the preservation of the mitochondrial granules and attractions sphere was a decided asset.

In this work only the testes were fixed and not the whole embryo, as was the custom, when dealing with the younger stages.

In the case of the youngest stages used in this investigation, the testes were allowed to remain in the killing fluid 24 hours but as there were signs of over-fixation in the sections, this time was reduced to 10 hours with much better results.

All the sections were cut 4μ in thickness, and following the acetic-osmic-bichromate fluid stained by Bensley's anilin-acid fuchsin—Wright's blood stain method and the anilin-acid-fuchsin-methyl green method. There is no need of entering into detail in regard to these staining methods since they have been described by Bensley ('11), Cowdry ('12), and Swift ('15).

Following Meves' fluid the iron hematoxylin stain was employed.

The following table will show at a glance the number of stages employed, their age, and the methods used in fixation and staining.

TABLE 1

METHOD		EMBRYOS OF 6 DAYS INCUBATION	7	8	9	11	13	15	17	20	CHICKS 3 DAYS OLD	6	10
Meves' fixation and iron-hematoxylin stain					1	1							
Acetic osmic-bichromate fixation	Bensley's anilin acid fuchsin and methyl-green stain			1	1								
	Bensley's anilin acid fuchsin and Wright's blood stain	2	2	2	2	3	2	2	2	2	2	2	1

THE INDIFFERENT GONAD

If the viscera are removed from the abdominal cavity of a 5-day chick embryo, the Wolffian bodies will be seen on the posterior wall on either side of the mid-line. On the ventromedial surface of each mesonephros, beginning at the cephalad extremity and extending caudad about two-thirds of the length of each Wolffian body, a narrow, white, rounded ridge will be observed. These narrow elongated elevations are the indifferent gonads. At this time they have a length of about 2 mm. and there is a noticeable difference in size in favor of the left gonad.

On examining a section through the anterior portion of the Wolffian body, the gonads will be seen to resemble two small abrupt hillocks on the surface of the mesonephros turned towards the mesentery, and, it will be noticed also that they have a broad connection with the mesonephros.

The microscope reveals, farther, that there are three distinct tissues in the gonads.

Clothing the free surface of each genital anlagen, and extending for some distance over the root of the mesentery, is an epithelium made up of two to three layers of tall columnar cells. This tissue is the germinal epithelium of Bornhaupt ('67) and Waldeyer ('70) and all later investigators. This epithelium is made up of columnar or rather cylindrical cells, which have oval or round deep staining nuclei and possesses a distinct basement membrane. This last structure is very noticeable and at this stage—5 days—can be followed over the germinal hillock as a regular curved line without any irregularities of any kind.

Under the germinal epithelium, which is thicker and more extensive over the left gonad, is another tissue—the stroma of the gonad. This tissue is nothing but embryonic mesenchyme, and as such is directly continuous with the same tissue in the Wolffian body and in the root of the developing mesentery. The cells of this tissue are loosely packed and have faintly staining nuclei. The cytoplasmic part of the cells appears as indistinctly bounded processes which anastomose with the processes of other

neighboring cells. The cells, then, are apt to be stellate in appearance and the whole tissue to resemble a loose syncytium. This stroma, more voluminous in the case of the left gonad, fills up the region between the concave germinal epithelium and the Wolffian body and, in fact, forms most of the area of the gonad.

This stroma is denser just under the epithelium, and from this region, if the sections happens to be right, a narrow cord may be seen to extend obliquely towards the Wolffian body. This cord, the result of a condensation of mesenchyme tissue, is known as a rete cord or cord of uro-genital union and must not be confused with the sexual cord, which arises later and in a different way. According to Firket ('14) there are 16 of these cords. It is not my intention to enter into the origin of these cords or to engage in the controversy as to their origin, for an excellent review of all the facts and literature will be found in Firket's ('14) article.

The third tissue present in the gonad is made up of primordial germ-cells.

The cells are easily seen, for their great size, large nucleus, and clear cytoplasm make them conspicuous in the chick as in all the other forms in which they have been observed. They merit, however, a more extended description.

The primordial germ-cells do not form a compact or continuous tissue in the indifferent gonad of 5 days, but are present, usually, as isolated cells or groups of several cells in the germinal epithelium and subjacent stroma (Swift, '15). They may be even seen in the root of the mesentery subject to the same arrangement.

These primordial germ-cells are, at a glance, seen to be different from the other tissues of the gonad. Although present in the epithelium and stroma of the genital hillock yet I have never seen a stage which could be called a transition between them and the surrounding cells, for in addition to their large size, great nucleus and clear cytoplasm, they possess even at this late date (5 days) many droplets of vitellus in the cytoplasm and a conspicuous attraction-sphere (Swift '14 and '15). The

cells of the germinal epithelium and stroma have none of the vitellus and inconspicuous spheres.

In fact the primordial germ-cells do not originate in the germinal epithelium as was believed by many of the older investigators, but at a distance from the site of the gonad. They arise in the germ wall entoderm (Swift, '14) and after an extended migration through the blood vessels (Swift '14 and von Berenberg-Gossler, '14) they reach the splanchnopleure entering into the formation of the mesentery, where they leave the vessels and then migrate in an amoeboid manner into the forming germinal epithelium.

During all their migration period and up to this time they divide very infrequently. That they do divide is proven by the increase of numbers from stage to stage (Firket, '14, and Swift, '14 and '15) and by the fact that they are frequently seen in small groups, which are probably the result of several successive divisions.

As was indicated in the short historical review of this article, it is still an open question as to the rôle played by the primordial germ-cells in the formation of the definitive sex-cells in the male.

DIFFERENTIATION OF SEX AND ORIGIN OF THE SEXUAL CORDS

The period of embryonic development in the chick from $5\frac{1}{2}$ to $6\frac{1}{2}$ days is of extreme interest and importance, for during that period the sexual cords appear and at its termination it is possible to tell the sex of the individual.

The sexual cords, which begin to appear at about the 132d hour of development, are the true sexual cords in the male or seminiferous cords, and in the female the medullary cords or cords of first proliferation. As the names imply, there is only one series of cords in the male while in the female there are two —the last series being known as the cortical cords or cords of second proliferation (Mihalkowics, '85, Hoffmann, '92, Firket, '14 and Swift, '15).

The cords of first proliferation are first noticed as buds or protrusions of the germinal epithelium into the underlying

stroma. The basement membrane appears wavy but is continuous around the swelling. The buds enlarge and elongate but remain attached to the germinal epithelium for some time (fig. 5, Swift, '15). These cords, which are at first small, are evidently of germinal epithelium origin, for the basement membrane of the latter structure is, for some time, continuous around the cord. Neither are they formed by infolding or invagination of the germinal epithelium, for in that case a tubular lumen would be present in each cord, and in some cases could be seen connected with the coelomic cavity. They are simply the result of increased local mitotic activity in the germinal epithelium. The primordial germ-cells which are present in the cords do not play any evident rôle in their formation, for none are ever found dividing just at the time of cord formation, while the peritoneal cells show signs of increased activity.

Although the cords begin to appear at $5\frac{1}{2}$ days, yet it is not until 12 hours later that the greatest activity of the germinal epithelium in their production occurs. At $6\frac{1}{2}$ days they have about ceased to appear.

At the end of the period of cord production the interior of the gonad seems to be made up nearly entirely of sexual cords. They are close together, separated from one another by a little stroma; a majority of the cords are no longer attached to the germinal epithelium but are still surrounded by a definite basement membrane.

The cords have become autonomous, for, although no longer attached to the germinal epithelium, they are growing rapidly as is indicated by the rapid increase in diameter of the cord and size of the gonads.

The cords have a definite orientation—they are straight and extend from the germinal epithelium down towards the Wolffian body.

The primordial germ-cells are during this period found in three situations in the gonad, in the germinal epithelium, in the sexual cord, and in the small amount of stroma between the cords.

When the embryo chick has reached the 156th hour of development ($6\frac{1}{2}$ days), the formation of cords of first proliferation ceases rather abruptly, and about this time it is possible to determine the sex of the individual.

In determining the sex of the embryo there are several criteria which are of value.

1. The relative size of the gonads. It has been known for a long time that the left gonad in the female grows rapidly while the right does not, so that in a short time after sexual cord formation, the left gonad or ovary has far outgrown the right. In the case of the male individual both gonads continue to grow. This criterion is of value, but too much reliance can be placed upon it especially during the 6th day, for in all cases, male as well as female, the left gonad is the larger. The left gonad is the larger during the indifferent stage and in the male is the larger even in the adult (Etzold, '91).

2. Thickness of the germinal epithelium. This and the following criterion I believe to be the most important. In the male the germinal epithelium is thin at the end of sexual cord formation and continues so, while in the female the germinal epithelium over the left gonad or ovary still consists of the several layers of columnar cells. In the male the epithelial cells become very quickly a single layer in thickness and the individual cells cuboid in character. This is an excellent test.

3. Number of primordial germ-cells in the germinal epithelium. In the germinal epithelium of the left female gonad the number of primordial germ-cells appears undiminished, while in the epithelium covering the male gonads there is hardly a single one to be seen (Hoffman, '92 and Swift, '15). In the case of the male they all appear to have gone into the sexual cords, and in the female to be remaining quiescent until the formation of the cortical cords, in the evolution of which they play so important a role (Swift, '15).

4. Attachment and growth of the sexual cords. The cords remain thin and attached to the germinal epithelium for a long time in the male gonad, while in the female they increase rapidly in diameter and become detached early in their evolution.

This rapid increase in size of the cords accounts for the more rapid growth of the embryonic ovary.

This last criterion is of less weight than the preceding, and is of importance only when used in connection with the others.

THE EVOLUTION OF THE SEXUAL OR SEMINIFEROUS CORDS OF THE EMBRYONIC TESTIS

The next three embryonic stages studied, namely, 7, 8, and 9 days, may be described together, since they are characterized in common by the same facts, and the changes although occurring, are not abrupt or great.

During this period the sex of the individual can be easily ascertained, either with or without the aid of the microscope. In the latter case the first criterion mentioned above, relative size of the gonads, is amply sufficient, and with the microscope a single glance at the germinal epithelium, the sexual cords, and the number of primordial germ-cells in the epithelium is all that is needed.

Both testes are slowly increasing in size but in all cases the left is found to be the larger. They are becoming rounder and in the process the broad connection with the Wolffian body is being narrowed; in other words, they are being constricted or pinched off of the Wolffian body.

The germinal epithelium is reduced to a single layer of cuboidal cells, and by the end of this period of 7 to 9 days of development, all connection with seminiferous cords is severed. This is brought about by a condensation of the mesenchyme under the epithelium to form the tunica albuginea.

The seminiferous cords, which will in the future claim most of our attention, make up the greater part of the testis. They have a definite orientation from the epithelium obliquely toward the narrowing attachment of the testis with the Wolffian body. Each cord is, of course, not entirely straight, but wavy. They are separated one from another by a very thin layer, one might almost say film, of stroma and a number can be followed at one time across the field of the microscope.

The cells of the seminiferous cords are of two kinds—the ordinary peritoneal cells, issue of the germinal epithelium, and the primordial germ-cells. The former are the more numerous and, at this period, can be easily seen to have definite membranes. They still preserve their cylindrical form, acquired in the formation of the germinal epithelium, and the dark staining round or oval nucleus.

The primordial germ-cells, although not numerous, are as conspicuous as when observed in the capillary of a 21 somite chick or the root of the forming mesentery (Swift, '14). They are a little smaller and have lost the characteristic vitellus but in all other ways are unchanged. They are still large, have the same clear cytoplasm, large round nucleus and conspicuous attraction sphere. From 2 to 8 of the primordial germ-cells are present in a single cord and there are no signs of division. In 9 embryos of this age studied I was not able to find a single primordial germ-cell in mitosis. This last fact is of interest when a comparison is made with the primordial germ-cells in the germinal epithelium of the ovary of the same age. It will be recalled (Swift, '15) that during the 8th and 9th day in the female the germ-cells are in an extremely active condition and that the formation of the cortical cords is well under way.

As regards the stroma only a few lines are necessary. Only under the germinal epithelium is the connective tissue development considerable. In that region there is a broad band of tissue, the albuginea. In the rest of the gonad, between the seminiferous cords, the small amount of stroma still remains embryonic in nature, forming a kind of mesenchymal syncytium. It resembles the mesenchyme of the mesonephros from which it was originally derived and with which it is still connected.

Four embryos aged 11 days were studied. Considerable change has taken place. The testes have increased greatly in size, are rounder, but still have a narrow attachment to the Wolffian body. On examining a section through the testis it will be noticed immediately that the stroma has increased in amount between the seminiferous cords (fig. 1), and that the

layer under the epithelium has thickened. In fact most of the increase in the size of the testes is due to the increase in amount of connective tissue. The cells of this tissue are still mesenchymal in character; cell boundaries are hard to determine and the cytoplasmic processes of the cells unite forming a kind of syncytium. The nuclei are round or oval (fig. 1).

The seminiferous cords no longer run in a regular way as described above, but form a kind of network. On studying them one receives the impression that they have grown considerably in length and as a result have had to become convoluted in order to adapt themselves to a slower growing space. In all four embryos studied several peculiarities were found to be common in regard to the seminiferous cords. In the first place a cord runs only a short distance across the field before being lost. This indicates folding (fig. 1). In the second place there are more cords present in that part of the testis next the Wolffian body, and here the net formed by the cords is more compact. In the region towards the germinal epithelium there is always a single cord which tends to run parallel to the germinal epithelium separated from it by the developing albuginea and from the other cords by a thick layer of stroma.

The seminiferous cords are surrounded by a definite basement membrane (fig. 1), which they acquired when given off from the germinal epithelium.

The cells present in the cords are principally of the peritoneal type, although an occasional primordial germ-cell can be seen (fig. 1). The cell walls of the peritoneal cells can be seen but are not so distinct as formerly. Peritoneal cells are occasionally seen dividing but the primordial germ-cells are still quiescent. In this stage the increase in the cords is one of length but not of diameter.

In the 13 day embryo several important changes must be recorded.

There has been a great increase in the amount of stroma (fig. 2) and as a result of this, in the size of the testis, which is now almost separated from the mesonephros. In an embryo four days younger the stroma was present simply as a thin film be-

tween the seminiferous cords; two days later there had been some increase in the connective tissue, especially in the region under the germinal epithelium, but in these embryos of 13 days development the area of the mesenchyme far exceeds that of the

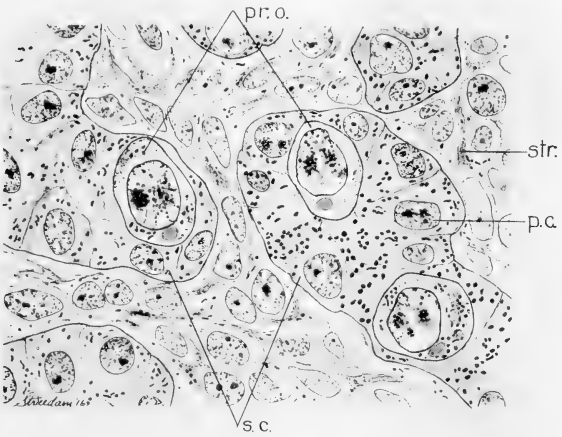

Fig. 1 Portion of a transverse section through the left testis of an 11 day chick embryo. This section shows the seminiferous cords, composed of peritoneal cells and containing several primordial germ-cells. The cords are separated from one another by a mesenchyme-like connective tissue.

The figures illustrating this article were drawn by Mr. A. B. Streedain. Zeiss apochromatic objective 1.5 mm., and compensating ocular 6 were employed for all the figures. The camera lucida was used in making all the drawings and magnification calculated at table level in all cases. The figures were reduced by one-fourth in reproduction giving a magnification of 1125 diameters for all the illustrations. The figures were drawn from preparations fixed in Bensley's acetic-osmic bichromate mixture and stained with Bensley's anilin acid fuchsin —Wright's blood stain. All the sections were cut 4 μ in thickness.

ABBREVIATIONS

int.C., interstitial cells
L., lumen of seminiferous cord
M.C., mitochondrial crescent
p.c., peritoneal cell
pr.o., primordial germ-cell
pr'.o'., primordial germ-cell in mitosis.
S., cell of support
S.C., seminiferous cord
Sp., spermatogonium
Sp'., spermatogonium in mitosis
Str., stroma

cords (fig. 2). In addition a few interstitial cells are present in the stroma between the cords. These interstitial cells are very numerous in later stages, so that an account of them and their development will be deferred until those are described.

The seminiferous cords have a larger diameter, and now form an open net with large interstices (fig. 2). The cords anastomose with each other in every conceivable plane and direction, so that their original orientation is completely lost. This tangle of cords now occupies the entire testis, the area of mesenchyme under the germinal epithelium, described in a previous stage,

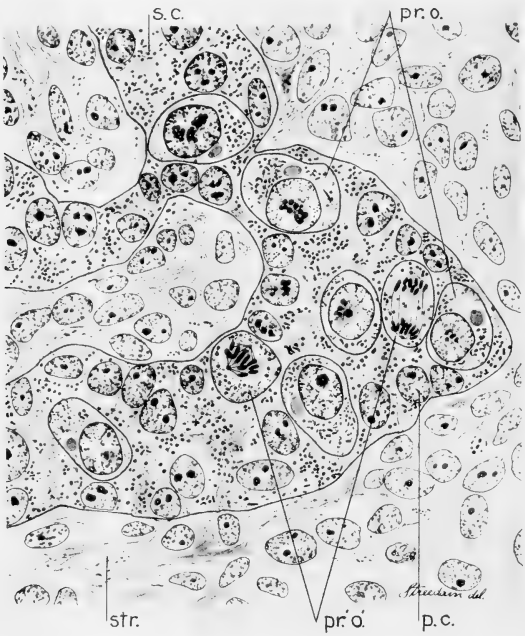

Fig. 2 Portion of a transverse section through the left testis of a 13 day chick embryo. This section shows a seminiferous cord containing many primordial germ-cells. Two of the germ-cells are dividing.

having disappeared. The cords stain more intensely now, stand out more sharply against the light connective-tissue background and contain peritoneal cells and primordial germ-cells. There has been an important change, however, for the latter are much more numerous than in any preceding age (fig. 2). This increase in number of the germ-cells is evident even when examining the section under the low power microscope, for they are seen as clear spots thickly sprinkled throughout the cords. On using the higher powers this is confirmed, and, in addition, it is seen that the primordial germ-cells are actively dividing (fig. 2). In a single section through the testis from 4–10 are found in mitosis. The increase in the size of the seminiferous cord is due to the increase in the number of the germ-cells for the peritoneal cells are quiescent.

Semon ('87) in the chick and Popoff ('09) in the chick and a number of other forms, described the 'ureier' and 'les ovules mâles' (primordial germ-cells) as having a definite position near the center of the seminiferous cords, while the peritoneal cells occupied the periphery. I have not found that to be the case (figs. 2 and 3). In all stages up to and including the 15 day stages examined, I find them to be scattered evenly throughout the cord—some at its center and some at the periphery. Later on they are all found at the periphery of the cord.

A few words are necessary in regard to the structure of the primordial germ-cells. As will be seen from all the figures illustrating this article there is little change in the primordial germ-cells or their tissue. In fact, there is little change to record in all their history from origin until they become oogonia or spermatogonia, unless it is a gradual loss of vitellus and a progressive slight decrease in size (Swift, '14 and '15). However, with the beginning of division in the female line of germ-cells, which resulted in the oogonia, there was a change in the arrangement of the mitochondria (Swift, '15). While the primordial germ-cells were quiescent in the germinal epithelium of the embryonic ovary, up to the 8 day stage, the mitochondria were evenly scattered in the cytoplasm (Swift, '15). At 8 days the germ cells began to divide, and in all the later stages the mitochon-

dria were found grouped around the attraction-sphere in a characteristic manner. It is a striking and suggestive fact that the same change occurs in the male line. In all the primordial germ-cells in the sexual cords of the male chick, up to and including the 11 day stage, the rod shaped and granular mitochondria are evenly distributed (fig. 1). This arrangement also

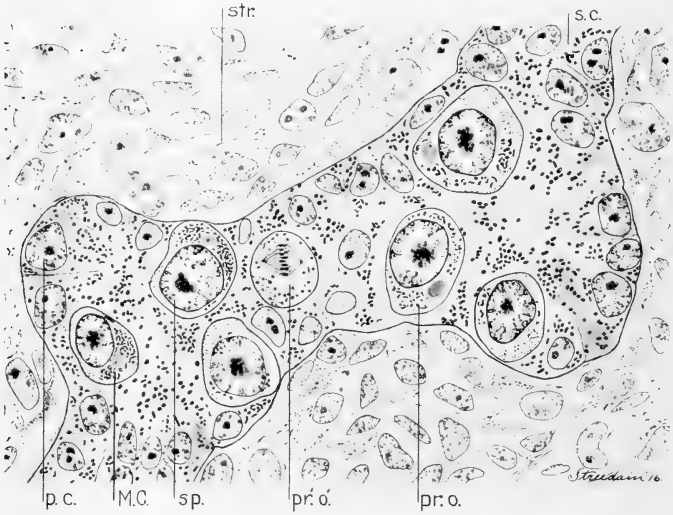

Fig. 3 Portion of a transverse section through the right testis of a 15 day chick embryo. This section shows a massive seminiferous cord, composed of primordial germ-cells, spermatogonia and peritoneal cells. One of the germ-cells is dividing. The spermatogonia, at the left, contain the characteristic mitochondrial crescent which serves to identify them.

holds for the germ-cells of the 13 day embryo and for the few present in the 15 day stage.

Thus, beginning with the 13 day embryo, a new mitochondrial arrangement is noticed; the mitochondria are grouped around the attraction-sphere, so that they form a granular crescent or cap on the nucleus, depending upon the plane of the section (figs. 3, 4, 5 and 6). As was stated above and in a former

article (Swift, '15) this same arrangement was observed in the oogonia of the chick and it is my belief that any cell in the male which has this character is a spermatogonium. Certainly all the spermatogonia of much later periods possess this distinguishing character. I believe, then, that spermatogonia formation

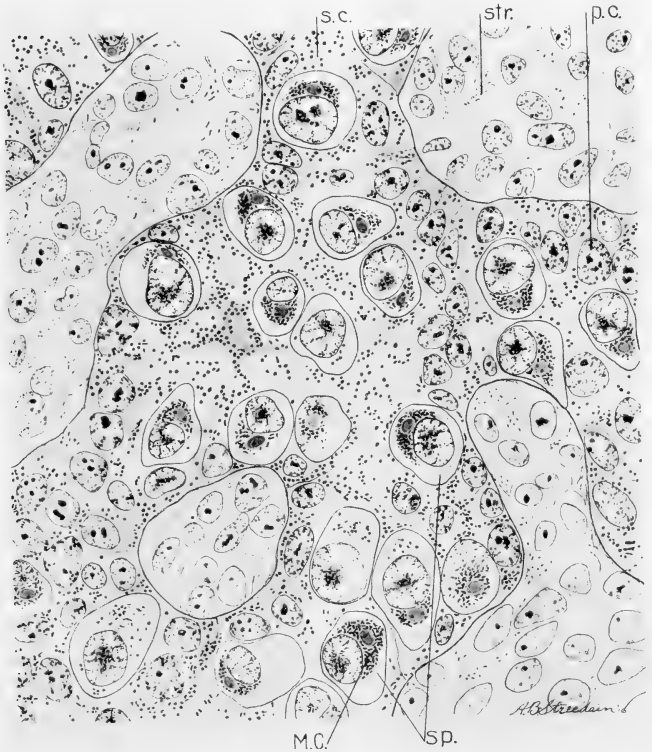

Fig. 4 Portion of a transverse section through the left testis of a 17 day chick embryo. This section of a massive seminiferous cord contains peritoneal cells and spermatogonia. All the spermatogonia contain the mitochondrial crescent. At this age practically all the primordial germ-cells have given way to the spermatogonia.

begins in the male chick embryo at 13 days as a result of primordial germ-cell division, and that the peculiar arrangement of mitochondria around the attraction-sphere, which will be taken up at greater length in a later chapter, distinguishes the spermatogonium from the primordial germ-cell.

