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ON THE PROPORTIONS, DEVELOPMENT AND ATTACHMENT OF THE TECTORIAL MEMBRANE

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ELEVEN FIGURES

Some years ago the writer (Hardesty '08) made an attempt to present something of the actual nature of the mammalian tectorial membrane based chiefly upon the study of teased fresh and unshrunken specimens. After learning something of the nature, shape and proportions of this structure in the fresh condition as compared with its appearance in the more usually employed sections of the cochlea, the writer became convinced that of the very numerous observations upon the membrane previously published practically all had been based upon the various more or less abnormal, rather than normal, appearances of it produced by the methods of its preparation for study. Experiments as to the action of the commonly used reagents upon the fresh tectorial membrane showed that under the usual treatment with alcohol in dehydration and the action of clearing reagents it suffers far more shrinkage and distortion than any other structure in the cochlea and perhaps in the entire body. This result of dehydration and clearing was very evident even after the action of fixing fluids which themselves were found to produce but little distortion. For this reason it was concluded that the usual sections of different embedded cochlea, and often different sections of the same cochlea, show the membrane, even in a given locality of its coil, varying greatly in its shape, size and in its position over the spiral organ (of Corti). The hope then suggested itself that something might be contributed to the knowledge of the actual character, shape and proportions of the tectorial membrane by studying it in the fresh condition, by

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comparing the fresh with teased out membranes acted upon by various fixing fluids, and by experimenting with methods of embedding for sections of the cochlea in which more normal appearances of the membrane might be retained. Cochleae of pigs were used wholly in that investigation, almost entirely from pigs at or near 'term.' Those of one young suckling pig were obtained. The paper made no attempt to cover the process of the development of the tectorial membrane further than to give a brief review of the various publications bearing upon it and then considered as already having well covered the process, and it gave one drawing showing one stage of the development for use in describing the assumed process by which its final structure is acquired and its adult position with reference to the spiral organ is attained.

Since the publication of the above paper dealing with the membrane in young animals, the writer has been occupied in an attempt to construct a model of the auditory apparatus in which the proportions, position and environment of the tectorial membrane in the cochlea are simulated and by which its behavior when subjected to the energy imparted to it by sound waves may be illustrated. In the construction of this model, a further detailed study of the proportions and position of the actual tectorial membrane seemed necessary, and in this study it became evident that the observations should be carried to the membranes of adult animals rather than confined to those in the cochleae of embryos and fetuses. Since several of the results of this further study seem of interest, though some of them are confirmatory of results previously published, and since relatively so few observations upon the adult mammalian cochlea have been recorded, it is the purpose of the present paper to give certain of the findings of a somewhat detailed study of the spiral organ of the adult mammal. The shape, proportions, position and attachment of its tectorial membrane are especially considered. Toward the end of the study, certain points in the question of the attachment and position of the adult membrane and the structure of its surfaces arose in comparing the findings with those recorded for the membranes in cochleae of embryos

and fetuses in the paper of the writer and in papers by others appearing since. These points made it desirable to again go over from the beginning the processes by which the tectorial membrane and spiral organ are developed. As bearing upon these points, it is deemed allowable to review here certain steps in the development as now found by the writer, and some drawings illustrating these steps are given.

Another purpose for which this paper is submitted is that it is a necessary anticipation of the study, begun earlier, involving the construction of the model mentioned above, with a view to illustrating the possible action of the membrane as a vibratory mechanism in the process of hearing. A description of the results of this attempt is now in preparation.

In my previous paper, using freshly obtained cochleae of pigs at and near 'term,' it was found that by very tedious and careful procedure it was possible to remove by teasing methods parts of and even the entire tectorial membrane in the fresh state. In the fresh state, the membrane was found to be most inconceivably flexible in nature, to cohere and to adhere to the teasing instruments with a most exasperating readiness, and to have a specific gravity practically no greater than that of the fluid in which it normally lies. It is transparent and its removal was best accomplished over a black stage of the dissecting microscope. Its behavior in the fluid in which it was teased under the dissecting microscope indicated that the fresh membrane possesses barely enough elasticity against stress applied to it transversely to enable it to gradually assume its coil when floating about in the fluid before coming to rest upon the bottom of the dish. Once in contact with the bottom it could not resume its coiled character. On the other hand, it appeared to possess considerably more elasticity against stress applied parallel with its length, more than enough to enable it to maintain its shape in transverse section and certainly enough to maintain its outspanning position just over the hair cells of the spiral organ, whatever the position of the head.

Under the compound microscope, study of both its whole thickness and of torn bits showed it to consist of very numerous fine filaments embedded in a seemingly gelatinous matrix. The membrane being of ectodermal origin, the matrix was considered as a jelly-like form of keratin. The varying directions, length and independent character of the filaments was described and the various other features of the membrane which seemed evident to the writer were compared as they agreed with or differed from the findings of others recorded in the literature. It was further found by experiment that the action of certain fixing fluids of themselves, those containing no alcohol and whose

ingredients produced no appreciable exosmotic diffusion currents in tissue elements, caused very slight distortion of the membrane. In Zenker's fluid, for example, the endosmotic and exosmotic effects upon the membrane appeared to be quite evenly balanced and measurements of the dimensions of tectorial membranes after its action indicated that practically no differences from the dimensions of the fresh had been produced. Cochleae could be left in this fluid long enough for decalcification of the fetal bony lamina and thus allow much greater ease and precision in removing this from the membranous cochlea preliminary to clipping the latter away in order to remove the tectorial membrane. After Zenker's fluid, the membrane in the fetus at term appeared more opaque, less flexible, less fragile, and more easily removable entire from its attachment upon the vestibular lip of the spiral limbus. Such fixed specimens could be washed in water, but attempts to dehydrate and clear them always resulted in shrinkage and distortion. Mounts for study had to be made in glycerine or glycerine-jelly.

Measurements of both the fresh and the thus fixed tectorial membranes gave an average length of the membrane, in pigs at about term, of 25.5 mm. In width and thickness it was found to decrease gradually and evenly from its end at the apex of the cochlea, where it is much the largest, to the narrow and thin end of its basal coil, which ceases to project in width beyond the hair cells of the spiral organ. In the first or apical coil, the width of the membrane was found to be such that its outer edge projects considerably beyond the outer hair cells of the organ.

In extent, the tectorial membrane was found to occupy the cochlear duct throughout and to be strictly coextensive with the spiral organ. Thus its length is less than the lengths of the scalae on its either side, especially less than that of the scala tympani. Each of its ends was found to be rounded and to terminate bluntly. Its axial edge thins suddenly and knife-like and is permanently attached upon the vestibular lip of the spiral limbus, being left thus attached by the ectodermal cells, which, at an early stage, ceased to produce it, leaving it adherent to the fibrous mesenchymal tissue comprising 'Huschke's teeth' and the remaining portion of the vestibular lip covered by it. Its outer edge was found to be bluntly rounded and slightly scalloped, as a result of the component filaments curving apex-ward around this edge from the basal surface, and frequently in small bundles, to form the edge. The apical surface was found to be convex and smooth. The immediate convex surface consisted of a thin layer representing the result of the first activity of the then young producing cells below, in which layer or first product the matrix at least had not been so completely produced as later, allowing the first formed ends of the filaments to become tangled or washed, as it were, into an irregular arrangement resembling a reticulum. The remainder of the filaments continued into the body of the membrane, untangled and evenly embedded in the later and more completely produced matrix of the

membrane. In transverse sections of the membrane, this immediate surface appears as a darker rind, or so condensed that the filaments cannot be traced in it, and it was referred to as the 'peripheral condensation.' A peripheral condensation likewise appears on the basal surface of the membrane in sections of embedded cochleae. There it was usually thinner and less uniform than on the convex surface but was considered as representing the last and likewise incomplete product of the activity of the cells producing the membrane—the surface left as the cells were ceasing to function and were being torn away from their product in receding from it. The appearance of condensation, or the darker rind, was considered as due to the action of the reagents upon the different character of these surfaces.

The basal surface of the removed membrane, viewed on the flat, showed three lines. The axial one of these was found to be the line of the imprint of Hüschke's teeth, or the line marking the outer margin of the attached axial edge (inner zone) of the membrane. The middle line was found to be 'Hensen's stripe,' a surface marking described by Hensen in 1863. This stripe was decided to be a very slight linear elevation occurring coincident with the line of the interlocking phalanges of the pillars of the spiral organ and thus adapted to the groove or space on the surface of the organ between the hairs of the inner and outer series of hair cells. A peripheral or outer line appeared to be the line of attachment of the outer margin of an otherwise detached or detachable thin layer of the basal surface which was described as an "accessory tectorial membrane." In the apical coils, this line, or outer edge of the accessory membrane, runs considerably axial to the outer edge of the tectorial membrane. This distance however gradually decreases towards the basal coil till the line comes to coincide with the outer edge of the tectorial membrane, or, in other words, the outer edges of the two membranes gradually come to coincide toward the basal end. The evidence of the existence and the description of the structure of this accessory membrane as well as the description of the arrangement of the filaments in the membrane proper were presented in some detail.

As to the attachment of the tectorial membrane, it was shown that the membrane is purely a cell product, wholly non-cellular from its beginning; that during its formation it is of necessity attached to the cells which produce it, and, at any stage of its formation, an incompletely formed region must be and is then attached to the cells completing that region; that its thin axial edge or zone, the region whose production ceased earliest, remains permanently adherent upon the vestibular lip of the spiral limbus; but that, after its production is completed, by actual observation of it in position in teased specimens and in trustworthy sections of it, by the study of the later stages of its formation by the cells concerned, and from the change in position of the spiral organ with reference to its basal surface, the conclusion was reached that the main body or entire outer zone of the membrane does, and by necessity comes to, project entirely free over the spiral

organ. Observations of others were cited both as agreeing and disputing that this outer part of the membrane is free, and it was advanced that all the disputing claims are based either upon studies of mammalian cochleae in stages of development before the production of the membrane is completed or upon studies of sections of dehydrated and embedded cochleae in which, due to shrinkage and distortion produced by the treatment, the membrane was shrunken and pressed upon and thus apparently attached upon the surface of the spiral organ.

It was finally urged in the paper that the tectorial membrane and not the basilar membrane (membranous part of the spiral lamina) is the chief vibratory structure in the mechanics of hearing. The principal of the considerations upon which this suggestion was based are the following: (1) The general acceptation that the cochlea is the peripheral organ of the auditory apparatus, that auditory impulses are aroused in the hair cells of the spiral organ, about which cells the telodendria of the auditory (cochlear) nerve fibers terminate, and that these impulses are initiated by the impingement of the hairs of the hair cells against the basal surface of the tectorial membrane. (2) That the tectorial membrane is far more flexible and by structure far more capable and qualified to serve as a vibratory mechanism than is the basilar membrane. (3) That the tectorial membrane varies in its proportions considerably more than the basilar, its thick and broad apical end gradually tapering to its narrow basal end, thus allowing greater possibilities of resonant activities and in wider range. (4) That in development and structure, the basilar membrane is not composed of individual nor independent fibers but is nothing more than a flat tendon whose component fasciculi are intimately connected with each other; and further that even if it were composed of independent fibers, being blanketed on its either side by continuous layers of tissue far thicker than itself, one of which layers is a syncytium, its fibers could not be thrown into vibration either individually or in groups. (5) That the tectoral membrane is always entirely coextensive with the spiral organ while the basilar membrane is not always so. (6) That the position of the tectorial membrane in the cochlea is more logical for the function than is that of the basilar membrane. The tectorial membrane may impart stimuli to the *peripheral* ends of the auditory cells and it also lies nearest the scala vestibuli, that is, to the fluid to which the foot of the stapes transfers directly the energy imparted by sound waves.

The theories in which the basilar membrane is considered the vibrating mechanism of the cochlea were, therefore, deemed untenable and an application of the 'telephone theory' to the tectorial membrane was suggested.

Since the publication of the above paper by the writer, three other papers dealing chiefly with the tectorial membrane have appeared.

The very instructive paper by Held ('09) deals with the development in the cochleae of the rabbit, chick and pigeon. He used embryos and various fetal stages of the rabbit before and after birth and used

the fixing fluid which was used chiefly here (see below) for fixing and decalcifying. He describes the tectorial membrane of the rabbit as composed of three layers in thickness and, during development, three zones in width. In the mature stage, he thinks the third or outer zone is questionably distinguishable, and that the only attachment of the membrane is that of its axial edge, or inner zone, upon the vestibular lip of the spiral limbus, the entire outer part being free. Certain of the findings in Held's paper will be referred to in place.

Vasticar ('09 and '10) gives what purports to be a study of the tectorial membrane from fresh and osmic acid fixed cochleae of the rabbit. As far as the anatomy of the membrane is concerned, beyond the statement that it is a "cuticular membrane of extreme delicacy," his descriptions of vertically implanted, independent filaments, like a vertically placed hair brush, the ends of the filaments sometimes showing an 'olivary corpuscle,' that the ends of the filaments give the basal surface the appearance of spiral striations, etc., are so far from structure actually observed and so fanciful as to render his conclusions worthless. He agrees with Coyne and Cannieu ('95) that the outer zone of the membrane is attached to the spiral organ, united by short cuticular ligaments to its cells as far over as the cells of Hensen, and he cites Corti, Claudius, Henle and Lowenberg as supporting this attachment of the outer zone, but states that Waldeyer, Hensen, Ranvier, Retzius, M. Duval, Tafani and others claim there is no attachment of the outer zone.

Prentiss ('13) deals with the cochlea of the pig, using stages from 4 cm. in length up to fetuses at about full term. He advances one new claim. The initial purpose of his paper seems to have been to support the frequently made assumptions of Shambaugh ('07, '08, '10, and '11) and to demolish by controversy suggestions offered by others as to the actual anatomy and probable action of the tectorial membrane. He states his best results were obtained by fixing with 2 per cent osmic acid and with vom Rath's osmic-picric-acetic mixture, claiming that the precipitation of the reduced osmium tetroxide gave a browning of the tectorial membrane by which "its cuticular structure" was sharply brought out. He decalcified before embedding with 5 per cent nitric acid in 80 per cent alcohol and used celloidin sections. A certain amount of decalcification must have been accomplished while in vom Rath's fluid. Later in his paper he refers to having dissected out tectorial membranes by "more favorable methods" than those employed by me and with results which did not support my observations. While in my first experiments in teasing out the fresh membranes I did "crush the bony labyrnth with a hammer," this was not done later and in doing it I doubt that the cochlea was jarred much more than by breaking the bone in another way, nor do I think that, as assumed by Prentiss, the blows necessarily disturbed the normal attachment of the membrane much more than considerably heavier blows on the skull which the function of hearing is able to sustain. While Prentiss's methods of dissection could have been

easily far better than mine, one is disappointed in that he gives no description whatever of his more favorable methods and none of the results obtained with them. Through the pages of his paper, he progressively describes the structure of the tectorial membrane as attached by 'delicate threads,' as composed of 'lamellae' (supporting Shambaugh), "parallel fibers or lamellae," "delicate parallel plates," 'a reticulum,' and finally advances the one new claim of his paper, namely, that the membrane is a honey-comb or chambered structure, each chamber corresponding to and produced by a separate cell and that thus the transverse dimensions of the chambers are the same as those of the cells of the greater and lesser epithelial thickenings or ridges of the earlier fetal stages, both of which ridges he claims produce the membrane. The producing cells being situated on the basal side of the membrane, the lengths of his chambers, curving axisward, must extend through the thickness of the membrane. He concluded that the content of his chambers (matrix, Zwischensubstanz, etc., of others) is a fluid "resembling the endolymph."

A claim suggesting Prentiss' contention as to the origin and structure of the membrane was advanced by Coyne and Cannieu ('95), and Prentiss states that I misquoted these authors when I cited their paper as one of those in which a fibrous character of the membrane was suggested. In citing Coyne and Cannieu at the place in question, I had no reference whatever to their idea, which I thought quite erroneous, as to the process by which the membrane is produced by the cells basal to it, but merely to the fact that they considered the structure of the membrane as "*un reseau.*" Vasticar ('11) quotes directly a later statement by Coyne in which "*d'aspect areolaire*" and "*la forme d'un reseau*" are again applied to it. I translated *reseau* as meaning reticulum. A reticulum is a net and, in anatomy, is a network whose meshes extend in all planes. A net is composed of threads, not plates nor lamellae, and the word does not convey the idea of walled pockets or alveoli, nor that of a honey-comb structure. Whether, as Coyne states, the meshes (*mailles*) of the reticulum conform to the sizes of the cells of the organ of Corti (*cellules sensorielles ciliees*), which he assumed secrete the membrane, was a question, however doubtful, not in mind at the time. Prentiss uses the paper of Coyne and Cannieu in support of his claim of the chambered structure. He is right in showing that my citing the paper at all in the relation I did was unfortunate. Support of the fibrous character of the membrane alone was sought, and while I find it is fibrous, the normal arrangement of the fibers is not in the form described in the paper.

Prentiss describes the walls of his chambers as coinciding with and continuous with the sides of the producing cells, the cavities of the chambers with the bodies of the cells. His figure 5, drawn from a section of the actual specimen in which the producing cells are cut longitudinally, is given to prove his chambered structure, but in this figure nearly half of the filaments of the membrane are given off from the ends of the cells instead of from their sides. Figure 9 of my paper

('08) represented a thin section after paraffin in which the membrane was cut in a plane parallel to but splitting Huschke's teeth. It showed the fibers near the teeth necessarily cut across, since they curve apexward toward the surface of the teeth, while the plane of section through the body of the membrane was more or less parallel in places with the course of the fibers. That the fibers in this section appeared as washed together, cohering to each other in anastomosing bundles, was explained by me as due to shrinkage and resultant agglutination. But Prentiss interprets this figure as showing his chambered structure, though the sections of the chambers he sees (the meshes of the apparent reticulum of bundles) vary from sizes for smaller than sections of the cells supposed to produce them could be, and the very various shapes of the meshes are questionably similar to what would be the shape of the cells so sectioned. Prentiss' figure 8, a section of the membrane similar to my figure 9, shows some of the larger meshes (sections of his chambers) about ten times the size of the smaller meshes. The cells of neither of the epithelial thickenings or ridges vary so in size. His section merely indicates even more agglutination than mine. As to the endolymph filling his chambers, Prentiss states that I "could not demonstrate by special stains the presence of a matrix which would hold the fibers together." The presence of the seemingly gelatinous or soft keratinous matrix, in which the fibers are embedded, seemed to me one of the most evident features of my preparations of teased tectorial membranes, and its presence and apparent character was described by me as well as by others previously. That I was unable to determine by stains a definite micro-chemical character for it is true.

I described in my teased preparations from the pig a structure which I called an "accessory tectorial membrane." Prentiss states that this probably represented the reticular membrane or lamina of the spiral organ stripped off in the teasing. Whether this structure is an accessory membrane or not, I have found that it likewise appears on the basal surface of the membrane of the adult hog and in places not torn. Prentiss' statement indicates first, that he has never seen it at all in his 'dissections' and second, that his idea of the lamina reticularis is rather peculiar.

My perhaps unnecessarily tedious experience in trying to determine the normal character of the tectorial membrane and in trying to get sections of it showing this character would lead me to suggest it rather impossible to get normal preparations after fixation with osmic acid, whose power of penetration is remarkably poor, or even with vom Rath's fluid, and to get normal appearances would be certainly impossible after decalcifying with 5 per cent nitric acid before embedding. To me, all of Prentiss' figures drawn from the actual specimens show considerable shrinkage, his figures 5 and 6 less than the others. In his figure 9, the distortion of the tectorial membrane, outward and out of shape is very evident. In his figure 10, given as representing the apical coil of a pig at about term, the outer edge of the membrane is badly

pressed axisward and pressed upon the spiral organ, and the spiral organ itself is pressed down upon the spiral lamina to an extent rendering it unrecognizable for this region of the coil. Except that it is pressed down in contact with a part of the spiral organ, there is no evidence whatever in this figure that the membrane is attached to the organ, other than that the hairs of the hair cells, represented in the figure as one simple filament from each hair cell and much longer than normal, are shown continuous into the membrane. I do not think that an insertion of the hairs into the tectorial membrane is ever seen in any stage of development except when the membrane has been crumpled or compressed upon the hairs, sticking the hairs into it. So far as I know, the only recent papers which claim this insertion as a normal attachment of the membrane are those of Prentiss and Shambaugh. Prentiss states that in all his preparations of the older cochleae the membrane was badly shrunken. To me they prove nothing as to the membrane beyond its mere existence, certainly nothing for argument as to an attachment of its outspanning zone.

To those of us suffering under the misapprehension that the apex of the cochlea is always directed toward the zenith, regardless of the position of the head, while the sense of hearing is being exercised, Prentiss devotes a drawing and some of his text to show that in the pig, while feeding for example, the tectorial membrane may be below rather than above the spiral organ and therefore must fall away from the spiral organ if not attached to it, and render the animal deaf while feeding. In my teasing out of the membrane, it appeared, as I stated, to possess a specific gravity but little greater than the fluid in which it lies and that it manifested a transverse elasticity amply sufficient to hold it in its position spanning over and close to the spiral organ, its broad axial edge alone being attached. Prentiss' dissected preparations, made better than mine, no doubt showed the same qualities.

MATERIAL AND METHODS

This study is made almost wholly upon cochleae of the adult pig and pig fetuses. Some cochleae of the adult ox were used for comparison and also some isolated sections of cochleae of the rat and guinea-pig were referred to. A few adult human cochleae were used but none could be obtained in sufficiently fresh condition for other than general comparison with those of the pig.

As is well known, the structures of the cochlear duct, especially the elements of the spiral organ, suffer maceration very quickly after death. Pig material obtained as promptly as three hours

after death shows beginning maceration or digestion of the organ to an extent usually rendering it very unsatisfactory. Pig embryos and fetuses kept in the uterus and therefore in the amniotic fluid, preserve somewhat longer, but in the older stages of these digestion of the elements of the organ begins remarkably quickly. Therefore, it was found very necessary to place the cochleae in the fixing fluid, directly from the freshly killed animals.

The human specimens were obtained at autopsy but were found even in the freshest case to which access could be obtained, to have suffered too much dissolution for trustworthy detailed study of either tectorial membrane or spiral organ. All the pig material and the cochleae of the ox were obtained and placed in fixing fluid at the slaughter-house. Fortunately, in the slaughter-houses of New Orleans, the hog is always split sagittally along the vertebral column and through the head immediately after evisceration. One has but to stand near the line of the passing carcasses, beyond the man who removes the brains, likewise split, and an abundant supply of adult labyrinths may be obtained from them within a few minutes after death. The bony labyrinth of the pig does not become fused to nor embedded in the petrous portion of the temporal bone as it is in man. In the pig fetus, it may be 'shelled out' with the fingers. In the adult hog it has developed considerably more bone than in the fetus, some of which excess is in the form of flattened bony processes extending adjacent to and parallel with the cranial surface of the temporal bone. The most evident of these processes extend from the region of the semicircular canals and, after pulling away the dura mater that may be left, a sharp screw-driver may be inserted under these processes, given a twist and the entire bone labyrinth is readily removed. The mesial surface of the bony labyrinth of the ox is likewise visible in the cranial wall but is more firmly planted in the temporal bone, though not wholly fused to it. Its removal requires a stronger screw-driver and greater effort, or even a chisel and mallet.

Vials, with blank labels on them, and a supply of fixing fluids were taken to the slaughter-house and the cochleae were dropped into fixing fluid as they were removed, the name of the fluid and the stage of the specimens being then indicated on the label.

A series of developing cochleae were finally obtained, beginning with those of fetuses of 3.5 cm. in length (crown rump measurement) and increasing by from 1 to 3 cm. up to fetuses at term. The latter stage varies in length between 20 and 30 cm. and had to be judged by the appearance of the fetus, chiefly by the amount of the hair on the body and the condition of the eyelids. The head of the fetus was removed and split sagittally, the brain cleaned out and, in the older specimens, the cochleae were broken out with the fingers. In the younger specimens, a square of the skull containing the cochleae was cut out, the surplus external tissue removed and the square dropped into fixing fluid. From certain of these, the cochleae could be removed under the dissecting microscope upon return to the laboratory; others, the youngest, were best carried through the procedure and sectioned entire, the plane of section determined by the landmarks of the cranial wall.

Specimens for the study of the adult tectorial membrane in the fresh conditions were placed in amniotic liquor, brought to the laboratory and used immediately. Occasionally physiological salt solution was used to which had been added a few drops of saturated solution of the bichloride of mercury, to check maceration. However, only sufficient fresh specimens were used to obtain a few sets of observations of the fresh tectorial membrane, it having been found in the previous study and verified here that certain fixing fluids, in themselves, distort the membranes very little if at all, and that the membranes may be removed much more easily and with less injury from fixed and partially decalcified than from fresh cochleae.

It was early found that with cochleae whose bony labyrinth was advanced, a greater percentage of normal appearances could be obtained if a small hole was made through the bone at the apex, before or within two hours after placing them in the fixing fluid. In doing this, very great care had to be taken not

to deeply penetrate the membranous labyrinth. A pair of very fine pointed, small bone forceps was found better for this than a drill-pointed needle. Even with fluids which decalcify as slowly as those used, the carbonic acid gas usually forms within the labyrinth faster than it can transfuse, and the resulting pressure of the confined bubbles will press the tectorial membrane upon and over the spiral organ, often pressing it in places beyond semblance of its normal shape and position. Such pressure may be relieved through a very small hole in the bone. Here, the holes were usually made immediately after return from the slaughter-house, holding the cochlea down in a Petri dish under the dissecting microscope and in sufficient of the fixing fluid to cover it.