At this time, also, it is necessary to say something about the continuity of the mitochondria in the germ line.

In this regard, I can say that they are always present in the primordial germ-cells, oogonia and spermatogonia, either as short rods or granules, depending somewhat upon the fixation. About the same amount seems to be present in the germ-cells at all stages, from origin in the primitive streak stage to oogonia and spermatogonia formation in the 8 and 13 day embryo respectively. Even in the oogonia and spermatogonia the number of mitochondria remains about as in the germ-cells, for the mass around the attraction-sphere is formed at the expense of the rest of the cytoplasm (figs. 4, 5 and 6).

In the 15 day chick embryo there are an immense number of interstitial cells in the stroma. They appear grouped in masses and cords in the stroma between the seminiferous cords. So numerous are they that it is possible to identify them in the section before removal of the paraffin. This can be easily done since they appear black, owing to the staining of their many fatty granules by the osmic acid.

The seminiferous cords branch and anastomose to form a net as described under the 13 day embryo. They contain, however, relatively and absolutely, many more spermatogonia, for the increase has been in that element owing to continued division of the germ-cells (fig. 3). The number of spermatogonia, compared with the preceding stage, is enormous. They are seen several hundred in a field, when the low power microscope is used. Numbers of mitoses in the primordial germ-cells and spermatogonia are present (fig. 3), and this is the stage in which they are found dividing most actively.

In regard to the 17 day chick embryo not a great deal need be said, for, in it, the findings closely resemble those described in the 15 day embryo.

The interstitial cells are present in greater quantity, and there has been some increase in the size of the cords; the amount of cord tissue, too, as compared with the stroma, has increased (fig. 4). Practically all the primordial germ-cells have been changed by division, giving rise to the spermatogonia (fig. 4). There is no evidence of division in the peritoneal cells but there number has not decreased. In certain regions of the seminiferous cord complex, it is evident that the spermatogonia are beginning to assume a position at the periphery of the cord, next to the basement membrane. This is very evident in the next stage. There is no evidence of a lumen beginning to appear in the cords. They still are solid, although it was at this age that Semon ('87), in the chick, described one as being present.

In the next embryo, that of 20 days development, there are some important changes to record.

The seminiferous cords are very massive and seem to be growing at the expense of the stroma, which is considerably reduced in quantity (fig. 5). The interstitial cells (fig. 5), too, are not as numerous as in the two preceding stages. The greatest changes, however, have taken place in the structure and orientation of the cords.

The spermatogonia, which hitherto have had no definite arrangement, being scattered helter-skelter throughout the cord, now begin to be arranged in a definite manner. They are placed against the basement membrane of the seminiferous cord in such a way that their long axes are at right angles to the long axis of the cord (fig. 5). Another striking fact is to be noted, that the attraction-sphere, with its mitochondrial body, is next to the basement membrane, while the nucleus is turned toward the axis of the cord (fig. 5). That is to say, the vegetative pole of the spermatogonium is next the basement membrane. The nucleus is excentrically placed, and always the greatest mass of cytoplasm, in which is placed the attraction-sphere, is towards the basement membrane (fig. 5).

The cords, also, are beginning to have a cavity. This cavity is far from being continuous and can be seen here and there only, in the central axis of the seminiferous cord (fig. 5). This

lumen is formed, not as one would suppose, by a fissure appearing between cells and then enlarging, but by a liquefaction of the cells in the central axis of the cord. When this destruction which involves chiefly the peritoneal cells, is complete, a slit appears in the debris and the lumen is formed (fig. 5). The peri-

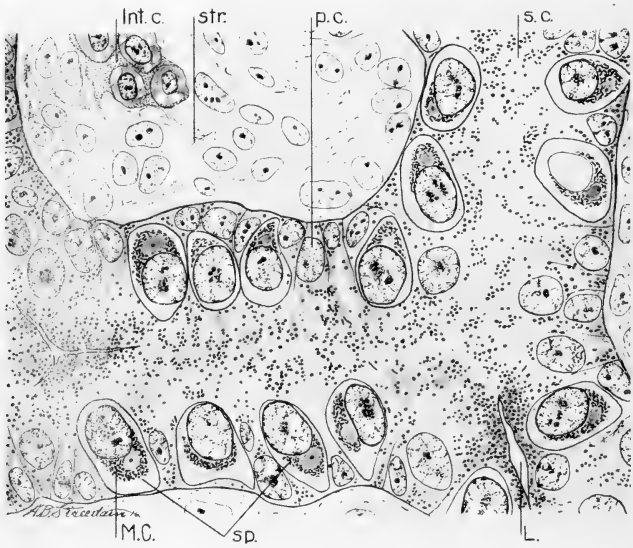

Fig. 5 Portion of a transverse section through the right testis of a 20 day chick embryo. This section shows a seminiferous cord in which a lumen is beginning to develop. The spermatogonia are next to the basement membrane, with elongating peritoneal cells between them. Notice that the mitochondrial crescents of the spermatogonia are next to the basement membrane of the cord. A small mass of interstitial cells is present in the stroma.

toneal cells are the principal sufferers because the spermatogonia have placed themselves along the wall.

I do not know positively through what agency they are enabled to place themselves against the basement membrane, but suppose that it is through their ability to move like an

amoeba. They or rather their ancestors, the primordial germ-cells, had this power in their early history, and it may have been in abeyance during their sojourn in the germinal epithelium and sex cords, only to be assumed again at this time. Color is lent to this theory by the fact that frequently, at this stage, the end of the spermatogonium turned toward the basement mem-

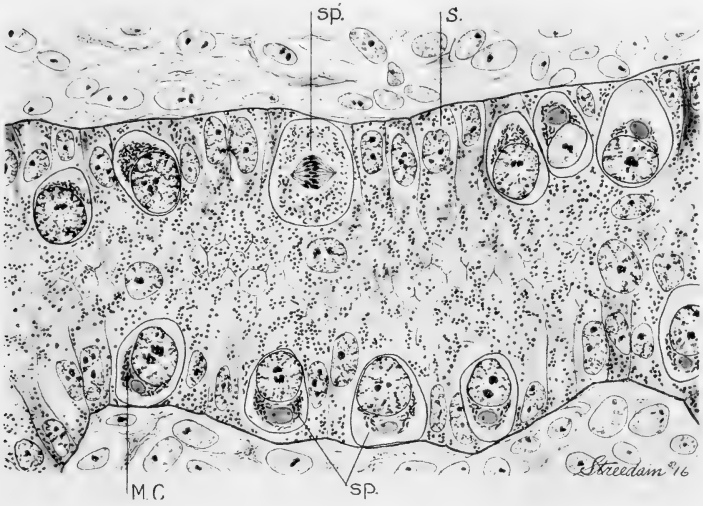

Fig 6 Portion of a transverse section through the left testis of a chick 3 days old. This section shows a seminiferous tubule containing spermatogonia and supporting cells. The spermatogonia are next the basement membrane and have a definite polarity—nucleus near the central portion of the tubule and mitochondrial crescent at the basement membrane. The supporting cells are descendants of the peritoneal cells.

brane is pointed, as if it were forcing its way in that direction between the intervening cells (fig. 5).

The cords, although forming a network, are again beginning to have a definite orientation, that is, they run obliquely from the germinal epithelium towards the remains of the Wolffian body.

In this, and the next stage to be described, the position of the peritoneal cells is also of great interest. In general there are one to three of them between adjacent spermatogonia and their long axes, too, are at right angles to the long axis of the cord (figs. 5 and 6). Their nuclei are placed next the basement membrane, while most of the cytoplasm is towards the central axis of the cord or tubule (figs. 5 and 6). This is the reverse of the condition in the spermatogonia.

In this stage and in the next we begin to have an idea of what obtains in the adults which have been studied. The spermatogonia at the basement membrane of the tube and between them the supporting cells—cells of Setroli—which are derived from the peritoneal cells.

In the next stage—a 3 day chick—the stroma is greatly reduced in amount as are the interstitial cells also. Most of the testis is taken up by the seminiferous cords and tubules, which are arranged in the way described in the 20 day chick embryo.

Most of the seminiferous cords have by this time acquired a cavity and may now be called tubules. In these seminiferous tubules nearly all the spermatogonia are against the basement membranes, as described before (fig. 6). The peritoneal cells have lengthened, or been pressed out, so that they begin to look like the supporting cells of an adult seminiferous tubule (fig. 6). In this as well as in the last stage, the spermatogonia are occasionally seen dividing (fig. 6). In these rare cases the mitotic figure is arranged, not like that which gives origin to the spermatocyte, but so that the line of cleavage between the daughter cells is at right angles to the long axis of the seminiferous tubule (fig. 6). In this way both daughter spermatogonia are kept in their proper position, next the membrane, and the number of peritoneal cells, or cells of support, which is still too large, is reduced by pressure (fig. 6).

In the 6 and 10 day chicks there are no changes of any moment to record, except that there is a progressive increase in the size of the lumen of the seminiferous cords and a reduction in the amount of stroma and in the number of interstitial cells. The cavity in the seminiferous cord, even at 10 days is slit-like

and is very far from resembling the large cavity pictured by Semon ('87) in stages of about this age.

THE INTERSTITIAL CELLS AND THE MITOCHONDRIAL CRESCENT OF THE SPERMATOGONIUM

It is not possible to enter into any account of the various opinions which have been advanced and held as regards the origin of the interstitial cells of the testis. For the various theories on this subject the reader is referred to Sainmont's article which appeared in 1905. However, to give a slight historical background a few leading articles will be cited.

Tourneux ('79) identified the interstitial cells in the testes of various animals and described them as being differentiated connective tissue cells.

Nüssbaum ('80) thought that the interstitial cells were derived from cell columns, which were given off by the germinal epithelium in early embryonic history.

Plato ('97) described the different stages which exist between the connective tissue cells and the differentiated interstitial cells. He studied the question in the testes of the cat and various other animals. In connection with this work he brought forward a rather novel theory which ascribed a nourishing function to the interstitial cells. He believed that they manufactured fat, which was passed through the basement membrane of the seminiferous tubule into the cells of Sertoli and used as food in spermatogenesis.

Allen ('03) and Whitehead ('04) believed that the connective tissue elements of both testis and ovary of the pig and rabbit, from which the interstitial cells were differentiated were derived from the peritoneum. Allen says: "In early stages they (the connective tissue elements) are not distinguishable from the cells which make up the sex-cords, except that the latter are marked off from the stroma by their membrana propria."

Whitehead "found himself in accord with the conclusion of Allen, that the interstitial tissue of the testis is derived from the peritoneum, meaning thereby the mesothelium of the genital ridge."

There are, then, two main opinions held as to the origin of the interstitial cells; the one school, including Kölliker, Tourneux ('79) and Plato ('97), hold that they arise from the connective tissue cells; the other group, to which belong Nüssbaum ('80) and von Bardeleben, believe that they come from the general epithelium. Allen ('03) and Whitehead ('04) must be classed as holding the latter, and Sainmont ('05) the former opinion.

In the male chick embryo the interstitial cells are first evident in the 13 day testis. They are not numerous and are evenly scattered throughout the testis. Usually they appear as single cells but at times 2–6 may be found together. The cells themselves have various forms, sometimes cubical, sometimes polyhedral and frequently fusiform. The nucleus is round or oval, stains deeply and usually has a central position (fig. 5). The cytoplasm of these cells also stains strongly and appears dense (fig. 5). The interstitial cells are remarkable, however, because of the immense amount of fat which they contain. This fat in the stained specimen has been dissolved out, but the vacuoles, which contained it are present, and occupy no small part of the cytoplasm (fig. 5). If the section be examined carefully intermediate stages between the connective tissue cells and the interstitial cells can be seen. That is, certain of the connective tissue cells will be seen containing small vacuoles and arranged at times in small groups.

In the 15 and 17 day embryonic testes there is an immense amount of interstitial cell tissue. It is arranged in masses and cords of varying sizes between the seminiferous cords. The interstitial cells of these and later stages can be easily seen before the section is stained and while it is still in paraffin. The interstitial cell cords and masses, owing to the contained fat, stain black with osmic acid and hence show up dark against the lighter background. Sainmont ('05) described a rich plexus of capillaries in relation to these masses of interstitial cells in the cat, but in the chick there seems to be no increase in vascularity around them.

Beginning with hatching and continuing until the chick is 10 days old there is a progressive decrease in the amount of inter-

stitial tissue as compared with the true connective tissue and the seminiferous cords. I do not know whether there is an actual decrease, or only apparent, due to the great increase in the size of the testis. In any event I was not able to find any signs of degeneration in the interstitial cells.

It is an interesting fact that the first appearance of the interstitial cells in the testis of the 13 day embryo is synchronous with the appearance of the spermatogonia, and that they only reach their maximum development when all the primordial germ-cells have become spermatogonia.

There is no doubt, in the chick, that they arise from the connective tissue of the testis, which in its turn is derived from the general mesenchyme of the mesonephros. That they arise from the connective tissue elements by simple differentiation is proven, first, by the presence of transitional forms at the time of their origin, and secondly, by the very rapid increase in the numbers of the interstitial cells in the absence of any evidence of division.

In a previous paper (Swift, '15), and in a preceding page of this article, a short account was given of the arrangement of the mitochondria around the attraction-sphere of the oogonia and spermatogonia respectively. I shall hereafter call this body, made up of sphere and mitochondria, the mitochondrial crescent.

As has been previously stated, the mitochondrial crescent consists of attraction-sphere and mitochondria, and occupies that part of the cell in which the cytoplasm is most voluminous; in other words, it occupies a part of the vegetative pole of the cell. The whole body in one plane, appears like a crescent fixed on, or capping the nucleus, the attraction-sphere in the middle enclosing the centrosomes, around it a thin clear area, and extending down on either side the arms of the crescent composed of mitochondria (figs. 3, 4, 5 and 6). In case the knife does not section the nucleus, but passes through the sphere, then the mitochondrial crescent appears as a circular mass, composed of attraction-sphere, clear area and surrounding circle of mitochondria (fig. 4). In nearly all cases the cytoplasm which suspends the mitochondrial portion of the mitochondrial cres-

cent appears denser and stains darker than that of the rest of the cell (figs. 4, 5 and 6).

This mitochondrial crescent appears in cells which are the result of primordial germ-cell division, in other words, in the oogonia and spermatogonia. It appears in a few cells in the female at 8 days and in the male at 13 days and this marks the ending of the primordial germ-cell line and the beginning of the oogonial and spermatogonial generations. By 17 days in the male (fig. 4) every cell of primordial germ-cell lineage possesses it, thus indicating that all are spermatogonia. When the spermatogonia place themselves against the basement membrane, when the cavity begins to appear in the cords, they do so in such a manner that the mitochondrial crescent is against the basement membrane (figs. 5 and 6).

This mitochondrial crescent, whose appearance and formation I have just described, is the same structure which D'Hollander ('04) described in the oocytes of the female chick. He, however, called the central sphere and its surrounding clear area the yolk nucleus of Balbiani and the mitochondrial portion the 'couche vitellogène' or 'couche palléale.' His methods did not demonstrate the mitochondria, but in the region of the mitochondrial crescent which they occupy his text described, and his figures showed a denser zone of cytoplasm. This denser zone may be dissolved mitochondria or it may be more condensed cytoplasm, which seems also to be present in my preparations (fig. 4).

There is, of course, a great deal of dispute as to the nature of the yolk nucleus—its cytological structure and its rôle in the cell.

Mertens ('94), in his study of the yolk nucleus of Balbiani in the oocytes of birds and mammals, came to the conclusion that two very different entities are described under the one name, first certain elements found in the cytoplasm which originate in the nucleus and, secondly, the attraction-sphere, which van Bambeke called the 'couche palléale' and Van der Stricht the 'couche vitellogène.' At the present moment I am not prepared to say anything positively as to the origin of the denser

material in the mitochondrial portion of the mitochondrial crescent, but I am very certain that the attraction-sphere and its surrounding body of mitochondria ought not to be called the yolk nucleus of Balbiani for it does not have a nuclear origin.

The denser ground substance in the mitochondrial portion of the mitochondrial crescent may be derived from the nucleus and fall into the class of a true yolk nucleus or a chromidial substance in the sense of R. Hertwig.

It may be that the mitochondrial portion of the mitochondrial crescent plays a rôle in the oocyte in the formation of vitellus, as has been suggested, acts as a true 'couche vitellogène,' but as to its function in the male, I cannot even surmise.

SUMMARY

1. In the male chick the true sexual cords or seminiferous cords originate from the germinal epithelium during the sixth and seventh days of development and are the result of localized activity of the epithelium. Nearly all the primordial germ-cells present in the germinal epithelium are carried down into the seminiferous cords but they play only a passive rôle for at this time they show no evidences of cell division.

2. The sexual cords remain attached to the germinal epithelium for only a short time, and continue to grow, after formation of the albuginea, as a result of division of the peritoneal cells.

3. At the end of the seventh day of development the sex of the individual can be easily told, for in the male the gonads are of nearly equal size, while in the female the left gonad is much the larger. In the male the germinal epithelium remains thin after the formation of the sexual cords and contains very few primordial germ-cells, while in the female the epithelium of the left gonad or ovary continues to be thick and contains many primordial germ-cells.

4. During the eighth and ninth days of development the gonads increase slowly in size and the thin sexual cords make up most of the volume of the testes. They are separated from one another by a thin layer of stroma and have a definite orientation from germinal epithelium obliquely down towards the

Wolffian body. When the embryo is 11 days old the stroma begins to increase in quantity and the seminiferous cords commence to meander and to anastomose with each other.

5. Up to the thirteenth day of development the primordial germ-cells in the sexual cords do not divide, their numbers remaining about the same as when they left the germinal epithelium. Beginning at this stage they divide actively and continue to do so for the next four days. The primordial germ-cells give rise to the spermatogonia, which are from now on very numerous in the sexual cords. The spermatogonia differ from the primordial germ-cells in possessing the mitochondrial crescent. This occupies part of the vegetative pole and consists of attraction-sphere containing centrosomes, and mitochondria, which in section are seen to extend over the nuclear membrane, capping the nucleus. The arms of the mitochondrial body may embrace more than one-half of the nucleus. This mitochondrial body is suspended in dense cytoplasm and is exactly comparable to the yolk nucleus as described by D'Hollander and Van der Stricht in the oocyte. I have also described it in the young oogonia of the chick. The interstitial cells appear in the stroma on the thirteenth day but reach their greatest development during the seventeenth day. They are simply differentiated stroma cells, since they do not divide and transitional forms can be seen.

6. Cavities begin to appear in the network of seminiferous cords during the twentieth day. These cavities are formed, not by fissures between the cells, but by liquefaction of the cells in the central axis of the cord. At the twentieth day the spermatogonia are found against the basement membrane, with the nucleus towards the central axis of the cord and the mitochondrial crescent near the basement membrane. They probably reach this position by amoeboid migration. The elongated cells between the spermatogonia are derived from the peritoneal cells of the seminiferous cords.

7. The primordial germ-cells give rise to the spermatogonia and the coelomic cells of the germinal epithelium produce the supporting cells of the seminiferous tubule.

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THE MORPHOGENESIS OF THE FOLLICLES IN THE HUMAN THYROID GLAND

EDGAR H. NORRIS

Institute of Anatomy, University of Minnesota

SEVENTEEN FIGURES

CONTENTS

I. INTRODUCTION

In spite of numerous investigations, many questions concerning the development of the thyroid gland are still unsettled. This applies particularly to the morphogenesis of the thyroid follicles. The various and contradictory views in the literature on the development of the follicles are doubtless due in part to lack of adequate series of successive embryonic stages (especially of human embryos) available for study. The greatest difficulty, however, has arisen from the use of inadequate methods of investigation. The method hitherto almost exclusively used, that of direct observation of the microscopic sections, is insufficient. By the use of reconstruction methods, however, it has been found possible in the present study to reach a satisfactory solution of this difficult and important problem, at least regarding some of the more fundamental features.

This study was undertaken in the Anatomical Laboratory of the University of Minnesota at the suggestion of Prof. C. M. Jackson, under whose supervision the work was conducted. I wish to thank Dr. Jackson for his valuable aid and criticism.

II. LITERATURE

The literature concerning the thyroid follicle will be considered in chronological order. First, it is desirable to mention briefly the various views which have been held concerning the morphology of the adult (human) thyroid follicle. Then follows a brief statement of the conclusions concerning follicle development (including also prefollicular stages), which have been arrived at by the observers who have worked upon human material. A few observations, made on lower forms, which have seemed especially pertinent to the present problem, are also included. Unless otherwise indicated, however, all statements refer to human material.

The follicular structure of the adult human thyroid gland has long been known. According to Boéchat ('73), Lalouette (1750), who was the first to describe the minute structure of the thyroid gland, found vesicles which seemed to communicate with each other. Bardeleben ('41) is said by Zeiss ('77) to have been the first to describe the adult thyroid follicles as isolated structures. Five years earlier, however, Jones ('36) described the thyroid follicles in considerable detail as completely closed vesicles. Although there has been considerable disagreement concerning the structure of the adult thyroid follicle, the majority of the later observers have, like Jones, described the vesicles of the adult gland as closed, spheroidal bodies. Cruveilhier ('43), Virchow ('63), and more recently Boéchat ('73), Zeiss ('77), and Hitzig ('94), however, have followed Lalouette in describing the follicles as forming a system of branched and communicating cavities within the gland. Still others, like Streiff ('97), have maintained that both branching forms and isolated vesicles occur in the adult gland.

Jones ('36), who was perhaps the first to describe the microscopic structure of the human fetal thyroid, found that in a fetus of four and one-half months the cells of the gland had become partially arranged into solid, globular masses; but no vesicles were observed at this stage.

Remak ('55) described in chick embryos in the wall of the primitive saccular, epithelial thyroid anlage the formation of thickenings which become separated and later give rise to the thyroid follicles. He also thought that the original saccular anlage might persist for some time and form new secondary vesicles by a process of constriction. He described similarly the origin of secondary follicles, both by constrictions and by solid budding, in the thyroid of pig fetuses four inches and above in length.

Peremeschko ('67) described the division of primary into secondary follicles in mammalian fetuses. Colloid is described as arising partly by secretion and partly by colloid metamorphosis of epithelial cells.

W. Müller ('71) described a developmental stage in which the thyroid consists of a network of cylindrical tubes. Such tubes were found in a 24 mm. fetus and in decreasing numbers in later fetuses and even in a three year old child. These tubes arise from solid epithelial cords by the development of a central lumen. The segmentation of the tubes with the formation of the gland-vesicles is produced by ingrowth from the mesoblast.

Horcicka ('80) found the thyroid gland of a four months' fetus to be made up for the most part of solid cell masses with a beginning of lumen formation in the central cells of these masses. Typical gland structure is found after the fifth fetal month.

Wölfler ('80) described the formation of follicles from solid masses of epithelial cells. Toward the end of the fetal period and after birth the peripheral cells of the groups dispose themselves in a circle. The central cells become at first granular, then degenerate and disappear in the pale, granular mass which fills the lumen of the vesicle thus formed.

L. Stieda ('81) noted that the anastomosing epithelial cords ('Epithelstränge') of the embryonic, mammalian thyroid are at first always solid, but that in the ends of these cords lumina appear, and the resultant vesicles are gradually constricted off.

Baber ('81) described the fully formed follicle as spheroidal in form, but observed also branching follicles which are probably giving rise to secondary follicles by a process of division.

Wölfler ('83) described the process of later development in the human thyroid gland as centrifugal. He distinguished a cortical and a medullary portion, which are respectively youngest and oldest, least developed and most developed portions.

His ('85) described in the thyroid gland of an embryo (Zw) between 16.5 and 22 mm. in length cells grouped to form acini or tubes. The inner ends of the cells have a light, colloidal appearance.

Biondi ('89) found that the (postnatal) thyroid vesicle discharges its contents, collapses and finally rearranges itself in the form of a number of small acini which repeat the process. He held that the colloid arises by cell secretion, and not by cell degeneration.

Ribbert ('89) described a centrifugal growth of the thyroid in embryos and newborn. Follicles are formed by the outgrowth of solid buds or sprouts from the old follicles.

Lustig ('91), who studied the thyroid gland in the pig and other animals, affirmed that colloid and follicles appear synchronously as the result of the degeneration of the central cells of the preëxisting solid masses.

Podack ('92) found well formed follicles in a fetus of five months. In some parts of the gland the follicular structure is only suggested and many cell-masses and cell-cords are present.

Marshall ('93) found that the thyroid in chick and frog embryos presents a stage in which the gland is made up of communicating, epithelial tubes. In the rabbit he described the presence of out-growths, some solid and some hollow, from the primitive epithelial anlage. In the human embryo: "At an early stage the lobes are excavated by a number of detached cavities, which become the vesicles of the adult thyroid."

Zielinska ('94) found the structure of the thyroid in newborn children variable both in size and number of the follicles, and also in the amount of solid cell masses. The relations "errinern an acinöse Drüsen und erwecken den Gedanken, dass hier ein sich verästelnder Drüsenkanal vorliegt, als dessen Endbläschen die solide Zellhaufen gelten können."

Hürthle ('94) described in the thyroid of young dogs scattered masses of interfollicular epithelium, in which new (primary) follicles arise by the secretion of colloid into the angles between adjacent cells.

Anderson ('94) described secondary follicles (postnatal) arising from the collapsed epithelium of emptied follicles in various mammals. The new lumina are formed by cell-secretion of chromophile spherules.

According to L. R. Müller ('96), the origin of small secondary follicles from the larger follicles, as described by Ribbert ('89) is clearly evident, even in the human adult.

Tourneux and Verdun ('97) in a careful study of the branchial derivatives in the human embryo described the transformation of the (median) thyroid plate (and later of the lateral thyroid anlage) into a richly anastomosing network of solid epithelial cords by ingrowth of vascular connective tissue in a 14 mm. embryo. This network was likewise observed in embryos of 19 mm. to 37 mm. in length. At 37 mm., the cords become varicose, and follicles develop by the formation of a cavity within each of the enlargements. A similar process of morphogenesis is described in the rabbit embryo by Soulié and Verdun ('97).

Streiff ('97) made wax reconstructions of normal, adult human thyroid tissue, and found it to be made up of closed follicles, ovoidal or spindle shaped. Branched forms due to budding or to secondary fusion were also described; some of these more complex forms he thought may represent persistent branching, a continuation of the embryonic process. He concluded that the thyroid arises as a branched tubular gland, the follicles being formed by constriction of the tubes.

Schreiber ('98), in a fetus of three months, found the thyroid gland for the most part arranged into follicles which contained much colloid.

Kürsteiner ('99) in fetuses from 8 to 30 cm. in length found the thyroid lobules made up of round or elongated, solid or hollow follicles. The lumina are few in number up to about 20 cm., but in the older fetuses they are numerous and evenly

distributed throughout the gland. Some branching vesicles were also noted at 17.5 cm.

Prenant ('01) (p. 13) stated that in the embryonic thyroid the solid epithelial cords are transformed into a network of tubes from which the follicles arise by a process of constriction.

Von Ebner ('02) found numerous well developed follicles in older fetuses and newborn. Between the follicles are found, even in the adult, frequent solid strings and nests of epithelial cells, which are in the majority during development.

Elkes ('03), who studied the thyroid in fetuses from four and one-half to six and one-half months in age, found that it presents both solid cords and well developed follicles in variable number. In the newborn the earlier follicles have largely disappeared, leaving only a few at the periphery of the gland.

Hertwig ('10) (pp. 444–446) described in the embryonic thyroid anastomosing epithelial cylinders. These become tubular; varicose dilations are by ingrowth of the adjacent connective tissue cut off to form the permanant follicles.

Isenschmid ('10) found that in the thyroid of children the gland grows not only by the increase in the size of the follicles, but by the formation of new follicles by two methods: budding and division. He found no evidence that follicles are formed from solid cell-masses (interfollicular epithelium) retained from the embryonal period.

According to Hesselberg ('10): "Die Ausbildung der Thyreoidea in der fötalen Periode erfolgt durch Zerfall der ursprünglich soliden Zellplatte in solide Zellstränge. Diese schnüren sich zu Bläschen ab, die zuerst am kaudalen Pol auftreten." The normal structure of the thyroid is established from the fourth fetal month on. Desquamation of epithelial cells was found in about half of the cases from the seventh to the ninth fetal month, and the follicles are almost entirely obliterated in the newborn. During the first week of postnatal life the follicles are reformed and increase in number by a process of budding.

Prenant and Bouin ('11) give an account of the development of the median thyroid anlage similar to that given by Prenant ('01).

Broman ('11) described in the differentiation of the thyroid anlage a tubular stage transformed by constrictions into beaded chains and finally into separate follicles.

According to Grosser ('12), the thyroid anlage begins to separate into solid cords in the human embryo of 8 mm. In the 50 mm. fetus the cords, especially in the periphery, appear beaded. The beaded cords become divided into separate cell-masses, the anlages of the follicles. The lumina may appear as independent cavities (no tubular stage), before the follicles are detached, or they may arise later even in early postnatal life.

Simpson ('12) referred to the tubular structure of the thyroid and describes the gland of a seven months old child as tubular in character.

Aschoff ('13) stated that in the developing thyroid gland connective tissue separates round epithelial balls from the anastomosing cords, and it is in these 'balls' that the follicular lumina develop.

Sobotta ('15) described the first lumina as appearing in the peripheral parts of the lateral thyroid lobes in a fetus 50 mm. in length. The final breaking up of the cell cords into single groups, which will later form follicles, progresses very gradually, so that the final structure of the gland is arrived at only after birth. Interfollicular epithelium persists, which may later give rise to follicles.

Kingsbury ('15) in describing the early development of the thyroid states that lumina (follicular cavities) appear within the cell cords in fetuses of 32 mm., but colloid is not demonstrable until 40 mm.

III. MATERIAL AND METHODS

This study is based upon the collection of human embryos in the Anatomical Laboratory of the University of Minnesota. Several of the series used are in excellent condition for histological study. The embryos of the collection have been variously fixed and stained. The additional glands specially prepared have, for the most part, been fixed in Formol-Zenker; embedded in paraffin, sectioned at 10 μ; mounted serially and

stained with alum-haematoxylin and eosin, or iron-haematoxylin and eosin.

Besides the specimens mounted in the collection, other glands from newborn children and from children in the early years of life were obtained at autopsy. These were used merely for purposes of comparison, and have neither been listed below, nor discussed in this paper.

The following table shows the materials used in this work. The embryos and fetuses used are arranged in the order of their crown-rump lengths. 'Minn. E. C.' refers to the Minnesota Embryological Collection. An asterisk (*) following the number signifies that the thyroid gland alone was sectioned. Otherwise the entire embryo was available in serial sections.

The ordinary reconstruction methods, both plastic (Born's wax-plate method) and graphic, were utilized in the present study. In all cases where a determination of the follicular form or structure was attempted, special precautions were observed in making the reconstructions as accurate as possible.

The drawings for reconstruction were made with the camera lucida on transparent paper. After the drawings were completed, those of successive sections were superimposed upon a tracing-table, and each epithelial structure in the section given a letter or number. The drawings were controlled by careful microscopic observations, to determine the frequently complicated relations of neighboring follicles. By this method it was possible to determine with certainty what the limits of any particular mass or follicle might be.

SERIAL NO.	MINN. E. C. NO.	C. R. LENGTH IN MM.	FIXATION	SECTION THICKNESS IN MICRONS
1	H 6	6.0	Zenker	10
2	H 13	7.5	Alcohol	15
3	H 60	11.0	Alcohol	20
4	H 68	11.0	Bouin	15
5	H 134	12.0	Alcohol	20
6	H 1	15.0	Alcohol	12
7	H 23	15.0	Alcohol	10
8	H 18	15.5	Formalin	10
9	H 28	16.0	Alcohol	10
10	H 62	16.0	Formalin	20
11	H 58	17.0	Formalin	20
12	H 260	18.0	Formalin	15
13	H 24	19.0	Zenker	12
14	H 2	20.0	Picro-sulphuric	15
15	H 7	20.0		10
16	H 265	21.0	Formalin	20
17	H 3	22.0	Zenker	12
18	H 15	22.0	Alcohol	12
19	H 64	23.0	Alcohol-formalin	20
20	H 304	24.0	Zenker	20
21	H 56	24.0	Alcohol	20
22	H 5	25.0	Zenker	15
23	H 21	26.0	Alcohol	15
24	H 29	26.0	Alcohol	12
25	H 99	26.0	Alcohol-formalin	20
26	H 48	27.0	Bouin	12
27	H 10	29.0	Alcohol	15
28	H 375	29.0	Formalin	10
29	H 259	30.0	Formalin	20
30	H 108	30.0	Zenker	10
31	H 57	31.0	Formalin	20
32	H 16	33.0	Zenker	15
33	H 313	35.0	Formalin	20
34	H 122	39.0	Formalin	20
35	H 8	41.0	Formalin	12
36	H 12	41.0	Zenker	15
37	H 121	46.0	Formalin	60
38	H 115	50.0	Formalin	40
39*	H 290	56.0	Formalin	10
40*	H 85	60.0	Formalin	10
41	H 26	65.0	Formalin	30
42*	H 285	66.0	Formalin	10
43*	H 81	83.0	Alcohol	10
44*	H 286	86.0	Zenker	10
45*	H 75	90.0	Formalin	10
46*	H 267	115.0	Formalin	10
47*	H 34	120.0	Formalin	10
48*	H 49	126.0	Bouin	10
49*	H 187	158.0	Formalin	10
50*	H 381	163.0	Formol-Zenker	10

IV. MORPHOGENESIS OF THE THYROID FOLLICLE

a. Prefollicular period

A brief consideration of the thyroid gland in the prefollicular period is essential to an understanding of the follicular development. For it is during this prefollicular period that the anlages of the primitive follicles are derived from the original epithelial mass; and, to a certain extent at least, the size, form, and arrangement of the earlier follicles are thereby predetermined.

The earlier well-known stages in the development of the (median) thyroid anlage in the human embryo are not considered in the present paper. The original thyroid diverticulum becomes detached and transformed into a small epithelial plate, well shown in the 6 mm. embryo (No. 1 of the present series). This and several succeeding prefollicular stages of the thyroid were carefully reconstructed by Born's wax-plate method, but for the present purposes it is unnecessary to figure or describe these models.

As is well known, the (median) thyroid epithelial plate soon presents irregularities, as shown in the 7.5 mm. embryo (No. 2), and rapidly becomes transformed into what appears in cross-sections to be (as heretofore almost universally described) a network of anastomosing epithelial cords.

With the details of this process of transformation the present study is not concerned. One feature of the end result, however, which comes out clearly in the reconstructed models, is that the cord-like appearance seen in the sections is largely an illusion. Fundamentally the plate-like structure of the thyroid anlage persists for a considerable time, although somewhat modified by a complicated process of fenestration, splitting, and budding during the growth of the primitive epithelial plate. The resultant structure consists essentially in a mass of irregular, branching and fenestrated plates, for the most part longitudinally arranged (parallel to the long axis of the body), so that in cross-section they appear as 'cords' of epithelial cells (fig. 1). This type of structure, with varying degrees of complexity is found in the various prefollicular stages of embryos from about 10 mm.

to 22 mm. in length. (Numbers 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 of the present series.)

Such a description, however, does not apply to the so-called lateral thyroid anlage (ultimobranchial body). As described by Tourneaux and Verdun ('97) and others, this body long remains

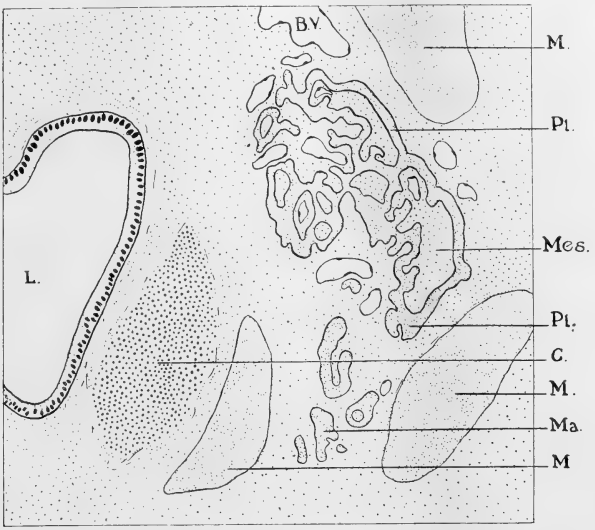


Fig. 1 Semi-diagrammatic drawing (camera lucida outlines) of a transverse section through the left lateral lobe of the thyroid gland and neighboring structures from a human fetus 22 mm. long (No. 18). This shows the complex interrelation of the smooth epithelial plates (*Pl.*) (which appear as 'cords' in the section) and the vascular mesenchyme (*Mes.*) found in the interspaces between them. The apparently detached epithelial masses (*Ma.*) shown are sections through projections from plates located in the upper part of the isthmus of the gland. *B.V.*, blood vessel; *M.*, muscle; *L.*, larynx; *C.*, cartilage. × 133.

as a compact, deeply staining mass of epithelium (the 'inner condensation' of Kingsbury '14) on the dorso-medial aspect of each lateral lobe. Whether in the human thyroid the lateral anlage later atrophies or finally becomes transformed into per-

manent thyroid tissue is still uncertain. This region is therefore purposely excluded from the following descriptions, which apply primarily to the process of morphogenesis in the structures derived from the median thyroid anlage.

The thyroid gland in the final stage of the prefollicular period is shown in a fetus of 22 mm. (No. 18). This stage will be described in some detail, in order to make clear the subsequent process of morphogenesis of the thyroid follicles. When studied in sections, the thyroid at this stage is seen to be made up apparently of epithelial 'cords' which are interrelated in a complex manner (fig. 1). A loose, anastomosing network is thus formed, the interspaces of which are filled with a vascular mesenchymal tissue (fig. 1). The 'cords' are, in general, only two cells in width, a feature characteristic of the thyroid plate and its derivatives at various stages.

Upon reconstruction (figs. 11 and 12) it is found that the epithelial network seen in sections, and described by so many observers as consisting of 'rods' or 'cords,' is merely a section of flat, slab-like plates, or bands, representing portions of fenestrated plates. It is true that some few of the epithelial masses are actually cord-like in form, but by far the greater number are better described as bands or plates. A definition of terms is necessary at this point. The term 'plate' will be used to signify a structure of relatively slight thickness, presenting expansive surfaces which are more or less smooth, and which may or may not be perforated (fenestrated). A band is a narrow plate. Therefore a fenestrated plate may be considered as made up of a number of anastomosing bands. There is great variability in the way in which these plates and bands are arranged. Some are mere slabs, which may or may not be perforated; others form irregular prisms or rounded cylinders, which open at both ends into the surrounding mesenchyme. The length and width of these plates is also quite variable. In three respects, however, they are in general agreement. They are two cells thick; present fairly smooth surfaces; and are longitudinally placed. Within these epithelial bands (fenestrated plates) the primitive follicles of the thyroid gland develop.

b. Follicular period

A thyroid follicle will be defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells. This definition includes all the features of the follicle which may be regarded as absolute and constant. The size and shape of the follicle may vary, and great differences are found in these respects in follicles of the same gland as well as in follicles of fetuses at different stages. Typically the thyroid follicle may be considered spherical or spheroidal in shape; but, as will appear later, this type is subject to considerable variation. The term primary follicle will be used to include those follicles developing independently of preexisting follicles. The follicles derived from preexisting follicles, by budding or otherwise, are termed secondary follicles.

In the present series, the first primary thyroid follicles appear in a fetus 24 mm. in crown-rump length (No. 20). In this fetus the thyroid gland has essentially the same structure as has that of the last (No. 18) described in the prefollicular period, i.e. it is made up chiefly of longitudinally placed epithelial plates or bands, only two cells in thickness. But in this stage, the plates, which have in previous stages been characterized by comparatively smooth surfaces, now present surfaces which are more or less roughened by the appearance of scattered hillocks or mounds (figs. 13 and 14). They are placed very irregularly with respect to one another, and may appear for the first time in any part of a plate, at its periphery or in a more central region.

When studied in cross sections (figs. 2 and 3) it is found that these hillocks are the immediate anlages of the early thyroid follicles. It is further seen that the hillocks are apparently produced by the concurrence of four different processes in the epithelium. The first process is that of cell rearrangement, the second that of cell proliferation, the third that of absolute cell growth, and the fourth that of lumen formation. These processes, although described separately, may occur simultaneously.

The first departure from the two-celled plate arrangement, in the process of follicle formation, is found in a rearrangement of

the cells of the plate (fig. 3). The cell outlines can be made out only with difficulty in most cases. But in those places where they can be seen, they bound cells which are more or less columnar in form. The nuclei are ovoidal or elliptical in outline and are placed with their long axes perpendicular to the surface of the plate. Here and there along the course of the plate (fig. 3) it will be noticed that some of the nuclei have shifted their axes and have changed their relative positions. Certain of the nuclei have rotated through an arc of 90 degrees so that their

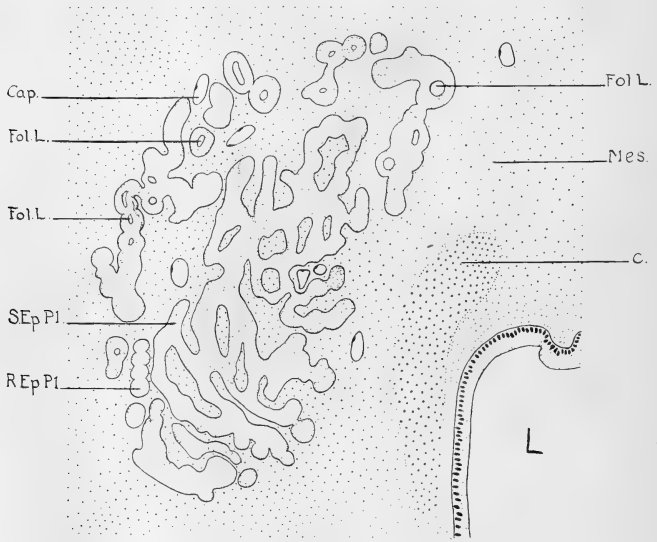


Fig. 2 Semi-diagrammatic drawing (camera lucida outlines) of a section through the right lateral lobe of the thyroid gland and neighboring structures of a human fetus 30 mm. long (No. 30). Shows epithelial plates developing rough surfaces; also early follicles in various stages of separation from the plates. Note the general increase in width of the plates as compared with those shown in figure 1, and the swellings and constrictions, giving 'beaded chain' appearance. *Cap.*, capillary; *Fol.L.*, follicle lumen; *S.Ep.Pl.*, smooth epithelial plate; *R.Ep.Pl.*, rough epithelial plate; *Mes.*, mesenchyme; *C.*, cartilage; *L.*, larynx. × 133.

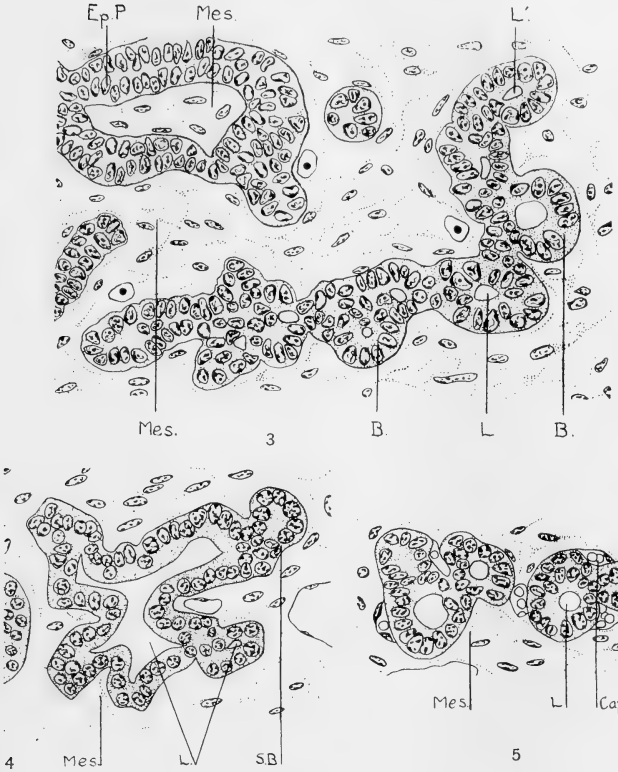


Fig. 3 Small portion of a cross section of the thyroid gland in a human fetus 30 mm. long (No. 30), magnified to show the cell structure. The location of the section is indicated (*a–b*) in figure 14. Note the appearance of lumina (*L.*) in buds (*B.*) from the surface of the plate as well as in swellings along its course (*L'*). In the upper left hand corner a section through one of the hollow epithelial prisms is seen (*Ep.P.*). *Mes.*, mesenchyme. × 400.

Fig. 4 Drawing of a section through follicle '*d*' in figure 16. Note the irregular form of the follicle, both the lumen (*L.*) and the wall. *Mes.*, mesenchyme; *S.B.*, solid bud. × 400.

Fig. 5 Drawing of a section through an epithelial plate taken at the level marked *a–b* in figure 7. *Mes.*, mesenchyme; *L.*, lumen; *Cap.*, capillary; *Ep.T.*, epithelial tag. × 400.

long axes, in their final position, are at right angles to their original position in the plate. As a result of this shifting process, little circlets (really spheres) of nuclei are formed in the plate.

This shifting of nuclei is but the visible expression of the changed position of the cell. For while it is impossible to observe the cell boundaries in most cases, it is hardly probable that the nuclei shift their axes independent of the cytoplasm; moreover, the few faint cell-boundaries which may be made out show the same changes in position as do the nuclei. Further, it is usually found that at the point from which a nucleus has shifted toward the center of the plate a slight depression appears on the surface of the plate, indicating that the cytoplasm has shared equally with the nucleus in the movement. From these three facts it may be concluded that the first process manifested in follicle formation is the shifting of the axes of certain cells of the epithelial plate through an arc of 90 degrees. There is no evidence that the depressions on the surface of the epithelial plate are due to invasions or activity of the adjacent mesenchyme (fig. 3).

This process results in the transformation of the smooth surfaces of the bands (fenestrated plates) into surfaces which are somewhat roughened. The irregularities are apparently not, at first, due to swellings on the plates, but rather to the slight indentations produced by the shifting of certain cells toward the center of the plate as above described. When studied in cross sections (fig. 2) such a plate appears as a sort of beaded chain, with alternate swellings and constrictions. But, as noted above, the initial swellings due to this process are only apparent and are actually not greater in thickness than is the plate in other parts of its extent where indentations have not yet occurred.

The extraordinary cellular activity of the epithelium at this stage is clearly manifested by the large number of mitotic figures to be seen. There is no section of the gland in the 24 mm. fetus (No. 20) which does not present several cells in process of mitosis. But the localization of these mitoses is even more significant than is their number. It will be noticed that the nuclear figures are usually found only in those places in the

epithelial plates where actual thickenings on the plates are being formed. Therefore the little mounds which appear on the plates, as the immediate anlages of the early follicles, may be formed not only by the rearrangement of the already existing cells of the epithelial plates, but also by the formation of new cells as well. Consequently, it can easily be seen how the apparent swellings on the plates, produced by the rearrangement of the existing cells, may be transformed into actual swellings, by the absolute increase in number of the cells found in a localized area. These swellings become roughly spheroidal in form.