Of the several fixing fluids tried, two were found to give the best results both with cochleae to be teased and cochleae to be embedded for sections. A fluid was desired which would serve both as a fixing and a decalcifying fluid and which would produce neither shrinkage of the tectorial membranes nor swelling. Gilson's mercuro-nitric mixture, for example, invariably gave distorted membranes in cochleae of late fetuses and of the adult, though fair preparations in sections of cochleae from quite young fetuses were obtained after it. The very small percentage of alcohol contained in this mixture was not deemed sufficient to produce the distortion by extraction of water from the membrane, but some other of its actions did prove unsatisfactory with the older stages. Decalcification after fixation by various fluids was tried with discouraging results. With both the nitric and hydrochloric acid decalcifying fluids, proven with other material, either maceration or distorting shrinkage effects resulted in the preparations. Decalcification after embedding the fixed cochlea in celloidin gave better but not satisfactory results. The celloidin imprisons bubbles of gas both within as well as outside the cochleae and those imprisoned within distorted the tectorial membrane in numerous places along its extent.

Zenker's fluid and the fluid employed by Held ('09) gave the best results. In Zenker's fluid, the swelling action of the acetic acid in it, causing the tissues to take up water, seems to be coun-

terbalanced by the action of the potassium bichromate and bichloride of mercury contained, and both these, in addition to the acetic acid, are slow decalcifiers, as well as fixing agents.

The mixture employed by Held was here made by adding 40 cc. of the commercial (40 per cent) formaldehyde and 50 cc. of glacial acetic to 1000 cc. of a 3.5 per cent aqueous solution of potassium bichromate. This mixture, is at first the color of the bichromate solution, but by warming or after standing a few hours it turns a greenish brown as the result of oxidation processes. It is recommended as best applied after this change begins. While the reaction between the formaldehyde and potassium bichromate must set free some formic acid, and while formic acid, acetic acid and even formaldehyde to a less extent, acting alone, cause the tissues to take up water and thus produce swelling, the mixture with the excess of bichromate seems to produce no swelling of the tectorial membrane nor of the elements of the organ of Corti.

An advantage of both this fluid and Zenker's is that specimens may remain in them for a long period without injury, and it is necessary for the cochleae to remain subjected till the desired decalcification has occurred. Here, the cochleae, brought from the slaughter-house in vials of fixing fluid, were suspended in large amounts of the fluid contained in low cylinder jars with cap covers. The jar, holding about one liter, was filled two-thirds full of the fluid and the cochleae suspended in it near the surface that they might be surrounded by fluid more free from the salts resulting from the decalcification, which salts sink to the bottom. Also they were suspended that they might be subjected to less pressure than if lying on the bottom. The string by which a cochlea was suspended hung over the edge and outside the jar, held in place by the cover, and to the outer end of the string was attached a label when necessary. Adult cochleae required three to four weeks for complete decalcification, the fluid being renewed twice a week. Decalcification sufficient for microtome sections can be judged by testing with a needle, being careful to pierce the specimen in the region of the vestibule. The thinner walls of the bony labyrinth of the cochlea

decalcifying first, it is always the thicker bony regions which give trouble with the sectioning knife.

An additional advantage of the bichromate-formalin-acetic mixture is that specimens fixed in it do not require a period of washing with water preliminary to dehydration for embedding. All the drawings given here were made intentionally from sections of cochleae fixed in this fluid.

Dehydration required especial care. Tectorial membranes fixed without distortion are often badly disfigured by the treatment preparatory to embedding. Here no grade of alcohol of more than 10 per cent greater strength than the preceding was used. Further, it was found best to make up the desired grades of alcohol and let each stand before using long enough for all the fine bubbles of air resulting from the mixture with water to pass off. Otherwise these bubbles will collect upon and possibly form within the cochleae. The specimens were transferred direct from the fixing fluid to 20 per cent alcohol. Then, allowing them to remain in each grade from 3 to 12 hours according to convenience, they were subjected in succession to 30, 40, 50, 60, 70, 80, 90, 95 per cent and absolute alcohol. All clearing agents preparatory to embedding in paraffin, especially xylol, were found especially injurious except with the very youngest stages studied. The absolute alcohol was in all cases allowed to act at least 6 hours and then replaced by ether-alcohol and the specimens embedded in celloidin in the usual way.

The celloidin blocks were hardened with chloroform (not with water) and, to obtain transparency of the celloidin, the blocks were first placed in 95 per cent alcohol for an hour or so before the 80 or 70 per cent alcohol in which they were sectioned. Some of the blocks were transferred from the chloroform to cedar oil in which they remained long enough to become cleared, and then sectioned with the knife flooded with cedar oil. The latter procedure, though excellent for orientation, because of the transparency of the blocks, and for ease in sectioning, requires more time before and after sectioning than sectioning in alcohol and it did not seem to give any better results.

For transverse sections of the tectorial membrane, and remainder of the spiral organ, it is necessary that the edge of the knife passes parallel with the axis of the cochlea, and for transverse sections of the shortest or apical turn of the coil of the membrane, only those sections can be used which pass through the actual apex of the cochlea and the diameter of the cochlear nerve at its base. Celloidin sections varying from 10 to 20 micra were studied. The 3.5, 5.5, and 9 cm. stages were also embedded and sectioned in paraffin for thinner sections.

For staining, the sections were passed through the gradually decreasing grades of alcohol and the most generally satisfactory results were given by Delafield's hematoxylin 6 hours or overnight, washing in water, and both decolorizing and counterstaining with Van Giesen's picric acid-fuchsin mixture. Thence the sections were both washed and dehydrated with the increasing grades of alcohol, cleared in creosote and mounted in balsam.

All the drawings here given, except figures 10 and 11, were first outlined in detail under the new Edinger drawing and projection apparatus. By it, inequality of magnification of parts given by the camera lucida are avoided. The outlines of the nuclei and those of many of the cells could be traced. All except figures 10 and 11 were outlined with the same combination of lenses and the same adjustment of the apparatus and thus they are drawn to scale. The drawings were completed with the identical section projected for the outline placed, in each case, under the microscope and studied under high power for corrections and insertion of details.

PROPORTIONS OF THE ADULT TECTORIAL MEMBRANE

The hog, ox and man belong to those species of mammals which possess the flat type of cochlea and the component structures in their cochlea are remarkably similar in form and character.

Wiedersheim ('93) gives the coil of the cochlea of the hog as having 4 turns, that of man as having nearly 3 turns and that of the ox, $3\frac{1}{2}$ turns. Gray ('07) gives the hog as having $3\frac{1}{2}$ turns

and man, $2\frac{3}{4}$ turns. He did not examine the cochlea of the ox. Wiedersheim must have taken into consideration the long basal end of the cochlea which does not take part in the coil. This end represents the direction of the first outgrowth of the cochlear pouch of the embryo. As shown by Streeter ('07) for the human, this first growth of the cochlear duct is straight, the coiling taking place in its further extension. In dissections of decalcified cochleae of the adult hog, it was found here that this basal end is of considerable length, comparable to nearly one-fourth of the basal, the longest turn of the cochlea, and that it not only does not take part in the coil but that its tip curves slightly in the opposite direction as well as basal-ward. Including the uncoiled basal end, the pig's cochlea, were it all coiled, would comprise about 4 turns.

The cochlea of the ox is broader and slightly less flat than those of the hog and man. Measurements taken inside the bony labyrinths gave, as diameters of the basal turn in vertical sections passing through the apex and therefore including little or none of the uncoiled basal end, for the ox 8.4 mm., for the hog 5.8 mm. and for the human 6.7 mm. And, as heights of the cochleae, measurements from the apical side of the scala vestibuli in the apical turn to the basal side of the scala tympani in the basal turn gave for the ox 6.7 mm., for the hog 4.4 mm. and for the human 4.9 mm. In other words, these measurements indicate that the coil of the membranous labyrinth of the ox is approximately 8 mm. broad at the base by 7 mm. in height; that of the hog, 7 mm. broad by 4 mm. in height, and that of man, 7 mm. broad by 5 mm. in height. The figures given are averages computed from measurements under the compound microscope of sections of 5 different cochleae of the adult hog, 4 of the ox and of 2 human cochleae. The sections of the human were not so satisfactory for the purpose as were the others, owing to imperfect decalcification at the time they were cut. However, they were such that the indication that the human cochlea is relatively more flat than those of the hog and ox may be suggested as correct. The two scalae appear relatively larger in the ox than in the hog and relatively larger in the human than in either.

The cochlea of the pig at term appears to be slightly smaller than that of the adult hog. Averages of measurements taken in the same way of cochlea of fetuses at about full term gave 4.8 mm. as the width of the coil at the base by 3.4 mm. in height. Comparison indicates that the greater size of the adult is in large part at least due to an evident increase in size attained by the two scalae. Very probably the total length of the cochlea has increased but very little in the adult.

The *length* of the tectorial membrane, is somewhat less than that of the cochlear duct which carries it. It does not extend to touch the blind apical end (caecum cupulare) of the duct nor does it extend quite to the end of the caecum vestibulare, the basal end of the duct. And the duct is not so long as the scalae on its either side, especially the scala tympani. It appears from the best that could be gathered from various sections and teased cochleae that the tectorial membrane is quite strictly co-extensive with the spiral organ. Its exact length not being the point in mind at the time the dissections of the adult hog were being made, only three membranes of the adult were teased out sufficiently intact to determine their lengths. Two of these were decided each to have a length of nearly 27 mm. and the outer about 26 mm. The teasing of these was done after fixation, which is better for measuring since the membrane does not stretch so readily. All three membranes were broken, but the pieces were all saved and could be arranged in order on the slide. Accurate measurement, of especially the apical or most curved turn of the coil, is difficult. It has to be done in segments. Much straightening and attempts to straighten the membrane usually increase the sources of error. The average length obtained from measurements of seven membranes of fetuses near full term and two from young suckling pigs, recorded in previous paper of the writer, was 25.5 mm. Some of those measurements were obtained by the use of a string laid upon outline drawings of the entire membrane, whole or in pieces, projected under known magnification. Allowing for error in measuring it can only be said that the tectorial membrane of the adult hog is probably

a little longer than that of the fetus at term, but hardly more than 1 mm. longer.

The *width* of the tectorial membrane varies considerably. It is widest in the apical turn of its coil and tapers thence gradually and regularly to the basal end, the tip of which is its narrowest part. Its position is over the apical side of the spiral organ, its axial zone being attached upon the vestibular lip of the spiral limbus. Its widest portion spans over and projects beyond the spiral organ to an extent of more than one-fourth of its total width. Its narrowest part barely spans the outer hair

TABLE 1

Giving in micra averages of the total width, the width of the attached axial zone and the width of the outspanning zone of the tectorial membrane of the adult hog obtained from measurements, taken at the intervals of the coil specified, from median vertical sections of five cochleae and of six teased out membranes

		1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
Sections of cochleae	Total width	554.2	622.5	477.7	302.4
	Attached axial zone	193.8	206.7	206.7	190.0
	Outspanning zone	360.4 apical end	415.8	271.0	112.4 basal end
Teased out membranes	Outspanning zone	462.0	413.5	243.3	68.5
	Total width	666.0	619.9	450.3	250.5

cells of the organ. Figures 1 to 4 are given to show its varying proportions and its position. These four figures are drawings to scale of the membrane and its environment as it appears sectioned in one side of a median vertical section of the cochlea of an adult pig. Thus the figures represent consecutively sections of the membrane across the 1st, 3rd, 5th and 7th half turns of its coil.

Measurements of the width of the membrane taken at the above intervals of its coil from stained median vertical sections of five different cochleae of the adult hog and measurements at the same intervals of six membranes teased out from the adult are recorded in table 1. Short segments of the long basal turn

of the coil of three of the latter membranes were lost in their removal, but in these cases the loss did not preclude the desired measurements, the basal ends being saved.

In explanation of table 1, it should be noted that, with the different vertical sections employed, the measurements of the width of the membrane could not be taken across the same identical regions of the coil. The celloidin blocks were not oriented on the microtome so that the median vertical sections always involved the same diameter of the cochleae and, therefore, the measurements of the ends of the membrane had to be taken in each case at varying unknown distances from the actual tips of these ends. Owing to the fact that the basal end of the membrane is not coiled, no median vertical section of the cochlea can pass through this end transversely. Manifestly oblique sections of either end could not be used for measurements of width, and transverse sections of the basal end must of necessity have passed considerable distances from its actual tip. With the teased out membranes coiled on the slide, the micrometer scale could be arranged as a radius involving the widest part of the apical end and crossing the other turns at the specified intervals. Afterward could be taken a separate measurement of the basal end, transversely across its narrowest part. The actual termination of each end of the membrane and of the apical end especially, is bluntly rounded. Measurements of the ends were taken near but not involving the rounded part.

Further, it seemed manifest in comparing the teased preparations with the sections that, in the sections the membrane had suffered some shrinkage. Practically all the shrinkage in width occurs in the thicker outspanning zone and especially toward the apical end where this zone is more voluminous. This explains some of the differences in the width of this zone shown in table 1 between the measurements from the sections and those of the teased out membranes. In all the sections of the adult cochleae, the first half turn of the membrane seemed to conform less in shape to what the teased preparations indicated it should be than in other regions of the coil. In the cochlea from which figures 1 to 4 were drawn, the outer edge of the outspanning zone in

the first half turn is considered distorted and retracted axisward by shrinkage (compare *OZ*, fig. 1, with fig. 2). In the table as well as in the drawings, it appears less in width in this than in the third half turn. This explanation of the shape of the section of the apical end as due to shrinkage is supported by the study of the tectorial membranes of fetal pigs described in my previous paper. The depressed apical surface of the outer edge of this zone as shown in figures 3 and 4 is also probably the result of shrinkage.

The attached axial zone (*AZ*, figs. 1 to 4) varies but slightly in width in the different turns of the adult cochlea. In the teased preparations, its width could be easily measured from the fact that the line of imprint of Huschke's teeth (*Ht*, fig. 2) upon the basal surface of the tectorial membrane could be seen. In these, its width was found to conform quite closely to the widths obtained by measurements from the sections (table 1). In figures 1 to 4, the width of this zone appears practically uniform throughout and table 1 shows the maximum variation to be but 16 micra, the zone being narrowest in the basal end just as is the outspanning zone. It will be seen below that the attached axial zone increases somewhat in general width between the fetus and the adult hog.

It must be remembered that the basal turn of the coil is much the longest of the turns, has the greatest radius of curvature, and that, therefore, there is a much greater distance or extent of the membrane between the sections of the 7th and 5th half turns than between the 5th and 3rd half turns, and especially greater than between the sections measured of the 3rd and 1st half turns, which latter involve the apical or shortest turn of the coil. Thus, though the proportions of the tectorial membrane may decrease uniformly from the apical to the basal end, one must expect greater differences between the sections and measurements which are taken transversely at the greater distances apart.

To sum up the studies made as to the width of the membrane, with computations from table 1, it may be advanced (1) that the membrane is widest at its apical end and decreases gradually

in width toward its basal end, the tip of which is its narrowest part; (2) that the actual total width of its apical end in the teased out preparations is about 415 μ or 2.7 times greater than its basal end; (3) that its attached, axial zone varies very slightly in width, being but very little narrower in the basal end; and finally (4) that, as indicated in figures 1 to 4, its great variations in width occur almost wholly in the width of its thick outspanning zone. As computed from table 1, this zone in the teased out membrane may be about 390 μ wider in the apical than in the basal end, or the apical 6.8 times the width of the basal end. The same computation applied to the measurements of the sections of the membrane give the outspanning zone in the 1st half turn 3.2 times the width, and in the 3rd half turn 3.7 times the width it has in the 7th half turn. That the differences in the width of this zone do not appear so great in the sections as in the teased out membranes, and that its 1st half turn appears less wide than its 3rd half turn, is explained as due to shrinkage of the apical end, especially in the first half turn, during the preparation of the material for sectioning, and to the differences in the regions of the membrane at which the measurements of its width had to be taken in the sections. The measurement of the 7th half turn especially was in the sections of necessity some distance from the tip of the basal end and therefore could not involve the narrowest part of the outspanning zone. Computed from the measurements of the teased out membrane, the width of the outspanning zone in the apical or shortest turn of the coil appears to decrease only about 1 per cent, while in the second whole turn the decrease is 41 per cent, and between the 5th half turn and the basal end, the longest interval between measurements, the decrease is 72 per cent.

This study of the proportions of the membrane suggests throughout that conclusions as to its actual functional shape based upon its appearance in even the best of sections of embedded cochleae are subject to considerable error.

The *thickness* of the tectorial membrane had to be measured wholly from the sections. Obviously, the teased out membranes could not be used for this. Measurements through

thickest part of the outspanning zone at the different intervals are given in table 2. The thickest part usually appeared to be in the region of the small ridge on the basal surface known as 'Hensen's stripe' (*HS*, figs. 1 to 3).

TABLE 2

Giving in micra averages of the thickness of the thickest part of the tectorial membrane of the adult hog obtained by measurements at the intervals specified in median vertical sections of five cochleae

1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
168.6	137.5	112.4	58.5

It may be seen from table 2 that, just as the outspanning zone is widest, so is it thickest in the apical end of the coil of the cochlea. After whatever shrinkage that may have resulted in the preparation of the sections, computations from the table indicate that in the apical end this zone may be 110 μ thicker or about 3 times as thick as it is at the 7th half turn. Between the 1st and 3rd half turns its thickness appears to decrease about 19 per cent, between the 3rd and the 5th half turns about 18 per cent, and between 5th and 7th half turns, the longest interval between measurements, the decrease is about 48 per cent. The smaller amount of decrease between the 3rd and 5th half turns is probably due to irregularities of shrinkage, but this cannot be determined from the preparations used. The drawings, figures 1 to 4, made from a single cochlea show the decrease in thickness between the 1st and 3rd half turn to be least and that the decrease increases progressively between the 3rd and 5th and the 5th and 7th half turns. One would assume from studies of the teased out unshrunken membranes that this latter form of decrease in thickness is the normal one.

The *volume* of the tectorial membrane may be computed approximately from the areas of its transverse sections. Considered the chief vibratory mechanism in the auditory apparatus, variations in the volume of the membrane, or the 'load' it carries, in different regions are the most important of the proportions. Most all of its volume being carried in its outspanning zone,

this zone must determine almost wholly the possibilities of vibration of the different regions of the membrane as well as of the membrane as a whole. The axial zone being attached upon the vestibular lip of the spiral limbus and varying very little throughout the length of the membrane, can hardly have much to do with the variations in "natural vibration frequency" of which the membrane may be capable. In trying to compute the areas of the transverse sections of the outspanning zone, it was thought fairer to use for one dimension its width as found in the teased out and unshrunken membranes. Other dimensions had to be taken from the sections regardless of whatever shrinkage the zone had suffered in them. Using the widths obtained from the teased out membranes as the widths of parallelograms, the depths used were averages obtained from six measurements of the thickness of the zone taken upon the transverse sections of the different regions of the coil at about equal intervals between the outer edge of the zone and the edge of Huschke's teeth (*HT*, figs. 1 to 4). The first measurement was taken in each case near the outer edge and the last at the edge of Huschke's teeth. Of the several cochleae used for the purpose, these sections were used which were judged as passing nearest the apical end of the membrane. The areas of section of the outspanning zone in each region were thus obtained by multiplying its width in the teased out membrane by the average of its thickness in the sections.

Proportional volumes of the two ends and variations in the volume of the zone were most desired, and for such, realizing that by any further procedure the results could be only approximate at best, it was considered most feasible merely to multiply the area obtained for the section of the zone in each region by a given short length, say one millimeter of the length of the membrane. The volumes thus obtained vary as the areas of the sections. The areas obtained are as follows:

Transverse section of apical end	61,723.20 square micra
Transverse section of 3rd half turn	43,293.45 square micra
Transverse section of 5th half turn	18,442.14 square micra
Transverse section of basal end	1,719.35 square micra

The volumes of the different regions, computed in the above way, represented by these areas, may be found to show the following proportional relations: (1) A given short length of the apical end of the outspanning zone of the tectorial membrane may be 41.7 times the volume of the same length of the basal end of the zone. The volume of the same length of the 3rd half turn may be 25.2 times, and that of the same length of the 5th half turn may be 10.7 times the volume of that length of the zone in the basal end. (2) Taken from the basal end toward the apex, it may be found that between the basal end and the region of the 5th half turn, the longest interval between measurements, the volume of a given short length of the outspanning zone may increase 90.7 per cent; between the 5th and the 3rd half turns the volume of the same length may increase 57.4 per cent, and between the 3rd half turn and the apical end, the volume of the same short length of the zone may increase 29.8 per cent.

Just as does the width and thickness, so, as to be expected, does the volume of the zone appear to increase progressively from the apical toward the basal end. That the percentage of increase toward the basal end of the cochlea is progressive rather than regular must be due to the fact that, beginning with the basal end, each turn of the coil is longer than the turn apical to it and, therefore, there were greater lengths of the zone between the transverse lines at which the above measurements were made in each succeeding coil from the apical toward the basal. In studies made here of the teased out membranes of the adult hog and in the studies of the membranes of pig fetuses at term, published in the writer's previous paper, the tectorial membrane, and therefore its outspanning zone, viewed as a whole appeared to increase in width with uniform regularity from the basal into the apical end. If the increase is irregular at all, the impression may be obtained that it is less rapid in the basal than in the other turns of the coil. Drawings illustrating the appearance of the teased out membrane are given in the previous paper.

The above variations in the proportions of the tectorial membrane, assumed at least to approximate the normal adult, are

thought to further support the suggestion that the tectorial is far more adapted as a vibratory mechanism in the auditory apparatus than is the basilar membrane. Whether in accord with the Helmholtz theory, which involves sympathetic resonance, or with a modified telephone theory, the much greater variation of the tectorial membrane allows for it a much greater scale of activity than seems possible in the basilar membrane. Measurements of the assumed vibratory width of the basilar membrane, for example those by Kolmer ('07), show that its width (length of its supposedly existing vibrating fibers) at the apical end of the spiral organ is only about 1.8 times its width in the basal end. Averages of measurements here made in cochleae of adult hogs, taken from the line at which the auditory nerve fibers (*AF*, fig. 2) disappear into the spiral organ to the outer angle of the scala tympani, give the basilar membrane a width of 257.9 μ at the apical end and a width of 184.8 μ at the basal end (fig. 4), thus showing it to be about 1.4 wider at the apical than at the basal end. When the measurements were taken at the level of the floor of the spiral sulcus (about at the line *AF*, fig. 1), the width of the thus questionably vibrating part of the spiral lamina was found to be only about 1.9 wider at the apex than at the base. It may be noted in the figures that this latter measurement includes in the basal coils some of the bony spiral lamina. As seen above, the width of the assumed vibratory part of the tectorial membrane, its outspanning zone, is 6.8 times greater at the apical than at the basal end. The basilar membrane does not consist of independent fibers and thus of fibers capable of resonant activity. Ayers ('91) described it as consisting of four layers of fibers, one of which runs at right angles to the other three. In my former paper, the main or radially arranged part of the basilar membrane was shown to be of the nature of a flat tendon, the tendon fasciculi (fibers of the earlier descriptions) being abundantly connected with each other by smaller collateral bundles. Vasticar ('12) said that it consists of six layers. He, however, included the layer of epithelioidal tissue, or syncytical mesenchyme, on the basal surface of the basilar membrane proper and the endothelium lining the

scala tympani as layers of the membrane. The basilar membrane proper and the layer of epithelioidal syncytium upon its basal side vary very little in thickness in the different regions of the coil and the little variation is irregular.

POSSIBLE VIBRATION OF THE SPIRAL LAMINA

Variations of the spiral organ (of Corti)

In the adult hog the spiral organ increases in both width and thickness in passing from the basal toward the apical end of the coil of the cochlea. Near the basal end (fig. 4) it may be seen that the spiral organ proper is much thinner and narrower than in the apical region (figs. 1–2). Reading the sections of the organ consecutively in both sides of the median vertical sections of the cochleae, or even reading figures 1 to 4, which represent the organ in one side of a cochlea, it is suggested that the increase in the size of the organ occurs uniformly from base to apex. Measurements of the width and thickness of the spiral organ as it appears in section in one side of five median vertical sections gave the averages recorded in table 3.

TABLE 3

Giving averages in micra of the width and thickness of the spiral organ, as measured in one side of vertical median sections of cochleae of five adult hogs

	1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
Width	223.3	221.1	208.7	130.0
Thickness	113.1	104.4	85.2	55.4

The measurements of width of the spiral organ, averages of which are given in table 3, were taken from the level of the surface of the epithelium lining the internal spiral sulcus (*ISS*, fig. 2) on the axial side of the organ to the level of the surface of the cells of Claudius (*CC*, fig. 2). The measurements of thickness of the organ were taken from the basilar membrane proper through the middle of the outer hair cells to the vestibular surface of the organ. As shown in figures 1 to 4, the vestibular surface of the organ inclines axisward appreciably in the api-

cal turns, the outer sustentacular cells being higher and forming a thicker ridge than in the basal end. Thus, measurement through the middle of the outer hair cells will give an approximate average of the thickness in each case. The degree of the axial incline of the surface of the organ decreases toward the basal end, at which it is almost absent. Attention is further called to the fact that in the basal end of the coil of the adult cochlea (fig. 4), both the epithelium lining the internal spiral sulcus and that known as the cells of Claudius are considerably thicker than in the more apical regions. The axisward incline of the outer rods of the organ increases slightly in passing from the basal toward the apical end. This is not so evident in figures 1 to 4 as it was in other sections of adult cochleae. Gray ('00), in suggesting a modification of the Helmholtz theory of hearing, noted that the rods of the spiral organ and the hair cells became smaller in passing from the apex to the base of the cochlea.