The third process referred to above is the absolute increase in size of the cells. While the cells are shifting their axes and proliferating, they are also growing in size. This fact results in the appearance seen in figure 2, where the solid, two-celled plates are found in some cases to be no greater in cross section than the one-celled plate which surrounds the follicle lumen. It might be thought that the cells do not actually increase in size, but only increase in height by a closer crowding together. But a study of figure 3 will show that such is not the case. For the cells are not more closely packed together in the newly formed follicles than they are in the two-celled plate.

This progressive increase in the height of the cells corresponds to the progressive stages in the differentiation of the two-celled plate into newly formed follicles. So that the thyroid gland of a 30 mm. fetus (No. 30) presents in different regions epithelial cells varying greatly in height. The lowest cells are found at the beginning of the process, in the two-celled plate; the highest being found at the other extreme, in the completely formed follicle.

Three of the four processes above mentioned as apparently involved in the evolution of the follicle from the epithelial plate have now been reviewed in detail. The formation of the lumen remains to be considered. Just preceding the appearance of the lumen, the spherule (in which it is about to develop) appears in cross section as a circlet of very tall columnar cells, whose nuclei are peripherally placed. This arrangement results in the formation of a striking picture. The nuclei are regularly placed

at the periphery of the circle and form a dark band, which surrounds an expansive, clear, central cytoplasmic portion. The magnitude of this cytoplasmic area and the sharp contrast between the two portions (in the stained preparations) are usually striking features (fig. 3). It is in the center of this cytoplasmic area that the lumen makes its appearance as a tiny spherical space outlined by a definite and regular margin. It is as though the cells had but drawn a little apart, so that their central ends, instead of remaining in contact one with another, might be separated by an interval. The relation of the early lumina to one another is well shown in figure 6, which is a graphic reconstruction of a plate. It is important to note that no tubular stage is found in the process of lumen formation. The lumina appear as absolutely independent spaces.

As the lumina first appear they apparently have no content; but undoubtedly they contain some substance which is not stained with the ordinary methods, and which increases in amount with the size of the follicular cavity. Certain of the larger lumina (not all of them), which are perhaps older, are found to contain a hazy, granular substance. Typical colloid does not appear until later, in the 60 mm. stage (No. 40).

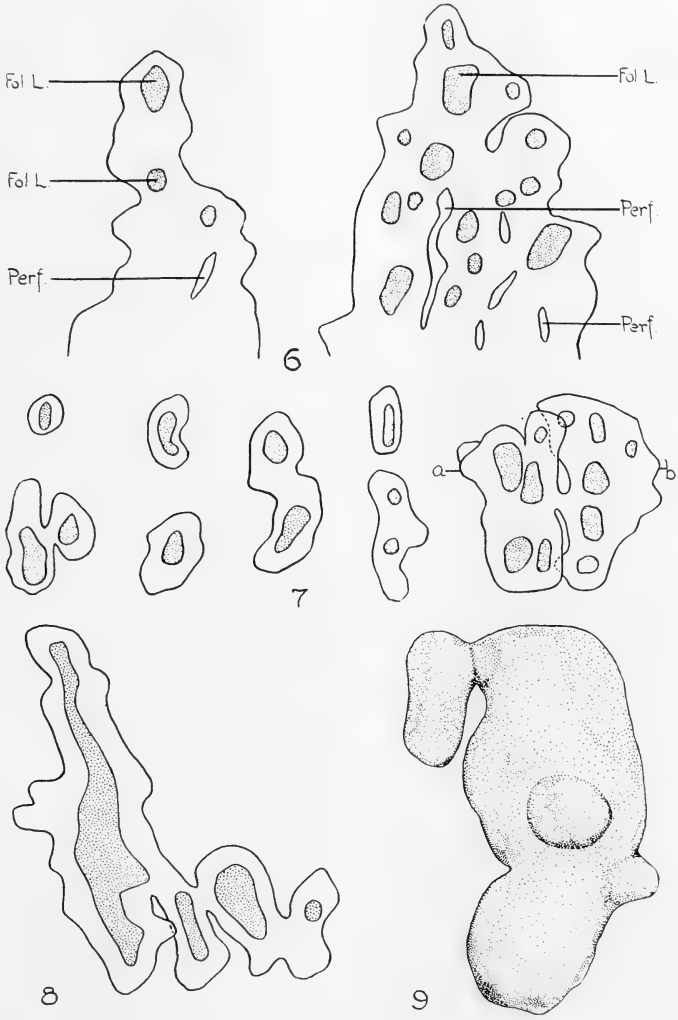
The various possibilities as to methods by which the follicular lumen may arise will be considered later in the discussion.

Fig. 6 A graphic reconstruction (surface view) of parts of two fenestrated epithelial plates from the thyroid gland of a human fetus 30 mm. long (No. 30) to show the relative position of the lumina (*Fol.L.*) as they appear in the plate. Lumina indicated by stippled areas. Note that in all cases these early lumina are quite distinct and never connected with one another. *Perf.*, perforation. × 267.

Fig. 7 A graphic reconstruction of a number of epithelial masses from the thyroid gland of a human fetus 60 mm. long (No. 40). Note the various degrees in the breaking up of the plates and the relative positions of the lumina. *a–b* indicates level of section in figure 5. × 267.

Fig. 8 A graphic reconstruction of a follicular complex shown in the lower part of figure 17. Lumina indicated by stippled areas. Note the solid buds. × 267.

Fig. 9 Model (reconstructed by Born's wax-plate method) of a large follicle from the peripheral part of the lateral lobe of the thyroid gland in a human fetus 60 mm. long (No. 40). The three buds shown have lumina communicating with the lumen of the main follicle. × 267.


Fol L.
Fol L.
Perf
6
Fol L.
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From the time of their first appearance, considerable variability in the size of the lumina found in any particular gland is to be noted. The first isolated follicles are found in the more peripheral parts of the thyroid gland, and it is in these regions that they first attain large size and considerable complexity. For some time after the formation of follicles has begun, all stages previously described may be found in different parts of the same gland, a considerable portion of which retains the irregular plate-like type of structure characteristic of the prefollicular stages.

In the foregoing account, the cell masses in which the lumina develop have been described as spherules whose cross section is circular in outline. While this is true for typical follicles and in most cases, some variation within comparatively narrow limits is found. Ovoidal or somewhat irregular follicles occur, but these are not more numerous than would be expected in a rapidly growing tissue.

The foregoing descriptions of the early primary follicles have been taken in large part from observations made on two fetuses, one of 24 mm. (No. 20) and one of 30 mm. (No. 30). The excellent condition of these specimens has made possible studies of considerable detail. The members of the series (Nos. 21, 22, 23, 24, 25, 26, 27, 28, 29) intervening between these two, although not favorable for such intensive studies, show substantially similar structure. These stages may be summarized very briefly. The comparatively smooth epithelial plates of the prefollicular period have been transformed into plates with rough surfaces. The roughenings on the plates are the early indication of the follicles about to be formed. With the progressively increasing number of follicles the plates are transformed into irregular bands, which in turn give rise to groups of solid or hollow masses of cells. In the 30 mm. stage, however, the thyroid gland is still largely made up of anastomosing bands (fenestrated plates) (figs. 3, 6, 13, 14), although some entirely isolated follicles are found.

While the fetus is increasing in length from 30 mm. to 50 mm. the thyroid gland presents merely a continuation of the process

above described; so that by the time the 50 mm. stage is reached the band or plate formations are relatively insignificant, while the isolated epithelial masses make up the greater part of the organ. All stages in the breaking up of the bands or plates may still be found, however. At this stage (50 mm.) the gland is therefore characterized by the presence of few epithelial plates or bands, very many isolated, solid cell-masses (anlages of follicles) and relatively few well developed follicles.

At 56 mm. (No. 39) the thyroid gland presents slightly more follicles than at 50 mm. Not only are the follicles increased in number, but they also show some changes in both size and form. In the previous stages the follicles have ranged from 16 to 55 μ in diameter (average diameter about 40 μ), and have been typically spherical in form. But in this stage many of the follicles at the periphery of the gland have enlarged considerably and have departed from the more usual spherical form of previous stages. This condition is more strikingly seen in the next fetus of the series (60 mm., No. 40) where both increase in size and change in form of the follicles are very evident (fig. 15). The number of follicles at this stage appears about equal to the number of solid epithelial masses. Figure 7 is a graphic reconstruction of a number of epithelial masses from which follicles are being formed and of certain isolated follicles. A study of sections at this stage will show follicles situated at the peripheral portions of the lobe whose diameters are three or even four or five times as large as those of greatest magnitude located more centrally. The average diameter of the centrally placed follicles is about 15 microns, while the average diameter of the follicles in the peripheral regions is approximately 55 microns. There is much less absolute variation in the size of the central follicles than in those of the periphery. Centrally placed follicles range from 13 to 17 microns, while peripherally placed follicles range from 20 to 103 microns in diameter. It will further be seen that these same large peripheral follicles are quite irregular in form. Instead of being circular in outline, they have a more or less regularly elliptical or ovoidal form, and may present a number of solid or hollow buds. Figure 9 rep-

resents a reconstruction of a typical one of these large budding follicles. Figure 7 is a graphic reconstruction of a number of epithelial masses found in the thyroid gland of the 60 mm. fetus (No. 40) to demonstrate stages in the breaking up of the plates and the formation of isolated follicles, which are present at this time.

The 65 mm. fetus (No. 41) is the first member of the series in which practically no epithelial plates or bands are found. From this stage on through the remainder of the series the thyroid

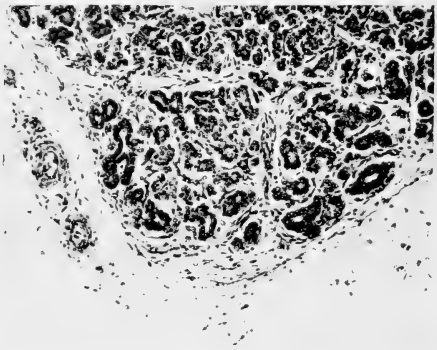


Fig. 10 Microphotograph of a portion of a cross section of the thyroid gland in a human fetus 86 mm. long (No. 44). Note the numerous branching follicles, also the elongated follicles in various stages of division by constriction. This section is in the region of the reconstruction shown in figure 15. × 100.

gland is made up almost entirely of independent, solid or hollow, more or less irregular epithelial cell-masses. The stages (Nos. 42 and 43) included between this embryo and the one of 86 mm. (No. 44) may be passed over briefly with the statement that the processes of budding and formation of new follicles as previously described are advancing rapidly parallel with the increasing length of the fetus.

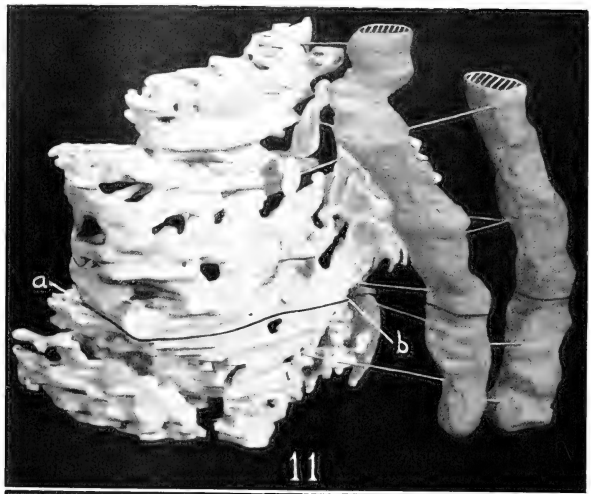
The 86 mm. fetus (No. 44) deserves special attention for it exhibits what appears to be the culmination of the budding processes referred to above. A study of figure 10 reveals the ex-

treme complexity of the gland at this stage. The hollow and solid epithelial masses are about equal in number. The extreme variability in the form of the follicles is the most striking feature (figs. 4, 10 and 16), and the reconstructions reveal more clearly the complexity of these structures than is apparent in sections. Elongated follicles with numerous hollow or solid buds, as well as numerous varieties of other more or less complex arrangements are to be seen. The whole picture is one of active growth. There is great variability in the size of the follicles, the largest being for the most part located in the peripheral zone; but the extreme variation in the follicular form makes absolute measurements for comparison of follicles of little value.

This condition of the gland prevails in the remaining members of the series, up to and including the 158 mm. fetus (Nos. 45, 46, 47, 48, 49). There are but two differences to be noted. In the first place, the relative number of follicles is increasing from stage to stage, so that by the time the fetus is 158 mm. in length, the follicles in number are far in excess over the solid, interfollicular masses. Secondly, the number of irregular and complex forms, although still occurring, is becoming fewer (fig. 17), and the spheroidal follicles are relatively numerous.

The thyroid of the 163 mm. fetus (No. 50) has quite a different structure from that described for the members of the series just preceding (Nos. 44, 45, 46, 47, 48, 49). The irregular and branching follicular complexes are comparatively few in number, while small, spherical follicles make up almost the whole of the tissue. There are, however, a few solid, interfollicular epithelial masses still present at this stage. The picture has changed from one in which the epithelial structures, instead of being complex in their form, have become relatively simpler in character and for the most part are organized into small follicles.

My material from this stage on up to and including newborn children shows to a greater or less extent the process of epithelial desquamation in the thyroid follicles as described by Elkes ('03), Hesselberg ('10), Isenschmid ('10) and others. Whether this process is physiological or pathological is as yet undeter-



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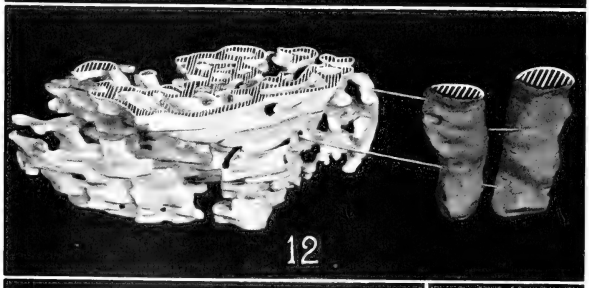

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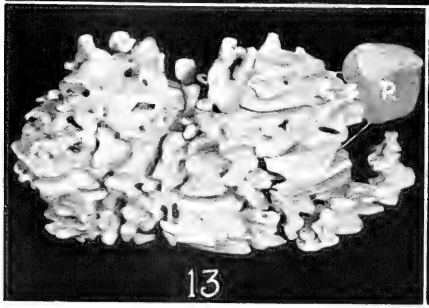

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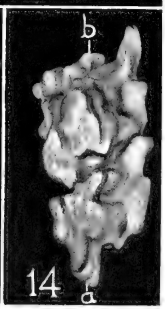

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mined. Because the material at hand is not sufficient to warrant the drawing of any conclusions in this matter, it is not listed with the materials nor discussed in this paper.

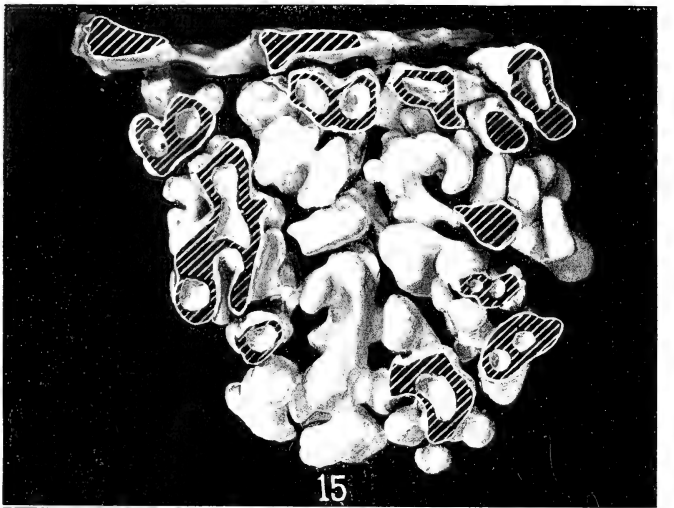
In four of the fetuses studied (Nos. 40, 42, 43, and 44) a number of cyst-like follicles located in all cases in the lower and posterior (dorsal) part of the lateral lobe, were observed. Some of these follicles measure as much as 200 microns in diameter. The size of these structures is not much greater than that of some of the larger normally appearing follicles. But in structure they are quite different, having walls made up of very much flattened epithelial cells, whose nuclei cause the cells to bulge and are separated from one another by much greater distances than is the case with the nuclei in the more usual follicles. The lumina of these cysts are quite regularly circular in outline and in many cases contain a granular substance. It is as though a follicle had been greatly distended, the cells of the wall being stretched and flattened.

Fig. 11 Model (reconstructed by Born's wax-plate method) of the left lateral lobe of the thyroid gland from a human fetus 22 mm. long (No. 18). Antero-lateral surface view to show the perforated (fenestrated) plates, which present a relatively smooth surface. Line *a–b* indicates the level of the section shown in figure 1, and the level at which the model was divided to show the relations pictured in figure 12. The carotid arteries are shown on the right hand side of the figure. × 150.

Fig. 12 The lower section of the model pictured in figure 11. The upper portion has been removed in order to demonstrate the relations to the gland mass of the appearances found in cross section. The separation of the two portions is made at the level of the section shown in figure 1 and indicated (*a–b*) in figure 11. × 150.

Fig. 13 Model (reconstructed by Born's wax-plate method) of the upper portion of the left lateral lobe of the thyroid gland from a human fetus 30 mm. long (No. 30). Plates are more broken up than in figure 11 and present surfaces which are relatively rough and irregular. The upper parathyroid (*P.*) is shown. Lateral view. × 100.

Fig. 14 Model (reconstructed by Born's wax-plate method) of a part of a plate from the thyroid gland of a human fetus 30 mm. long (No. 30) at a higher magnification, to demonstrate more exactly than shown in figure 13 the appearance and relations of the mounds forming on the surface of the plate. The plane *a–b* indicates the position of the section shown in figure 3. Lateral view. × 265.



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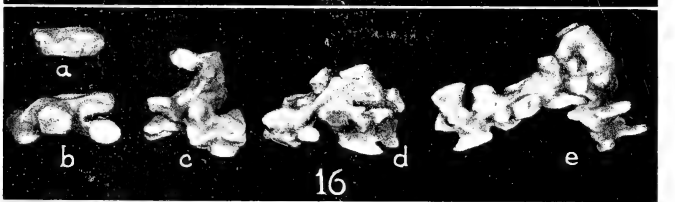

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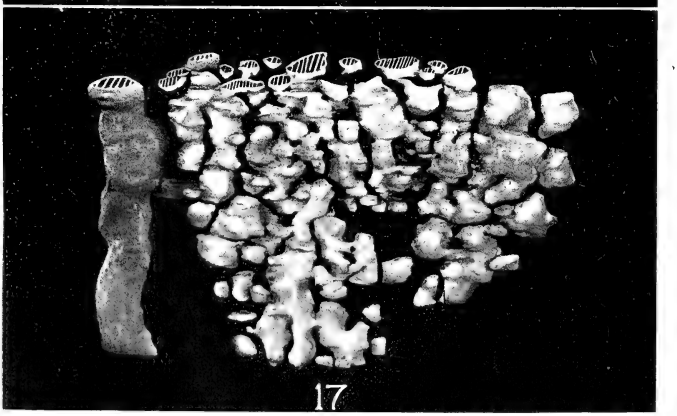

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V. DISCUSSION AND CONCLUSIONS

Remak's theory of the derivation of the thyroid follicles directly from the primitive saccular thyroid anlage has not been confirmed. In the prefollicular stages, the thyroid is by recent investigators quite generally described as assuming the form of irregular, anastomosing 'cords' or masses of epithelium. This undoubtedly appears to be the case when sections of the gland are observed (figs. 1 and 2). But the reconstruction methods used in the present investigation reveal a surprisingly different condition. It is found that, as a matter of fact, in the great majority of cases the cords are illusions and in reality are merely sections of fenestrated epithelial plates longitudinally arranged.

As to the further steps in the process of morphogenesis of the follicles from these anastomosing 'cords', widely divergent views have been held, as noted in the section on 'Literature.' While differing considerably in detail, these views may be classified according to their principal features. The morphogenesis of the primary follicles and of the secondary follicles will be considered in their order.

According to the apparently most widely accepted view, based perhaps largely upon preconceived ideas or theories concerning the evolution and morphogenesis of the thyroid gland, there are two distinct stages in the transformation of the epithelial 'cords' into follicles. First the anastomosing 'cords' acquire lumina, so that the gland becomes a more or less definite network of hollow epithelial tubes. The tubes then become constricted (by

Fig. 15 Wax reconstruction of a portion of a peripheral lobule of the thyroid gland of a human fetus 60 mm. long (No. 40). This model is from the region shown in figure 10. The structure placed across the top of the figure is a blood vessel. The oblique lines designate cut surfaces. Viewed from above. × 270.

Fig. 16 A series of follicles (*a, b, c, d, e*) reconstructed by Born's wax-plate method to show the varying degrees of complexity. All are taken from the thyroid gland of a human fetus 86 mm. long (No. 44). × 135.

Fig. 17 Wax reconstruction (Born's method) of a peripheral lobule of the thyroid gland in a human fetus 158 mm. long (No. 49). The variable form and mutual relations of the follicles are evident. The structure at the left of the figure is a blood vessel. The oblique lines designate cut surfaces. × 130.

ingrowth of vascular connective tissue) into spheroidal segments, each of which becomes a small sac or follicle whose cavity represents a portion of the originally continuous lumen of the tube. This view has been advocated by W. Müller ('71), Marshall ('93 (in chick and frog), Streiff ('97), Prenant ('01), Hertwig ('10), Prenant, Bouin and Maillard ('11), Broman ('11) and others. Some like Streiff ('97) and Simpson ('12) have described this branching tubular condition as persisting in part throughout fetal life and even in postnatal life.

Other investigators, however, have described the lumina of the primary thyroid follicles as appearing directly and independently, with no preceding tubular stage. The anastomosing solid cell-cords are usually described as becoming varicose, with successive enlargements and constrictions, so as to present an irregular beaded chain appearance. Sooner or later each of these spheroidal swollen masses acquires a lumen and becomes separated so as to form an independent follicle. This method of follicle formation (with no tubular stage) has been described by Tourneux and Verdun ('97), Soulié and Verdun ('97), Grosser ('12), Aschoff ('13), Sobotta ('15) and Kingsbury ('15).

It is impossible to decide from direct observations of sections which of the preceding theories is correct. By reconstruction methods, however, both graphic reconstruction and wax-plate models, evidence has been secured in the present investigation which definitely disproves the tubular theory and establishes in the human thyroid the independent origin of the lumina of the thyroid follicles. The follicles, however, appear not in epithelial 'cords' as described by earlier observers, but in the fenestrated epithelial plates above mentioned. The view that the thyroid is a modified branching tubular gland (Zielinska, Streiff, Simpson, and others) therefore obtains no support from its morphogenesis, aside from the initial stage of the primitive diverticulum.

In the glands studied in the present series the first follicles appear in a fetus of 24 mm. in length (No. 20). This is earlier than the time of appearance described by most observers. His ('85), however, described follicles in a fetus (Zw) whose absolute

length is not recorded, but is placed in series between fetuses of 16.5 and 22.0 mm. in length. Kingsbury ('15) described follicles in a fetus of 32 mm.; Tourneux and Verdun ('97) in one of 32.4 mm.; and Grosser ('12) and Sobotta ('15) in fetuses of 50 mm. These are the only cases found in the literature where the presence of early follicles has been noted in fetuses of definite length. Several observers refer to the age of fetuses in which the thyroid follicles appear, but in terms too indefinite to be of value for comparison.

Although by definition the prefollicular period ends abruptly with the appearance of the first follicles, it is not true that the structure of the gland undergoes any corresponding sudden change with the advent of the follicular period. The epithelial bands (fenestrated plates) are only gradually replaced by the primary follicles and structures characteristic of the prefollicular period may be present through a considerable part of the follicular period, at least until the fetus has attained a length of 65 mm.

Concerning the first three processes (rearrangement of the cells, cell proliferation, and increase in the size of the cells) involved in the development of the follicles from the epithelial plate, no further discussion is necessary. The fourth process, however, that of lumen formation, calls for further consideration. The follicular lumen might arise in various ways, which have been suggested by earlier investigators. Most of the workers, however, do not mention the process by which the lumen of the follicle is formed.

Wölfler ('80) and Lustig ('91) have described the formation of lumina in the solid cell masses by a degeneration of the more centrally placed cells. The present investigation does not support this view, however. In the first place none of the so-called central cells have been found; and secondly no evidences of degeneration have been observed in the primary follicles.

It might be supposed, as Hürthle ('94) and Anderson ('94) have suggested, that the lumen is formed by the accumulation of colloid between the angles of the cells which compose the solid mass. Such a process would leave a colloid-containing space

surrounded by epithelial cells. But in no case in the present observations was 'colloid' found within the very early follicles; although the accumulation of some other (precolloidal?) secretory product between the angles of the cells, might result in lumen formation. The fact is that the smaller lumina, which are probably the most recent in formation, have been universally found to be devoid of any demonstrable content, and that some of the larger and supposedly older lumina do contain a stainable substance. As to the nature of the precolloidal substance or substances, nothing definite is known.

One might suggest (as thought by His?) that the lumen could be formed by a degeneration or liquefaction of the central ends of the cells which later form its outline. In this case it would be expected that the early follicles would present a lumen outlined by an irregular or ragged margin. Without exception, however, the lumina of the early follicles are clearly outlined and marked off by a very sharp margin.

Having studied the way in which the follicle forms within the plate, it is of further interest to determine the method by which the follicle frees itself from the plate and comes to take up an isolated existence. Earlier observers, in describing the formation of follicles from anastomosing rods or tubes, have laid emphasis upon the activity of the adjacent mesenchymal tissue as the factor operating in the separation of the follicles. In the present study no definite morphological evidence appears in favor of this view. For as shown in figure 3 there is no special differentiation of the mesenchyme or increased vascularity in the regions in which follicles are being separated off from the plate. The evidence seems rather to indicate that the follicles themselves are the active agents in their separation. Thus as certain of the cells leave their original positions to assume a position more nearly in the center of the plate, the indentations previously described appear on the plate. These may be considered as weak points. And as the cells increase in number between these indentations it is not difficult to see how the increased pressure due to the increased mass might force certain follicles out from the row in which they formed and thus iso-

late them from the parent plate. The vascular mesenchyme doubtless takes some slight part in the process, however.

As previously pointed out, the primary follicles may not in every case be at once separated completely from the plate. Instead of being in all cases sharply outlined and spheroidal in form, small portions of the plate (epithelial tags) may be left hanging to the follicle wall. The significance of such epithelial tags is readily understood when it is considered how easily they might be mistaken for epithelial buds arising from the primary follicle in which secondary follicles were about to form. These structures will be mentioned later in the discussion of the secondary follicles. It has been seen that there is no sharp line of demarcation between the prefollicular and follicular periods. Similarly, in the origin of follicles, the period of primary follicle formation is not sharply marked off from that of secondary follicle formation.

The number of thyroid follicles is apparently not absolutely established or finally limited at any stage of development (as is the case, for instance, with the glomeruli of the kidneys). In the earlier stages, the number of follicles increases by the formation of additional primary follicles. Soon, however, these primary follicles begin to give rise to secondary follicles (at 56 mm.). In later stages the formation of primary follicles apparently ceases, although their occurrence even in the adult has been claimed, e.g., by Hürthle ('94) and Sobotta ('15), the newformed follicles being all secondary in character. Various methods of secondary follicle formation have been described.

1. Origin from buds or sprouts. Ribbert ('89), L. R. Müller ('96), Streiff ('97), Isenschmid ('10) and others have described this process. The bud is usually described as a local thickening of the follicle wall, which continues to increase in size by the proliferation of cells, until a solid bud, projecting into the stroma, is formed. Directly, through the concentric rearrangement of the cells, the form of the lumen can be made out, even while the young follicle is still in contact with the mother-follicle.

2. Origin from collapsed follicles. Biondi ('89) Anderson ('94), and others have described the process as follows. After filling

the vesicle discharges its contents, collapses and finally rearranges itself in the form of a number of small acini which repeat the process.

3. Origin by fusion of follicles. Streiff ('97), v. Ebner ('02), Isenschmid ('10) and others have observed follicles which are apparently formed by the secondary fusion of two or more preexisting follicles.

4. Origin by division of follicles. Isenschmid ('10) has described the formation of daughter-follicles by the growth of an epithelial spur across the lumen of the mother follicle; and Peremeschko ('67) has noted the formation of secondary follicles by the constriction of the parent follicle.

In discussing my observations concerning the formation of secondary follicles in comparison with those of earlier observers, it may be said at the outset that nothing to support either the second or the third methods just outlined has been noted. These, however, have been described chiefly by previous observers upon postnatal material. The other two methods (budding and division of follicles), however, are in general agreement with the findings of the present investigation.

There appear to be three general methods by which secondary follicles arise in the fetal thyroid, the third of which might be regarded as a modification of the second. But each of these types is subject to a wide degree of variation, so that many intermediate and modified forms are found.

1. Solid epithelial buds may develop on the follicle wall (figs. 4, 8 and 16*d*). These may become separated from the parent follicle while in the solid state, or they may develop lumina while connected with the wall of the mother-follicle, and subsequently be constricted off. This method is essentially that advanced by Ribbert ('89), L. R. Müller ('96), Streiff ('97), and Isenschmid ('10). It is difficult, especially in the earlier stages, to distinguish these solid buds from the 'epithelial tags' representing persistent portions of the original epithelial plates remaining attached to the earlier primary follicles.

2. Hollow buds whose cavities are continuous with that of the mother lumen are also found (figs. 4, 9, 10). It might be sug-

gested that these were originally solid buds whose lumen appeared independently and later established a secondary connection with the lumen of the parent follicle. While it is difficult to disprove such an occurrence, it would tend to reduce rather than to increase the number of follicles and seems improbable. The parent follicle apparently sends off extensions or branches, which represent both the wall and the lumen and gradually become constricted off to form new follicles. All possible stages in such a process, from the slightest outpouching of the wall to the finally separated follicle are easily observable.

3. The third method of secondary follicle formation is the simple division of the parent follicle somewhat as described by Peremeschko. The process appears similar to that of hollow bud formation. The follicle first takes on the form of an elongated ellipse which becomes constricted about its center. No cases of division by the ingrowth of epithelial spurs as described by Isenschmid (for postnatal stages) have been observed in my fetal material, though such may possibly occur.

Having considered the primary and secondary follicles separately it remains to consider them in their relations to each other and to the gland as a whole.

The formation of the secondary thyroid follicles begins when the fetus has reached a length of about 56 mm. From the 70 mm. stage on the formation of secondary follicles progresses with such rapidity that the total number of follicles is very greatly increased within a short period of time, and the relative number of primary follicles becomes progressively smaller.

It might be thought that such elongated, irregular, branched and budding forms as appear in large numbers in certain of the older members of the series (especially in the 86 mm. fetus, No. 43) are derived from irregular, branching bands (fenestrated plates) retained from the prefollicular period. But the evidence disproves that view. After the appearance of the first follicles, the bands and plates are being progressively broken up until the gland parenchyma in a fetus of 65 mm. is practically devoid of such structures, and is almost entirely made up of isolated solid or hollow masses of cells. So closely does this

resolution of the plates parallel the increasing length of the embryo up to 65 mm. that it is very improbable that individual variations could explain the presence of these structures in such great numbers at the relatively late stage of 86 mm. Further, as shown in figure 16, forms of all degrees of complexity may be found from simple spheroidal follicles to those of extreme complexity. The conclusion is therefore reached that these branching structures, which are better described as follicular complexes than as follicles, are developmentally only follicles which have grown excessively and attained a high degree of complexity.

Such a rapid increase in the number of thyroid follicles as occurs in fetuses between 65 and 158 mm. in length might be expected to produce a marked increase in the size of the gland. But according to Jackson ('09) the growth curve for the prenatal thyroid gland shows no remarkable increase in the size (weight) of the gland during this period. These two observations, which at first may appear contradictory, are readily explained when the size of the follicles is taken into account. The secondary follicles formed by budding and division of the primary follicles are very small and arise from follicles which are in most cases relatively of much greater size. So that while the number of follicles is greatly increased during this period, the gland mass is not correspondingly larger. As previously described, the formation of secondary follicles becomes less rapid before the fetus has reached 163 mm. in length. From this point on, the number of thyroid follicles apparently increases but slowly, the subsequent growth of the gland being due rather to the increase in the size of the individual follicles than to a further increase in their number.

The significance of the large cyst-like follicles described in four of the fetuses is uncertain. Kürsteiner ('99) has described the presence of similar follicles in four fetuses. The remarkable regularity with which they were found, in his cases as well as in those of the present series, located in the lower and posterior (dorsal) part of the lateral lobe of the gland, is a striking fact. Possibly they may be related to the cysts of the thyroid gland, frequently met in pathological conditions of postnatal life.

VI. SUMMARY

By methods of reconstruction (both graphic and wax-plate), the complicated process of morphogenesis of the follicles of the prenatal human thyroid gland has been worked out and several mooted points definitely established.

1. The so-called 'cords' forming the anastomosing network in sections of the thyroid (median anlage) in the later prefollicular stages represent chiefly sections of epithelial bands, two cells in thickness, and forming irregular, fenestrated plates.

2. The frequently described stage in which the 'cords' are transformed into an anastomosing set of epithelial tubes from which the follicles are derived does not exist. The process of follicle formation gives no evidence or indication that the thyroid has been derived from a branching tubular gland.

3. The primary thyroid follicles arise directly as isolated and independent structures from the epithelial plates of the prefollicular period, by the rearrangement of cells, cell proliferation, increase in the size of the cells, and lumen formation.

4. The primary follicles appear in fetuses about 24 mm. in length. The epithelial bands (fenestrated plates) have practically disappeared in a fetus of 65 mm., but a few solid interfollicular epithelial masses are still present in fetuses 163 mm. in crown-rump length.

5. Secondary thyroid follicles are formed from preexisting follicles apparently by three methods: by solid buds; by hollow buds; and by constriction of the parent follicle.

6. The first secondary follicles appear in fetuses about 56 mm. in length, but are formed most rapidly in stages when the fetus is between 80 mm. and 158 mm. long. After 163 mm., the growth of the gland probably takes place largely by the increase in size of the individual follicles, rather than by increase in their number.

7. Large cystic follicles were observed in the lower and posterior (dorsal) parts of four glands from the older fetuses. Their significance is uncertain, as is likewise the apparent involution of the follicles with desquamation of epithelium observed in the later fetal and newborn stages.

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THE EFFECTS OF LOW TEMPERATURE UPON THE DEVELOPMENT OF FUNDULUS

A CONTRIBUTION TO THE THEORY OF TERATOGENY

WM. E. KELLICOTT

From The Marine Biological Laboratory, Woods Hole, and the Biological Laboratory, Goucher College

There have recently appeared several embryological contributions of importance, based upon the development of the familiar minnow, Fundulus heteroclitus. Stockard ('09, '10, '13) and more recently Werber ('15, a) have described a large number of abnormal and monstrous embryos resulting from chemical treatment of the fertilized eggs and have made several suggestions regarding the modus operandi of the treatments used and the causes of monstrous development. And in the works of Stockard ('15) and Reagan ('15) advantage is taken of the possibility of causing, also by chemical treatment, certain embryonic abnormalities, in attacking a group of mooted questions in normal embryogeny and histogenesis.

During the summer of 1915, at The Marine Biological Laboratory, I was able to carry out a rather extended series of experiments on the production of monstrosities in Fundulus by a method which involved no direct chemical stimulation and which thus permits me to test certain previously suggested causes of abnormal development in the species studied. The method is so simple and so markedly effective that it seems desirable to make a brief report upon it and to point out its bearings upon the general theories of teratogeny, although at this time I shall not attempt to give an extended analysis either of the precise results obtained or of the exact mechanism of the disturbance of normal development.

In a word the method consists simply in subjecting the fertilized ova or young embryos to the temperatures of the ordinary household refrigerator, namely, 8–13°C. for a few hours or days.

In connection with the work on Teleosts in the Embryology Course at The Marine Biological Laboratory, we have for several seasons made a practice of placing the eggs, at various stages of development, in the refrigerator in order to slow their development and thus secure certain stages at hours convenient for study. It was noticed that a slight reduction in temperature merely slowed development without leading to any serious disturbance in morphogenesis, while a few hours at a temperature below 12–14°C. were followed, upon returning the eggs to the laboratory, by a considerable mortality and by very frequent abnormality (the eggs of Tautogolabrus (syn. Ctenolabrus), not Fundulus, were then used). In fact abnormalities in nuclear and cytoplasmic arrangements were to be noted while the eggs were still at the lower temperature. It was not until last summer, however, that the more precise examination of the results of this treatment was undertaken. While these experiments were in progress Loeb ('15) reported certain observations on the effects of much lower temperatures upon the development of Fundulus. His results will be discussed and compared with my own later in this paper. My apparent neglect to test certain points of disagreement is due merely to the fact that his paper did not come to my attention until some time after the spawning season of Fundulus was entirely past, which made such an attempt impossible.

The eggs were inseminated in the usual manner, i.e., dry, with the addition of testis teased in only a few drops of seawater. They were placed in finger bowls with a half-inch or so of water; the bowls were covered to prevent evaporation, and placed on the shelves of the refrigerator. The range of temperatures used was secured merely by using higher or lower shelves, in compartments alongside or below the ice compartment. The water was renewed daily, or in some instances every other day, the fresh water being at the refrigerator temperature. That covering the bowls with glass plates did not

materially affect the oxygenation of the eggs is sufficiently indicated by the normal development of similarly handled controls in the room temperature. The possible effect of darkness in the refrigerator was controlled in only one instance, when no effects were observed.

If the temperatures used be not too low (11–13°C.) the only marked immediate effect seems to be the retardation of normal development, which is resumed at its usual rate when the ova are returned to the ordinary temperatures of the laboratory (20–24°C.). It is a simple matter thus to prolong the age of the early blastula from the normal average of about five hours, to more than two weeks, that is some sixty-five times, and probably it might be prolonged much more. Such blastulas, replaced in the room, may develop and hatch quite normally, or they may form embryos showing marked abnormalities, indicating that underlying their normal appearance earlier there was an actual disturbance of some kind. One can not always say by superficially inspecting the entire blastula, or later stage, whether it is completely normal or not.

Subjection to a lower temperature (8–10°C.) for a few hours or days, is usually followed either by death or by abnormal development, normal development after such treatment being found in only a small percentage of the total treated. It should be noted, however, as a point of some importance, that in every experiment where development continued at all, after removal from the refrigerator, at least a small percentage of the embryos developed normally.

Considerable variation was observed among different lots of eggs, some showing few abnormalities after a treatment that resulted in very few normally hatching larvæ from other lots of eggs. It should also be noted that in one, and in only one, control, did the total proportion of abnormal embryos equal the minimum observed after subjection to a low temperature for more than five hours; and in no other case did the abnormalities in the control even approach the minimum in treated lots.

Eggs placed in the refrigerator within two to five minutes after insemination usually proceed to form a germ-disc which differs

from the normal in being smaller, that is in containing less protoplasm, and in being markedly more convex. This increase in convexity may simply indicate a decrease in surface tension directly due to the lower temperature. If the temperature be not lower than about 12°C. cleavage continues slowly and with little or no apparent abnormality, save that the blastomeres, like the germ-discs, are smaller and unusually convex, and therefore more than normally separated from one another. Stages of eight to sixteen cells may be found twenty-four to thirty-six hours after fertilization.

At a temperature of about 8–10°C. the effects are quite varied. In the first place, in a considerable and widely variable proportion of the eggs no true germ-disc is formed, cleavage rarely occurs, and after a time the protoplasmic parts become wholly vacuolated, giving no sign of living processes, either during continued treatment to cold or after their removal to a higher temperature. Other eggs, however, are not killed by this treatment; a few may form small germ-discs and cleave regularly once or twice during twenty-seven to thirty hours. Or cleavage may become very irregular, blastomeres later showing wide differences in size and extensive divergences from the typical arrangement. Many instances were noted where the germ-disc was very imperfectly formed, the thin protoplasmic cap remaining spread over one-fourth to one-third of the egg, with occasional mounds, cell-like though without cell-walls, scattered irregularly over it. In still other instances there were no true cellular structures whatever present, even after several days, and aggregations of nuclear substance might be seen scattered promiscuously through a cytoplasmic mass of irregular form. Several eggs were noted in which the protoplasm had collected in several distinct and widely separated regions. After several days all these eggs showed at least two kinds of material derived from the cytoplasmic part of the egg; one was dark and granular, quite opaque but still apparently active, the other clear and vacuolated and apparently no longer living.

It should be noted that throughout this paper the appearances described are those given by microscopic examination of total,

unfixed eggs. The results of the detailed study of sections of such eggs, which will perhaps enable me to determine more precisely the effects of the cold, will be reported separately at a later time.

Upon removal to room temperature, after having been some hours or days in the condition just described, many eggs failed to develop, and after some hours longer died without undergoing any apparent structural changes. On the other hand, most of those eggs in which there were present considerable masses of the darker granular material showed some processes of development, exhibiting before their death every degree and form of abnormality, from irregular protoplasmic masses, suggesting cellular structures, to hatched larvæ, well-formed though usually abnormal in some respect. In a very few instances (three were noted) normal larvæ resulted from the development of these dark, irregular, non-cellular masses.

When the eggs were allowed to develop normally for a few minutes (fifteen to thirty) before refrigeration, the general results did not seem to be markedly different, although there are some indications that fewer embryos developed normally.

Two lots of eggs were allowed to develop normally for twenty-two to twenty-three hours after insemination before they were placed in the refrigerator at about 11°C. At this age and at room temperature, the germ-ring is formed and just commencing its extension around the yolk. From a fourth to a third of these were dead after twelve hours in the refrigerator. Most of the remainder, after eleven days in the cold were alive, and upon transference to the room developed, some into normally hatching larvæ, others into larvæ with various defects and abnormalities, some very pronounced. Upon removal after twenty-one days many died and nearly all of the survivors developed very abnormally; but two hatched normally after ten to eleven days in the laboratory, i.e., thirty-two to thirty-three days after fertilization.

One lot of eggs was allowed to develop for forty-three hours before refrigeration at about 10°C. At this age the germ-ring is just closing, the embryo is well established and the optic vesicles

in some instances just forming, in others clearly formed. After eight and one-fourth days at 8–10°C. these were removed to the room; nearly all were alive but development had continued very slowly so that none showed beating hearts (normally the heart begins to pulsate about twenty-four hours after the establishment of the optic vesicles and the closure of the germ-ring). Some were already abnormal at the time of their removal, many others became abnormal as development in the room continued, but about two-thirds hatched normally after eight to eleven days longer.

It is interesting to compare certain of these observations with those of Loeb ('15) who found that treatment with a temperature somewhat lower than any I used, namely 7°C., was followed by no abnormalities in development. At that temperature Loeb found that "the newly fertilized eggs can live for weeks . . . . without being injured" and that they "developed very slowly but no abnormal embryos were observed, although some of the eggs were kept at a temperature of 7°C. for four weeks" (p. 62). Loeb also used temperatures much lower than any with which I worked and found that even at 0–2°C., if the treatment were not prolonged more than four to seven and one-half hours, and if stages from the time of insemination to about four cells were used, only 20–30 per cent became abnormal upon transference to the room; longer treatment increased the mortality considerably, none surviving forty-eight hours at this temperature. If the eggs were first allowed to develop fifteen hours at a normal temperature (stage given as about one hundred and twenty-eight cells, although at the usual laboratory temperature of 22°C. this stage is reached about five hours after insemination) Loeb found that after two days at 0–2°C. normal development was still possible, although he does not state that abnormal development did not also occur in some individuals. But he found that if the embryo is once formed before treatment, it survives weeks of subjection to 0–2°C. "without any injury." "As soon as it is put back to room temperature it continues to develop" (p. 59).

I am entirely at a loss to interpret the difference between Loeb's observations and my own on this point. It is just possible, referring to the latter instances mentioned above, that at a temperature of only 0–2°C. the living processes of the embryo are so completely stopped that when development is resumed at a higher temperature no disturbing effects are to be seen; in other words not even abnormal development may proceed at so low a temperature. But this possibility seems largely negatived by the observation that extremely harmful results follow similar treatment of uncleaved or four cell stages. And no such interpretation seems possible for his results at 7°C., which should be more nearly comparable with my own at 8–10°C. I observed no instance of normal development occurring after eggs had been three weeks at 8–10°C., although several lots of eggs were tested and among the same lots normal development sometimes followed briefer treatment; and abnormalities were very common after treatment for only twenty-four hours, in some lots even for only five and one-half hours. Two lots of eggs, treated in just the same manner as the others, showed no abnormalities upon development after twelve hours at 8–10°C. It seems quite unlikely, however, that all of Loeb's material could have been so unusually resistant, in view of the rarity with which such lots came under my observation. I may add in passing that Werber ('15, b) notes that he too was able to secure abnormal embryos by treatment with temperatures much higher than those used by Loeb, but he gives no details either of his experiments or results.

It would not be profitable to attempt to describe in detail the abnormalities observed, nor even to enumerate all of them. In general it may be said that the embryos resulting from this treatment showed every degree of abnormality, from irregular masses of protoplasm, alive though exhibiting few of the characteristics of organisms, up to completely normal hatched larvæ. Among several hundred embryos examined there was found almost every conceivable kind of disturbance. Every characteristic that could be observed externally with the lower powers of the microscope showed some degree of abnormality in

some embryo. Certain organs, such as those of the circulatory system—the heart, vessels and blood-cells—and the eyes, were especially subject to abnormality, others such as the ears and covering ectoderm were rarely affected; yet *no structure was found which was not affected in some degree in some embryo.*

By way of general support for this statement I may mention just a few of the observed abnormalities, without mentioning the details of the treatment in individual instances.

In those cases where no definite embryo might be said to have formed, such conditions as the following were noted: formless, non-cellular but 'living' protoplasmic masses; protoplasmic masses with varying degrees of cellular structure; irregular protoplasmic masses (probably cellular) showing suggestions of organs, such as brain fragments, lenses, portions of optic cups, groups of somites, masses of erythrocytes, rhythmically contractile cells arranged either as flat sheets or as tubular 'hearts,' scattered pigment cells of the usual types, endothelial cells over the surface of the yolk, fragments of notochordal tissue.

Among those cases where a more or less complete embryo was formed (connected with the preceding condition by various gradations) a few of the abnormalities noted were the following: two separate and complete embryos on a single yolk; absence of head; absence of tail; large malformed head; short stumpy tail; sinuous body and tail; short deep body and tail; malformed pectoral fins; localized ectodermal proliferations; some regions of the brain not closed dorsally; absence or hypertrophy of various regions of the brain; various degrees of anterior approximation and fusion of the eyes; ventral fusion of the eyes; absence of one eye, the other remaining normal in size and position; eyes of unequal size; optic cups not closed; various degrees of albinism never quite complete; absence of certain types of pigment cells; atypical concentrations of pigment cells; greatly dilated pericardial cavity; heart abnormally placed, posterior or lateral to its normal location; two hearts dissimilar in size and form, asymmetrically located and with different rates of contraction; absence of heart; heart short and 'telescoped;' heart elongated and thread-like; heart long and dilated; heart flat and plate-

like; heart undifferentiated into chambers; only the sinus end of heart contractile; well-marked rhythms in rate of contraction of heart, sometimes occasionally stopping; no circulation, either on account of absence, or proximal or distal closure of the heart; few vessels over the yolk; yolk-vessels abnormally arranged; heart thread-like but with a good and complete circulation; very few erythrocytes but abundant plasma circulating freely; erythrocytes in masses on postero-dorsal surface of yolk; large masses of erythrocytes antero-ventral to heart or along the anterior margin of pericardial cavity (after hatching this latter mass was in a median ventral position); dense mass of erythrocytes collected in tail, *i.e.*, caudal aorta and vein anastomosing at base of tail.