The resonance theory, elaborated by Helmholtz, was based upon erroneous anatomical descriptions of the basilar membrane by others. Requiring that the membrane be composed of separate fibers of varying length and free to exercise sympathetic vibrations in response to sound waves of varying length imparted to the endolymph, the theory must be abandoned. The basilar membrane not only does not consist of independent fibers, but it is blanketed on both its sides by thick continuous layers of other tissue, the thickest of which is the cells of the spiral organ itself. The telephone theory of hearing, suggested by Rinne in 1865 and Voltolini in 1885, elaborated by Rutherford in 1886 and further by Waller in 1891 and Meyer in 1898, was likewise applied to the basilar membrane. Denying the possibility of the selective or sympathetic resonance required by the Helmholtz theory, it assumes that the vibrations producing sound act upon the basilar membrane as a whole; that the vibration frequencies induced in the tympanic membrane by given sound waves are repeated by such extents of the basilar membrane, beginning at the basal end, as the resistance offered by the components of the apparatus and the inertia of the membrane will allow. At one time in its development, the tele-

phone theory was applied to the cochlea as a whole instead of being confined to the basilar membrane.

Any application of the telephone theory, either to the entire cochlea or to the basilar membrane requires, of course, agitation of the spiral organ and in such a way that the hairs of the hair cells are involved with the basal surface of the tectorial membrane. In other words, the theory must include the possible vibration of the entire membranous portion of the spiral lamina.

I do not desire to dispute the possibility that energy imparted to the cochlea by sound waves may throw into vibration the membranous spiral lamina. On the contrary, the possibility seems to me supported in my experiments with the model referred to above, and by studies of the character of the basilar membrane, and especially by the above observation that the cellular part of the spiral organ increases in bulk in passing from its basal to its apical end. Since the base of the stapes imparts the wave motion to the cochlea at its basal end, since the higher pitches are known to be mediated by the basal end of the cochlea, and since sound waves of greater vibration frequency (higher pitch) are known to be 'damped out' more readily in overcoming resistance than those of lesser vibration frequency, it seems indeed suggestive that the membranous spiral lamina not only gradually increases in width or bulk but also that its chief load, the cellular spiral organ, itself increases from the basal toward the apical end. But, on the other hand, I beg to emphasize the fact, very evident to me, that the tectorial membrane is far more adapted for the vibratory activities required. It is infinitely more flexible than the basilar membrane, or membranous spiral lamina, especially when both are in their position. In my previous study I had opportunity to compare the two when both are teased out and found that even then the tectorial membrane as compared with the lamina is as a strip of thin paper compared with a board. The arrangement of the fibers in the structure of the tectorial membrane, giving it sufficient elasticity against stress applied longitudinally to enable it to retain its position close upon the spiral organ but giving it practically no resistance to stress applied transversely, renders it

peculiarly adapted for undulatory motion. The vibrations are imparted by the base of the stapes by way of the fenestra vestibuli (ovalis), first to the fluid in the scala vestibuli, which scala is on the apical side of the spiral organ. The tectorial membrane projects over the apical side of the spiral organ and therefore is in the logical position for being most readily disturbed by the motion imparted. Finally, among other advantages, the tectorial membrane varies far more in its proportions than does the membranous spiral lamina and especially more than the basilar membrane. In width, its vibratory or outspanning zone is about 7 times wider in the apical than in the basal end while the width of the apical end of the supposedly vibrating part of the spiral lamina is only about 1.4 times its width in the basal end. The differences between the volume of the two ends of the tectorial membrane very evidently exceed those of the lamina to a much greater extent than do the differences in width, and variations in volume are the most important for the functions ascribed to the membranes in the telephone theory.

It has been computed that the actual force of the vibrations of the tympanic membrane, produced by sound waves, is increased about 30 times as repeated in the vibrations of the base of the stapes, and that the amplitude of certain waves as imparted to the tympanic membrane may be reduced as much as 76 times in their transference to the base of the stapes. Both force and amplitude are decreased in overcoming resistance in the cochlea. It is quite possible that very strong sound stimuli may throw into vibrations both the tectorial membrane and the membranous spiral lamina while the less strong and more ordinary stimuli affect the more adapted tectorial membrane alone. If the two were equally affected by the given strong stimuli, their resultant movements would be parallel and the required stimulation of the hair cells in the way usually supposed would not occur. If, however, the position and greater flexibility of the tectorial membrane should result in greater excursion or amplitude of vibration in it than in the lamina, then the hair cells could be stimulated in the way supposed and in accord with the vibration frequency of the wave motion applied. It

might be advanced that vibration of both the tectorial membrane and the membranous spiral lamina in case of very strong sound stimuli would be an economical arrangment in the structure of the cochlea. By such, the tectorial membrane, whose motion alone actually stimulates the hair cells, may in these cases be kept within working distance of the protruding hairs, or by such may be obviated injuriously forcible impingement of the tectorial membrane upon the hairs, which might result from extraordinarily great excursions produced in it by the strong stimuli, were the lamina to remain undisturbed. In the more ordinary and less strong sound stimuli, amplitudes and vibration frequencies must occur which are incapable of throwing the lamina into vibration at all but which may affect the tectorial membrane, its excursions decreasing, with the strength of the stimuli, to the functional limit of the auditory apparatus.

I hope to discuss more fully the probable action of the tectorial membrane with a description of experiments with the model referred to above.

STRUCTURE OF THE TECTORIAL MEMBRANE

In the study of the tectorial membrane of the adult hog, I have found little reason to modify the conclusions drawn in my previous paper as to structure from its study in cochleae of fetal pigs. It consists of fibrils or filaments of very varying lengths imbedded in a gelatin-like, probably keratinous, matrix. The directions in which its fibrils are arranged result from the gradual changes in position and the increase and decrease in number of the individual cells which produce its fibrils. The greater epithelial ridge, which produces the membrane, grows thicker, wider and longer for a considerable period after it has begun production, and then recedes in thickness and width and finally disappears as a producing structure. The axial side of the ridge ceases to grow and its cells cease to produce the membrane earlier than the outer side of the ridge. Thus the fibrils of the axial side of the membrane are the shorter and merely curve, from the basal side axisward, due to the growth in width of the

ridge, and apexward from the cells producing them. The fibrils of the outer side of the membrane are longer and curve outward, apexward and axisward from the basal side, claimed as due to first the great increase in width and then the decrease in width of the greater epithelial ridge, both of which changes in width occur after the beginning of the membrane.

From the study of the adult, I see no reason for describing the tectorial membrane in more than two zones, the attached axial zone and the outspanning zone. To let Hensen's stripe mark the boundary of a third zone is, I think, unnecessary other than that the stripe forms the axial boundary of that width of the membrane which projects beyond the interlocked phalanges of the pillars of the spiral organ. A 'border plexus' or a thin outermost zone does not exist in the adult nor in the older fetuses. The outer edge of the membrane is bluntly rounded and, in contour, slightly scalloped. The fibrils forming the edge curve from the basal surface outward, around the edge and then apexward and axisward. The appearance described by me as an "accessory tectorial membrane" in the fetus is present on the membranes of the adult hog. In one specimen of the teased out membrane it appeared partly lifted away from the basal surface in places but in none of the mounts from the adult was it so completely lifted away from the main body of the tectorial membrane and so separately visible as in the specimen from the fetus described and illustrated in the previous paper.

Held ('09) described the thickness of the tectorial membranes of the rabbit as composed of three layers: A 'Decknetz' on the apical surface; a middle fibrillar layer on the main body of the membrane composed of fibrils embedded in a matrix (Zwischensubstanz), and a thin homogeneous layer bounding the basal surface. All three layers come together in the outer edge of the membrane which he calls 'the selvage' and which he describes as blunt and irregularly fibrous. By irregularly fibrous is meant that bundles of fibrils curve around the selvage giving it the lobed or scalloped appearance in contour mentioned above. His descriptions of the course of the fibrils and their abundance conforms in the main with mine.

Upon looking at Held's figures of his Decknetz I felt sure at first that he was describing the structure I referred to as an accessory tectorial membrane with its regularly arranged fibrils washed together and cohering in irregular bundles, making his net, and that either he had mistaken it to be on the apical surface or I had been mistaken in thinking it on the basal surface of the tectorial membrane. In his description of it and in his figures, the outer border of his Decknetz is shown to be thicker and its meshes finer than in other parts. The 'accessory membrane' might appear thus if its fibers were disarranged by the treatment. He thinks the 'bars' of his net are assembled from finer fibrils and states that the direction of the bars in places conforms with the direction of the fibrils in the fibrillar layer or main body of the membrane below. However, he describes his Decknetz as extending on the apical surface of the membrane from and usually involving its outer edge, over the entire outer zone and upon the axial or inner zone which is attached upon the vestibular lip of the spiral limbus. While one of the two systems of fibers in my accessory membrane appeared to conform with the direction of the fibers in the main body of the tectorial membrane, the latter being crossed at very acute angles by the other system, only in the basal turn did the outer edge of the accessory membrane seem to extend to the outer edge of the main body, and only in the basal turn did its very delicate axial edge seem to me to extend further axisward than the line of Hensen's stripe. As seen by Held, his Decknetz is considerably wider. He states that he could not follow it completely over the attached axial zone because of the density of this region.

Held's homogeneous layer bounding the basal surface of the tectorial membrane is, I think, nothing more than the finely granular and faintly fibrous pale staining layer found by Rickenbacher ('01) for the guinea-pig after decalcification with nitric acid and staining with eosin. After the procedure used by me, both in the previous and in the present studies, to obtain sections of the membrane, the stain I used shows a thin more densely staining layer bounding both the apical and basal surfaces. I described this as a peripheral condensation of the substance of

the membrane (*PC*, figs. 1 to 3) and explained it as representing, on the apical surface, the product of the first activities of the then young producing cells, and on the basal surface as the last product of the then declining activities of the producing cells. That it appears condensed as compared with main body of the membrane, I thought due to the different actions of the reagents upon these superficial and less completely formed products of the beginning and waning functions of the cells. As noted by Held for his homogeneous layer, the peripheral condensation is not so noticeable along the axial side of the basal surface. Here the maturely active cells are probably torn from their product more suddenly than from the thicker part of the outspanning zone. Also, as noted by Held, the very thin extreme axial edge of the attached axial zone appears dense in the preparations. This edge corresponds to only the earlier product of the activities of the cells, for here production of the membrane ceases in the early fetus and the cells sink back into inactive form while the remaining are still forming the 'peripheral condensations.' I have called attention to sections of the apical surface cut parallel to it, showing a coarse reticular arrangement and have suggested that the reticulum in these sections represented the first formed ends of the fibers of the membrane, not so adequately embedded in matrix as in the main body, washed together and cohering in anastomosing bundles. In the transverse sections, these form part of the peripheral condensation. If this suggestion is possible, it could explain Held's Decknetz of the apical surface.

Held did little more than look over the figures given in my paper. He kindly gives a 'Zusatz' at the end of his paper in which he states that he did not know of my paper till after his manuscript had been closed, and that he thought some of my figures defective, two of them 'artificial monstrosities' of the tectorial membrane resulting from swelling. Held had never studied the tectorial membrane of the hog, which I think is similar to the human. All the drawings of the present paper are intentionally made from projections of sections of cochleae fixed and prepared for sectioning according to the identical

procedure used by Held for his sections of the cochleae of the rabbit. In so far as possible, I judge my preparations were no more shrunken than were his. Judged from measurements and the appearance of the teased out membrane, I am quite sure they were not swollen. I have not studied the tectorial membrane of the rabbit. He frequently referred to the tectorial membrane of the adult rabbit but, unfortunately, does not figure it. His figure 17, six days after birth, is the oldest stage he exhibits in the rabbit. The rabbit being one of the rodents in which parturition is comparatively premature, his six day old rabbit was scarcely more developed in than a pig fetus about term.

ON THE DEVELOPMENT AND ATTACHMENT OF THE TECTORIAL MEMBRANE

The essential steps in the development of the spiral organ and its tectorial membrane were worked out by Corti himself in 1851 and by Kölliker in 1861. Many other papers have dealt with phases of the process, repeating and adding to the findings of Corti and Kölliker, the most recent being those of Held and Prentiss. Permission is asked here merely to review the process as to its bearing upon two points: the position which the tectorial membrane acquires in its maturely functioning condition and the attachment it retains. As indicated in my previous paper, both these points have been touched upon repeatedly without agreement in results. The most recent papers fail to agree upon them. I have here tried to contribute quantitatively something as to the growth changes in the width of the membrane, and as to the growth changes resulting in its adult position with reference to the cells of the spiral organ and also resulting in the one attachment it retains in the hog.

In the hog, the length of the fetus is but an approximate criterion of the stage of the development of its cochlea. Fetuses obtained in California, for example, I have found average longer at a given stage of the spiral organ than do fetuses from hogs raised in Louisiana. The hog brought to the slaughter-house averages larger in San Francisco than in New Orleans, due no doubt to differences in breed and feeding, for very probably

the Louisiana hogs average older and smaller at killing than those in California. All the fetuses here used were obtained in New Orleans.

In fetuses of about 5 cm. (fig. 5), the coiling outgrowth of the cochlear pouch has already acquired nearly three turns. Only the first indications are then appearing of the liquefaction of the mesenchyme, the progress of which gives the scalae on the two sides of the cochlear duct. Along the basal side of the cochlear duct the epithelium has already become much thicker than that of the apical side, but as yet there is no differentiation of the epithelium into the greater and lesser epithelial ridges of the later stages. Upon the axio-basal surface of the epithelium of the cochlear duct appears the beginning of the tectorial membrane (*TM*, fig. 5), being produced by the thicker epithelium. In the sections of embedded material, this product appears densely but finely fibrillar. The fibrils may be seen continuous with the apical ends of the long cylindrical cells, but in the whole product they appear very much tangled and matted together as though an interfibrillar substance were insufficient in either amount or quality to individually support them. When compared with the body of the membrane of the later stages, one receives the impression that the fibrils here, which represent the apical ends of the fibrils of the later stages, are smaller than in the later stages. As may be noted by comparison with the figures following, the extreme axial edge of the young membrane (*AZ*, fig. 5), though very thin, is almost as thick as it is in the later stages. The cells producing this edge never become higher, never become so actively productive of the membrane as those of the remainder of the thick epithelium, and they grade into the non-productive cells of the duct. The outer edge of the young membrane is likewise thin at this stage, the cells producing it grading likewise from the less productive into the non-productive cells. However, the outer edge of the thicker epithelium grows in width and activity along with the rest of the thickening. The entire thickness of the tectorial membrane so far produced in figure 5, only represents a part of the width of what I have called the peripheral condensation on the apical

surface of the sections of the further developed membrane, or it perhaps represents a part of Held's Decknetz.

In the outer side of the thick epithelium, there usually appears at this stage a lighter area (*L*, fig. 5) resulting from the nuclei here being placed farther away from the apical ends of their cells. Comparison with later stages (figs. 6 and 7) suggests that this area represents the outer margin of the greater epithelial ridge.

In fetuses of about 8 cm., the increase in number of the cells in the epithelium of the basal side of the cochlear duct has rendered the thicker epithelium there still thicker and wider, and has also resulted in a second but lesser thickening along the outer edge of the first. At this stage the cells of both the greater and the lesser epithelial thickenings, or ridges, are engaged in the production of the finely fibrillar, tangled structure characteristic of the first and seemingly imperfectly formed part of the tectorial membrane. The cells of the lesser ridge, which later become differentiated into the elements of the spiral organ, seem to retain but a very short time this tendency to produce fibrils similar to that of their adjoining neighbors of the greater ridge, along with whom they have developed. While producing fibrils, they are never as actively productive as even the cells of the extreme axial edge of the greater ridge, and their product is more sparse and less mature than even the first product of the greater ridge. They represent the edge of the productive grading into the non-productive cells, and, as they differentiate into the cell elements of the spiral organ, they cease production altogether.

In figure 6, from a pig fetus of 9 cm., the first evidence in my series of the differentiation of the elements of the spiral organ is shown. The first to be distinguished is the series of inner hair cells (*IAC*). While in some sections of this stage and earlier, there may appear shrinkage cracks between the cells of either of the ridges, the beginning of the spiral tunnel has not appeared at this stage, though Prentiss represents the tunnel in his figure of the second turn of a pig of 5.5 cm. Figure 6, as does each of the figures following, represents a section through the 3rd half

turn (the first half of the 2nd turn) of the coil. Development and differentiation begins in the basal end and precedes toward the apex. Therefore a section through the basal turn would show a more advanced stage of development.

The tectorial membrane in figure 6 has thickened so that a main body and an axial edge may be easily distinguished. The beginning of the invasion of the axial edge of the greater ridge by the mesenchymal tissue to produce the vestibular lip of the spiral limbus may be seen. Soon the cells of this axial edge, involved in mesenchyme, will cease to produce tectorial membrane, the cessation at the extreme edge having already begun. The outer edge of the body of the membrane is bluntly rounded and coincides with the outer edge of the greater ridge producing it. Attached upon this outer edge of the membrane are the few fibrils produced by the cells of the lesser ridge.

The amount of the fibrils or fibers produced by the lesser ridge reaches its maximum in pigs between 8 and 14 cm., depending wholly upon the extent to which the elements of the spiral organ have differentiated. The change of character of any of the cells of the ridge into that of any of the various elements of the spiral organ once established, those cells cease to produce the fibrils, become devoted to a different function. From the first they are never more than the outer border of less active cells grading into the non-productive type of the rest of the duct. These fibrils are always more sparse and appear to be supported by a far less amount of the interfibrillar matrix than those of the membrane proper. Further, the amount of the fibrils varies considerably in different individuals in the same stage of development. Figures 6 and 7 (*LF*) show an amount maybe a little greater than an average in my preparations from pig fetuses. Prentiss must have found a greater amount in the pig than any of my preparations show, especially in his figure 6 (pig of 13 cm.), which shows the product extending well over upon the cells of Claudius and relatively thick, considering that it shows the effect of considerable shrinkage and agglutination. His argument strongly urges that a large outer zone of the tectorial membrane is produced by the cells of the spiral organ

(and the cells of Claudius?), which zone he claims remains permanently attached in the adult to the cells producing it.

Held describes and figures for the rabbit fetus these tangled fibrils arising from the lesser epithelial ridge and later attached to the cells of the spiral organ, and calls them 'Haftfasern.' He thinks that a part of his Decknetz and a narrow outermost zone of the membrane is formed from them. In none of his figures from the rabbit do his Haftfasern show an abundance and arrangement similar to that of the membrane. On the contrary, his figures for his fetuses up to near term, show what I consider the outer edge of the tectorial membrane proper to terminate bluntly at the axial side of the series of inner hair cells. He states that his narrow outermost zone (formed from the fibrils in question) is not distinguishable in the older stages and he thinks it a retrogressive structure, for later, when the whole outspanning zone of the membrane is becoming free, he finds only a thin relatively coarse net upon the outer edge (selvage) of the membrane.

Rickenbacher, for the fetal guinea-pig, shows a mass upon the lesser epithelial ridge, after the hair cells have differentiated, considerably greater than I have seen in the pig. He described it as of the nature of his granular, pale staining layer in which fibrils appear, stating that in guinea-pigs of 5.5 cm., the tectorial membrane proper ceases at the immediate axial side of the inner hair cells and that the pale staining mass becomes a fibrous 'Deckschicht' upon and produced by the spiral organ, and that it becomes a process of the tectorial membrane proper (p. 395). In another place he states that it adds a small outer zone to the membrane. It is later detached from the spiral organ, he agreeing that the outspanning zone of the developed tectorial membrane of the guinea-pig is free.

Prentiss charges that I misrepresented Rickenbacher in using his name as one who agreed that "there is no good reason to assume that the cells giving rise to the organ of Corti ever have anything to do with" the development of the tectorial membrane. In again going over Rickenbacher's paper, I find Prentiss' charge a true one, and I cannot conceive of my reason at that time for using his name as I did in the sentence quoted by Prentiss. It is barely possible that his name was

used instead of that of another author, Hensen probably, for while only in the passage of his paper incorporated by Prentiss does Rickenbacher state at all definitely that he considers a part of the tectorial membrane proper *developed from* the lesser epithelial ridge, yet in several places one may infer that he does so consider it. In the passage incorporated by Prentiss, the part in question is referred to as follows: "Die schmale Randzone ist eine sekundäre Bildung, welche an dem kleinen Epithelialwulst *abgesondert* wird." (Italics are mine). In my paper, as stated, no attempt was made to enter fully into the processes of development of the membrane. Sections from relatively few early fetuses were examined. The purpose was to learn something of the nature of the membrane already developed. In the few sections I had representing the stages of the differentiation of the spiral organ, the fibrils over the lesser ridge must have been exceptionally sparse, for I considered them as artefact and omitted to show them in the one figure offered dealing with development. Granular masses and filaments are usually seen in the sections adhering upon other parts of the epithelium lining the cochlear duct, especially in its angles, and I described the fibrils I saw as a thin, frayed reticulum, sticking upon the lesser ridge and continuous with the tectorial membrane, composed of coagulum filaments resulting from the action of the fixing fluid upon the endolymph. I considered Rickenbacher's pale staining substance over the spiral organ as artefact, most especially his detailed presentation of it in his figure 15, for in this he showed it as adhering to the young spiral organ by five stout processes, four of which each blended upon and surrounded only the hairs of the inner and outer hair cells. Between these few attaching processes were shown what were evidently shrinkage spaces, though he called them 'intercommunicating spaces in which the endolymph circulates.' He called the processes 'fiber bundles,' which was perhaps true, but the predominant structure of his mass appeared to be granular and I considered it artefact, as it was, especially the arrangement of his fiber bundles. In the present study, I cannot agree for the pig with any of his interpretations of his Deckschicht. I agree with him that the tectorial membrane becomes free from the spiral organ.

Up to pigs of about 16 cm., the greater epithelial ridge grows thicker and wider and the activity with which the membrane is produced by it increases. The outer third of the greater ridge becomes thicker than the axial two-thirds, the position of its nuclei giving the impression that its long cylindrical cells have been forced apexward by growth pressure. Figure 7, from the 3rd half turn of a pig of 14 cm., shows this but to a less extent than may occur at 16 cm. The rapidly growing, outspanning zone of the tectorial membrane coincides with the surface of the

greater ridge throughout. Its outer third cups around or clasps the more elevated outer third of the greater ridge, and its rounded edge is curved basalward to terminate at the inner hair cells of the young spiral organ (figs. 7 and 8). The cells comprising the outer margin of the greater ridge are directed outward, diverging toward a direction parallel with the surface of the spiral organ and thus maintaining the position approximately vertical to the basal surface of the enclasping outer edge of the membrane. The bluntly rounded outer edge of the membrane is frequently torn loose in the preparation of the sections. Then it may be straightened outward over the spiral organ, either projecting free from it or often pressed down upon it when distorted. Quite often, when torn free or distorted by pressure, the edge appears shrunken to a flattened, dark staining projection. Even when undisturbed and undistorted, the outer edge may project over the inner hair cell, as in figure 7. Then the fibrils supplied to this edge by the reclining cells of the outermost part of the greater ridge may be traced to curve into and around the edge, contributing to its rounded contour.

The fibrils produced by the lesser ridge and attached to the now thickened outer edge of the tectorial membrane, appear drawn more straight by the growth of the membrane and appear continuous with the 'peripheral condensation' of the apical surface of the outer edge. These fibrils and the apical peripheral condensations are considered above as homologous in that both represent the product of the first activities of the cells, a product less completely formed or organized than the body of the tectorial membrane later produced by the cells of the greater ridge. The fibrils, early formed by the lesser ridge, must contribute but a very small part of Held's Decknetz, for it is hardly possible, considering the physical character of the tectorial membrane and the very evident delicacy of the fibrils, that they can be pushed around and upon the apical surface of the membrane. In figure 8, which represents a section of the 3rd half turn of the cochlea of a pig of 19.5 cm., is indicated, I think, the process of rupture and disintegration of the few and always loosely associated fibrils produced by the lesser epithelial ridge.

The further increased thickness of the outer edge of the tectorial membrane, added to it from the basal side, has begun to draw into bundles and tear apart the sparse fibrils. From the preparations of stages below and above that represented by figure 8, I think that the cells of the lesser ridge, now the more advanced spiral organ, ceased to produce fibrils in pigs of about 12 to 14 cm., and never actively produced them. Being at the beginning involved with those produced by the greater ridge, the fibrils on the lesser ridge and later spiral organ remain continuous with the outer edge of the tectorial membrane proper but they contribute practically nothing to it. When produced in unusually large amount, they may produce the appearance of the loose plexus attached to the freed outer edge of the membrane observed by Held in the late fetus, or they may produce the "border plexus of Lowenberg" described in late fetuses by others. But, they partially if not entirely disintegrate. There is no evidence of them upon the clean, rounded, outer margin of tectorial membrane of the adult pig or even of the pig at full term; they do not show on the fully developed spiral organ, unless they may possibly contribute something to its lamina reticularis.