Many more abnormalities might be mentioned and of course innumerable minor details of abnormal character might be cited from the material. What variety of conditions might be revealed by the thorough study of sections of these embryos can only be imagined.

One further point might be mentioned. Stockard observes ('15, p. 26) that embryos developing without a circulation are not able actually to hatch. As a rule I had a similar experience but one exception was so remarkable that it seems worth noting. After eleven days at 10–11°C., one lot of eggs which had developed in the laboratory for twenty-three hours before cooling, showed many normal embryos, for the most part at a stage when the optic vesicles were just forming. Others were grossly abnormal or entirely dead. Among those appearing normal upon removal from the cold, many abnormalities appeared later and one of these, which was found hatched twelve days after removal, or twenty-four after fertilization, had no circulation whatever, although the heart was present and pulsating weakly. This, however, was not its only defect. It was below average size, had but a single median anterior eye and correspondingly the nose and upper jaw region were, as usual in cases of cyclopia, narrow and elongated, the tail was short and ended in an undifferentiated mass, pigment cells were largely massed on the ventral surface of the yolk. It seemed unable to direct its swim-

ming movements (it was probably blind) and both in swimming and in resting it turned on either side instead of maintaining a normal position. This fish hatched sometime during the twenty-eight hours preceding the notice of its having hatched, and after living in this condition for thirty hours longer, its heart seemed somewhat more feeble, its fin motions became slower and it was killed.

That fish lacking so physiologically important a process as the circulation may develop to the point of hatching is alone a most remarkable fact. But that such an organism can actually hatch from its egg-membrane and, in spite of the other abnormalities noted, remain alive and active for between thirty and fifty-eight hours, can not fail to arouse many questions regarding the general problem of embryonic adaptations. If a fish can live for weeks, and then hatch and remain active for hours longer, entirely lacking a whole physiological system commonly regarded as so essential, one may certainly be permitted to doubt whether slight details in the arrangement of this or of other parts, may have the functional importance often assumed.

In connection with the observation of the irregular, only partially cellular protoplasmic masses mentioned above, it is interesting to notice that we have here an illustration of the fact that the protoplasmic substance of a highly specialized form may still be capable of existence as protoplasm, though not showing any of the normal morphogenetic processes characteristic of the living substance at that age; and may remain able for days and weeks to carry on some of its vital processes. Yet in spite of the fact that these protoplasmic masses were so widely aberrant in form and appearance, exhibiting none of the usual morphological characteristics of the protoplasmic portions of fish-eggs, nor of any other kind of vertebrate egg, save the primary differentiation into nuclear and cytoplasmic materials, they were still undoubtedly living. They remained free from bacterial infection, form and appearance slowly changed, coagulation did not occur, in many instances for weeks. Moreover it is not true that in all such cases death was the inevitable result, for when this condition was not too long maintained, such irregular,

largely though not wholly non-cellular masses occasionally gave rise to true embryos exhibiting the usual developmental processes. Such embryos usually showed various degrees and kinds of abnormality, but some few ultimately produced normally hatching larvæ. These masses were therefore more than merely living; their later history shows that even in that state there must have remained present, in many instances, some kind or remnant of an underlying construction or organization that determined either directly or through some regulatory process, development of parts, at least, of Teleost embryos, and in a few instances of essentially normal Fundulus embryos.

My chief object in describing, at this time, the effects of low temperature upon the development of Fundulus is not to give a morphological or histological description of the malformations and abnormalities produced, but to suggest the bearings of these results upon certain current hypotheses as to the real causes of such defects and the way in which the unusual physical conditions may have affected the morphogenetic properties of the ovum. I shall not attempt to review extensively the various suggestions as to the causes of monstrous development, but some of the more recent hypotheses only may be examined from the point of view of the results described here. For this purpose I shall refer chiefly to the suggestions made by three recent workers in this field.

As a preliminary word I should note that such observations as these show that "the idea that the low temperature only retards the chemical reactions underlying development" (Loeb '15, p. 59) is, for the Fundulus egg, true only to a certain point. When temperatures are lowered below say 12–14° C. the orderly developmental processes are not only slowed but may be actually modified so that some of the consequent processes are rendered abnormal. This may, of course, be due primarily to the fact that certain processes are slowed more than others, but the result is a disturbance of normal development which is different from a mere slowing down of the entire mechanism, the component processes remaining normally related to one another. Apparently the precise temperature when this effect is produced

varies with same internal or fluctuating condition of the egg and also with its stage of development.

We should also point out here that Lillie and Knowlton ('97) found that eggs of the frog (Rana virescens) at 2–3° C. always developed abnormally, if at all, the abnormalities usually appearing in the region of the blastopore.

In his classic "Study of the Causes Underlying the Origin of Human Monsters," Mall ('08) included an extensive survey of the more important results in the whole field of experimental teratology, particularly among the vertebrates. He concludes that even in such dissimilar instances of teratogeny as those in the toad resulting from fertilization with X-rayed spermatozoa (Bardeen, '07), those in the Teleost following chemical treatment during cleavage (Loeb, '93, Stockard, '06, '07) and the human instances described by himself, "although the methods employed are very different, the principle involved and the results obtained are much the same" (p. 24). And, "In general the methods employed by experimental teratologists is to subject the eggs to various insults which affect the nutrition and impair the growth of the embryo" (p. 52). "A monster is due to the influence of external substances which retard the growth of the embryo, usually one portion more than the other" (p. 36). In brief Mall's conclusion regarding the causes of human monsters is that "faulty implantation of the ovum, which naturally affects the growth of the embryo" (p. 25), by interfering with the normal nutritional relations leads to monstrous development; "that certain parts of the embryo are more susceptible to insults than others" (p. 32), and consequently it is these that are affected first (p. 16), though subsequently the faulty implantation must be remedied so that the embryo may continue to grow (p. 25). "But in order to produce a finished monster the nutrition must not be impaired too much" (p. 31). Many other quotations from the same work might be added to show that the words nutrition and growth are used with their customary significance, implying only the supply of materials and energy necessary for the usual processes of extension of at least partially differentiated structures or anlagen. Disturbances of

the general chemical reactions underlying development but not directly related to the income of the embryo, such as nuclear differentiation, mitosis, distribution of nuclear and cytoplasmic substances, specification of protoplasmic areas, et cetera, are evidently not included under the terms nutrition and growth as used here.

Essentially the same idea is contained in Stockard's hypothesis of the causes of the defects observed in Fundulus after chemical treatment, namely, that such treatment tends to lower the developmental vigor of the embryo, this lack of vigor being subsequently shown by the failure of certain structures to develop normally. This hypothesis is formulated chiefly in connection with the explanation of optic defects, particularly cyclopia. "A certain amount of energy is necessary for differentiation of the eye to take place . . . . but when the required energy for any reason is not available the eyes are incapable of any differentiation" ('13 b, p. 271). In the absence of any other evidence for such a deficiency in available energy on the part of the embryo, such an explanation comes to little more than a statement that the eyes do not develop. I can not be certain whether or not it is intended that this explanation should be applied to the other abnormalities observed by him, regarding which, however, no other suggestion is made. But as I shall show there are some difficulties in making a general application of this idea in explaining all of the defects noted in his experiments with various chemical substances.

Using a butyric acid or acetone treatment, Werber ('15 a, '16) secured not only abnormalities similar to those recorded by Stockard, but also a wide range of the same general types, and in many instances the same specific types, as those which I have found to follow the action of a low temperature. Werber believes that there are some factors common to the morphogenesis of all the diverse abnormalities and monstrosities observed in a given experiment. He applies the term 'blastolysis' collectively to the factors involved and believes that there occurs, as a consequence, a destruction or dispersal of parts or all of the germ's substance.

My own findings reported here certainly tend strongly to confirm the idea that the causes underlying abnormal development are to be sought in some derangement of the fundamental developmental mechanism or relations of the diverse components of the very early stages of the organism. In the first place it should be noted that by using a low temperature as the abnormal stimulus I have eliminated the necessity of supposing that there are any specific chemical alterations, such as precipitating or solvent effects, due to the use of chemical stimuli, which is so important an element of Werber's hypothesis. There may be some osmotic changes in the cooled eggs and there certainly is an increase in oxygen tension, but McClendon ('12) was unable to find that such conditions affected the frequency of cyclopia in Fundulus. On the other hand some of the effects of cold may be directly observed in the eggs during treatment, so that the character of the disturbance is not wholly left to be inferred from the study of later development.

As mentioned above, eggs which were placed in the refrigerator within a few minutes after insemination were found, some days or weeks later, whether a few cleavages had appeared meanwhile or not, to contain at least three classes of materials. 1) Not only nuclei of the usual appearance but also irregular, large and small masses and fragments of nuclear material scattered indiscriminately through the cytoplasmic parts of the egg. There were ordinarily no cellular outlines corresponding with these masses of nuclear substance. 2) Masses of granular cytoplasmic substance somewhat resembling the material of the greater part of the normal uncleaved germ-disc. 3) Masses of clearer, possibly protoplasmic substance usually in the form of vacuoles, slightly resembling in physical appearance that mass of clear cytoplasm that in normal development forms, for a brief period preceding cleavage, a lens-shaped disc on the lower side of the central part of the germ-disc. I am not yet in position to identify these forms of cytoplasmic substance with the two chief forms observable in normal development; but the similarity is suggestive. Eggs in this state are still capable of some sort of developmental process when returned to ordinary temperatures,

but abnormalities are very frequent and show the remarkable range of characters described above.

It seems, then, that the causes of the observed abnormalities in development may be referred to these conditions: (a) abnormalities in nuclear composition and in the distribution of differentiated nuclear substances; (b) abnormal distributions and associations of two more evidently differentiated cytoplasmic substances; (c) abnormal associations between nuclear and cytoplasmic substances, either or both of which may be abnormal in its own composition as compared with the corresponding materials in the regularly developing egg.

In a word this means that the organization of any part of the early organism may be disturbed, and that such disturbances are the causes of abnormal and monstrous development. That development can occur at all under such circumstances indicates remarkable regulatory properties of the egg substance. The complete lack of specificity in the effects of the cold indicates that the disturbance is profound and that it affects the fundamental organization of the ovum rather than any especially differentiated representative substances or anlagen, which, moreover, have been shown by other evidence not to exist in the Teleost ovum (Morgan, '93, Sumner, '04).

Going a step back of these observations to the query as to just how the low temperature can produce such disturbances, we can at this time make suggestions only in very general terms. It is evident that not all of the processes of development or parts of the developmental mechanism are affected similarly, for that would lead to development normal in all save its rate. Some physical or chemical processes or structures must be more extensively interrupted or altered than others, as a result of the lowered temperature. They are thrown out of their normal relations to the other processes or parts of the mechanism and sooner or later a whole train of consequences may become evident. It is very important to note, however, that the substances or processes thus affected are not to be thought of as specific tissue—or 'organ-forming substances' nor as differentiated, cellular or formed rudiments or anlagen, but as elements or factors

in that whole complex mechanism which as an entire system epigenetically gives rise to such substances or rudiments. It is quite likely that, in part at least, these altered reactions are such as may lead to disturbances of the mitotic processes of maturation and fertilization, such as were described in such great variety in Ascaris by Sala ('95) or of nuclear division during cleavage, as described by Conklin ('12) in Crepidula, whereby abnormal nuclear structures may be formed. It is also not unlikely that the observed failure to form cell-walls in many parts of the cytoplasm, or the abnormal location of cell-walls when formed, may open the way to the possibility of abnormal nuclear and cytoplasmic associations. Another possibility lies in the physical slowing of the translocatory movements of the gradually differentiating cytoplasmic materials. In eggs subjected to cold immediately after fertilization the normal flowing together of the cytoplasm to form the germ-disc and the subsequent rearrangement and redistribution of different cytoplasmic substances, often appear superficially to be seriously interrupted. But all such conditions are themselves to be regarded as consequences of antecedent modifications of some more elementary chemical or physical organizational processes as yet beyond analysis and description.

The great variety in the results following treatment is just what would be expected on the basis of such a disorganizational effect. Both nuclear and cytoplasmic materials are themselves so complex, and the complete system of relations, both material and energetic, between them and among the various component parts of each, which as a whole we term the 'organization' of the ovum, is so extremely complicated, that it affords almost unlimited possibilities for modification and disturbance. Without knowing very much more than we do about the physics and chemistry of this organization and about its regulatory capacity, it would seem largely or wholly a matter of chance, what would be the precise later results of any single modification or simple group of disturbances in this system. The results of such disturbance are entirely unpredictable in individual instances at present.

We should recognize, as preliminary to much of the discussion that follows, that either the morphological or the chemical extent of the initial disturbances may not be the only condition correlated with the extent of the later derangements. A comparatively slight modification in an essential or highly important organizational factor would have a more marked result in later development, than would a more extensive alteration in factors of lesser importance. The quality of the disturbance rather than its extent is primarily involved. It is further likely that no relation could be determined between the extent of the initial disturbance and the final effect on account of the possibility of regulatory action and because comparatively slight initial alterations might give a wholly abnormal trend to a long series of consequent processes finally resulting in very pronounced abnormalities.

With this general statement of the essential nature of the disorganization hypothesis we may turn to an examination of the widely current nutrition hypothesis, based largely upon the results following chemical treatment, in order to draw attention to certain difficulties in its application to some of the observed facts and to inquire whether the suggestions made here avoid any of these difficulties without creating others.

If any justification seems necessary for the attempt to criticize, from the viewpoint of the results of the action of low temperatures, the hypothesis based upon chemically prod ced effects, it is to be found in the essential identity of the consequences of these different modes of treatment. Indeed I should go further and bring under this same point of view the abnormal types of development following certain other experimental conditions, such as heterogeneous hybridization, and the subjection of gametes or zygote to radium radiations, which will be discussed later.

The nutrition hypothesis as stated by Mall, and to some extent adopted by Stockard, has for some time now largely been held to account for such illustrations of teratogenesis as those described here, but it seems open to certain serious objections. In the first place it might be pointed out that the work of Pack-

ard ('14) on the effects of radium radiation upon the eggs and sperm of Nereis, has largely rendered untenable the suggestion of Mall that the effects of such treatment are nutritional in character. Packard concludes upon very clear evidence that there are strong reasons for believing that the radium radiations act indirectly upon both the chromatin and cytoplasm of either or both germ cells or the zygote, by bringing about in these, destructive chemical processes. Development is thus rendered abnormal both by the destruction of the normal chemical and physical mechanism of early development, and also possibly by the toxic presence of the abnormal substances thus formed and present in the cleaving egg. That is, the abnormalities found in later development may be referred to abnormal nuclear and cytoplasmic behavior during the cleavage processes, before the specific germ-layer or tissue differentiations are inaugurated. This clearly removes the results from the category of nutritional effects and affords an explanation of the same general character as that which I have suggested above, with this difference, however, that I am not inclined to stress the possibility that the effects are due to the presence or absence of specific chemical substances, but rather hold them to be due to unusual combinations of differentiated materials both nuclear and cytoplasmic, in a word to a disturbance of the 'organization' of the ovum or of certain parts of it; and this difference seems to me of quite an essential character.

How such results as those of Lewis ('09) and Spemann ('03, '04) and some of Stockard's ('13 b) who caused cyclopia in Fundulus and Amblystoma by the actual physical destruction or even removal of the differentiated anterior end of the central nervous system, can be interpreted as due to a nutritional effect is even less clear than the possibility of such an interpretation of the effects of radium upon the spermatozoa previous to fertilization. To compare and identify the cyclopia in Fundulus resulting from such removals of already differentiating structures, with the cyclopia resulting from chemical treatment of four to eight cell stages, involves the assumption that during early cleavage in Fundulus there is already present some repre-

sentative, definitive, organized rudiment of the anterior tip of the central nervous system, and further that it possesses a sensitivity to general nutritional disturbances which is also specific, *i.e.*, not shared equally by other rudiments which must also be assumed to be present at this time. But it is well known that the Teleost ovum is of the indeterminate type, and injury or removal of whole blastomeres of the normal cleavage group causes no later defects or abnormalities in the developing embryo. The assumption of specificity in the action of nutritional effects in connection with cyclopia, is further negatived by the great variety of the results following chemical treatment. I have already pointed out the great variety of abnormalities and monstrosities observed by Stockard, Werber, and others, appearing under the same experimental conditions. The only suggestion of actual specificity is that of Stockard ('10, p. 369) that treatment with certain percentages of alcohol gives, among the surviving embryos 90–98 per cent with abnormal eyes, generally cyclopean. But he does not say that other defects may not also be found in these or other embryos similarly treated, indeed on the contrary he mentions a great variety of ear, brain and other defects which may either accompany eye-defects or appear independently of them, under similar treatment. Moreover, the expression 'eye defects' covers a number of different conditions, not all of which can be referred to the cause which he assumes for cyclopia, namely, the inhibition of the development of the median anterior tip of the central nervous system. To find a great variety of abnormalities among embryos subjected to the same treatment leads definitely to giving up the idea of specific reactions of the rudiments of such structures to the unusual conditions. To relate the appearance of such a variety of abnormalities during later development of the embryo, to the effects of the treatment upon some specific parts of the materials of the cleavage group is, therefore, to assume the existence of differentiations during cleavage which have been shown not to exist, and then to require further that each or any or all of these may be specifically affected, which is to say that there is no specificity at all in the action or in the reaction.

Stockard ('13 b) explains "the fact that a number of eggs when subjected to the same solution do not all respond in a like manner" merely by regarding this as "a typical case of differences in individual resistance and vigor which is observed among any one hundred individuals of any living species" (p. 282). But the kinds of differences found here are not at all such that they may be regarded as illustrations of that normal fluctuation in all characteristics which represents the reactions of organisms to the incidence of environing conditions. It is probably true that eggs and embryos do differ in those complex conditions which we summarize in the words 'resistance and vigor.' But these qualities do not determine whether an embryo shall or shall not have eyes, hearts, pigment cells, and so forth. We are dealing with a wholly and fundamentally different phenomenon.

An important objection to this nutrition hypothesis, it seems to me, is that there is little or no actual evidence given that the nutritional conditions of the egg or embryo are directly affected. The evidence offered is that defective and monstrous embryos result from various methods of chemical treatment; but it is these defective and monstrous embryos that are to be explained. In the few instances where there is evidence of abnormal relations between embryonic and vitelline portions of the egg, it is more reasonable, in view of the great variety of other conditions found, to regard this too as a result of a primary disturbance, rather than as the cause of a variety of conditions which may also occur in its absence.

The suggestion as to the responsibility of abnormal nutritional relations as the cause of abnormal and monstrous development was made primarily in connection with the human embryo and then extended to other forms. It is quite possible that in the human and other placental mammalian embryos such effects may in some cases be found to be specific and to be related to nutritional abnormalities due to disturbances in the parental organism. However, the mammalian embryo develops for some hours or days before implantation occurs and becomes effective as a nutritional factor so that it is only subsequently to that time that the effects of faulty nutrition might be exercised.

Embryonic differentiations having been begun before that time, the way might be opened to a specificity of action upon some part requiring a large supply of energy and material. The later the abnormal conditions act, the more likely are they to be specific in their action. But in the absence of definite proof of such a cause, it is on the whole much easier to interpret monstrosities, even among the Mammals, as resulting from organizational disturbances produced by the presence or absence of some definite chemical environment, whether that be nutritional or not. And such a chemical stimulus might be operative not only after, but also before implantation, when the extent of the resulting abnormality might be very great, so great as to result in the formation of a 'complete monster.' I should not, however, oppose the idea that mammalian monsters may be due, in some cases to nutritional defects: in this connection I should merely suggest that the observed facts do not exclude the possibility of a general organizational disturbance as the cause of monstrous development in this group, and that even in the placental mammalian embryo, although with less likelihood than in oviparous forms, the nutritional disturbance, when it is known to exist, may itself be a result rather than a cause of the deranged organization.

But it seems, in the light of subsequent observations, that it was a mistake to extend this interpretation of the causes of abnormal development so generally to other classes of vertebrates and to say that such abnormalities result from conditions "which affect the nutrition and impair the growth of the embryo" (Mall '08, p. 52) or which "tend to lower the developmental vigor of the embryo" (Stockard, '13 a, p. 83), or that "a certain amount of energy is necessary for differentiation of the eye to take place . . . . but when the required energy for any reason is not available the eyes are incapable of any differentiation" (Stockard, '13 b, p. 271). Such a statement seems to leave unexplained such cases as have been observed both by Werber and myself, where portions of eyes, fragments of optic cups, lenses, or even fairly complete eyes, may be found either without other true tissues or organs being differentiated, or with scattered parts of other organs and bits of tissue.

The difficulty of explaining such an abnormality as monophthalmia asymmetrica, or other asymmetrical abnormalities, on this nutritional basis is also apparent. Stockard recognizes this and merely suggests that "It might be that at some critical point in development one of the future eye centers is affected after the growth centers had begun to localize in more or less lateral positions" ('13 b, p. 278). Such asymmetrical abnormalities offer no special difficulties of interpretation on the disorganizational hypothesis.

Stockard ('13 b, p. 281) has also noted that the earlier the treatment is administered during the development of Amblystoma the more extensive are the resulting abnormalities: "the developmental period of administration is of as high importance in determining the result as is the nature of the stimulus used." This is readily understood upon the hypothesis that the unusual stimuli act by disturbing the normal organizational relations of nucleus and cytoplasm, since a simple or localized disturbance at an early stage would be followed by much more widespread effects than would an equal disturbance at a later stage, when many of the differentiations might be already determined and the effects consequently more localized. After the essential differentiations of the organism have been made, the effects of external stimuli would be likely to have relatively slight morphogenetic results. (Compare the morphogenetic results of stimulation in embryo and in adult organisms.)

Stockard believes that "all of the eye conditions [in Fundulus] may be interpreted as arising through developmental arrests ('13 a, p. 83); and throughout his papers the abnormalities observed are continually referred to as 'defects,' an interpretation that is cited in support of the nutrition hypothesis. While most of the abnormalities observed have the nature of defects, if by defect we mean only failure to differentiate, yet not all of the abnormalities noted in Fundulus, to mention but this case, are of this nature. In my own observations I might mention for example, the development of two complete and separate embryos on a single yolk; the development of two separate hearts, not paired but in different regions of the embryo; the

proliferation of ectoderm cells, the formation of large masses of erythrocytes, et cetera. Apparently the only way to interpret such conditions as due to some defect is to have recourse to the interpretative method employed by some geneticists (with the difference, however, that there it may be clearly justified) and say that when abnormal development occurs it is due to a defect in that which would have kept it normal. But that would merely be to say that abnormal development occurs. And it should be recalled just here that Loeb ('15) noticed that eyeless embryos developing from a cross between Fundulus and Menidia reached that condition after passing through earlier stages in which the eyes appeared to be normal. In such instances anophthalmia is certainly not due to any original destruction of 'ophthalmoblastic anlagen.'

Loeb occasionally inclines toward this nutrition hypothesis in explanation of some abnormalities and points out that, since in most of his observed instances of anophthalmia circulation also is lacking, "the inference is possible that the anomalous condition of the eye may be due to lack of circulation" ('15, p. 67). But the large number of instances in which the eyes may develop normally in the complete absence of circulation renders such an inference untenable.

A rather significant test of the nutrition hypothesis, or at least of certain phases of it, can be found in those cases where the embryo develops without a heart or without a circulation. Here is certainly a profound disturbance of the nutritive relations of the entire embryo. Whatever the primary cause of such a lack, the conditions should, on this hypothesis, be accompanied or followed by marked and varied abnormalities. If nutritional disturbances so easily affect development as the general hypothesis requires, such embryos should certainly produce 'complete monsters' capable of but a brief existence. It is often the case that embryos lacking these organs are also defective in various other ways, but it not infrequently happens that such embryos may develop with a high degree of normality in all other respects and when removed from the egg membranes may continue to live and react almost normally for some time.

The main objections to the nutrition hypothesis of the causes of embryonic abnormalities and monsters as it has been stated, are, then, the following: it does not afford an interpretation of the results, such as those of Bardeen and Packard, following treatment of the sperm with radium radiations; it does not afford any suggestions as to the nature of the underlying disturbances through which the abnormalities are produced by the unusual conditions used or assumed to be present; it does not explain why the action of the experimental conditions during cleavage should not produce visible results until much later; it does not explain why monsters of the same parentage are diverse (see Mall '08, p. 12); it does not explain why the effects of treatment are greater when applied during the earlier stages of development; it does not explain the production of abnormalities which are not defects or developmental arrests; it does not avoid the necessity of assuming a degree of differentiation during cleavage and a specificity of the action of the external conditions, both of which have been shown not to exist.

On the other hand, the hypothesis suggested earlier in this paper, that the causes of abnormal and monstrous development are to be found in the disturbance of the normal organization of the fertilized ovum or cleavage group, as evidenced by the abnormal characters and distribution of the nuclear and cytoplasmic substances, avoids these objections and affords an easy interpretation of the observations mentioned.

It seems to have been premature to have assumed (Mall '08) that all the classes of embryonic abnormalities and monstrosities described by Mall, Bardeen, Spemann, Lewis and Stockard, are really of the same essential nature and due to similar causes. I see no reason for not admitting that such embryos may result from different causes in different cases, whether they be (1) the abnormal characters of the gametes before fertilization, or disturbances of the early cleavage processes as these concern both nuclear and cytoplasmic constituents, (2) the mechanical removal of differentiated rudiments, or (3) the lack of energy and materials ordinarily supplied through nutritional pathways, including the pathways within and among the parts

of the egg and embryo themselves; and I believe that these are not merely different phases of the action of a single principle, certainly not all due to a lack of 'developmental vigor' although that is a pretty general phrase and one that might cover a multitude of varied conditions, individually unlike and due to varied primary causes.

Turning now to a much briefer consideration of Werber's hypothesis, as stated above, I shall refer only to his latest papers ('15, '16). While admitting that the nutrition hypothesis may account for the production of certain types of human monsters, Werber rejects its general validity in favor of the view that modifications in the physical or chemical environment, for example in the blood of the parent organism, affect directly either the germ cells before fertilization or the fertilized ovum or later developmental stages ('15 a, p. 530). Thus he agrees with Spemann in opposing the hypothesis of Stockard mentioned above, regarding the cause of cyclopia, and believes that the eye-defects, which form the main subject of disagreement here, are really due to morphological defects of some kind, and not to an inhibition resulting from lack of developmental vigor or energy. He goes farther than this, however, and suggests that the substances used in his experiments, namely, butyric acid and acetone, caused "an elimination of materials of the blastomeres or of the germ-disc and probably also of the yolk-sac." "Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter" ('16). And further "This elimination of material may be due either to the precipitating or solvent effect respectively of the chemicals which were used" ('15 a, p. 559). From the examination of very extensive material he concludes that "either the blastomeres or the germ-disc had been blastolytically fragmented owing probably to both physical and chemical factors" (p. 559), and that whatever scattered parts survive this fragmenting and blastolytic process "may go on developing into a whole defective [*sic*] or a meroplastic, embryo, or even into an isolated organ" (p. 559). And in another place ('15 b) he adds that an increased imbibition of water following an increase in the per-

meability of the egg membrane is another factor in producing blastolysis. Thus through the operation of such factors together, some parts of the germinal substance may "be entirely destroyed owing to the increase in osmotic pressure, while the remainder may go on developing and eventually give rise to various monstrosities" (p. 240).

The first part of Werber's hypothesis, namely that the effects are due to a physical and chemical modification of the germ cells, cleavage group or germ-disc, is clearly in accordance with the more general disorganization hypothesis. But in the absence of direct evidence, which it would be extremely difficult to secure, of the destruction of certain specific materials and not of germinal substance in general, it seems more nearly in accord with the general conception of development to believe that what is effected is a disturbance or disarrangement of the constituents of nuclei or cytoplasm, or both, or of the normal relations between and among these materials. Otherwise, and in accordance with the latter part of Werber's hypothesis, it is necessary to assume in the germ cell or cleavage group, a whole series of unlike substances whose differentiations are already specific and definitely necessary, not as organizational factors, but as the rudiments or anlagen of the later differentiating tissues and organs of the embryo. It is here that I should take exception to the hypothesis, for there is as yet no direct evidence for the existence, in such early stages, of such differentiated rudiments. It seems much more likely that it is just the mechanism of differentiation that is disturbed by these abnormal environments, and not even the earliest formed results of the operation of such a mechanism, although it is quite possible that there may also be an added actual destruction of specific materials that as such are necessary to normal development. This difference between Werber's view and that stated here is not a minor one; it is the difference between the predeterminational and the epigenetic views of development.

The fact is an important one, that results exactly parallel to those of Werber (and Stockard) follow upon the mere lowering of temperature, a condition which eliminates the possibility of a

direct chemical action, such as precipitation or decomposition, and which can also produce disturbances in the arrangement of nuclear and cytoplasmic substances that are actually visible, but which may only be assumed to have destroyed any chemically and developmentally differentiated and specifically necessary materials. In this respect my experiments afford, I think, a valuable check on the results following chemical treatment and indicate the more general validity of the disorganization hypothesis.

And further, since I have observed several instances where eggs exhibiting rather extensive fragmentation and dispersal of protoplasmic parts, were still able to form normal embryos, I am led to believe that such conditions in themselves need not lead to abnormal development, but that some other condition must be primarily responsible. If certain essential organizational conditions may remain present or susceptible of restoration, development may proceed normally even though some considerable parts of the egg protoplasm may actually have been destroyed; a conclusion which is also indicated by the results of removal and pricking experiments on single blastomeres of the Teleost ovum (Morgan '93).

Most of Werber's discussion of the action of his mode of treatment centers about the actual mode of the production of eye-defects and of the respective merits of the 'fusion' and the 'inhibition' hypotheses. But such a discussion seems concerned rather with the after effects than with the real causes of the abnormality. That is, while it is very important to know the proximate causes of such a condition as cyclopia, neither of the suggested causes is really fundamental, and either fusion or inhibition may result from a primary organizational disturbance, in the same way that varieties of nutritional abnormality may also result from a similar underlying cause.

In one respect Werber seems to fall into the error made by Stockard in assuming that such conditions as cyclopia result from a specific effect upon a differentiated rudiment of the anterior end of the central nervous system, already differentiated in the early cleavage group. This leads him ('15, pp. 557–8) to

support Child's 'axial gradient' theory in explanation of the frequency with which there occur defects of the tissues and organs developing in the anterior end of the embryo. "When the egg is acted upon by a toxic substance, a restricted area at the anterior end of the embryo's median body axis becomes so altered chemically as to be eliminated from further development or it may go on developing to a certain point beyond which it is chemically unable to proceed" (p. 557). "The size of the injured area at the anterior end is probably subject to considerable variation," and thus the effects may be limited to the future interocular area, or they may include parts, varying in extent, of the potential optic anlagen, or one optic anlage only, and so on. In addition to these assumptions, it is further necessary to assume (p. 558) not only the existence of differentiated ophthalmoblastic anlagen in the very early cleavage group, but definite and symmetrically placed double ophthalmoblastic anlagen with different degrees of susceptibility to the chemical substances in solution. By means of these and other assumptions (p. 558) Werber is able to reconcile the two hypotheses of the causes of cyclopia mentioned above, but in accomplishing this he runs contrary to the demonstrated lack of specification or determination in the cleavage group of the Teleost. Eggs were treated in the one- to sixteen-cell stages, and as Werber himself remarks (pp. 531–2) it is probable "that it is mainly the initial effect of the toxic solution on the ovum that causes it to develop in an atypical manner." There is no anterior end of an embryo represented by any differentiated material in the sixteen-cell stage of the Teleost, no ophthalmoblastic anlage; but there is an organization or developmental mechanism capable of producing these parts, much later, a mechanism that is interfered with and upset, and there is no specificity in the result of the derangement. This lack of specificity is directly opposed to the application of the axial gradient hypothesis, for as a matter of fact, any part, posterior as well as anterior, may become abnormal following this or other modes of treatment.

The explanations for the observation that all of the organs of the anterior end of the Teleost embryo—eyes, brain, heart, etc.,

are the more likely to exhibit abnormality seem much less recondite. In the first place, from the mode of its formation by confluence (concrescence) the head end of the Teleost embryo is the first part to be formed and differentiated, and any disturbances of the normal processes of tissue and organ differentiation are much more likely to be exhibited first in the region earliest differentiating. And in the second place, the organs of the head region, especially the sense organs, brain and heart are in general more highly and therefore more extensively differentiated, their normal development involves the interaction of a larger number of factors, than in most other parts of the embryo where muscles and the connective tissues form the chief constituents. Hence a slight initial disturbance would produce a more frequent as well as more marked result in the anterior part of the embryo, where a more precise arrangement of the underlying conditions of differentiation is necessary, than in the more posterior parts where the tissues and organs are simpler and possess greater regulatory properties.

And it should not be forgotten that as a matter of fact every part of the embryo is subject to abnormality following treatment with chemicals or low temperature; no part has been found to be wholly free from abnormality in every case. This is an observation, by the way, which has a decided bearing upon the use of experimentally treated material as an aid to the solution of problems in the normal development of Fundulus. If any part, organ or tissue, whatever, may be affected abnormally by such treatment, an extreme degree of caution should be exercised in applying to the interpretation of the events of normal development, the evidence drawn from the histogenesis of embryos developing from treated eggs.

In conclusion I should like to refer briefly to the bearing of the hypothesis stated here, upon a group of observations of a wholly different kind. I refer to certain results of Teleost hybridization described by Moenkhaus ('10), Loeb ('12), and Newman ('14). Moenkhaus found that in such hybrids "development in its early stages proceeds normally, *i.e.*, when superficially viewed the deleterious effects of the two strange

sex products upon each other showing only at later cleavage and subsequently;" that gastrulation, *i.e.*, the formation of the germ layers—the most marked of the earlier differentiations of the embryo, was a period of high mortality; that numerous abnormalities appeared in the hybrid embryos surviving this period. He interpreted this to signify that the sex cells exercised a poisonous action upon each other, preventing normal development, and suggested as an analogy, merely, the toxic effects of transfused bloods. Loeb also noted that various abnormalities were not infrequent among heterogeneous hybrids, especially in respect to the circulatory system and eyes, and suggested that the small size of the embryos thus produced might be due to their failure to digest the yolk as rapidly as the pure bred embryos.

Newman similarly finds the period of gastrulation one of high mortality, but he shows that the effect of the 'foreign' sperm may frequently be detected even during the earlier cleavage period. He also refers to the variety of abnormalities that may appear during the later development of those embryos surviving the gastrulation period, and partially relies upon the nutrition hypothesis to explain them. However, since the problem he was investigating was an entirely different one, he does not attempt a careful analysis of the probable causes of abnormal development, and it would be unfair seriously to criticise his suggestion that death or abnormality during gastrulation is due to failure to establish nutritive relations with the yolk, which is to be regarded rather as a passing suggestion than as a definite opinion. I do not understand that he, or anyone, has demonstrated that this period is especially characterized by the establishment of such relations with the yolk.

It is of considerable importance from my point of view to note that Newman finds that one of the common effects of hybridization is a disturbance in the time relations of various processes of development, both acceleration and retardation being quite common consequences. This means that not only is there a disturbance of the normal morphological sequences in such hybrid organisms, but that the whole organizational, in-

tegrational mechanism is or may be affected. Several obscure relations noted by Newman might be profitably discussed from the viewpoint of the general hypothesis which I am suggesting, for example, his statement (p. 469) that "It is difficult to imagine what factors underlie this wide range of success of individual hybrids of the same parentage," an explanation of which I think these suggestions afford.

However, it is not my intention, at this time, to attempt a general application of this disorganizational hypothesis to such phenomena, and the work of Moenkhaus and Newman is mentioned in this connection chiefly to show that such observations are not counter to this general explanation of the causes of abnormal development. For it is, I take it, a strong point in favor of any suggested cause of a restricted group of phenomena, that it is not opposed by the facts concerning nearly related phenomena. Such seems to be the case with these suggestions as to the causes underlying the formation of abnormal and monstrous embryos in Fundulus; for it not only accounts for the results following treatment with low temperatures and chemical substances, but it is not opposed, to say the least, by observations on the causes of similar developmental phenomena following treatment of the germ cells by radium radiations and following hybridization, and further, it accords with what is known of the early development (cleavage) of the Teleost and with the current general conceptions of the developmental process.

SUMMARY

1. By subjecting the eggs of Fundulus, immediately after fertilization, to the temperatures of the ordinary refrigerator, many of them are caused to develop abnormally when returned to the laboratory temperature.

2. The abnormalities observed after such treatment cover a very wide range and no characteristic, externally observable, is found not to be affected to some degree in some embryo.

3. Similar treatment after the embryo has become well-formed also leads to similar results, though with lesser frequency.

4. The effects of the low temperature which may be actually observed in the treated individuals, take the form of irregularities in the distribution and combinations of both nuclear and cytoplasmic substances, and in the formation of cell-walls.

5. The results obtained by this method are essentially similar to those already known to follow chemical treatment and heterogeneous hybridization.

6. It is suggested that the cause of abnormal and monstrous development here and in other similar instances, is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual characters and distributions of the differentiated materials of the egg protoplasm.

7. This 'disorganization' hypothesis seems to afford a better explanation of many instances of abnormal and monstrous development among the vertebrates, than does the current 'nutrition' hypothesis, which is in many particulars opposed by the results reported here.

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THE DEVELOPMENT OF THE SPIRAL COIL IN THE LARGE INTESTINE OF THE PIG

PAUL E. LINEBACK

Harvard Medical School, Boston, Mass.

TWENTY-THREE FIGURES

The student of human anatomy who happens to examine the viscera of the adult hog will be greatly impressed by the spiral arrangement of the ascending colon. For the colon in the pig, after arising from the caecum which is almost entirely on the left of the mid-ventral line, passes at once to the left, and then swings around the abdominal cavity in voluminous coils. The small intestines are mostly hidden behind it, though they appear in the right iliac fossa, and altogether the colon is the principal object seen when the abdominal cavity is opened.

To describe the course of the pig's colon in detail is a difficult undertaking, which John Hunter skillfully attempted in the following passage (*Essays and Observations*, 1861):

> It makes five spiral turns like a screw, coming nearer the center; at the end of which it is bent back upon itself, passing between the former turns as far as the first: but in this retrograde course it gets nearer the center of the screw, so that it is entirely hid at last, then makes a quick turn upward, adhering to itself and to the left kidney, as high as the first spiral turn; from thence it passes across and close to the spine, and before the mesentery, adhering to the lower surface of the pancreas, and, as it were, encloses the fore-part of the root of the mesentery; it then passes down the right side before the duodenum, gets behind the bladder, and forms the rectum.

Hunter's description was cited by Owen who notes that "the spiral turns of the colon, above described, form one of the characteristics of the Artiodactyle order." To a certain extent this is true, but the cow and sheep, and doubtless other forms, present considerable modifications of the arrangement found in the

pig. These are all more or less adequately described in the text-books of veterinary anatomy. The spiral coil in the pig is clearly figured by Sisson, as seen both dissected and in situ, and he adds a diagram of its course.

The musculature of the pig's colon is also peculiar, in that it possesses two taeniae instead of three throughout most of its course, and it was while studying the development of these taeniae that my attention was diverted to the coil itself by Dr. F. T. Lewis. At his suggestion the following account of its embryological history has been prepared, and it is a pleasure to record my indebtedness to him for coöperating throughout this work. Such an extraordinary and conspicuous formation has not escaped previous study, but the existing descriptions are so meager that they should certainly be supplemented by further investigation. This was Martin's opinion when in 1889 he published the first of his papers containing most of the information now available.

In the Schweizer-Archiv für Thierheilkunde Martin presents a series of diagrammatic figures showing the probable evolution of the spiral colon in the sheep, including hypothetical drawings of some stages which he had not actually observed. They are accompanied by a brief description, containing references to corresponding stages in the cow, but little is said regarding the pig. In 1891 Martin supplied a new set of diagrams of the development in the sheep, and a fuller description. In brief, he considers that the colon, which previously has been quite straight, forms a loop, the apex of which soon becomes bent like a hook. The loop continues to elongate spirally, "as one of its limbs grows slower than the other, and thus we have the beginning of the spiral coil."

In the same year ('91) Bonnet published modifications of Martin's earlier diagrams which are clearer, but apparently more arbitrary. He records the formation of a "primitive loop of the colon" in embryos of the horse. This, he observed, has become somewhat S-shaped in a specimen measuring 10 cm., but it never makes more than one revolution. In the pig, Bonnet finds that a corresponding loop "winds itself spirally around

an imaginary axis and forms the colon-labyrinth, later shaped like a bee-hive, consisting of $3\frac{1}{2}$ concentric outer and $3\frac{1}{2}$ excentric inner convolutions."

In 1901 MacCallum described the development of the pig's intestine, intending to show that its coils are measurably constant. In a 32-mm. embryo, he states that the large intestine, "in the region where it turns to form the rectum" is thrown into 'irregular twists.' The complexity of this rectal group of coils is said to increase in later stages, but its further evolution is not described in detail. However, it is clear that MacCallum failed to find a primitive loop, produced from an otherwise straight colon, which elongated and grew into a helicoid spiral, as described by Martin and Bonnet; and in the following study it will be shown that the course of development is more complicated than these authors have represented.

Beginning with an embryo of 12 mm., it will be found, upon dissection, that the intestine has formed its primary loop extending into the umbilical cord, and that torsion has not yet occurred. The intestine at this stage may therefore be compared with that of human embryos of 7–10 mm. In both forms, the large intestine occupies the greater portion of the posterior limb of the loop, beginning at the small bulbus coli, which marks the future caecum (fig. 1). The apex of the loop in the pig is more persistently attached to the vitelline duct than in man, and this duct has been cut across in figures 1 to 4. Moreover the length of the primary loop in the pig is greater than in human embryos. In the pig the distance from the base of the loop to its point of attachment to the yolk-stalk is between $\frac{1}{3}$ and $\frac{2}{3}$ of the length of the entire embryo. (For example, in figure 1 it is $\frac{1}{4}$, and in the reconstruction of a 12-mm. pig by Lewis it is $\frac{1}{3}$). In human embryos possessing a primary unrotated loop, as reconstructed by His, Elze, and Lewis, the length of the loop is between $\frac{1}{7}$ and $\frac{1}{10}$ of that of the whole embryo. It is not improbable that the distinctly longer and more slender loop in the pig provides for the more extensive intestinal convolutions, characteristic of the pig in the stages immediately following.

In pig embryos of 24 mm. the torsion of the primary loop has begun, so that the large intestine passes across the left side of the duodenum and becomes the anterior limb of the loop (fig. 2). It extends from the freely-projecting caecum in a remarkably straight course, free from all convolutions, to the right-angled bend where it descends to the rectum. The other limb of the loop, which forms the small intestine, has become three times as long as the colic limb and is thrown into many convolutions, arranged in linear series. Together they form a striking and characteristic figure quite unlike anything seen in human development. By referring to Mall's reconstructions of the intestines of two 24-mm. human embryos (1897, Tafeln 21 u. 22) or to Johnson's more comparable drawing of a 22.8-mm. specimen (Lewis, '12, p. 321) the differences will be apparent.

According to Mall ('97) the convolutions of the human small intestine are quite constant, and there are six which are primary. With many secondary subdivisions, he found that these could be identified in the adult. Following Mall, MacCallum studied the development of the coils in the pig and likewise found that in "embryos of the same size the coils are constant in their arrangement and definite in their position." But this conclusion ought not to be accepted without further investigations. In MacCallum's figures of embryos of 23 and 25 mm., there is a well-marked stretch of small intestine toward the apex of the loop, which is quite free from coils. No such interval is shown in figure 2, and having found it but once in many embryos dissected, I must regard it as exceptional. The four primary groups of MacCallum are not apparent in my specimens, and the individual coils in figure 2 cannot be homologized with those in MacCallum's reconstructions.

The torsion of the primary intestinal loop is carried much further in the pig than in man. The human intestine rotates through an arc of approximately 180°, so that the original posterior limb becomes anterior and vice versa. That is, it accomplishes such a rotation as is nearly completed in figure 2 and stops at that point. But the pig's intestine goes further, performing a complete revolution, as shown in figures 3 and 4.

After this rotation of 360°, the limb of the loop which was originally posterior has again become posterior (as seen by comparing figs. 1 and 4), but the anterior limb now crosses it twice. As duodenum, it passes down on the right side of the colic or posterior limb, then bends to the left beneath the colon, and finally passes upward crossing the colon a second time, but now on its left side. Figure 3 is an interesting intermediate stage in this process. The first rotation, of 180°, has been completed, even to the apex of the primary loop (which is not the case in figure 2), and the second rotation of 180° has occurred in the proximal part of the loop, but not distally. The rotation extends from the proximal portion of the loop outward, and the process has been completed in the distal part of the loop in figure 4.

Martin observed a similarly complete rotation in the sheep and described it as follows:

> The more the small intestine forms coils, the more it crowds the recurrent colic limb dorsally and at the same time to the right and caudally, until it is surrounded by a ring of coils of small intestines; and the *half axial rotation* about the mesentery in a 56-day embryo is transformed into a *complete rotation*. Thereby the relation of duodenum to the large intestine becomes changed. Earlier only a simple crossing took place, but now there is an encircling.

In the pig, MacCallum described the rotation in connection with the various groups of coils which he recognized. Thus he states that 'Group D,' which includes those coils of the small intestine which are nearest the caecum, "has rotated posteriorly, dorsally, and to the right. . . . . It thus moves past Group C and carries the caecum with it, so that the beginning of the large intestine lies dorsally and posterior to Group D." Correspondingly a group of coils of the large intestine (Group E) is said to "rotate through three-quarters of a circle." Although it is apparent that the rotation was observed, its description is unnecessarily involved, since it is based on groups of coils of questionable distinctness, rather than on the limbs of the primary loop.

The formation of coils in the large intestine begins in pig embryos of about 30 mm. At 35 mm. (fig. 4), they are well developed and are gathered in a knot between the caecum and the splenic flexure. This flexure it should be noted, is the only one which is found in the pig, so that the pig's ascending colon corresponds with both the ascending and transverse colons of human anatomy. A sinuous condition, preceding the formation of distinct coils, is seen in the 30-mm. embryo, figure 3. Similarly MacCallum found coils of the colon in pigs of 30 and 32 mm., and at 40 mm. he states that they 'form a conspicuous mass.'

The transformation of the mass of coils into the well-arranged spiral of the adult may be followed in special dissections, and from a large number of such preparations ten have been chosen for illustration (figs. 5 to 14). The final condition is shown in figure 14, representing the colon from an adult, and this is preceded by a figure of the spiral four weeks after birth (fig. 13). The other drawings are from embryos ranging from 50–120 mm. Throughout the series, the small intestines have been cut away near the colic valve, but in figures 5 to 11 a short piece of the ascending portion of the duodenum has been retained for orientation. Around this the colon makes a characteristic bend, and then, at the splenic flexure, it becomes the descending colon, which is free from coils or kinks even in the adult. In all the dissections the rectum has been cut away, and the entire mesentery has been removed. The coils of the colon have separated from one another, but only so far as necessary to show the course of the tube. This necessitates a slight displacement of some of the flexures, but none has been omitted or radically altered.