In figure 8, the beginning of the cessation of production and the recession of the cells of the greater ridge is well advanced. The attached axial zone of the membrane ceases earliest to grow, of course. The invasion and growth of the mesenchymal tissue to form the vestibular lip of the spiral limbus soon involves the epithelial cells, which never become so high in the region, pocketing them in an inactive condition. Cessation of activity of the cells of the greater ridge proper begins under Huschke's teeth and progresses outward. The activity seems to wane, the last product being less complete than earlier, then the cells begin to recede, their fibrils torn asunder as the space between them and the basal surface of the membrane increases. The receding cells decrease in number. The cells of the outer and thickest part of the greater ridge remain active longest, produce the thickest part of the membrane and its outer edge. With the recession and reduction of the outer part of the ridge, the membrane has attained its adult proportions. The greater ridge grows pro-

gressively wider and thicker toward the apical end of the cochlea and remains active longer at the apical end, thus producing the progressively broader and thicker apical end of the membrane. Figure 9, drawn from a section of the 3rd half turn of the cochlea of a pig of 22 cm., suggests the persistent activity of the outermost part of the greater ridge. In it, the final retrogression of the producing cells (*GR*) is represented. This retrogression seems to occur after the cells of the axial two-thirds of the ridge have completed their retrogression and decrease in number to form the cells lining the spiral sulcus. The indentation in the basal surface of the membrane over the disintegrating cells is thought to be artefact, for tracing backward through the stages suggests that very little if any of this region of the membrane is contributed by the outer part of the greater ridge. The process by which the spiral organ assumes its position under the outspanning zone of the membrane is about completed and, in the process, the cells which produced the outer regions of the membrane have been carried axisward from their product. Between the membrane and the organ and disintegrating cells, there is, in some of the sections of this stage, a suggestion of delicate fibrils, some of which seem to be drawn axisward. So, if that portion of the membrane which now lies over the disintegrating cells is contributed to by the cells at all, it is most probably only a few delicate fibrils drawn axisward, parallel against and contributing to the peripheral condensation of the basal surface.

Attention is called to the fact that the spiral organ has increased in size between the stage of 19 cm. (fig. 8) and 22 cm. (fig. 9). Though its growth is not quite completed, it has approached the thickness possessed by the adult in this turn of the cochlea. The growth increase in the thickness of the organ is indicated in table 4 in which averages of the thickness are given in micra. The measurements were taken from the surface of the organ, through the middle of the outer hair cells, to the basilar membrane.

Cochleae from one litter of pigs averaging about 21 cm. showed the differentiation of the spiral organ completed in the 3rd half

TABLE 4

Giving in micra averages of the thickness of the spiral organ (of Corti) as found for the pig in the various stages of development and the regions of the coil of the cochleae specified

SPECIMENS		1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
Number used	Sizes pig				
2	16 cm.	34.6	30.8	30.8	38.5
4	19.5 cm.	52.4	53.9	60.1	52.4
2	22 cm.	69.3	84.9	61.6	46.2
5	near term	102.0	89.2	71.3	52.0
5	adult	113.1	104.4	85.2	55.4

turn. The cochlea from which figure 9 was made came from a pig of 22 cm. and the two of this stage measured for the results recorded in table 4 were chosen from this lot of pigs. The spiral organ was some thicker in one of the two than in the other. The variations in the thickness of the 7th half turn, shown in table 4, are due largely to the varying distances from the actual basal end of the organ at which the plane of section passed, for at 19 cm., in my specimens, the differentiation of the organ in the basal region is about completed. Examination of the various stages, and the measurements, show that with the beginning of the differentiation of the spiral organ from the lesser ridge, the organ begins to increase in thickness throughout the cochlea, and that, though the increase takes place most rapidly in the stages before full term, it seems to continue after birth. The increase is greatest in the apical end.

The growth increase in the width of the organ could not be accurately measured in the 1st and 3rd half turns till above pigs of 22 cm., because, as shown in figure 9, its differentiation was not complete. It is usually completed throughout in pigs of full term. The varying width of the organ in the adult is given in table 3.

As suggested in the figures, the cells of the greater epithelial ridge finally all sink till they are represented only by the relatively few, broad, fattened cells lining the internal spiral sulcus. The outermost five to eight of the cells of the greater ridge retain a portion of their high cylindrical form and become the

inner supporting cells of the spiral organ. As such, they retain to an extent their outwardly reclining direction. In the recession and disintegration of the cells, the decrease in number is such that the cells (nuclei) in the greater epithelial ridge of the apical turns at 15 to 16 cm. are twenty to twenty-five times the number of the cells which line the internal spiral sulcus and comprise the inner fourth (the smaller supporting part) of the mature spiral organ. Held and others before him have shown for other mammals that the retrogression and disintegration of the cells of the greater ridge begins at the axial edge of the ridge and proceeds outward, thus freeing from attachment first the axial border of the outspanning zone of the tectorial membrane; and Prentiss notes that the "inner half of the spiral organ" (inner supporting cells) is derived from the greater ridge.

The inner supporting cells, during their differentiation from the cells of the greater ridge recede slightly. Then they increase in both height and size in the increase in the size of the spiral organ so evident in the apical coils of the adult hog. However, as shown in the figures and as will be noted below, the outer supporting cells (cells of Hensen) increase in height and size considerably more than the inner during the growth of the apical regions of the organ. The cells of Claudius also decrease slightly in height and increase in width between the early fetus and the adult form of the spiral organ. The decrease in height seems to result from loss in their distal ends, for the nuclei are situated earlier in the middle and proximal (or basal) ends of the high columnar cells and later in the distal ends of the low columnar and cubic cells. In the adult, the cells of Claudius appear to increase considerably in height in passing from the apical to the basal end of the cochlear duct. It may be suggested that both the spiral organ and the cells continuous with it, though their differentiation is completed earliest in the basal end, do not undergo so extensive differentiation in the basal as in the apical end; (compare figures 1 to 4). Further, from study of the different stages of development, it seems probable that at least four cells of the lesser ridge take part in forming the boundary of the tunnel of the spiral organ in a given section

of it: (1) Each of the pillars of the organ appears to be produced by a separate cell which is entirely used up in the process and its nucleus disappears; (2) the 'foot cells,' one bracing against the inner side of the basal end of each pillar, seem to be derived separately, and though they may aid in nourishing the pillars, they probably have little or nothing to do with their production.

Figures 10 and 11 are given to illustrate respectively the actual and the more usually apparent relation of the basal surface of the growing tectorial membrane to the cells of the greater epithelial ridge producing it. Each of the figures represents a few cells of the outer side of the middle third of the greater ridge, figure 10 from the 5th half turn of a pig of 15 cm. and figure 11 from the 3rd half turn of one of 14 cm. The letter *J*, indicating the line of junction between the membrane and cells, is placed on the axial side of each drawing. Figure 10 shows an appearance seldom found in the sections of embedded material, but the appearance is, I think, the correct one. By close observation of the specimen from which it was made one may note from 3 to 8 fibrils given off from the apical end of each cell. The fibrils pass almost vertically from the surface of the cells and then curve axisward in the part of the membrane previously formed. Each fibril shows a slight, elongated enlargement in its immediate junction with its cell. The number of fibrils observed from each cell probably depends upon the planes in which the cells are split by the section more than upon variation in the sizes of the cells. The cells average about 7 μ in diameter. Held computed for the rabbit from 33 to 38 fibrils per each 100 μ of the surface line of section of the greater ridge. If 5 fibrils be considered as the average per cell, then each 100 μ of surface of the ridge in the pig may involve about 70 fibrils. Taking into consideration the third dimension of the cell, as many as 25 fibrils may be given off by each cell.

That, beginning in the apical surface of the membrane, the fibrils course from the axial side outward and then basalward to their cells of origin is due to the fact that the greater ridge increases greatly in width outwardly while the fibrils are being produced. Later, toward completion of the membrane, as the

cells begin to recede along the axial side of the ridge and the ridge begins to decrease in width, the last produced ends of the fibrils are drawn axisward in the basal surface of the membrane. This drawing axisward is done only by the cells of the thicker and more persistent outer part of the greater ridge, the drawing, I think, extending in the basal surface only about as far axisward as Hensen's stripe, which it helps to form as well as helping to form the basal peripheral condensation as seen in the sections. The fibrils thus drawn axisward must form the arrangement on the basal surface of the outer part of the teased out membrane described by me as an "accessory tectorial membrane."

The increase in the number of cells in the greater ridge during its increase in width results, of course, in an increase in the number of fibrils and thus in the width of the membrane as compared with the earlier stages. If the claim made by Prentiss that the tectorial membrane is a honey-comb or chambered structure, each chamber coinciding with and produced by a cell, were true, then in the sections would be found occasionally strips as wide as the cells, walls of the chambers parallel with which the plane of section had passed. Such strips have never been seen. On the contrary, the fibrillar character is one of the most evident possessed by the membrane.

In sections in which the condition similar to that shown in figure 10 could be seen, the region of junction (*J*) appeared lighter than the earlier formed part of the membrane. This is interpreted as indicating that the interfibrillar matrix here has not, as yet, been completely formed. Figure 11 represents the condition, some degree of which is the usual appearance in all sections of dehydrated cochleae while the membrane is being formed. It is interpreted as the result of the action of the reagents, used in the preparation for sectioning, upon the last and not yet perfectly formed part of the membrane. The matrix in this region must be in a stage more readily shrunken by dehydration and clearing than in the earlier formed part of the membrane. The fibrils appear agglutinated into irregular bundles with spaces between them more or less free. In the area drawn the bundles were formed for the most part, one on

the end of each cell, similar to the way cilia often appear in brushes in sections of the uterine tube and the ducts of the testis, for example. When shrinkage and agglutination are less evident than in figure 11, the bundles are smaller and may be formed indifferently upon and between the ends of the cells. When more shrinkage has occurred the bundles may be larger and the shrinkage spaces may extend over several cells. The spaces are similar to those Rickenbacher pictured and considered as designed for the circulation of endolymph. Figures 10 and 11 are not camera drawings.

The *attachment and functional position* of the tectorial membrane are interrelated. The outspanning zone, after it is produced, becomes entirely free, first from its slight attachment to the young spiral organ (lesser epithelial ridge) and later from its parent cells, and the process by which it becomes free is the chief of the processes by which it later attains its functional position over the spiral organ. Held ('09) quite fully reviewed the ideas advanced by the different authors as to how the auditory hair cells, developed in the lesser ridge at the outer edge of the growing membrane, acquire their much more axial position well under the membrane, and I cited most of them in my former paper. I think all the processes possible have been suggested. When the younger stages are compared with the stages after birth (compare figures 7 and 8 with figure 2) it is seen that the spiral organ may become so shifted in its relative position that its hair cells come to stand under the middle of the basal surface of the outspanning zone of the membrane, and that the outer edge of the membrane may project in the 3rd half turn (and no doubt more in the 1st) well beyond the organ and even over the first 10 or 12 of the cells of Claudius. Under the narrow basal end of the membrane (fig. 4), the shifting of position is barely sufficient to bring the outer edge over the outer hair cells of the organ. In this region the greater epithelial ridge completes the membrane earlier, is active during a shorter period, and increases in width during production much less than in the apical turns. It may be noted further that in the more apical turns the outer supporting cells increase in height so much more

than the inner that the apical surface of the spiral organ becomes markedly inclined axisward.

The ideas advanced as to how the spiral organ attains its relative position under the membrane include: (1) that the organ becomes shifted axisward bodily along the basilar membrane; (2) that growth in width and thickness of the vestibular lip of the spiral limbus serves to tip and project the membrane, attached upon it, over the spiral organ; (3) that the increase in the size and height of the outer supporting cells (cells of Hensen and Deiters) presses the hair cells axisward, causes the apical ends of the pillars to lean axisward and actually slightly displaces axisward the feet of the pillars; (4) that the membrane is produced in its functioning position with reference to the organ instead of there occurring a shifting of position of either the organ or the membrane.

The latter idea, that the tectorial membrane is produced in situ without changes in its position relative to the hair cells, was naturally one of the first to be advanced. Early discarded, it has been revived by Held and adopted by Prentiss. It requires, of necessity, that in the apical turns, the thicker, outer two-thirds of the outspanning zone be produced by the spiral organ, largely after its elements have been differentiated, and by the cells of Claudius (compare figure 7 with figure 2). A stage of development has never yet been described and, I think, never been seen, certainly not in the pig, in which this thicker (and, in the apical turns, by far the greater) part of the membrane was being produced by the cells of the differentiating spiral organ and the cells of Claudius. In the pig, and in the published figures from other mammals, the few sparse, loosely arranged and unsupported fibrils described as produced by the cells of the lesser ridge, attain their maximum amount long before the differentiation of the elements of the spiral organ is completed. They are produced, I think, most rapidly in the pig before the differentiation is well under way. The cells of the lesser ridge have never been seen in a relation to the growing membrane similar to that of the cells of the greater ridge seen in all specimens, and further, what is most evidently to be the whole out-

spanning zone of the membrane is found during its production, in all trustworthy preparations of the pig, overlying the cells of the greater ridge only and associated with them only. The idea is very simple but I am convinced the actual anatomy in development does not support it. From my study of the specimens I, as yet, find no evidence that the few fibrils seen coming from the lesser ridge and continuous with the outer edge of the membrane proper can contribute appreciably, if at all, to the bulk of the membrane. I think they disintegrate for the most part at least, leaving the outer edge of the functioning membrane clean and bluntly rounded, its fibrils curving from the apical surface around the edge and axisward in the basal surface. Both Rickenbacher's and Held's figures, from other animals, show what I consider the outer edge of the later tectorial membrane extending only to the inner hair cells.

Comparison of the different stages suggests that each of the other three ideas is tenable in part, two of them especially; that the relative position of the organ to the basal surface of the membrane does become shifted and that three factors may enter:

(1) The growth increase in thickness of the vestibular lip of the spiral limbus can have nothing to do with inducing the membrane to span the spiral organ. Averages obtained from measurements in several cochleae of each of six stages from 13 cm. to the adult show that in all the turns of the cochlea, the lip acquires its adult thickness in the fetus of 14 to 15 cm. At this stage the membrane proper is still being produced in all the turns of the cochlea and does not anywhere project over the spiral organ. The thickness of the lip in the different half turns in the pig of 14 cm. and the older stages, including the adult, were found to be strikingly similar. Further, the vestibular lip attains less thickness throughout in the apical turn than in the basal, while the membrane projects over and beyond the organ more in the apical turns. So it is hardly probable that the membrane is tipped outward by increase in thickness of the vestibular lip of the spiral limbus. The width of the vestibular lip, while difficult to measure definitely, likewise seems to increase very little between 14 cm. and the adult. The small

increase in width is mostly attained in that part of the lip upon which the axial zone of the tectorial membrane is attached, that is, from the insertion of the vestibular (Reissner's) membrane to the edge of Huschke's teeth, and the edge of Huschke's teeth projects outward farther in the full term fetus and adult than in the earlier stages (compare figures 2 to 7). The average widths of this part of the lip, which is most of it, are given in table 5. The greater variations in the averages for the width at the 1st half turn are due to the varying distances from the tip end of the lip at which the knife passed, for the lip narrows suddenly in terminating in the hammulus. The slight increase in width,

TABLE 5

Giving in micra averages of the width of the vestibular lip of the spiral limbus from the insertion of the vestibular membrane to the edge of Huschke's teeth in the different sizes of the pig and the different turns of the cochlea specified

SPECIMENS		1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
Number used	Length				
3	13 cm.		125.7	125.5	151.0
4	14 cm.		159.6	152.0	159.6
4	15 cm.	152.0	190.0	171.0	186.2
2	16 cm.	68.4	174.8	182.4	155.8
3	19.5 cm.	133.0	186.2	182.4	184.3
2	22 cm.	161.7	209.0	197.6	186.2
6	near term	179.4	216.6	214.5	202.2
5	adult	193.8	206.7	206.7	190.0

shown in the table, between 13 cm. and 22 cm. can have little to do with other than the growth of the attached axial zone of the membrane. At 13 and 14 cm. the part of the lip in the 1st half turn is not sufficiently outlined to measure. Most of the increase seems to occur after 22 cm., that is, between this and the fetus at term and the adult, when the membrane is becoming and has become detached from its parent cells and from the spiral organ. This latter increase in the width of the lip may contribute a slight projection of the tectorial membrane outward and over the spiral organ. The width of this part of the lip is about the same as the width of the attached axial zone of the membrane.

(2) As seen in the drawings, the spiral organ grows in thickness between the time the tectorial membrane begins to span over it and the adult stage. The greatest increase in thickness occurs in its outer side by the increase in the length of the outer sustentacular cells, and the apical ends of these curve axisward. As a result the apical surface of the organ is made to incline axisward. The outer pillar of the organ also increases in length more than the inner pillar, and as a result of this, and perhaps of the increase in the length of the outer supporting cells also, the apical ends of the two pillars are forced axisward. At their differentiation, the apical ends of the pillars incline strongly outward (see fig. 8, 19 cm.) in conformity with the pressure of the cells of the outer part of the greater epithelial ridge. It seems evident, therefore, that the apical surface of the organ is forced axisward during growth and therefore under the tectorial membrane to a slight extent. The normal spaces between the elements of the spiral organ, including the large Nuel's space, no doubt result in part from this movement of the organ axisward. Nuel's space, however, is present in considerable size before the upgrowth of the outer supporting cells has started (compare figures 8 and 2). This space increases in passing from the basal to the apical end. Any movement of the surface possibly produced in this way is, however, not enough to account for the change in relative position of the organ evident in the apical turns. In the basal end, the great increase in the height of the outer supporting cells does not occur, thus the apical surface of the organ is not inclined axisward, though, the pillars do not lean outward as might be expected. The change in the relative position of the organ with reference to the basal surface of the tectorial membrane is less in the basal end and progressively increases in passing toward the apical end.

(3) To accomplish the very marked shift in the position of the spiral organ with reference to the basal surface of the tectorial membrane, especially in the apical turns, some process is necessary by which the entire organ is moved axisward. During differentiation of its elements, the spiral organ is situated

along the outer edge of the membrane and is even attached to this edge by the sparse fibrils formed by its cells before their differentiation is completed (fig. 7). In the adult the hair cells in the apical turns come to stand under the middle of the wide outspanning zone (fig. 2).

Pressure displacement of tissue in the direction of the least resistance is common in organogenesis and, in the adult, such displacement is a common observation of the surgeon. Comparison of the stages of the pig in the different turns of the cochlea shows that the feet of the pillars separate during the growth of the organ. The separation is greatest toward the apical end. Hensen ('71 and '73, cited from Held) found by measurement in the ox that the feet of both pillars are moved axisward. In the apical turn he found that the foot of the outer pillar shifted about 37 μ and that of the inner pillar about 95 μ, thus not only indicating actual movement but also accounting for the separation of the feet during the enlargement of the organ. I do not know between what stages of ox fetuses the measurements were made. If two elements of the spiral organ move axisward at all, it is possible that all the elements may move. If the elements move at all, it is possible that they may move sufficiently to account for the change in the relative position of the organ. During the rapid increase in the width of the greater epithelial ridge, the differentiating spiral organ is moved outward.

The cells of the greater ridge, when it is widest (in pigs from 14 to 16 cm.), are about twenty-five times greater in number than the cells, derived from the ridge, which later line the internal spiral sulcus. The cells lining the sulcus in the adult are broader than the cells of the greater ridge, but they average certainly not more than three times as broad. As shown above, at from 13 to 16 cm., the outer third of the greater ridge is thickened into a rounded elevation, its cells and nuclei being displaced apexward and its outermost cells being forced in the outward direction (figs. 7 and 8), indicating considerable growth pressure. In the later recession and disintegration of the cells of the greater ridge, as the production of the tectorial membrane is com-

pleted, the pressure must not only be relieved, but, from the marked decrease in number of cells, there may result an almost negative pressure.

Measurements show that the space occupied by the width of the greater epithelial ridge increases throughout the coils of the cochlea up to pigs of 15 to 16 cm. (when the ridge is widest) and thereafter it begins to decrease very perceptibly. The measurements were taken from the membrana propria of the epithelium of the greater ridge, at its most axial extension under Huschke's teeth (*S*, figs. 1 and 7), to the apical end of the inner

TABLE 6

Giving in micra averages of the width of the developing and developed internal spiral sulcus, measured from the membrana propria of the epithelium under Huschke's teeth to the apical ends of the inner hair cells of the spiral organ, in the different pigs and the different regions of the cochlea specified

SPECIMENS		1ST HALF TURN	3RD HALF TURN	5TH HALF TURN	7TH HALF TURN
Number used	Length				
3	9 cm.		231.0	238.7	192.5
3	11.4 cm.		284.3	292.6	223.3
3	13 cm.		277.2	292.6	227.2
3	14 cm.		300.3	269.5	231.0
4	15 cm.	254.0	330.0	297.3	274.8
2	16 cm.	261.8	323.4	261.8	177.1
3	19.5 cm.	241.8	289.5	257.2	175.6
2	22 cm.	231.0	250.3	216.4	161.8
6	near term	193.9	231.2	234.1	193.9
5	adult	188.9	232.5	219.6	130.9

hair cell of the spiral organ. The averages of the measurements are recorded in table 6. As in the other tables, some of the variations in width of the greater ridge, and internal spiral sulcus, evident in the 1st and 7th half turns are due to the varying distances from the apical and basal ends of the cochlear duct at which the knife passed. In the specimens from pigs of 9 to 11.4 cm., the 1st half turn was not completed, and in those from 13 to 14 cm., the differentiation of the vestibular lip of the spiral limbus in this turn was not sufficient for the measurement. In the 3rd half turn of the 9 cm. pig (see fig. 6) this differentiation had not taken place, and thus this

measurement is only approximate. Individual pigs of a given length vary in the degree of development of the cochlea, especially if from different litters.

It may be seen from table 6 that between pig fetuses of 9 and 15 cm. the width of the greater epithelial ridge increases appreciably, and between 15 cm. and the adult the width of the space occupied by it decreases. The increase and the decrease is evidently greatest in the apical part of the cochlea. The decrease in the 1st and 3rd half turns may be as much as one-third of the width of the greater ridge when at its maximum size and activity. During the development of the spiral organ from the lesser epithelial ridge, the apical ends of the inner hair cells are in line along the outer edge of the growing tectorial membrane. In the 3rd (and no doubt the 1st) half turn of the adult organ, these ends of the hair cells are situated along about the middle of the width of the outspanning zone of the membrane (fig. 2). In other words, it is suggested that in the apical coil of the cochlea, after the tectorial membrane is about completely produced and while the spiral organ is enlarging, the inner hair cells, and therefore the organ, may be moved axisward a distance of about half the maximum width of the greater epithelial ridge, the maximum width of the ridge representing approximately the width of the outspanning zone of the membrane produced upon it.

It is suggested that a movement axisward of the organ of about one-third the width of the outspanning zone may be induced by the pressure of the growing cells of Claudius and the enlarging supporting cells on the outer side of the organ, and be allowed by the retrogression and decrease in number of the cells of the greater ridge on its axial side. The remaining one-sixth of the distance estimated for the apical coil may be accomplished by the other but less effective processes mentioned, namely, the inclination axisward of the apical surface of the organ. Table 6 shows that the movement is not so much in the basal part of the cochlea and figures 3 and 4, the 5th and 7th half turn of the adult, show that the distance of the inner hair cell from the outer edge of the membrane is less in the basal coils than in the

apical turn. At the basal end, the greater ridge remains narrower and produces the narrow end of the membrane. Where the greater ridge is widest, there the membrane is produced widest, the reduction in the cells of the ridge is greatest, the enlargement of the outer supporting cells is greatest, and there, as a result, the change axisward of the spiral organ is greatest.

Prentiss states that measurement "shows no important change in the position of the spiral organ from the 13 cm. to the 18.5 cm. stage, nor later in the newborn" pig. He allows the inference that he measured "the distance between the inner angle of the cochlea and pillar cells," but gives no records of measurements. His averages for the 13 and 18.5 cm. stages and those for the newborn pig would have been interesting for comparison with like measurements that could be made in the specimens used here. On page 434 he describes the greater ridge as increasing in width by growth and multiplication of its cells, "carrying the spiral organ outwards," while he denies in other passages the possibility of the spiral organ being carried axisward.

With the completion of the retrogression and disintegration of the cells of the greater ridge and the simultaneous movement axisward of both the entire spiral organ and its apical surface, the thick, outspanning zone of the tectorial membrane, of necessity, becomes free. The few loosely arranged fibrils, produced by the lesser ridge before its completed differentiation into the spiral organ, may persist and remain attached to the outer edge of the membrane till torn asunder in the freeing process. There is no evidence of them on the membrane of the adult. In the fetus at term, when the process of freeing is being completed, it is possible that remnants of them may at times adhere as a delicate plexus to the outer edge of the membrane.

It is possible that the outer margin of the tectorial membrane, which, during its production, cups around the outer part of the greater ridge, may straighten outward slightly upon being freed. If so, such behavior would contribute slightly to the projection of the membrane beyond the spiral organ.

The thin axial zone remains attached upon the vestibular lip of the spiral limbus. The cells producing this are never thick,

do not undergo the growth disturbances undergone by the greater ridge proper, and the fibrous mesenchymal tissue added about them in the formation of the lip aids them in retaining the attachment. This, I am convinced, is, in the pig and probably all mammals, the only attachment of the tectorial membrane after acquiring its functioning form.

The freeing of the outer zone may not be an absolute necessity for the mediation of auditory impulses. In the birds the sensory cells, corresponding to the hair cells of the mammal, are dispersed in a simple epithelial ridge producing both them and the tectorial membrane, and the ridge not receding and disintegrating, the membrane remains attached to its parent cells. In the mammal, the otolith membranes of the maculae are homologous to the tectorial membrane, though simpler, and these remain attached in part to their parent epithelium, situated in which are the special sensory cells. Experiments with the model I have tried to construct, imitating the tectorial membrane and its environment, suggest that, were the outspanning zone attached, it could be disturbed by vibratory disturbances in the endolymph, though not so readily nor so definitely as when the zone is free. However, in the actual anatomy of the mammalian cochlea it is evident that this zone is free. Held ('09) cites Preyer for the observation that auditory reflexes do not begin in the guinea-pig till one-half hour after birth, and both Held and Rickenbacher found that the outspanning zone of the membrane is freed at about the time of birth. More suggestive are the observations of Kreidl and Yamase ('07) upon rats. As is known, the young of these rodents, unlike the guinea-pig, are born in a comparatively fetal stage of development. They found that auditory reflexes do not begin in them till 12 to 14 days after birth. Held found in rabbits, which, like the rat, are born in a fetal condition, that the outer zone of the tectorial membrane is not free at 6 days after birth, though it is entirely free in the adult stage. He does not describe cochleae of rabbits between 6 days after birth and his stages in which the zone is free.

SUMMARY

1. The bony labyrinth of the adult pig, as well as that of the pig fetus, is not fused with nor enveloped by the petrous part of the temporal bone and thus may be readily removed.

2. A fixing fluid which also slowly but sufficiently decalcifies is best used for normal appearances of the cochlear structures, and, especially with cochleae of adult pigs, it is necessary to make a small hole in the bony labyrinth to relieve pressure of gas formed within during decalcification.

3. Measured within the outer wall of the bony part of the coil, the diameter of the basal coil of the cochlea of the adult ox is 8.4 mm.; that of the adult pig, 5.8 mm.; and that of man, 6.7 mm. The height of the cochlea, from the apical side of the scala vestibuli in the apical turn to the basal side of the scala tympani in the basal turn, was found to be, for the adult ox, 6.7 mm.; for the adult pig, 4.4 mm., and for the human, 4.9 mm. The human cochlea seems to be slightly more flat than those of the ox and pig. The cochlea of the pig at term, exclusive of the outer bony wall, is slightly less in size than that of the adult pig. The slight difference is apparently due to an increase in the size of the two scalae in the adult.

4. The length of the tectorial membrane of the adult pig is estimated from measurements to be about 26 mm. or less than 1 mm. longer than in the fetus at term.

5. The attached, axial zone of the tectorial membrane of the adult pig is of practically the same width throughout the entire length of the membrane.

6. As to the proportions of the outspanning zone of the tectorial membrane of the adult, indicating its adaptedness to serve as the chief vibratory structure of the auditory apparatus: (a) it is widest and thickest at its apical end, tapering gradually and evenly to its basal end, which is its narrowest part; (b) the width of the apical end of the outspanning zone appears from averages of measurements to be 6.8 times its width at the basal end; (c) in thickness, the apical end of the outspanning zone averages in sections of it 3 times its thickness at the 7th half

turn of the coil; (d) in volume, a given short length of the apical end of the outspanning zone may be more than 41.7 times the volume of the same length of the basal end of the zone. Between the basal end and the 5th half turn, the longest interval between measurements in section, the volume of the outspanning zone may increase 90.7 per cent; between the 5th and 3rd half turns, the increase may be 57.4 per cent, and between the 3rd and the 1st half turns, it may be 29.8 per cent. These variations in the proportions are urged in support of the suggestion that the tectorial membrane is far more adapted to serve as the vibratory structure than is the basilar membrane.

7. In the adult pig, the spiral organ (of Corti) increases appreciably in both thickness and width in passing from the basal to the apical end of the cochlea.

8. That the membranous spiral lamina (basilar membrane) may be thrown into vibration by certain strong stimuli is not denied, and that the chief load carried by it, namely, the spiral organ, increases in passing from the basal to the apical end of the cochlea is considered as suggestive of its possible vibratory behavoir. The superior advantages of the tectorial membrane are enumerated and some of its possible activities compared.

9. Pig fetuses of a given length vary very much in the stages of development of the cochlea, especially if from different litters. For a given stage of development, it seems that fetuses obtained in Louisiana average smaller than fetuses obtained in California.

10. The cells of the lesser epithelial ridge, which differentiate into the elements of the spiral organ, at an early stage produce a delicate film of loosely arranged, imperfectly embedded fibrils. These have been erroneously considered by some as increasing to form an outer zone of the tectorial membrane; by others as forming a permanent attachment of the membrane to the spiral organ. It is urged that the cells of the lesser ridge, at first grading from the outer edge of the greater ridge, never actively engage in the production of fibrils, and, as they differentiate into the elements of the spiral organ, they cease the production altogether. They contribute little, if anything, to the formation of

the adult tectorial membrane, though their few fibers may remain attached to its outer edge till torn asunder in the later processes. The membrane is produced by the greater ridge.

11. With the differentiation of the spiral organ from the lesser epithelial ridge, the organ begins to increase in thickness and, though most of this increase occurs in the stages before full term, some of it seems to occur after birth. Growth changes of the organ occur least in the basal end of the cochlea. It is suggested that at least four cells of the lesser ridge take part in a given section in forming the elements comprising the walls of the spiral tunnel.

12. The outermost part of the greater epithelial ridge becomes thicker by growth pressure than the remainder, is active for a longer period, produces the thicker part and outer edge of the tectorial membrane, and its outermost cells, in the process of recession and disintegration of the ridge, seem to differentiate into the inner supporting cells of the spiral organ.

13. In the production of the tectorial membrane, each cell of the greater epithelial ridge may contribute an average of 25 fibrils to the membrane. Each fibril seems to show a slightly elongated enlargement at its junction with its cell. In the region of the immediate surface of the ridge, that of the product of the most recent activity of the cells, the interfibrillar matrix does not appear as abundant, or so completely produced, as in the older body of the membrane. This less completely formed part of the membrane shows shrinkage effects of the reagents in all sections of the cochlea.

14. With the growth in width of the greater epithelial ridge in the early stages, the differentiating spiral organ (lesser ridge) situated along the outer edge of the growing tectorial membrane, is carried outward. The developed spiral organ acquires its position well under the basal surface of the tectorial membrane almost entirely by being carried axisward during the completion of the membrane. As the cells of the greater ridge recede from the membrane they disintegrate to about one-twenty-fifth of their greatest number, thus more than relieving all growth pressure on the axial side of the spiral organ. During this proc-

ess, the outer supporting cells of the spiral organ increase in size and height, producing growth pressure on the outer side of the membrane and producing an inclination axisward of the apical surface of the organ. In the apical turn, where these changes are greatest, the hair cells of the organ may be carried axisward a distance nearly half the width of the membrane. The upgrowth of the outer supporting cells also forces axisward the apical ends of the elements of the spiral organ and in this way contributes a small part to the shift in the relative position of the hair cells. A slight increase in width of the vestibular lip of the spiral limbus may contribute a still smaller part by extending the membrane outward.

15. The outspanning zone of the tectorial membrane is, and of necessity becomes, free with the completion of the recession and disintegration of the cells which produce it, and by the movement axisward under it of the spiral organ. Such of the few fibrils produced earlier by the lesser ridge as may persist are of necessity torn away in the changes in position and all of them probably disintegrate.

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PLATES

REFERENCE LETTERS

AF, lamina of peripheral auditory nerve fibers
AAZ, attached axial zone of tectorial membrane
BM, basilar membrane
CC, cells of Claudius
DC, supporting cells of Deiters
E, endothelium lining scala tympani
ES, epithelioidal syncytium
F, auditory nerve fibers to outer hair cells
GR, greater epithelial ridge or thickening
HC, outer supporting cells (of Hensen)
HS, Hensen's stripe
HT, Huschke's (auditory) teeth
ISC, inner supporting cells of spiral organ
ISS, internal spiral sulcus
IAC, inner auditory hair cells
J, region of junction between fibrils and producing cells
L, lighter outer margin of young greater ridge
LF, fibrils produced by cells of lesser epithelial ridge
LM, liquefying mesenchymal tissue
LR, lesser epithelial ridge or thickening
NS, Nuel's space
OZ, outspanning zone
PC, peripheral condensation of incomplete structure
SG, spiral ganglion
SL, spiral ligament
SP, spiral prominence, stria vascularis
ST, scala tympani
SV, scala vestibuli
TM, tectorial membrane
VL, vestibular lip of spiral limbus
VM, vestibular (Reissner's) membrane

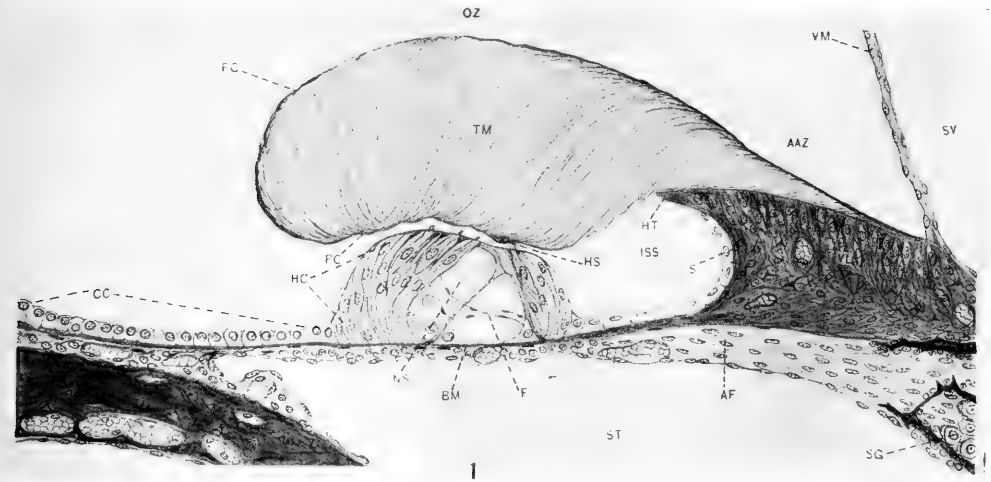
PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 9 are reproduced to scale

1 Section across the 1st half turn of the spiral organ and its tectorial membrane, cochlea of adult hog.

2 Section across 3rd half turn of spiral organ of adult hog; same cochlea as figure 1.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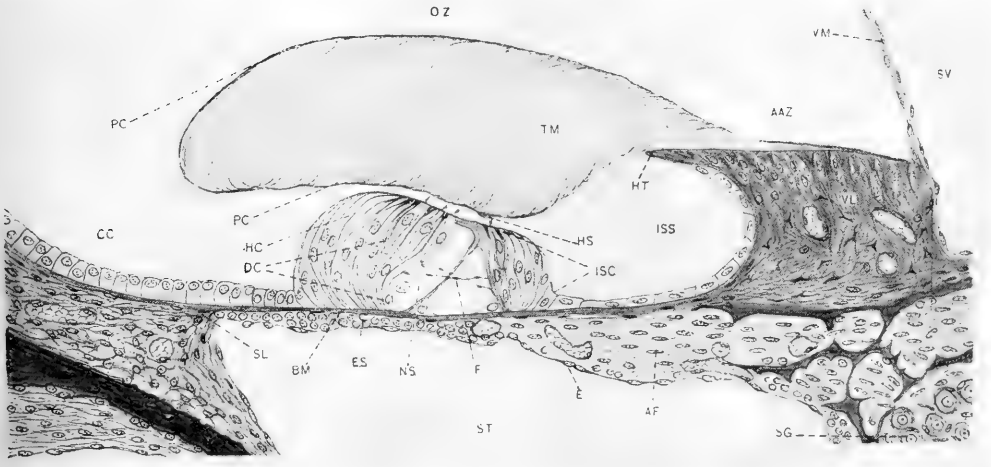
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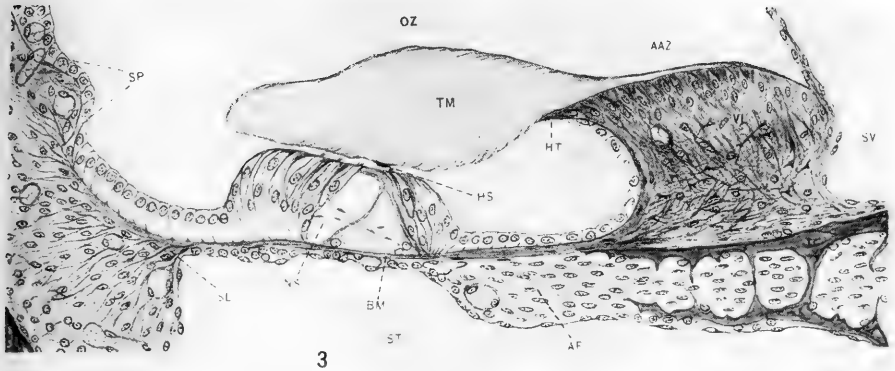
2

PLATE 2

EXPLANATION OF FIGURES

3 Section across 5th half turn of spiral organ of adult hog; same cochlea as figures 1 and 2.

4 Section across 7th half turn of spiral organ of adult hog; same cochlea as figures 1 and 2, and 3.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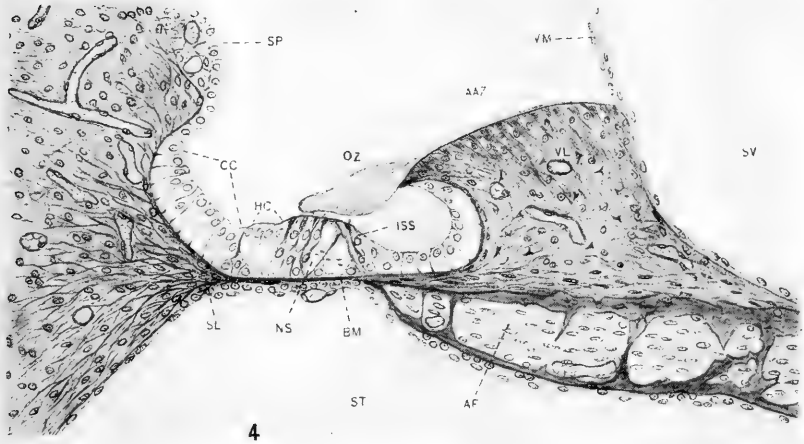
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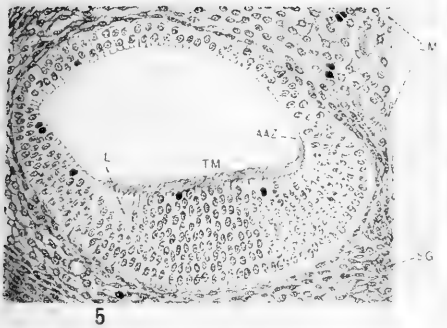
PLATE 3

EXPLANATION OF FIGURES

5 Section of 3rd half turn of cochlear duct of pig of 5.5 cm., showing thickening of epithelium of axio-basal side and beginning of tectorial membrane.

6 Section of 3rd half turn of cochlear duct of pig of 9 cm., showing lesser epithelial ridge and the fibrils produced by it, and advancement in production of the tectorial membrane.

7 Section of 3rd half turn of developing tectorial membrane and differentiating spiral organ of pig of 14 cm.



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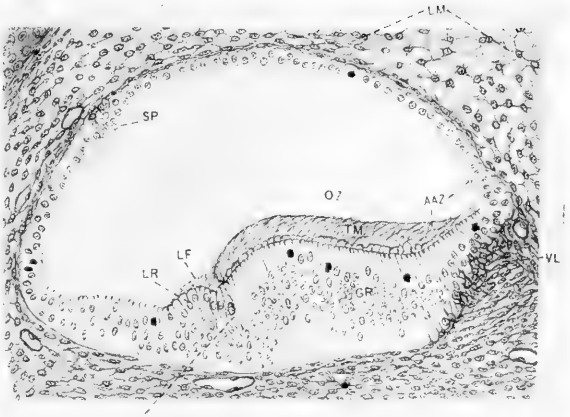

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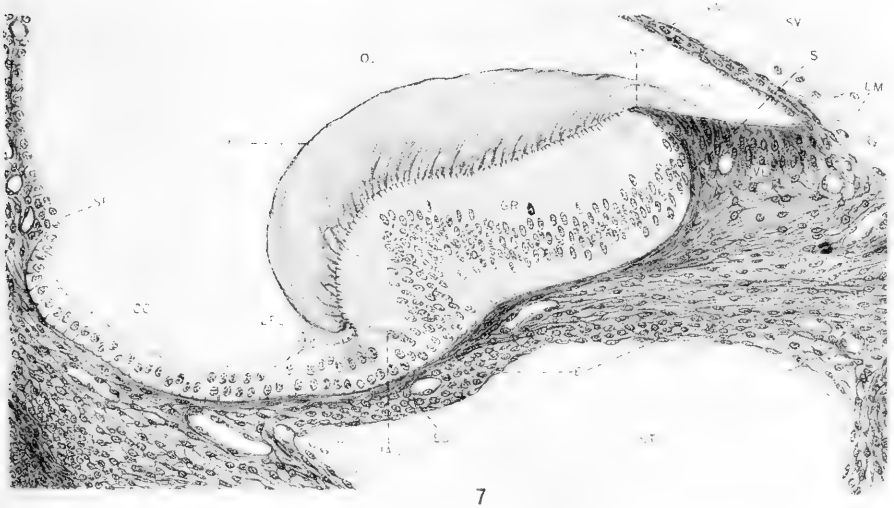

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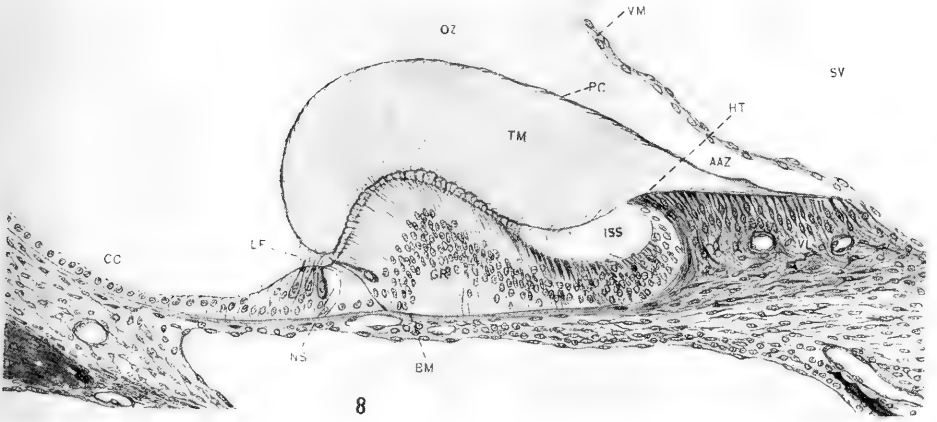
PLATE 4

EXPLANATION OF FIGURES

8 Section across 3rd half turn of developing tectorial membrane and differentiating spiral organ of pig of 19.5 cm. The recession of the cells of the axial side of the greater epithelial ridge is well under way.

9 Section across 3rd half turn of spiral organ and its tectorial membrane of pig of 22 cm. The recession and disintegration of the cells of the outer part of greater epithelial ridge is nearing its completion, leaving the inner supporting cells of the spiral organ.



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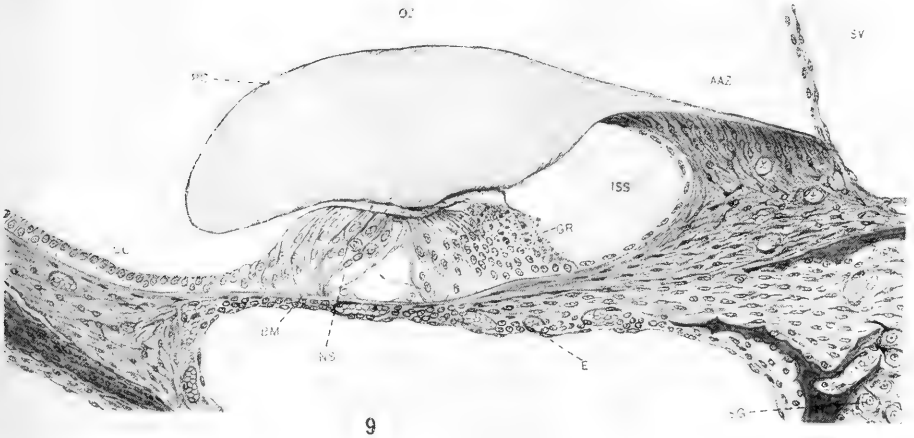

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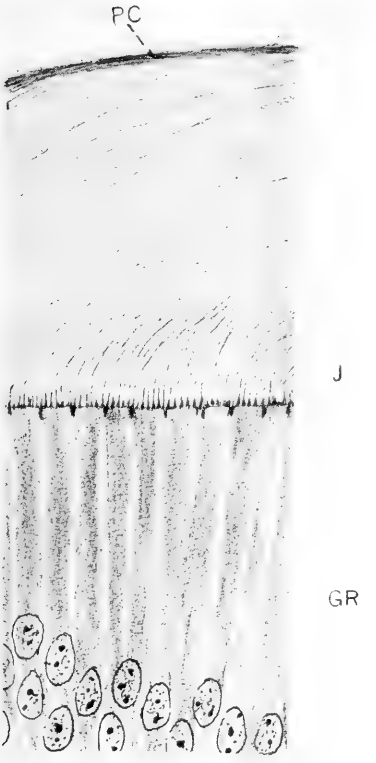
PLATE 5

EXPLANATION OF FIGURES

10 Drawing from the outer part of the middle third of the greater epithelial ridge in the 5th half turn of the cochlea of a pig of 15 cm., showing, at *J*, detail of the relation of the cells of the ridge to the fibrils and tectorial membrane they produce.

11 Drawing from the outer part of the middle third of the greater epithelial ridge in the 3rd half turn of the cochlea of a pig of 14 cm., showing, at *J*, a usual appearance in sections of the relation shown in figure 10, interpreted as due to shrinkage produced by the reagents used in preparing material for sectioning.



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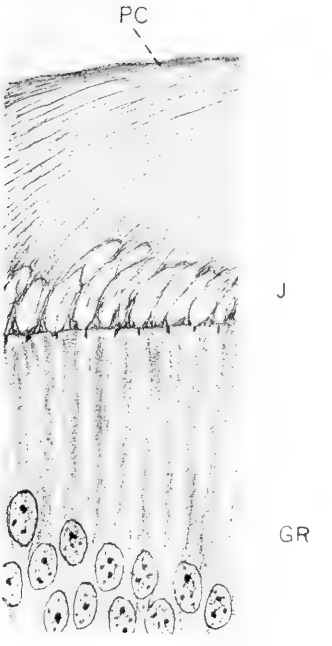

11

EFFECTS OF ACUTE AND CHRONIC INANITION UPON THE RELATIVE WEIGHTS OF THE VARIOUS ORGANS AND SYSTEMS OF ADULT ALBINO RATS

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TWO FIGURES

CONTENTS

As emphasized by the author in a previous paper (Jackson '13), there is great need of a series of comprehensive growth norms, which would serve as a basis for experimental work and give a better insight into the growth process in animals. Through the

investigations of Donaldson, Hatai, Jackson, Lowrey and others, considerable progress has been made toward the establishment of a growth norm for the albino rat. Since the normal growth process and the relative weights of the organs are frequently modified by malnutrition from various (sometimes unsuspected) causes, it is clearly of great importance to know the effects produced by inanition. In drawing conclusions from the results of any experiment upon animals, the possible effect of inanition should always be kept in mind. The present investigation upon this subject, which was begun at the University of Missouri, has been continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. The grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc.

MATERIAL AND METHODS

The material included 21 well-nourished adult albino rats (Mus Norvegicus albinus) of unknown age. Two of these (O 5 and O 6) were derived from the Missouri colony and 19 from a local Minnesota stock. Of the 21, 4 were females and 17 males. They had been fed chiefly upon grain (oats and corn) with occasional meat and vegetables, and were placed upon a bread and milk diet for some days before beginning the experiment. During the experiment they were kept in cages with wire-net bottoms, allowing the feces (which might otherwise be eaten) to drop through.

Fifteen of the rats were used for the acute inanition experiment. They were allowed plenty of water, but no food otherwise. They were weighed daily and the individual records kept. The initial body weights varied from 182 to 367 grams (table 3). They were killed after 6 to 12 (average 9) days,[1] the total loss in body weight varying from 25 to 39 per cent (average loss,

[1] Bell ('11) found that the wild brown (Norway?) rat in captivity would live only 3 or 4 days, with loss of 25 or 30 per cent in body weight, when water is supplied but food withheld. Among animals in general, the older or larger are able to withstand inanition longer than the younger or smaller animals, probably on account of less rapid metabolism in the former.

about 33 per cent). This represents moderately severe, but not extreme inanition, as a well-nourished adult albino rat will probably lose 40 per cent of his body weight before death, if kept warm. When pushed to the extreme, however, the rat is likely to die unexpectedly, with undesirable post mortem changes. These might affect not only the structure but also the weight of the organs, through congestion and coagulation of blood, and should therefore be avoided. Three of the rats included (table 3, rats Nos. M 9, M 13, and S 28) were found dead, and in this case merely the weights of the head, extremities, integument, skeleton and musculature (which did not appear to be materially affected) are recorded.