In order to understand the stages in embryonic development included in this series, it is necessary to have in mind certain features of the condition to be finally attained, which are presented in figures 15 to 19. The apical portion of the coil in the adult (fig. 14), and likewise in the more advanced embryos, presents the curious pattern shown in figure 15 (from an embryo of 200 mm.). The observer will be uncertain whether the point *a*

or the point *b* is the actual apex. If *a* is selected, the adhesions of the mesentery and of the adjacent coils may be torn apart so that the spiral may be unwound as shown in figure 16. It would then consist of two parallel limbs, which, in this specimen, make $3\frac{1}{2}$ revolutions. If *b* is taken as the apex, the coil may be unwound as shown in figure 17. Beginning at the valve of the colon as before, there are now only three revolutions. It is evident, however, that the unwinding shown in figure 16 is natural, and that the other is an 'artificial' dissection, for the adhesions yield more readily in the former and there is less tearing of the mesentery. Moreover, at the base of the spiral, the proximal and distal limbs of the coil early become adherent to one another and to the body-wall, establishing a fixed point. The basal limbs retain this position in figure 16, but have been separated in figure 17, so that the former is clearly the correct picture, and the true apex is at *a*.

Additional revolutions may take place without changing the apical pattern. If half a turn is added to the coil shown in figures 15 and 16, the bend *a* will be carried up between *y* and *z* toward *b*, and the conditions shown in figures 18 and 19 will result. In figure 19 the bend *ap*, which before unwinding the coil appears to correspond with *b* in figure 15, is clearly the true apex.

The number of revolutions actually produced varies, and small fractions, generally neglected, appear quite as often as whole or half turns. Hunter, in the passage cited, speaks of 'five spiral turns,' evidently referring to the five tiers shown in figure 14. In this specimen, however, beginning at the valve of the colon, there are but four revolutions, and this appears to be the normal number. Bonnet's statement that there are $3\frac{1}{2}$ can be applied to this specimen only by regarding *b* as the apex instead of *a*.

The length of the spiral portion of the colon in the young adult (fig. 14) is 2.6 meters. The distance from the colic valve to the apex is 1.4 meters, or 53 per cent of the total length. Thus the apex is finally located just beyond the middle of that part of the colon which forms the spiral.

In the following description the terms *proximal* or *outer* limb will be applied to the part of the colon leading from the caecum to the apex, and *distal* or *inner* limb to the part from the apex to the splenic flexure. The inner limb in the adult is of much smaller diameter than the outer, except toward the apex; and its revolutions, which closely accompany those of the outer limb, are hidden within the dome-shaped mass. Having in mind these relations and the descriptions of previous writers that a primary loop, with its apex at the middle point, simply winds up to produce the adult form, the conditions in the embryo may be carefully examined.

At 50 mm. (fig. 5), as in the adult, the colon may be divided into two nearly equal parts. The proximal half (in fig. 5 and in the following drawings) has been heavily stippled to contrast with the distal half. Beginning at the caecum, which at this stage points ventrally, the colon passes toward the dorsal body-wall, near which it makes a rather sharp turn and doubles back upon itself. After running ventrally it redoubles by a sharp turn and goes dorsally. This folding continues back and forth throughout the proximal half. Distally the colon consists of several short coils, irregularly arranged, which become adherent to the body-wall near the duodenum. Except at this fixed point, the colon at this stage is freely movable.

The most notable feature of the following stage (fig. 6, from an embryo of 55 mm.) is the elongation of the first loop in the proximal half of the colon. The proximal or outer half of this first loop is now clearly a portion of the basal convolution of the permanent spiral. In figures 7 and 8 (from embryos of 64 and 75 mm. respectively) the first coil has further elongated, accomplishing, in figure 8, one half of a revolution. In connection with this development, the caecum has shifted toward the left of the body where it becomes permanently located, and the entire colon has become twisted upon itself, duplicating the torsion of the primary intestinal loop of earlier stages. In other words, the part of the colon toward the caecum has come to cross the left side of the distal part of the colon, just as, in the 24-mm. stage (fig. 2), the large intestine crosses the left

side of the small intestine. In fact the arrangement shown in figure 8 might well suggest to a student of human embryology a large intestine surrounding coils of small intestine. Where the crossing takes place, the proximal and distal portions of the colon become adherent to one another and thus the basal ends of the ascending and descending portions of the future spiral become fixed. It is therefore from this basal portion outward that the future spiral is to be established, clearly necessitating a rearrangement of the irregular coils existing in the 70 mm. stage.

From the stages which have now been considered, it is evident that the method of development in the sheep described by Martin and accepted by Bonnet is not applicable to the pig. The colon does not present a simple primary loop, but shows many convolutions. It is true that among these the first or basal loop has a definite bend or apex as seen in figures 5 to 8, but this, unlike the apex in Martin's primary loop, does not become the apex of the spiral in the adult. If it did so, the proximal tenth of the colon in the 50-mm. pig must produce the proximal half of the adult spiral, and the distal nine-tenths would produce only the distal half; but there is no evidence of such unequal growth. It is therefore reasonable to suppose that the proximal half in the embryo, which has been heavily stippled, will produce a corresponding proportion in the adult. Accordingly the future apex may be approximately located at the transition between the dark and light stippling, at a point which in these stages has not been definitely established.

The continued advance of the spiral arrangement of the outer coil is clearly shown in figures 9 and 10 (embryos of 90 and 95 mm. respectively). No further torsion of the colon than that already recorded has taken place, but the winding up of the outer basal coil has advanced from $\frac{1}{2}$ a revolution in figure 8, through 1 revolution in figure 9, to 2 complete revolutions in figure 10. The way in which the bends of earlier stages are obliterated in this process is suggested in figure 9, where several are evidently about to be taken up in a well-rounded curve. The first of these, at *a*, has nearly disappeared. It occurs at a point $\frac{3}{25}$ of the distance from the ileo-colic junction to the

place where the colon comes into relation with the duodenum. In figure 8 the apex of the bend *a* is at $\frac{3}{20}$ of this distance, and accordingly the flexures labelled *a* in the two figures may be regarded as homologous. But in figure 9, *a* no longer marks the apex. The bend which it designates has been incorporated in the outer coil, together with the reversed bend *b*, and a new apex appears at *c* (which may fairly be compared with *c* in figure 8). Beyond this point, in figure 9, the proximal half of the colon still pursues a zig-zag course as in earlier stages, but it swings back and fourth through shorter arcs, and the transfer of the apex from *c* to *g* is already suggested. To accomplish this, the flexures *d*, *e*, and *f* are destined to pass through the condition at present exhibited by *a* and *b*. In the 95-mm. embryo (fig. 10) this has taken place, and slight irregularities in the outer coil are all that remain of the former to-and-fro oscillations.

The outer or ascending spiral develops in advance of the inner, descending spiral, as may be seen in the figures already examined. The coils in the distal half of the colon are quite disorganized in figures 5 to 8. Beginning in figure 9 (at *g*) and more extensively in figure 10, the apical portion of the ascending coil is accompanied by a descending coil, and thus the final relation between the outer and inner coils is beginning to appear. An apex is thus established which will not advance further by taking up flexures in its path, but chiefly through elongation, which it shares with the rest of the colon, and by becoming more tightly wound about its axis. However, it will be shown that a slight advance of the apex along the inner or descending limb is yet to occur, at the time when the characteristic apical pattern is produced.

The final stages are shown in figures 11 to 14. In the embryo of 110 mm. (fig. 11) three revolutions have been completed, and except for the loosely wound apex, the spiral appears finished.[1] The flexure *a* is destined to turn to the right and upward into the concavity of the flexure *c*, and thus it will form the apical pat-

[1] The counting of the revolutions in the figures will be facilitated by placing a straight edge from the ileo-colic junction to the apex.

tern already discussed. At the same time the apex will advance from *a* to *b*. This has happened in the 120-mm. embryo (fig. 12), and accordingly $3\frac{1}{2}$ revolutions are there present. The same is true of the coil from a pig four weeks after birth (fig. 13). Gradually the spiral becomes more compact and its coils more adherent to one another, and at the same time the portions of the inner coil which are visible on the exterior become buried. These changes are clearly shown in the figures. In the adult (fig. 14) the apex of the coil has rotated so that instead of pointing downward, it is directed toward the left, and thus four revolutions are completed. Less of the inner coil is exposed than at birth, and only half a turn can now be observed at the apex. With these relatively slight changes in the constitution of the coil, its general appearance has been transformed through the development of the sacculations, which at birth are scarcely indicated.

The principal morphological feature of the developing coil which the figures fail to suggest, is its increase in size and much greater increase in length. This can be shown by measurements; and at the same time the position of the apex can be more accurately located. The following table, therefore, includes the total length of the spiral part of the colon (from the colic valve to the contact with the duodenum), and also the distance from the colic valve to the apex of the coil. The apex in the earlier stages is temporary, and in the 95-mm. specimen (fig. 9) it must be chosen somewhat arbitrarily. When the distance from the colic valve to the apex of the coil has reached 50 per cent of the length of the entire spiral, the permanent apex has presumably become established. Accordingly, in the table, the distance to the apex is followed by the percentage, which it represents, of the spiral part of the colon. Measurements of the small coils are made with some difficulty, so that the results are only approximately correct; but even with these limitations, the measurements are found instructive.

Stage of development	*Spiral part of colon*	*From colic valve to the apex*
50 mm.	17 mm.	1.7 mm., 10 per cent
55 mm.	20 mm.	2.5 mm., 12 per cent
64 mm.	28 mm.	2.8 mm., 10 per cent
75 mm.	38 mm.	6 mm., 15 per cent
90 mm.	56 mm.	14 mm., 25 per cent
95 mm.	72 mm.	28 mm., 38 per cent
110 mm.[2]	101 mm.	51 mm., 50 per cent
120 mm.	121 mm.	68 mm., 56 per cent
4 weeks	1200 mm.	600 mm., 50 per cent
6 weeks	1635 mm.	845 mm., 51 per cent
young adult	2670 mm.	1430 mm., 53 per cent

[2] Average of two specimens.

The way in which the coil develops suggests the possibility of several sorts of anomalies, some of which were observed in a series of one hundred adults and one hundred embryos examined for this purpose. Five of the adults had coils with an additional half-turn, just as the embryo of 180 mm. shown in figure 18 has half a turn more than is usual atthat stage (cf. fig. 16). No adult showed less than four revolutions, and none showed reversals or other malformations of the spiral. Among the embryos, anomalies were more abundant. Figure 21 represents the colon of an embryo of 55 mm. which is placed beside a normal one (fig. 20) for comparison. In the anomaly the basal coil has begun to rotate dorsally and to the right, in the reverse direction. Three other specimens of coils reversed from the base were found among the one hundred examined. A second type of anomaly was observed in an embryo of 108 mm. (fig. 23, likewise placed beside a normal specimen, fig. 22). Here the spiral began to wind in the normal direction, but evidently encountered a sharp flexure which could not be taken up, so that a reversal occurs at the point *x*. Presumably at a stage corresponding with that shown in figure 8, such a bend as is there labelled *a* persisted, and the advancing apex took the direction of the flexure *b*. Accordingly, beyond the point *x* in figure 22 the coil is reversed. The inner coil has adapted itself to the outer throughout, and in its concealed portion it reverses its course opposite *x*. Another very similar anomaly was found in an embryo of 95 mm., in which the inner coil likewise re-

versed so as to accompany the outer in its abnormal course. Since six cases of partial or complete reversal were found in one hundred embryos and none among one hundred adults, the question arises whether the condition may be ultimately corrected, or whether like a volvulus, it may lead to fatal results. But the number of specimens examined is perhaps too small to be significant in this respect.

SUMMARY

In a series of drawings which largely explain themselves, an attempt has been made to present the development of the colon in the pig in greater detail than heretofore.

The torsion of the primary intestinal loop, which in man stops at 180°, proceeds to a complete revolution in the pig; in this it corresponds with the development in the sheep as described by Martin.

But the spiral coil in the pig does not begin as a single loop of the colon which simply winds up, as in the sheep, according to Martin and Bonnet. On the contrary it arises as a knot of kinks and coils. The first of these forms the basal portion of the outer limb of the permanent spiral.

Within the limits of the colon there then appears a rotation or torsion, so that the proximal part crosses the distal part, and the basal coil encircles the other convolutions in the way that the human colon encircles the small intestine.

The basal coil advances by taking up secondary flexures in its path until it makes two revolutions. By that time the apex of the coil is about midway in the course of the spiral part of the colon, and further growth of the spiral is chiefly by the coiling of the apex. In establishing the characteristic apical pattern, however, the apex advances half a turn further along the inner or descending limb of the coil.

In case the basal loop is turned in the wrong direction, or if having started normally it encounters bends which do not yield, complete or partial reversals of the spiral occur, six cases of which were found in embryos.

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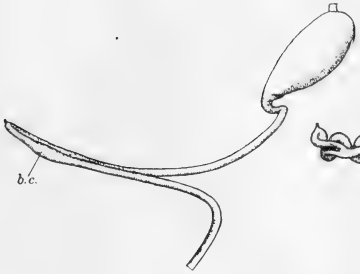


Fig. 1

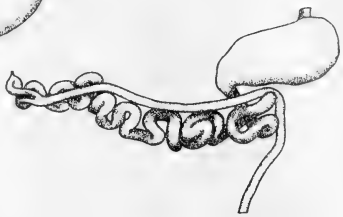


Fig. 2

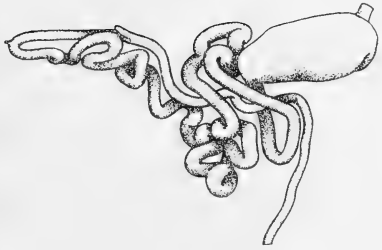


Fig. 3

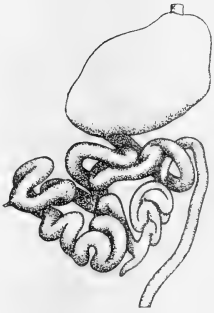


Fig. 4

All the figures represent dissections of the intestines of the pig.

Figs. 1–4 The stomach and the small and large intestines seen from the left side. × 14 diam. Figure 1, embryo of 12 mm.; figure 2, 24 mm.; figure 3, 30 mm.; figure 4, 35 mm. *b.c.*, bulbus coli.

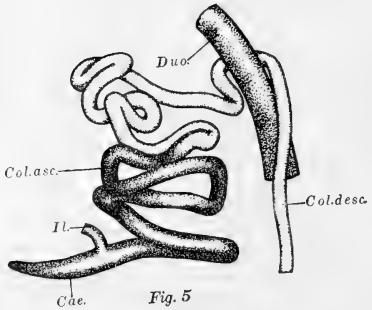


Fig. 5

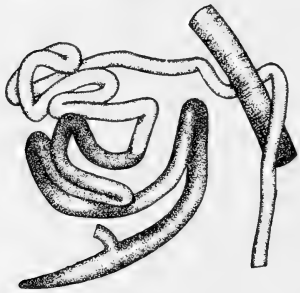


Fig. 6

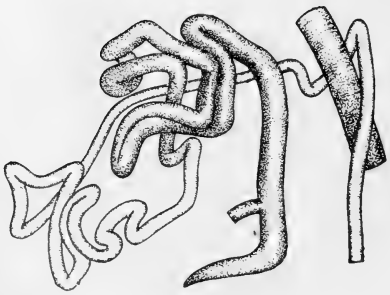
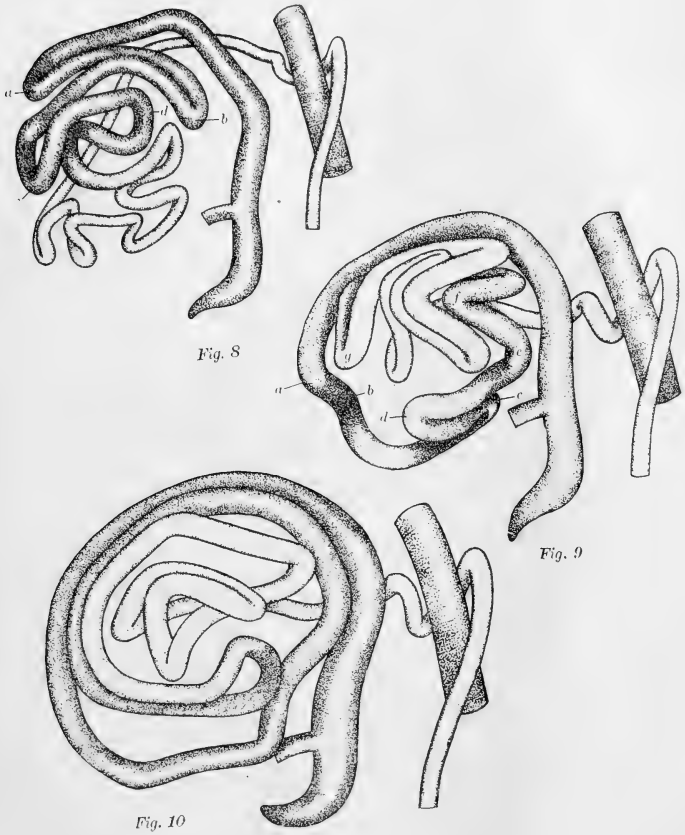


Fig. 7

Figs. 5–7 The coils of the colon, slightly displaced so that their course may be followed, seen obliquely in left-ventral view. Figure 5, embryo of 50 mm.; figure 6, 55 mm.; figure 7, 60 mm.: all × 14 diam. *Cae.*, caecum; *Col. asc.*, ascending colon; *Col. desc.*, descending colon; *Duo.*, duodenum; *Il.*, ileum.



Figs. 8–10 Same dissection and view as figures 5–7. Fig. 8, 75 mm.; figure 9, 90 mm.; figure 10, 95 mm.: all × 10 diam.

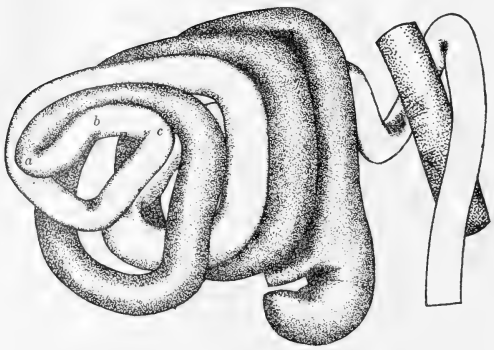


Fig. 11

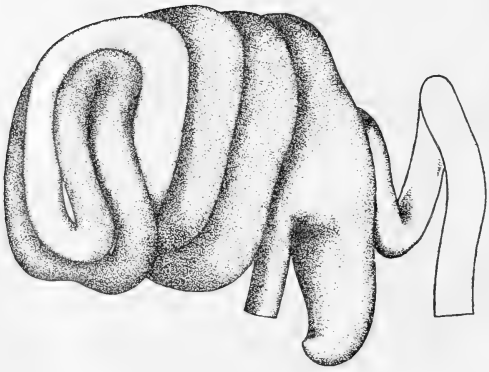


Fig. 12

Figs. 11–12 Late stages in the development of the spiral coil of the colon. Figure 11, embryo of 110 mm.; figure 12, 120 mm.: both × 7 diam.

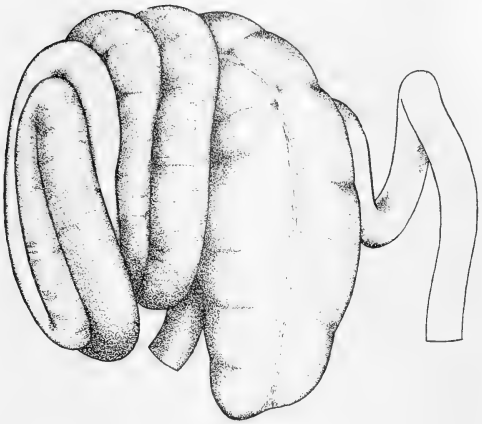


Fig. 13

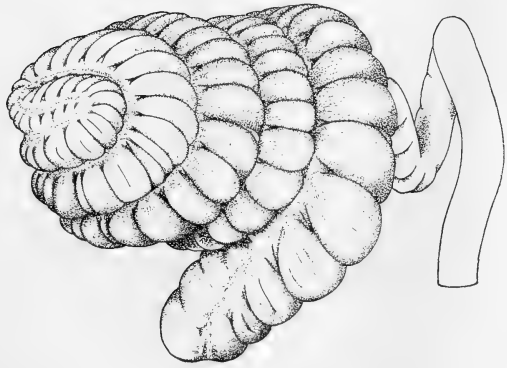


Fig. 14

Figure 13, from a pig 4 weeks after birth; $\frac{2}{3}$ natural size. Figure 14, from a young adult; $\frac{1}{3}$ natural size.

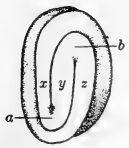


Fig. 15

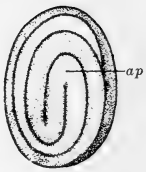


Fig. 19

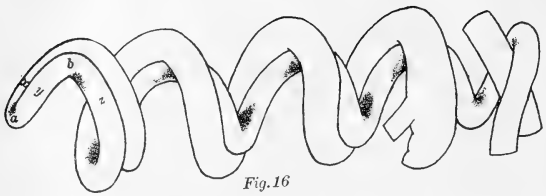


Fig. 16

Fig. 17

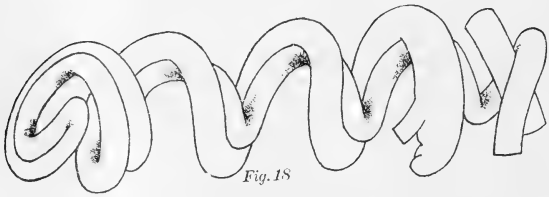


Fig. 18

Figs. 15–19 Sketches showing the arrangement of the spiral and its apex. Figure 15, the apical pattern, and figures 16 and 17, two methods of unwinding the coil, from an embryo of 200 mm. Figure 18, the spiral and figure 19, its apex, from an embryo of 180 mm.; the spiral here presents half a revolution more than that shown in figures 15 to 17. The letters mark points referred to in the text.

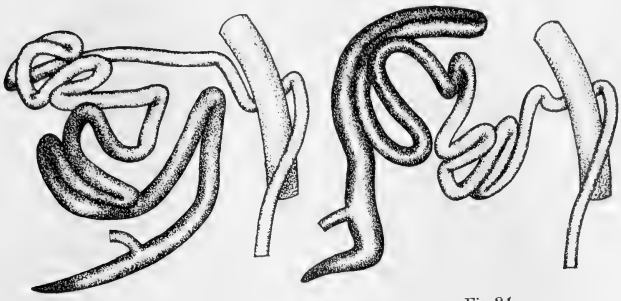


Fig. 20

Fig. 21

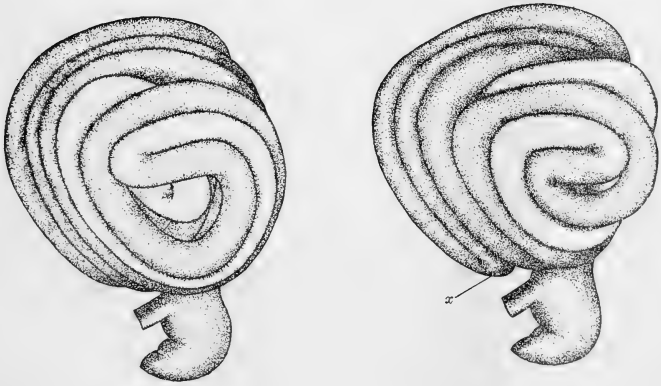


Fig. 22

Fig. 23

Figs. 20–23 Anomalies of the spiral. Figure 21, abnormal coil from an embryo of 55 mm., placed beside a normal specimen (fig. 20) for comparison: × 14 diam. Figure 23, an abnormal coil from an embryo of 120 mm., placed beside a normal coil (fig. 22) from an embryo of 100 mm.: × 7 diam.

SUBJECT AND AUTHOR INDEX