In the rats subjected to acute inanition, the daily loss in body weight was fairly uniform in some individuals, but varied greatly in others. As might be expected, the loss is usually (though not invariably) greatest on the first day of the experiment, probably due to the reduction in contents of the stomach and intestines. In most cases (11 out of 15) the loss in body weight is greater during the first half than during the second half of the experiment, though the difference is usually slight. There is no constant relation between the percentage loss in body weight and either the initial body weight or the length of the inanition period. In general, the loss in body weight of albino rats during acute inanition is very similar in extent and variations to that found by Chossat ('43) for pigeons. Chossat demonstrated that the loss in body weight is greatest in the first third of the inanition period, slightly less in the last third, and considerably less in the middle third.

In order to compare the results of chronic inanition, 6 rats (all males) were fed upon a bread and milk diet gradually decreased in amount, so as to reduce the body weight about 1 per cent (of the initial weight) daily. Water *ad libitum* was also supplied. This was continued until the loss in body weight amounted to an average of about 36 per cent, which required about 5 weeks (table 3). The amount of food (entire wheat (Graham) bread soaked in whole milk), required for this purpose varied somewhat in individuals, but was approximately 10 per

cent of the body weight daily. This is usually found insufficient for the maintenance of body weight in adult albino rats at ordinary room temperature. One rat (No. M 4) was found dead; the others were killed.

At the end of the experiment, in both acute and chronic inanition, the rats were killed by chloroform and the various organs and parts carefully dissected and weighed. The technique followed is that described by Jackson ('13) and Jackson and Lowrey ('12). The skeleton was first prepared as heretofore by carefully dissecting off the musculature, leaving the periosteum, ligaments and cartilages intact. This is recorded as 'ligamentous skeleton.' In most cases the periosteum and ligaments were then removed by maceration for about one hour in 1 per cent 'gold dust' solution, following which the bones were cleaned under water with forceps and camel's hair brush.[2] So far as possible, the cartilages, including the intervertebral disks, were left intact and included with the skeleton. The skeleton was then taken from the water (excess moisture removed by filter paper) and weighed as 'cartilaginous skeleton.' The skeleton was then dried for 30 days in a dust-proof case at ordinary room temperature. This was found insufficient to remove all moisture, so the skeletons were finally dried several days in an oven at about 90°C., until a constant weight was reached.

Portions of the various tissues and organs were preserved for histological study, which will be considered in a later paper.

As heretofore, in calculating the percentage weights, the net body weight (gross body weight less intestinal contents) is taken. The percentage weights of the organs are therefore slightly higher than they would be if calculated upon the basis of the gross body weight, but the difference is usually negligible, as the intestinal contents form only 2 or 3 per cent of the body weight during inanition.

[2] For this method of preparing the cartilaginous skeleton, I am indebted to Professor Donaldson of The Wistar Institute. He states (in a personal communication) that the fresh skeleton is apparently slightly reduced (about 3 per cent) in weight by the 'gold dust' treatment, but this difference is so small as to be practically negligible.

For purposes of comparison, the previous observations upon the normal weights of the various organs of the albino rat by Donaldson ('09), Hatai ('13), Jackson ('13) and Jackson and Lowrey ('12) were utilized. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of six steers, varying from very fat to thin, are cited through the courtesy of Professors Trowbridge and Moulton.

The averages given in table 3 are the arithmetical means of the corresponding individual observations in the acute and the chronic inanition series, respectively. In estimating the normals at corresponding initial body weights for comparison, however, merely the averages were used. That is, the absolute weight of each organ corresponding to the *average* body weight was estimated (from data already available for the normal rat), and the corresponding percentage weight calculated. This is not quite so accurate as it would have been if the corresponding normal for each individual had been estimated, and the mean of these taken for comparison with the averages in the acute and chronic inanition series. However, the difference is slight and apparently not sufficient in the present series to justify the more laborious method of making the individual estimates.

In view of the comparatively small number of observations, and the known variability, especially of some of the organs (Jackson '13), the conclusions reached in the present paper are by no means to be considered as final. It is believed, however, that they are sufficient to give an approximate idea of some of the more obvious and important changes in weight during inanition. As such they may be useful, even though limited in number, and may lead to further and more extensive investigations in the case of various individual organs. In general, the amount of variation found is sufficient to demand the exercise of caution in drawing conclusions from an insufficient number of observations, as is sometimes done in experimental work.

Although the literature on the subject of inanition is extremely large, comparatively few specific data directly bearing upon the question of the changes in the weight of the various organs are to be found. These are referred to later under the

appropriate headings. Papers dealing merely with histological changes are usually not mentioned. Extensive references to the literature on inanition will be found in the articles by Morgulis ('11) and Mühlmann ('99).

LENGTH OF BODY AND TAIL

With reference to the body weight compared to the body (nose-anus) length at the end of the inanition period, the normal relations as determined by Donaldson ('09) were used for comparison. As might be expected, the body weight following inanition, both acute and chronic, is found to be lower than normal for corresponding trunk length, since the skeleton is known to be in general but slightly affected by inanition. The difference, however, is surprisingly small. It is a noteworthy fact that the *initial* body weight of the animals used in he experi-

TABLE 1

	ACUTE INANITION SERIES: GRAMS	CHRONIC INANITION SERIES: GRAMS
Normal gross body weight (theoretical, Donaldson) corresponding to body length at end of inanition period, average (from individual calculations)	191	147
Actual gross body weight found at beginning of the inanition period, average (table 1)	255	214
Actual gross body weight found at end of the inanition period, average	170	136

ments is far greater than that normal for the body length at the *end* of the inanition period. (The length cannot be accurately measured in the living animal without anesthetics at the *beginning* of the experiment.)

Thus, as shown in table 1, the body length at the end of the inanition period corresponds to a body weight much nearer to the final than to the initial weight of the animals subjected to inanition.

There are two possible explanations for this surprisingly close approach to the normal in the body weight after inanition. It is possible that the rats at the beginning of the experiment were

somewhat too heavy for the normal at corresponding body length. This might account for the result, even on the assumption that the body length has remained constant during the period of inanition. On the other hand, it is more probable that there has been an actual slight decrease in the trunk length during inanition (probably due to shrinkage of the intervertebral disks), which would equally well account for the facts observed. Individual variations must also be kept in mind, as well as the possibility that the normal ratio of body weight to body length in the strain of rats used may differ somewhat from the normal, as determined by Donaldson.

The theory of a decrease in the trunk length during inanition is strengthened by the apparently changed ratio of tail length to body length. In a separate paper (Jackson '15) I have shown that the normal ratio of the tail length to body length in the albino rat increases from an average of about 0.36 in the newborn to 0.88 at 6 weeks (body weight 50 grams), decreasing slowly to about 0.85 at a body weight of 200 grams and to about 0.80 at 300 grams. (The lower ratio found in the heavier rats is partly due to the absence of females, in which the tail averages relatively longer than in the males.) Thus it appears that in rats with body weights corresponding to the initial weights in the inanition series, the normal average ratio of tail length to body length should be between 0.80 and 0.85. It is impracticable to make the actual measurements on the living animals, although this might be done by the use of anesthetics. The data given in table 3 show that at the end of the inanition period, however, the average ratio in the acute inanition series is 0.93, and in the chronic inanition series, 0.97. Thus it appears that inanition in adult albino rats tends to produce relatively long-tailed individuals, due probably to a shrinkage in the trunk length.

Hatai ('08) reached the opposite conclusion, viz., that underfeeding produces *short-tailed* individuals; but his observations were upon younger, growing rats, in which the conditions might be somewhat different. Morgulis ('11) in the salamander, Diemyctylus, likewise found a greater shrinkage in the tail during inanition, while Harms ('09) found the converse to be true in

Triton. This question is discussed more fully in the paper above referred to (Jackson '15), the conclusion being that inanition in young rats also tends to produce relatively long-tailed individuals.

HEAD

The head (fig. 1; table 3) in acute inanition averages about 11.2 per cent of the body weight, varying from 10 per cent in the larger rats to 13.9 per cent in the smaller. Normally (Jackson '13; Jackson and Lowrey '12) in rats corresponding to the body weight at the beginning of the experiment, the head should range from about 8 per cent in the larger rats to 10 per cent in the smaller, the average for the group being about 9 per cent. Thus we may assume that during the period of acute inanition the head has increased from an average of about 9 per cent to about 11.2 per cent of the body. This is an increase of about one-fourth in the relative size of the head.

Since the whole body has lost an average of one-third in absolute weight, it might appear at first glance that the head has remained nearly constant in absolute weight. This, however, is not true. If the body weight decreased one-third while the head remained constant in absolute weight, the *relative* (percentage) weight of the head would increase in the ratio of 2:3 or from 9 to $13\frac{1}{2}$ per cent. A study of the absolute weights of the head, compared with the normal, at the beginning of the experiment shows that the head has actually lost weight, but in much smaller proportion than the body as a whole. This is what is to be expected since, as will be shown later, the brain, eyeballs and skeleton in general lose but little or none in absolute weight during inanition; while the loss in fat (some of which is on the head) is great, and the loss in the integument and musculature is in nearly the same proportion as in the body as a whole.

The head of the 6 rats subjected to chronic inanition averages 11.4 per cent of the body weight, slightly higher than in acute inanition. The rats used in chronic inanition averaged smaller in body weight (table 3), and the normal relative initial weight of their heads should therefore be slightly higher, about 10 per cent (instead of 9 per cent, as in the acute inanition group).

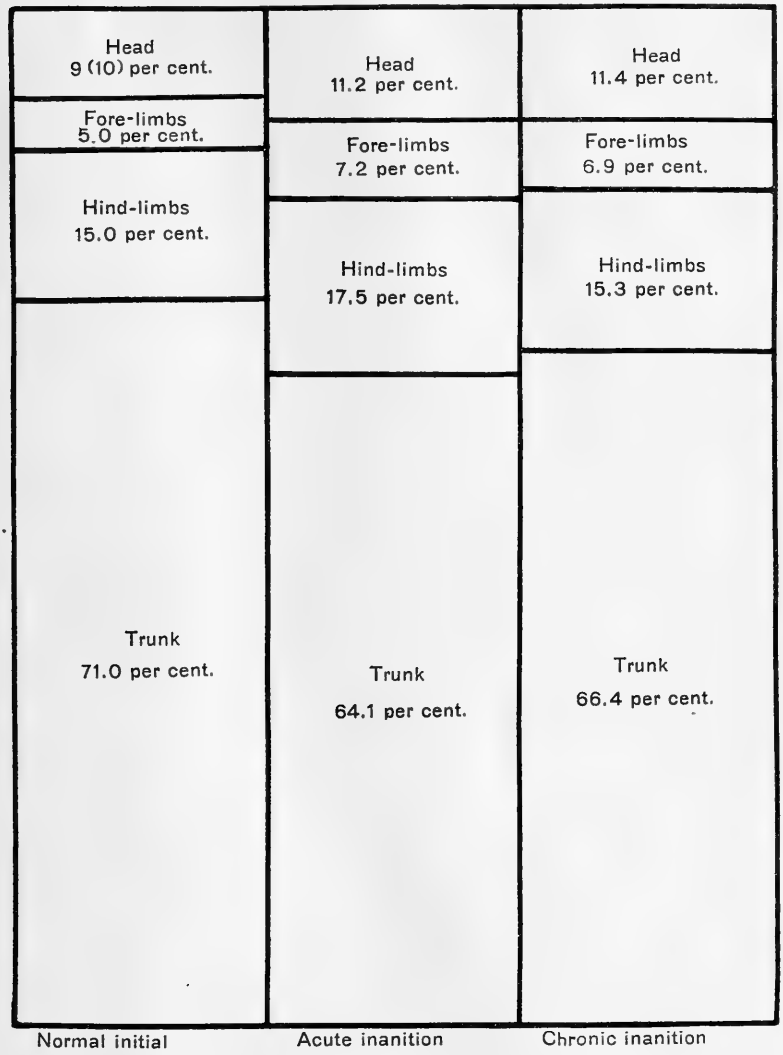

Fig. 1 Diagram representing the relative (percentage) weights of the head, trunk and extremities of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

EXTREMITIES AND TRUNK

The extremities were separated from the trunk at the shoulder and hip-joints, respectively. From figure 1 and table 3 it is seen that the *fore limbs* formed an average of 7.2 per cent of the body in the acute inanition series, and 6.9 per cent in the chronic inanition series. According to Jackson and Lowrey ('12), in the normal adult rat the fore limbs form about 5 per cent of the body. Consequently it would appear that there has been a relative increase in the weight of the fore limbs during inanition, especially during acute inanition. This increase is greater than would be expected, since (as will appear later) the skeleton is the only important constituent of the limbs which increases in relative weight during inanition, the integument and musculature remaining relatively constant. The conclusion as to the fore-limbs should be regarded as uncertain, however, because of: (1) the difficulty in separating the limbs (especially the skin) in a uniform manner; (2) the comparatively small number of observations upon which both the normal and the experimental average is based; (3) the apparent lack of agreement between the results for the entire fore limbs and for their components, skin, skeleton and musculature. It is possible, on the other hand, that the losses for skin, skeleton and musculature are not uniform in all parts of the body. Chossat ('13) has shown, for example, that the great pectoral muscles in pigeons lose relatively much more than the remainder of the musculature during inanition.

The *hind limbs* (fig. 1; table 3) in the normal adult form about 15 per cent of the body (Jackson and Lowrey). As might be expected, this is slightly increased, to 17.5 per cent, in the acute inanition series, probably on account of the relatively heavier skeleton. In the chronic inanition series, the hind limbs average 15.3 per cent, or about the same as normal. This may be explained as due to the relatively greater loss in the integument and musculature during chronic inanition (as will be shown later), this loss tending to counterbalance the relative increase in the skeleton.

The *trunk* (fig. 1; table 3) is measured by substracting the weight of the head and extremities from the net body weight.

It forms normally about 0.71 per cent of the adult body (Jackson and Lowrey). During inanition, the trunk becomes relatively smaller, averaging 64.6 per cent in the acute inanition series (64.1 per cent, corresponding to the average of 11.2 per cent for the head in a larger series), and 66.4 per cent in the chronic inanition series. The relative decrease in the trunk of course counterbalances the relative increase in the head and extremities.

INTEGUMENT

As shown in figure 2 and table 3, the integument (which includes the skin and appendages, hair and claws) is fairly uniform in its relative weight, averaging 18.9 per cent in acute inanition (or 19.1 per cent in those cases in which the viscera were weighed), and 17.8 per cent in chronic inanition. The average integument for normal adults (fig. 2) corresponding to the initial size of these rats forms about 18 per cent of the body weight (Jackson and Lowrey '12). It is therefore evident that during both acute and chronic inanition in adult albino rats the loss in weight of the integument is nearly proportional to that of the body as a whole, so the relative (percentage) weight remains almost the same.

In absolute weight, the integument has apparently decreased from about 45.9 grams, the normal at the average initial body weight in the acute inanition series (255 grams), to an average of 31.6 grams, as shown in table 3. This would correspond to a loss of 31.2 per cent in the weight of the integument. In the chronic inanition series, the corresponding decrease is from 38.5 to 23.7 grams, a loss of 38.4 per cent. Apparently, therefore, the loss in the weight of the integument is relatively slightly greater during chronic than during acute inanition.

The data found in the literature concerning the relative loss of the integument during inanition are not in agreement. In the dog, Aron ('11) states that the skin loses relatively more than the muscles (therefore, more than the body as a whole); Voit ('05 b) cites data showing a relative increase in the (fat-free) skin; while Falck's ('54) data show the skin relatively unchanged in weight. In the rabbit and cat, observations by Pfeiffer ('87), Voit ('66)

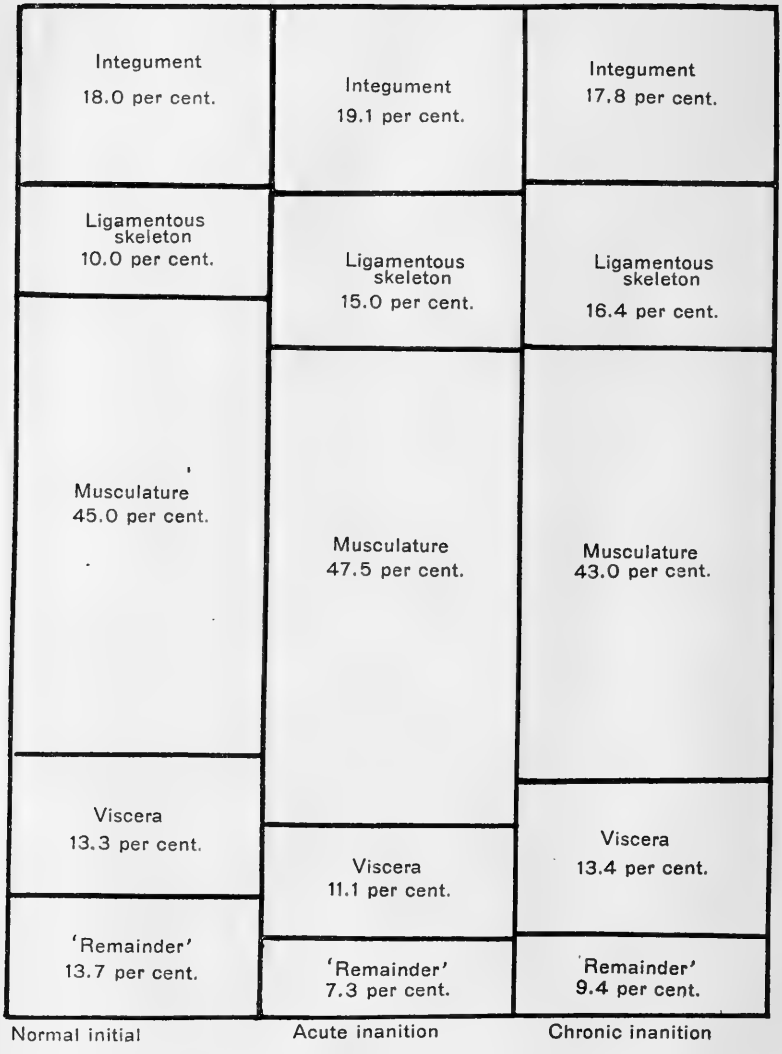

Fig 2 Diagram representing the relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') of adult albino rats. The first column represents the relations found in normal rats corresponding to the average initial body weight of those used in the experiments. The second column represents the relations found in the acute inanition series, and the third column those found in the chronic inanition series.

and Sedlmair ('99) show a relative increase in the skin during inanition. Much of the variation is doubtless due to the differences in the relative amount of fat present in the integument of the normal animal. The subcutaneous fat is not abundant in the rat. Some unpublished data from the Missouri Agricultural Experiment Station upon a series of steers show that the percentage of the hide increases from 5.5 per cent in a very fat animal to 8.5 per cent of the body in a thin animal.

SKELETON

The ligamentous skeleton (table 3; fig. 2) forms an average of 15.3 per cent of the body after acute inanition (15.0 per cent in the series in which the viscera were weighed), and 16.4 per cent after chronic inanition. Since the average for the normal rat corresponding to the initial body weight of the animals used is only about 10 per cent of the body weight (Jackson and Lowrey '12), it is evident that while the body weight has decreased one-third during the period of inanition, the ligamentous skeleton has decreased little or none in absolute weight. If the normal skeleton formed 10 per cent of the body, and remained constant while the body lost one-third in weight, the *relative* weight of the skeleton would be increased one-half, or to 15.0 per cent. This corresponds with the observations as closely as could be expected.

In terms of absolute weight, assuming that the skeleton formed 10 per cent of the normal initial weight, the skeleton apparently decreased from 25.5 to 25.4 grams in the acute inanition series (loss of 0.4 per cent); and *increased* from 21.4 to 21.8 grams in the chronic inanition series (*gain* of 1.8 per cent). No great stress can be laid upon the accuracy of these figures, however, on account of the small number of observations, and the variability and uncertainty as to the normal weight.

The cartilaginous skeleton, including the bones and cartilages after removal of the ligaments by maceration (as previously described), forms an average of 10.9 per cent of the body in seven cases of acute inanition, and 12.4 per cent in the chronic inani-

tion series (table 3). No published observations upon the normal cartilaginous skeleton are available for comparison. Professor Donaldson of The Wistar Institute, however, has very kindly sent me a series of observations upon the weight of the cartilaginous skeleton in normal albino rats. The average, in 10 cases with body weight varying from 194 to 426 grams, forms about 6.7 per cent of the (gross) body weight. In this series, however, I understand that the intervertebral cartilages were not preserved, so this figure is probably somewhat too low for use as a normal to be directly compared with my observations upon the cartilaginous skeleton in inanition. The normal adult cartilaginous skeleton, including the intervertebral cartilages, would *probably* form about 7 per cent (or slightly more) of the net body weight.

The dried cartilaginous skeleton (table 3) formed an average of 5.48 per cent of the net body weight in 7 cases of acute inanition, and 6.03 per cent in the 6 chronic cases. Thus it is evident that in each series the cartilaginous skeleton is composed of approximately half dry substances and half water. In the acute inanition series, the dry substance in the cartilaginous skeleton averages 50.5 per cent (range, 45.1 to 54.6 per cent). In the chronic series, the dry substance averages 49.2 per cent (range, 45.5 to 55.7 per cent). If case M 5 be excluded (which appears to be exceptional or erroneous), the average for the chronic series would be still lower, or 47.9 per cent. In any event, the dry content appears to be slightly lower and the water content correspondingly higher on the average in the chronic inanition series, which might be expected.

Data upon the normal composition of the skeleton of the rat for comparison are scarce. In one case which I have observed (at 10 weeks) the net body weight was 115 grams, ligamentous skeleton 9.91 per cent of body weight, fresh cartilaginous skeleton 5.57 per cent, and dry cartilaginous skeleton 2.97 per cent. In this case the dry substance formed 53.4 per cent of the cartilaginous skeleton. Lowrey ('13) in two albino rats (body weight 267.5 grams) finds the *ligamentous* skeleton forming about 9 per cent of the body weight, and containing 52.6 (52.1 to 53.1)

per cent of dry substance. (This would probably be slightly higher than the dry content of the *cartilaginous* skeleton, however, on account of the higher percentage of water in the ligaments.) Further data are necessary before final conclusions can be reached, but it appears that in the rat (as in other animals) the percentage of water in the skeleton is increased during inanition.

It is a well known fact that in general the skeleton loses comparatively little during inanition, and thus increases greatly in relative (percentage) weight. In a series of six steers, the skeleton varied from 10.6 per cent of the body in a very fat animal to 19.3 per cent in a thin animal. (Data from the Missouri Agricultural Experiment Station.) An apparent slight loss in absolute weight of the (cartilaginous?) skeleton has been observed during inanition as follows: pigeons, by Chossat ('43), 3 per cent; cats, by Voit ('66), 14 per cent; little or none by Sedlmair ('99); dogs, little or none by Voit ('05 a) and Falck ('54); rabbits, little or none by Gusmitta ('84), Weiske ('95) and Pfeiffer ('87).

Since the calcified framework is but little affected in volume during inanition, it is evident that there can be but little, if any, loss in absolute weight of the skeleton. It is at least theoretically possible that there may even be a slight *increase* in its weight, since the fat in the bone-marrow is replaced by a mucoid substance (Jackson '04) presumably of higher specific gravity. Examination of bones, therefore, invariably shows a marked increase in water content during inanition. As Sedlmair ('99) states (p. 33): "Es giebt kein einziges sicheres Beispiel für einen geringeren Wassergehalt der Knochen hungernder Tiere; alle Forscher (Chossat und Lukjanow an Tauben, Gusmitta, C. Voit und Schöndorff an Hunden, Weiske an Kaninchen) fanden darin einen höheren Wassergehalt." This would naturally vary in different bones, and in different animals, according to the relative fat content. Small animals, like the rat, have in general relatively much less marrow fat than larger animals.

MUSCULATURE

The musculature (table 3; fig. 2) forms an average of 47.6 per cent of the body in the acute inanition series, which is slightly higher than the average for the normal adult rat (which is about 45 per cent, Jackson and Lowrey '12). The musculature in the chronic inanition series is somewhat lower, the average being 43.0 per cent of the body weight. This would indicate a somewhat greater loss relatively in chronic inanition.

In terms of absolute weight, the musculature would appear to have decreased from 114.75 to 79.25 grams (a loss of 30.9 per cent) in the acute inanition series; and from 96.30 to 57.0 grams (a loss of 40.8 per cent) in the chronic inanition series.

There is much variation in the weight of the musculature following inanition, judging from data available in the literature. The statements usually refer to acute inanition. According to Gaglio ('84), while the body weight of the frog loses 56 per cent the musculature loses 85 per cent of its weight. The musculature appears to suffer a slight relative loss (decrease in percentage weight) in dogs (Falck '54; Voit '05 b) and cats (Sedlmair '99), while it remains nearly unchanged in the rabbit (Pfeiffer '87; Voit, '05 b). On the other hand, data from a series of steers slaughtered in the Missouri Agricultural Experiment Station indicate a relative increase in the musculature from about 33 per cent of the body weight in a very fat animal, to 44 per cent in a lean animal, due probably to the earlier loss of the fat. Voit ('66) finds a slight relative increase in the musculature of the cat. Lasarew ('97) in an extensive series of guinea-pigs subjected to various degrees of inanition found that the loss of the musculature is somewhat less than that of the body fat in the earlier periods, but greater in the later periods. While the body weight lost 10 per cent, the musculature lost only 7.28 per cent of its weight; but the loss of the musculature was much greater at later periods. This would perhaps explain the relatively greater loss of the musculature during chronic inanition.

VISCERA AND REMAINDER

The average for the total visceral group in the normal adult rat, according to Jackson and Lowrey ('12; based upon only a few observations) is about 13.3 per cent of the body weight. In the acute nanition series (table 3; fig. 2) the average found is 11.1 per cent; while n the chronic series it is 13.4 per cent. This would seem to indicate that the loss in weight is relatively greater in acute than in chronic inanition. As a matter of fact, the great majority of the individual organs, as will be seen later, show a greater relative loss during chronic inanition. The liver and spleen are exceptions, however, and the large bulk of the former overbalances the other viscera when all are grouped together. On the whole, there is not much change in the relative weight of the visceral group; but there is, however, much variation among the individual organs, as will be seen later.

Aron ('11) states that the organs lose more than the musculature during inanition in dogs. Data by Voit ('05 b) show a decrease in the relative size of the viscera in the rabbit, but not much change in the dog.

The 'remainder' is the amount obtained by substracting from the net body weight the weight of the skin, skeleton, musculature and visceral group. It includes the loss by evaporation and escape of liquids, a few small unweighed organs, and the dissectable masses of fat. In the normal adult rat, the 'remainder' forms about 13 per cent of the body weight (Jackson and Lowrey '12). In the chronic inanition series, the average is 9.4 per cent. In the acute series the average is 7.3 per cent. The decrease in the relative weight of the 'remainder' is probably due chiefly to loss of fat.

BRAIN

The average for the brain in the acute inanition series (table 1) is 1.18 per cent of the body weight. By means of the tables and curves constructed by Donaldson ('09), the (theoretical) normal weight of the brain can be derived, corresponding to any given body weight. This gives an average of 0.78 per cent for the assumed normal weight of the brain at the beginning of the

experiment (gross body weight, 244 grams). Since the average loss in body weight during the inanition period is one-third, it is evident that the brain has lost but little if any in absolute weight.

The theoretical average initial absolute weight of the brain is about 1.902 grams, forming 0.78 per cent of the (gross) body weight (Donaldson '09). According to Donaldson's ('08) table 1, the brain weight observed in six cases averaged 1.900 grams, the theoretical being 1.905 grams, at the body weight of 245 grams. If this absolute weight of the brain remained constant, while the body weight lost one-third (33.9 per cent), the final percentage weight of the brain would be increased about one-half, or to 1.17 per cent. The average absolute weight of the brain actually observed at the end of the period of acute inanition is 1.8046 grams, corresponding to an average of 1.18 per cent of the (net) body weight.[3] This weight is slightly less than would be expected if the brain remained constant in absolute weight, indicating a loss of about 5.1 per cent in the absolute weight of the brain during inanition.

The observations upon the brain in chronic inanition (table 3) indicate a similar condition. The average brain in this series weighs 1.743 grams and forms an average of 1.33 per cent of the (net) body weight. (This is the mean of the individual percentages; 1.743 grams would form 1.31 per cent of the average net body weight, 132.5 grams; or 1.28 per cent of the average corresponding gross body weight, 136 grams.) According to calculations from Donaldson's ('09) data, the average brain corresponding to the body weight at the beginning of the experiment (213.7 grams) should weigh 1.866 grams, forming about 0.87 per cent of the body weight (the percentage being higher than in the acute inanition series on account of the smaller average body weight).[4] This is slightly higher than the result to be expected on the assumption that the absolute brain weight has remained

[3] This is the mean of the individual percentages, as shown in table 3. The average brain weight, 1.8046 grams, would correspond to 1.13 per cent of the corresponding average gross body weight, 160 grams, at the end of the experiment.

[4] According to Donaldson's ('08) table 1, corresponding to a body weight of 215 grams, the average of 8 brains, both sexes, was 1.873 grams, the corresponding theoretical weight by formula being 1.871 grams.

constant, while the body weight has decreased about one-third (36.1 per cent). In other words, the results indicate a loss of about 6.6 per cent in the absolute weight of the brain during chronic inanition, which is slightly greater than the apparent loss (5.1 per cent) during acute inanition. A larger series of observations would be necessary, however, to draw any final conclusions with precision.

The data agree fairly well with the results of Hatai ('04) who found an estimated loss of about 5 per cent in the weight of the brain of young rats during chronic inanition. Donaldson ('11), however, found in still younger rats an actual *increase* of 3.6 per cent in the brain weight during chronic inanition (body weight held constant from age of 30 days to 51 days); while my own observations (Jackson '15) indicate little or no change in the brain weight under these conditions.

It has long been known that of all the organs of the body, the central nervous system apparently suffers least in weight (if at all) during inanition. Thus Chossat ('43) found no loss[5] in the weight of the brain in starved pigeons, with loss in body weight of about 40 per cent. Falck ('54) in dogs, and Lasarew ('97) in guinea-pigs, found little or no loss in the weight of the brain and cord during inanition. Bowin ('80) and Pfeiffer ('87) found an absolute decrease (but relative increase) in the brain weight of the rabbit. Voit ('66) in cats found a decrease of about 3 per cent in the weight of brain and cord; while the data of Sedlmair ('99) would even indicate an actual *increase* in their weight during starvation. Data from a series of steers slaughtered in the Missouri Agricultural Experiment Station show that the absolute weight of the brain and cord in thin animals is but very slightly less than in very fat animals with nearly double the

[5] Since Chossat's results are often misquoted, it may be noted that he found the average weight of the brain almost identical in the 8 starved pigeons and in 8 controls of nearly the same (initial) body weight; (brain weight average 2.27 grams for starved; 2.25 grams for corresponding controls). On account of the difficulty in determining the exact plane of separation between the brain and spinal cord, however, Chossat preferred to combine their weights, thus giving a slight decrease (about 1.9 per cent) in the absolute weight of the entire central nervous system during inanition.

body weight. The relative (percentage) weight is of course correspondingly higher in the thin animals.

In young animals during the period of active growth, the results of inanition (especially chronic inanition) are in general somewhat different from those in adults, since the tendency of the organs to maintenance is in the young animal complicated by the growth impulse (Jackson '15). Bechterew ('95) found a slight apparent loss in the absolute weight of the brain and spinal cord of the newborn cat and dog during acute inanition. Hatai ('04) found in a series of young albino rats subjected to chronic inanition for 21 days an estimated average loss of about 5 per cent in the absolute weight of the brain. Later (Hatai '08) in another group of young rats in which growth had been retarded by underfeeding, he found that in these stunted animals the brain and cord had a weight approximately normal for the corresponding body weight. Donaldson ('11), in still younger rats held at nearly constant body weight by underfeeding from age of 30 days to 51 days, finds an apparent *increase* of 3.6 per cent in the weight of the brain. My own observations (Jackson '15), however, indicate little or no change in the weight of the brain under these conditions.

On the whole it appears that in adults inanition, both acute and chronic, produces a slight loss in the absolute weight of the brain. In young, rapidly growing animals, however, it is doubtful whether any such loss occurs, especially n chronic inanition, where there is even a possible increase in brain weight.

SPINAL CORD

The spinal cord in the acute inanition series (table 3) has an average weight of 0.631 gram and forms an average of 0.41 per cent of the body weight. Calculations from Donaldson's ('09) data indicate that at the average initial gross body weight (244 grams) the spinal cord should weight 0.625 gram, forming 0.26 per cent of the body weight. The data would therefore seem to indicate not only no loss, but even a very slight *gain* of the spinal cord in absolute weight during the period of acute inanition. In an earlier paper, Donaldson ('08) in table 4 gives

the average weight of the spinal cord (at body weight of 245 grams) as 0.630 gram in 2 males, and 0.640 gram in 4 females. On the whole, therefore, we may conclude that the spinal cord during acute inanition undergoes little or no change in weight. Individual variations make comparisons with controls more or less uncertain, especially in a relatively small series of observations.

A somewhat different result is found in the chronic inanition series. Donaldson's ('09) data would indicate that the spinal cord in body weights corresponding to the average initial weight (214 grams) of this series would form an average weight of 0.593 gram or 0.28 per cent of the body weight. In an earlier paper, Donaldson ('08) in table 4 gives the weight of the spinal cord (at body weight of 215 grams) as averaging 0.590 gram in 5 males, and 0.630 gram in 3 females (corresponding theoretical weight by formula being 0.593 gram, sexes combined). The actual weight of the cord found at the end of the inanition period averages 0.569 gram, or about 0.43 per cent of the body weight. This would indicate a loss of about 4 per cent in the absolute weight of the spinal cord during chronic inanition. Thus in the spinal cord, as in the brain, there appears to be in the adult rat a tendency to greater loss in chronic than in acute inanition. In the young rat, however, this tendency is more than counterbalanced by the growth impulse, so that the spinal cord may gain in weight while the body weight is held constant (Donaldson '11; Jackson '15).

In any event, however, it is evident that the adult spinal cord, like the brain, shows but very slight if any loss in absolute weight during inanition, thus increasing markedly in relative (percentage) weight. This is in general agreement with the observations of Chossat ('43), Falck ('54), Lasarew ('97), Sedlmair ('99), Voit ('66), and Bechterew ('95) previously mentioned in the discussion of the brain.

EYEBALLS

The eyeballs in the acute inanition series (table 3) form an average of about 0.19 per cent of the body weight. In normal rats corresponding to their initial body weight, the average

should be about 0.12 per cent (Jackson '13). Since the body weight has decreased about one-third (average 33.9 per cent) it is evident that the eyeballs must have remained nearly stationary in absolute weight, thus increasing their relative (percentage) weight by about one-half. In terms of absolute weight, there would appear to be a reduction from 0.298 gram (the theoretical weight corresponding to the initial body weight of 244 grams, according to Hatai '13) to 0.285 gram, the final average weight of the eyeballs (table 3). This would indicate a loss of 4.4 per cent.

A similar condition is found in the chronic inanition series. The eyeballs here form an average of about 0.20 per cent of the body weight, while the normal for the initial body weight averages about 0.13 per cent. (The higher figures in the chronic inanition series are due to the smaller initial body weight in this series.) Thus it is evident that, with a loss of about one-third (average 36.1 per cent) in the body weight during inanition, the absolute weight of the eyeballs has changed but little. In terms of absolute weight there would appear to be a reduction from about 0.280 gram (the theoretical weight corresponding to the average initial body weight of 214 grams, according to Hatai '13) to 0.2638 gram, the final average weight of the eyeballs (table 3). This would correspond to a loss of 5.8 per cent in the weight of the eyeballs during chronic inanition.

That the eyeballs lose little or nothing in absolute weight during inanition, increasing proportionately in relative (percentage) weight, is also indicated by the data of Falck ('54) for the dog, and Sedlmair ('99) for the cat. Bitsch ('95) finds the (absolute) weight of the eyeballs in the dog usually *increased* during inanition, which he suggested may be due to oedema.

THYROID GLAND

The thyroid gland in the acute inanition series (table 3) forms an average of 0.023 per cent of the body weight. The normal for the initial body weights would average about 0.015 per cent (Jackson '13). Since the body weight has been reduced one-third during inanition, this would indicate that there has been no

loss in the absolute weight of the thyroid gland. In terms of absolute weight, the thyroid gland after acute inanition averages 0.0380 gram, which is almost identical with the theoretical weight corresponding to the average initial body weight of 244 grams (Hatai '13).

The results in chronic inanition are somewhat different. Here the thyroid gland forms an average of about 0.020 per cent of the body weight, the normal for the corresponding initial body weights being about 0.016 per cent. In this case the thyroid gland has apparently lost weight during chronic inanition, though relatively less than the body as a whole. According to Hatai ('13) the weight of the thyroid gland corresponding to the average initial body weight of 214 grams would be about 0.034 gram. The final absolute weight averages 0.0266 gram, indicating a loss of about 21.8 per cent in chronic inanition.

On account of the variability of the thyroid gland, however, and the difficulty in dissecting it out accurately, no final conclusions can be drawn from the limited number of observations at hand. As in the case of the brain, spinal cord and eyeballs, however, there appears to be a greater tendency to loss in weight of the thyroid gland during chronic inanition.

Excepting the observations by Falck ('54), indicating no change in the relative (percentage) weight of the thyroid gland during inanition in the dog, no data on this subject have been found in the literature. Traina ('04) cites data, however, upon the histological changes.

THYMUS

It is already known that the thymus in the albino rat normally undergoes a diminution in weight (due to age involution) after the age of 10 weeks (Jackson '13) or more precisely 85 days (Hatai '14). At the age of one year, it forms normally about 0.020 per cent of the body (Jackson). The thymus in the present acute inanition series forms an average of about 0.020 per cent of the body weight, while in the chronic inanition series the average is 0.021 per cent (table 3). The age of the rats used in the inanition experiments is unknown, but from the

body weight it is probable that few of them were less than a year old. Therefore while hunger is known to produce a marked involution of the thymus (Hammar), it is probable that in the present case the involution had already been produced by age, and was not caused by inanition. According to Jonson ('09), in young rabbits the weight curves of fat and thymus are similar in *chronic* inanition, but the fat decreases somewhat more rapidly in *acute* inanition.

HEART

The heart in the acute inanition series forms an average of about 0.43 per cent of the body weight, and about 0.42 per cent in the chronic inanition series (table 3). The normal for corresponding initial body weights would average about 0.43 per cent (Jackson '13) or about 0.38 per cent, according to Hatai's ('13) data. Thus it is evident that the heart during inanition has lost weight nearly in the same proportion as the whole body.

Using Hatai's ('13) curves to determine the normal at corresponding initial body weights, the absolute weight of the heart apparently decreases from about 0.925 to 0.6687 gram (loss of 27.7 per cent) in the acute inanition series; and from about 0.830 to 0.5577 gram (loss of 32.8 per cent) in the chronic inanition series. As in the case of the viscera previously considered, the loss is apparently slightly greater relatively in chronic inanition.

The statements in the literature generally indicate that the heart during inanition loses somewhat less in weight than the body as a whole, and increases accordingly in relative (percentage) weight. This would appear to be the case in man (Aschoff '11; Lustig '02), cat (Voit '66; Sedlmair '99), newborn kitten (v. Bechterew '95) dog (Falck '54), guinea-pig (Lasarew '97), rabbit (Bowin '80), and in thin compared with fat steers (data from Missouri Agricultural Experiment Station). The data of Chossat ('43) for pigeons, however, would indicate a reduction in heart weight relatively slightly greater than in the body as a whole.

LUNGS

The lungs of the rat are quite variable in weight, owing to the frequency of infection, which may affect their weight even when the lesions are slight. The average for the lungs in the acute inanition series is 0.61 per cent of the body weight, and 0.55 per cent in the chronic inanition series (table 3). Since the normal lungs corresponding to the average initial body weight form an average of about 0.60 per cent (Jackson '13), it appears (although the evidence is insufficient for final conclusions) that during inanition, both acute and chronic, in adult albino rats the lungs lose in weight in about the same proportion as the whole body, their relative (percentage) weight remaining nearly the same. Taking the normal weight of the lungs at corresponding body weights from Hatai's ('13) curve, there is apparently a decrease from about 1.40 to 0.968 gram (loss of 30.9 per cent) in the acute inanition series; and from about 1.24 to 0.743 gram (loss of 40 per cent) in the chronic series. Again, as in the case of the viscera previously considered, the loss appears relatively greater during chronic inanition.

Comparatively few data are found in the literature concerning the weight of the lungs in inanition. In pigeons, Chossat ('43) found a loss of 22.4 per cent in the lungs, compared with 40 per cent in the entire body weight. Thus their relative (percentage) weight is considerably increased, which was also observed by Voit ('66) and Sedlmair ('99) in the cat, and von Bechterew ('95) in newborn kitten. In the dog, however, Falck's ('54) data indicate a loss relatively slightly greater than that of the body, with a corresponding decrease in percentage weight.

LIVER

The liver in the acute inanition series (table 3) forms an average of about 2.88 per cent of the body weight. Since the normal for the corresponding initial body weights is about 4.5 per cent in the acute inanition series, and 4.3 per cent in the chronic (Jackson '13) it is evident that the liver has apparently lost in weight relatively more than the body as a whole. In the chronic

inanition series, however, the weight of the liver is more variable, and the average (3.98 per cent) is but little below the normal. As the liver is normally subject to great individual variations in weight (Jackson '13), however, caution should be observed in drawing final conclusions.

In terms of absolute weight, using my (Jackson '13) data for comparison, it appears that the liver decreases from about 10.98 to 4.587 grams (loss of 58 per cent) in the acute inanition series, and from 9.20 to 5.219 grams (loss of 43 per cent) in the chronic inanition series. It may also be remembered that in the series investigated by Hatai ('13), the average weight of the normal liver was found distinctly higher than in my series; and the loss in weight, estimated upon this basis, would be considerably greater.

With the exception of the adipose tissue, the thymus (in young animals) and occasionally the spleen, all investigators agree that the loss of weight in the liver is relatively greater than that in any other organ. Thus the liver decreases in relative (percentage) weight, as has been observed in man (Aschoff '11), white mouse (Cesa-Bianchi '09), pigeon (Chossat '43), rabbit (Pfeiffer '87), cat (Voit '66; Sedlmair '99), dog (Falck '54), newborn cat and dog (v. Bechterew '95), guinea-pig (Lasarew '97) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). While the loss is practically always relatively greater than in the body as a whole, the amount of loss is quite variable.

Since the loss in weight of the liver (unlike all viscera previously considered) is relatively greater in acute inanition it seems probable that the greatest loss occurs during the earliest stages of inanition, when the liver yields its store of easily available food material (glycogen, fat, etc.). Thus Lasarew ('97) found that during the early period of inanition in the guinea-pig, while the body weight lost 10 per cent, the liver lost 18 per cent of its weight, or relatively near y twice as much. Toward the end of the inanition period, on the contrary, the liver apparently lost relatively only half as much as the whole body.

SPLEEN

In the acute inanition series (table 3), the spleen forms an average of 0.21 per cent of the body. This is below the normal for the average corresponding initial body weight, in which case the average is 0.27 per cent. In the chronic inanition series, however, the average (0.30 per cent) is slightly higher than the normal (Jackson '13). The individuals in both acute and chronic inanition, however, are exceedingly variable, as seen in the table. This is also true of the normal spleen, which is one of the most variable organs in the body (Jackson '13). The number of observations is therefore insufficient for final conclusions concerning the effect of inanition upon the weight of the spleen in the albino rat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the spleen from about 0.645 to 0.3177 gram (loss of 51 per cent) in the acute inanition series; and from 0.570 to 0.4056 gram (loss of 29 per cent) in the chronic series.

That the spleen loses heavily during inanition, losing in relative as well as absolute weight, has been found in man (Aschoff '11; Stschastny '98), pigeon (Chossat '43), rabbit (Bowin '80), and cat (Voit '66; Sedlmair '99); while a decrease less marked than in the whole body (relative increase) appears in the dog (Falck '54) and in thin steers, compared with fat (data from Missouri Agricultural Experiment Station). Data from von Bechterew ('95) indicate a relative increase in the spleen of newborn kittens during inanition, but a decrease in puppies. These apparently conflicting statements are perhaps to be explained largely by the great variability of the spleen, making comparison with controls uncertain. In addition, however, there is the possibility that the loss in the spleen may vary according to the character and stage of inanition. Thus Lasarew ('97) in the guinea-pig found the greatest loss in weight of the spleen to occur in the middle period of inanition (second period of 10 per cent loss in body weight), during which the spleen lost 31 per cent in weight.

STOMACH AND INTESTINES

The stomach and intestines (including mesentery and pancreas) together with their content (table 3) form an average of about 5.2 per cent of the body weight in the acute inanition series, and 6.3 per cent in the chronic inanition series. Since the average normal for the initial body weights is about 9.0 per cent (Jackson '13), it is evident, as might be expected, that the stomach and intestines with contents have lost weight in relatively much greater proportion than has the body as a whole. The loss affects not only the contents, but the empty tract, which has apparently decreased from about 6.0 per cent (normal at the initial body weight) of the body weight to 3.25 per cent (acute inanition series) or 3.5 per cent (chronic series). There is apparently not much difference in this respect between the chronic and acute series. A relatively small part of the loss is in the mesenteric fat.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, there is apparently a decrease in the weight of the empty alimentary canal from about 11.65 to 5.01 grams in the acute inanition series, and from 10.70 to 4.54 grams in the chronic series. This would correspond to a loss of about 57 per cent in each series.

Data in the literature are very scarce concerning changes in weight of the alimentary canal during inanition. According to Falck ('54), the loss appears to be nearly proportional to that of the entire body (relative weight increasing from 8.4 to 8.7 per cent). This appears to be true also for the cat according to Sedlmair ('99), although a much smaller loss was found by Voit ('66). Unpublished data on a series of steers (Missouri Agricultural Experiment Station) indicate that the relative weight of the (empty) intestines (without fat) is variable, but tends to be greater in thin than in fat animals.

SUPRARENAL GLANDS

The suprarenal glands must be considered separately in the sexes on account of a difference in relative weight (Jackson '13). In normal rats corresponding to the initial body weights of the present series, the average for the suprarenal glands of the male is about 0.17 per cent of the body weight, and for the female about 0.26 per cent. The four females of the acute inanition series (none present in the chronic series) show an average of 0.30 per cent of the body weight (table 3), but the number is too small for definite conclusions. The males show an increase (from 0.17 per cent) to an average of 0.25 per cent in both acute and chronic inanition series (table 3). It would therefore appear that the loss in absolute weight has been less than in the body as a whole in the female with little or no loss in the male.

In terms of absolute weight, taking Hatai's ('13) curve for the normal, it appears that in the male rat the suprarenals have *increased* from about 0.0390 to 0.0396 grams (gain of 1.5 per cent) in the acute inanition series; and *decreased* from about 0.0360 to 0.0328 grams (loss of 8.9 per cent) in the chronic series. The apparent increase during acute inanition is probably not significant. A larger number of observations is necessary to determine the matter.

No data concerning the weight of the suprarenal glands during inanition have been found in the literature, although Traina ('04) cites several investigations on the histological changes.

KIDNEYS

The kidneys (table 3) in the acute inanition series form an average of 0.97 per cent of the body weight and in the chronic series 1.00 per cent. According to Jackson ('13) the normal for the initial body weights would average about 0.95 per cent (although Hatai's data would place it at about 0.84 per cent). It therefore appears that the kidneys have lost weight to nearly the same extent relatively as the body in general, thus gaining but slightly in relative (percentage) weight.

If Hatai's ('13) curve be taken as the normal, in absolute weight the kidneys would apparently decrease from about 2.04 to 1.520 grams (a loss of 25.5 per cent) during acute inanition; and from 1.80 to 1.317 grams (a loss of 26.8 per cent) in the chronic inanition series. If my data indicating a higher normal were taken, the loss would be correspondingly greater.

Considering the importance of the kidneys, there is in the literature a surprising lack of data concerning their weight during inanition. That they lose in weight relatively somewhat less than the body as a whole (thus gaining in percentage weight) is indicated in the pigeon (Chossat '43), cat (Voit '66) and dog (Falck '54). There appears also a slight relative increase in the kidney weight of thin steers compared with fat (data from Missouri Agricultural Experiment Station). On the other hand, Sedlmair's ('99) data for starved cats indicate no change in relative (percentage) weight in one case and a slight decrease in another.

GONADS

a. Female. The two observations recorded for the weight of the ovaries, 0.051 per cent and 0.033 per cent of the body weight are both above the normal average (0.025 per cent), but are of course entirely too few to be significant.

b. Male. At the age of one year (body weight, 210 grams) the normal testes and epididymi form an average of about 1.20 per cent of the body weight, of which approximately one-fourth (0.30 per cent) belongs to the epididymi, and three-fourths (0.90 per cent) to the testis (Jackson '13). Hatai's ('13) data would put the testis a little higher (about 1.05 per cent). In the acute inanition series (table 3) the average relative weight for the testes is 1.12 per cent, and for the epididymi is 0.39 per cent of the body weight. In the chronic series, the average for the testes is 1.02 per cent, and for the epididymi 0.33 per cent. It would therefore appear that during inanition the loss in weight of both testes and epididymi is relatively not very different from that in the body as a whole, and is (like that of the majority of the viscera) more marked in chronic than in acute

inanition. On account of the variability of these organs, however, more data are needed before final conclusions can be justified.

In terms of absolute weight, taking Hatai's ('13) data for the normal at the initial body weight, the testis would appear to decrease from 2.50 to 1.756 grams (a loss of 29.8 per cent) in the acute inanition series; and from 2.27 to 1.355 grams (a loss of 40.3 per cent) in the chronic inanition series.

Although numerous investigations have been made upon the histological changes in the gonads during inanition (Traina '04), the only observations found concerning their weight are that by Falck ('54) showing the relative (percentage) weight of the testis in the dog to remain apparently unchanged, and that by (Voit '66) showing a relative decrease in the testis of the cat.

HYPOPHYSIS

In the case of the hypophysis, as with the suprarenal glands, there is normally a sexual difference to be considered (Hatai '13). When the data in table 3 are compared with Hatai's chart 10, it will be found that the absolute weights of the hypophysis, in both acute and chronic inanition, correspond fairly well with those of the normal gland at the *final* body weight. That is, the weight of the hypophysis during inanition has apparently decreased in nearly the same proportion as the whole body, so the relative weight is but little changed. As calculated from Hatai's data, the (male) hypophysis would form about 0.0036 per cent of the body at the average initial body weight, as compared with 0.0043 per cent found in the acute inanition series, and 0.0045 per cent in the chronic series.

In terms of absolute weight, the (male) hypophysis has apparently decreased from about 0.0093 to 0.0069 gram, the average of the acute inanition series (loss of 26.1 per cent); and from 0.0079 to 0.0059 gram (loss of 25.3 per cent) in the chronic inanition series. A larger number of observations would of course be necessary to determine the result with precision.

No data have been found in the literature concerning the weight of the hypophysis during inanition.

DISCUSSION

The changes in the average relative weights of the various organs and systems as a result of inanition in the adult albino rat are summarized in table 2.

While no great emphasis can be laid upon the exactness of the figures shown in table 2, it is evident that with respect to relative loss in weight during inanition, the organs may be divided into three groups. In the first group, which includes the suprarenals, thyroid, skeleton, eyeballs, spinal cord and brain, and thymus, there is but little (if any) loss in absolute weight during inanition, and a corresponding increase in relative (percentage) weight. In general, there is a relatively greater loss during chronic

TABLE 2

ORGAN OR SYSTEM	NORMAL PERCENTAGE OF BODY AT INITIAL BODY WEIGHT	PERCENTAGE OF BODY AFTER INANITION		PERCENTAGE LOSS OF ORGAN DURING INANITION	
		Acute	Chronic	Acute	Chronic
Suprarenals (male)	0.0170	0.0220	0.0260	+1.5(?)	−8.9
Thymus	0.0200	0.0200	0.0210	0(?)	0(?)
Thyroid gland	0.0150(0.016)	0.0230	0.0200	0(?)	−21.8
Spinal cord	0.2500(0.290)	0.4000	0.4300	0(?)	−4.0
Ligamentous skeleton	10.0000	15.0000	16.4000	−0.4	+1.8(?)
Eyeballs	0.1200(0.130)	0.1900	0.2000	−4.4	−5.8
Brain	0.7800(0.870)	1.1700	1.3300	−5.1	−6.6
Kidneys	0.9500	0.9600	1.0000	−25.5	−26.8
Hypophysis	0.0036	0.0043	0.0045	−26.1	−25.3
Heart	0.4300	0.4400	0.4200	−27.7	−32.8
Testes	0.9000	1.0600	1.0100	−29.8	−40.3
Lungs	0.6000	0.6100	0.5500	−30.9	−40.0
Musculature	45.0000	47.5000	43.0000	−30.9	−40.8
Integument	18.0000	19.1000	17.8000	−31.2	−38.5
Whole body	*100.0000*	*100.0000*	*100.0000*	*−33.9*	*−36.1*
Spleen	0.2700	0.2100	0.3100	−51.0	−29.0
Stomach—intestines	6.0000	3.4000	3.5000	−57.0	−57.0
Liver	4.5000	3.1000	4.0000	−58.0	−43.0

inanition, which is especially marked in the case of the thyroid gland. The thymus, having already undergone age involution, is affected but slightly, if at all.

In the second group, which includes the kidneys, hypophysis, heart, testes, lungs, musculature and integument, the loss in absolute weight during inanition is more nearly in proportion to that of the whole body, so their relative (percentage) weight is usually not greatly changed. In all except the hypophysis, however, the loss is relatively greater during chronic inanition. Especially the lungs, testes, integument and musculature appear to lose markedly during chronic inanition.

In the third group, including the spleen, liver and alimentary canal, the loss in absolute weight is relatively much greater than in the body as a whole, so they decrease in relative as well as in absolute weight. The liver and spleen are exceptional, however, in that their loss is apparently relatively greater in acute than in chronic inanition. In fact, in chronic inanition the spleen apparently belongs with the second group.

The variability of the organs as to loss of weight during inanition has been explained in two ways: Manassein ('69) noted that those organs which are most active in the organism lose least during inanition. A more rational explanation is that of Paschutin ('81), according to whom the various organs lose in proportion to their storage content of available food supply. The various proteids, fats and carbohydrates are dissolved and carried away by the circulation at different times and with different degrees of rapidity. Thus the variability in the loss of weight in different organs and in different types of inanition would be ultimately explained primarily upon a chemical basis.

SUMMARY

The principal results of the present paper may be briefly summarized as follows:

1. During both acute and chronic inanition there is apparently a slight increase in the ratio of tail length to body length. This is probably due to a decrease in the trunk length during inanition.

2. The head and fore limbs during inanition lose relatively less than the body as a whole, and therefore increase in relative (percentage) weight. The hind limbs nearly maintain their original relative weight (slight increase during acute inanition), while the trunk decreases in relative weight.

3. Of the systems—integument, skeleton, musculature, viscera and 'remainder'—the integument and musculature lose relatively in nearly the same proportion as the whole body, slightly less during acute inanition and slightly more during chronic inanition. The skeleton nearly maintains its original absolute weight, and therefore increases markedly in relative (percentage) weight. There is a marked decrease in the 'remainder,' probably due chiefly to loss of fat. The visceral group as a whole undergoes little change in relative weight, showing a slight decrease during acute inanition. This decrease is due to the large size of the liver, which undergoes a greater loss in acute than in chronic inanition. The majority of the viscera, on the other hand, show a greater loss during chronic inanition.

4. As to relative loss of weight during inanition, the individual viscera may be divided into three groups: (1) the suprarenal glands, thyroid glands, eyeballs, spinal cord and brain lose but very little (if any) in absolute weight, and therefore increase correspondingly in relative (percentage) weight. The thymus has already undergone age involution, and is therefore unaffected. (2) The kidneys, heart, lungs, hypophysis and testes lose more nearly in proportion to the entire body (in general, somewhat more during chronic inanition), and therefore do not change greatly in relative (percentage) weight. (3) The spleen (in acute inanition), liver and alimentary canal (both empty and with contents) lose relatively much more heavily than the whole body, and therefore decrease in relative (percentage) as well as in absolute weight.

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TABLE 3

Acute and chronic inanition

RAT NO. AND SEX	LENGTH OF BODY	LENGTH OF TAIL	RATIO OF TAIL TO BODY	INANITION PERIOD	ORIGINAL GROSS BODY WEIGHT	FINAL GROSS (AND NET) BODY WEIGHT	LOSS OF BODY WEIGHT	HEAD		FORE-LIMBS	
								Weight	Percent of body	Weight	Percent of body
	cm.	*cm.*		*days*	*grams*	*grams*	*per cent*	*grams*		*grams*	
S 18 f.	17.5	17.5	1.00	Acute, 8.0	196.0	121.0(117.2)	38.0	16.2	13.9		
M 8 m.	17.1	17.3	1.01	10.0	221.0	132.0(128.9)	40.0	14.1	10.9	9.2	7.1
O 6 f.	18.0	15.8	0.88	12.0	193.0	132.0(129.5)	32.0	17.4	13.4		
M 7 m.	16.6	16.0	0.96	9.0	216.0	134.0(131.2)	38.0	14.2	10.8	8.5	6.5
M 9 m.	15.3	16.5	1.08	6.0	190.0	137.0(134.0)	28.0	14.8	11.1	8.7	6.5
O 5 f.	20.0	18.0	0.90	8.0	182.0	136.0(135.0)	25.0	16.4	12.1		
M 10 m.	18.1	17.2	0.95	9.0	221.0	142.0(139.4)	36.0	14.8	10.7	10.3	7.4
M 1 m.	20.5	19.5	0.95	9.0	248.0	168.0(165.2)	32.0	17.0	10.3	13.8	8.4
M 2 m.				11.0	254.0	170.0(167.2)	33.0	18.9	11.3	12.7	7.6
S 26 m.	20.5	18.0	0.88	8.0	284.0	174.0(171.5)	39.0	18.7	10.9		
S 16 f.	19.5	17.5	0.90	9.0	267.0	190.0(186.0)	29.0	20.3	10.9		
S 25 m.	20.5	19.0	0.93	9.0	328.0	202.0(198.3)	35.0	22.1	11.1		
S 27 m.	21.5	18.0	0.84	8.0	320.0	223.0(219.0)	30.0	23.6	11.0		
M 13 m.	21.3	19.6	0.92	6.0	333.0	240.0(237.0)	28.0	23.7	10.0	16.8	7.1
S 28 m.	23.0	19.5	0.85	10.0	367.0	243.0(240.0)	34.0	24.6	10.3		
Average (acute)	19.2	17.8	0.93	Acute, 9.0	255.0 (244 for viscera)	170.0(166.0) 160.0(157.4) for viscera	33.1 (33.9 for viscera)	18.47	11.2 (10.7 for limbs and trunk	11.4	7.2
M 3 m.	17.5	16.5	0.94	Chronic, 36.0	188.0	125.0(122.1)	33.5	13.9	11.4	9.8	8.0
M 12 m.	17.3	17.1	0.99	33.0	200.0	128.0(124.6)	36.0	14.2	11.4	7.8	6.2
M 5 m.	19.0	18.5	0.97	36.0	205.0	129.0(126.6)	37.0	15.2	12.0	10.4	8.2
M 4 m.	17.5	17.0	0.97	41.0	205.0	134.0(129.2)	35.1	13.5	10.5	7.4	5.7
M 6 m.	17.5	16.7	0.95	35.0	220.0	138.0(134.1)	37.2	15.3	11.4	9.5	7.1
M 11 m.	19.0	18.6	0.98	33.0	264.0	163.0(158.5)	37.5	18.3	11.5	9.8	6.2
Average (chronic)	18.0	17.4	0.97	Chronic, 35.7	213.7	136.2(132.5)	36.1	15.1	11.4	9.1	6.9

TABLE 3—*Continued*

RAT NO. AND SEX	HIND-LIMBS		TRUNK		INTEGUMENT		LIGAMENTOUS SKELETON		CARTILAGINOUS SKELETON		DRY SKELETON	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.					23.06	19.7	20.30	17.4				
M 8 m.	22.5	17.4	83.1	64.5	25.50	19.8	21.60	16.7	16.15	12.5	7.321	5.68
O 6 f.					24.20	18.6	19.50	15.1				
M 7 m.	23.9	18.2	84.6	64.5	24.80	18.9	19.70	15.4	15.19	11.6	7.224	5.51
M 9 m.	23.8	17.8	86.7	64.7	25.20	18.8	19.50	16.4	14.81	11.1	7.094	5.30
O 5 f.					22.00	16.3	15.20	11.3				
M 10 m.	24.3	17.5	90.0	64.7	27.30	19.6	21.70	15.6	15.05	10.8	7.325	5.27
M 1 m.	28.6	17.3	105.8	64.1	32.20	19.5	26.00	15.8	19.30	11.7	10.516	6.37
M 2 m.	27.6	16.5	108.0	64.6	35.00	21.0	21.00	12.6	14.00	8.4	7.741	4.64
S 26 m.					33.36	19.5	29.34	17.1				
S 16 f.					32.48	17.5	28.13	15.1				
S 25 m.					39.78	20.2	25.48	12.9				
S 27 m.					40.63	18.5	33.03	15.1				
M 13 m.	42.6	18.0	153.9	64.9	46.70	19.8	37.60	15.9	24.37	10.3	13.324	5.62
S 28 m.					41.43	17.3	40.01	16.7				
Average (acute)	27.6	17.5	101.7	64.6 (64.1)	31.60	18.9 (19.1 for viscera)	25.40	15.3 (15.0 for viscera)	16.98	10.9	8.748	5.48
M 3 m.	18.5	15.2	79.9	65.5	19.50	16.0	18.50	15.2	14.00	11.5	6.511	5.34
M 12 m.	20.7	16.6	81.9	65.5	21.40	17.1	21.40	17.1	14.60	11.7	7.156	5.72
M 5 m.	19.0	15.0	82.0	64.6	24.50	19.3	22.50	17.8	16.65	13.2	9.310	7.33
M 4 m.	15.9	12.3	92.4	71.4	18.90	14.7	18.20	14.1	16.10	12.5	7.318	5.67
M 6 m.	21.1	15.7	88.2	65.8	25.50	19.1	21.90	16.3	16.20	12.1	7.690	5.74
M 11 m.	26.5	16.7	103.9	65.3	32.70	20.6	28.30	17.8	21.52	13.5	11.015	6.38
Average (chronic)	20.3	15.3	88.05	66.4	23.70	17.8	21.80	16.4	16.5	12.4	8.167	6.03

TABLE 3—*Continued.*

RAT NO. AND SEX	MUSCULATURE		TOTAL VISCERA		REMAINDER		BRAIN		SPINAL CORD		EYEBALLS	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	55.64	47.6	12.82	11.0	5.37	4.3	1.833	1.57	0.5800	0.50	0.2660	0.23
M 8 m.	59.60	46.2	14.03	10.9	8.20	6.4	1.704	1.32	0.5652	0.44	0.2594	0.20
O 6 f.	58.70	45.3	14.12	10.9	13.08	10.1	1.802	1.39	0.6340	0.49	0.3180	0.25
M 7 m.	66.90	51.0	13.55	10.3	6.20	4.4	1.738	1.33	0.5710	0.44	0.2124	0.16
M 9 m.	67.80	50.6	(viscera not weighed)									
O 5 f.	65.80	48.7	18.69	13.8	13.40	9.9	1.650	1.22	0.5520	0.41	0.3120	0.23
M 10 m.	65.30	47.0	16.30	11.8	8.80	6.2	1.768	1.27	0.5794	0.42	0.2416	0.17
M 1 m.	75.00	45.5	16.80	10.2	13.70	8.7	1.734	1.05	0.6010	0.36	0.2838	0.17
M 2 m.	78.10	46.8	17.90	10.7	14.50	8.7	1.842	1.10	0.5542	0.33	0.2942	0.18
S 26 m.	79.49	46.4	16.50	9.6	12.70	7.4	1.773	1.04	0.7200	0.42	0.2470	0.15
S 16 f.	91.80	49.4	23.25	12.5	10.23	5.5	1.811	0.97	0.6990	0.38	0.3206	0.17
S 25 m.	99.02	50.0	18.22	9.2	15.25	7.7	2.005	1.01	0.7780	0.39	0.3222	0.16
S 27 m.	100.10	45.7	25.60	11.7	21.90	10.0	1.995	0.91	0.7380	0.34	0.3440	0.16
M 13 m.	122.00	51.4	(viscera not weighed)									
S 28 m.	103.50	43.7	(viscera not weighed									
Average (acute)	79.25	47.6 (47.5)	17.30	11.1	10.30	7.3	1.8046	1.18	0.6310	0.41	0.285	0.19
M 3 m.	54.20	44.4	19.34	16.0	10.50	8.4	1.794	1.47	0.5840	0.48	0.2580	0.21
M 12 m.	56.50	45.2	15.78	12.6	9.50	7.7	1.707	1.37	0.5394	0.43	0.2680	0.21
M 5 m.	54.30	42.8	15.27	12.1	10.00	8.0	1.704	1.34	0.5990	0.47	0.2990	0.24
M 4 m.	51.10	39.6	18.60	14.5	22.40	16.9	1.852	1.44	0.6060	0.47	0.2368	0.18
M 6 m.	58.60	43.7	18.61	13.9	9.50	7.1	1.578	1.18	0.4950	0.37	0.2548	0.19
M 11 m.	67.40	42.4	17.49	11.0	12.60	8.2	1.821	1.15	0.5908	0.37	0.2662	0.17
Average (chronic)	57.00	43.0	17.51	13.4	12.40	9.4	1.743	1.33	0.5690	0.43	0.2638	0.20

TABLE 3—*Continued.*

RAT NO. AND SEX	THYROID GLAND		THYMUS		HEART		LUNGS		LIVER		SPLEEN	
	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	0.0276	0.024	0.0072	0.006	0.5690	0.49	0.7870	0.67	2.921	2.50	0.1150	0.10
M 8 m.	0.0320	0.025	0.0326	0.025	0.5170	0.40	0.7440	0.58	2.714	2.10	0.2420	0.19
O 6 f.					0.7210	0.56			4.457	3.44	0.3590	0.28
M 7 m.	0.0290	0.022	0.0331	0.025	0.5000	0.38	0.8150	0.62	2.263	1.73	0.2180	0.17
M 9 m.												
O 5 f.					0.7350	0.54			5.751	4.26	0.4980	0.37
M 10 m.	0.0264	0.019	0.0386	0.028	0.5298	0.38	0.7820	0.56	3.537	2.54	0.3494	0.25
M 1 m.	0.0196	0.012	0.0238	0.014	0.6992	0.41			5.264	3.19	0.3644	0.22
M 2 m.	0.0248	0.015	0.0338	0.020	0.6690	0.40	1.3420	0.80	5.419	3.24	0.4190	0.25
S 26 m.	0.0402	0.024	0.0186	0.011	0.6410	0.38	1.0220	0.60	2.931	1.71	0.1630	0.10
S 16 f.	0.0392	0.021	0.0616	0.033	0.8306	0.45	1.0130	0.55	7.641	4.11	0.4390	0.24
S 25 m.	0.0928	0.047	0.0219	0.011	0.7850	0.40	1.1240	0.57	3.914	1.97	0.2670	0.14
S 27 m.	0.0486	0.022	0.0230	0.011	0.7980	0.37	1.0900	0.50	8.228	3.75	0.3790	0.17
M 13 m.												
S 28 m.												
Average (acute)	0.0380	0.023	0.0294	0.020	0.6687	0.43	0.9680	0.61	4.587	2.88	0.3177	0.21
M 3 m.	0.0202	0.017	0.0190	0.016	0.6762	0.55	0.5262	0.43	6.297	5.16	0.3714	0.30
M 12 m.	0.0250	0.020	0.0268	0.021	0.4756	0.38	0.6680	0.53	4.321	3.46	0.3650	0.29
M 5 m.	0.0158	0.013	0.0308	0.024	0.5510	0.43			3.046	2.40	0.1802	0.14
M 4 m.	0.0174	0.014	0.0168	0.013	0.4942	0.38	0.7460	0.58	7.039	5.45	0.4170	0.32
M 6 m.	0.0400	0.030	0.0462	0.034	0.5710	0.43	0.8820	0.66	5.958	4.44	0.6184	0.46
M 11 m.	0.0410	0.026	0.0256	0.016	0.5780	0.36	0.8940	0.56	4.654	2.98	0.4818	0.30
Average (chronic)	0.0266	0.020	0.0275	0.021	0.5577	0.42	0.7430	0.55	5.219	3.98	0.4056	0.30

TABLE 3—*Continued.*

RAT NO. AND SEX	STOMACH-INTESTINES (FILLED)		STOMACH-INTESTINES (EMPTY)		SUPRARENALS		KIDNEYS		TESTIS		EPIDIDYMI		HYPOPHYSIS	
	Weight	Percent of body	Weight	Percent of body	Weight	Percent of body	Weight	Percent of body	Weight	Per cent of body	Weight	Percent of body	Weight	Per cent of body
	grams		*grams*		*grams*		*grams*		*grams*		*grams*		*grams*	
S 18 f.	8.360	7.15	4.530	3.88	0.0468	0.040	1.075	0.92	(0.0600)*	(0.051)*				
M 8 m.	6.655	5.16	3.585	2.78	0.0380	0.029	1.374	1.07	1.754	1.36	0.4666	0.36	0.0066	0.0051
O 6 f.	8.270	6.38	4.680	3.60	0.0330	0.025	1.374	1.06						
M 7 m.	6.509	4.96	3.729	2.84	0.0414	0.032	1.235	0.94	1.675	1.28	0.4860	0.37	0.0060	p.0046
M 9 m.														
O 5 f.	8.720	6.46	7.250	5.37	0.0420	0.031	1.484	1.10						
M 10 m.	6.860	4.94	4.290	3.08	0.0344	0.025	1.410	1.01	2.179	1.57	0.5590	0.43	0.0068	0.0049
M 1 m.	6.090	3.69	3.280	1.99	0.0414	0.025	1.513	0.92	1.502	0.91	0.4490	0.27	0.0070	0.0043
M 2 m.	7.040	4.22	4.290	2.57	0.0338	0.020	1.505	0.90	1.124	0.90	0.4680	0.67	0.0066	0.0040
S 26 m.	7.100	4.15	4.550	2.66	0.0398	0.023	1.557	0.91	1.905	1.11	0.7640	0.45	0.0076	0.0045
S 16 f.	11.980	6.44	8.610	4.63	0.0416	0.022	1.634	0.88	(0.0616)*	(0.033)*			0.0146	0.0078
S 25 m.	8.540	4.31	5.070	2.56	0.0458	0.023	1.929	0.97	1.479	0.75	0.4120	0.21	0.0048	0.0024
S 27 m.	10.320	4.71	6.270	2.86	0.0424	0.019	2.150	0.98	2.430	1.11	0.7210	0.33	0.0096	0.0044
M 13 m.														
S 28 m.														
Average (acute)	8.040	5.21	5.010	3.25	m0.0396	0.025	1.520	0.97	1.756	1.12	0.5407	0.39	m0.0069	0.0043
					f0.0409	0.030							f0.0146	0.0078
M 3 m.	10.480	8.60	6.530	5.40	0.0252	0.021	1.252	1.04	0.682	0.56	0.3060	0.25	0.0058	0.0048
M 12 m.	7.060	5.70	3.660	2.90	0.0306	0.025	1.418	1.13	1.829	1.46	0.4400	0.35	0.0052	0.0042
M 5 m.	6.030	4.80	3.660	2.90	0.0320	0.025	1.153	0.91	1.201	0.95	0.3910	0.31	0.0058	0.0046
M 4 m.	8.950	6.90	4.220	3.30	0.0496	0.038	1.465	1.13	1.113	0.86	0.3430	0.27	0.0062	0.0048
M 6 m.	8.480	6.30	4.580	3.40	0.0270	0.020	1.235	0.92	1.708	1.27	0.6160	0.46	0.0060	0.0045
M 11 m.	9.080	5.70	4.580	2.90	0.0326	0.021	1.379	0.87	1.597	1.00	0.5410	0.34	0.0064	0.0040
Average (chronic)	8.35	6.30	4.540	3.50	0.0328	0.025	1.317	1.00	1.355	1.02	0.4400	0.33	0.0059	0.0045

*Ovaries.

ON THE DEVELOPMENT OF THE NEURO-MUSCULAR SPINDLE IN THE EXTRINSIC EYE MUSCLES OF THE PIG

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TWELVE FIGURES

Since Hassal in 1851 first pointed out the presence of spindle-shaped nerve endings in muscle, nearly all anatomists and neurologists have at one time or another in their work become deeply interested in them. The problems which they offer seem to fall naturally into the three great groups—separate yet intimately associated—which divide the research that has been done upon most of the integral parts of the body, namely, the structural or histological studies; the functional side, including both physiological and pathological aspects; and finally, the embryological development. The sensory nerve endings in muscle were first approached from the histological side, obviously because methods for such investigation were at hand, and an adequate histological picture of the adult structure is the only sound basis for further advance. This was furnished first by Kölliker in 1862 and during the next year by Kühne, to whom we owe the term 'muscle spindle.' Since then, with the development of better ways for demonstrating the finer structure of the nervous system, such as the vital methylene blue method and the use of silver nitrate as a neuro-fibril stain, additional points have been added by various workers, among whom are Golgi, Dogiel, Sihler and Huber. They seem to have left little to be desired as to the structure of the ending. I can think of one question, however, that others have asked which has never been answered. Do the sensory endings in muscle have an intermediate substance comparable to that in the motor endings—the receptive 'substance' of

Langley—which is acted upon by curare, and which does not degenerate with the nerve when the latter is cut? Although there may not be complete evidence to warrant an affirmative answer to this question, one of the findings of my work upon the development of the muscle spindle makes it essential to consider such a structure as a possibility, if not a probability. I will take this question up more fully later, when I come to the actual description of the neuro-muscular spindles in the embryonic muscle. Following the demonstration of the unique spindle in the muscle with its apparently highly specialized structure, naturally the next question to be settled was, what function has it? This was admirably answered by Ruffini and Sherrington, the former reasoning out its necessary action from its histological structure; the latter confirming his conclusions by that now well-known experiment of cutting the ventral roots of the nerves to one of the limbs of a cat and finding that the intrafusal nerve fibers did not participate in the Wallerian degeneration which affected all the motor nerves of the limb muscles. Prior to Sherrington's work any ideas as to the function had been largely a matter of conjecture. Still, Charles Bell, as early as 1826, had believed in the entity of a distinct muscle sense—the so-called "sixth sense of Bell"—for which he believed an anatomical basis would some day be found. The final phase of the work—the embryological development of the muscle spindle—seemed to me neglected, perhaps because it had not impressed anyone as being very important, or more likely, because the material was not conveniently to be had for this study.

The few statements bearing upon the sensory nerve endings in embryos that I have been able to find in the literature are in articles by Bardeen, Kölliker, Schultze, Felix, and indirectly by London and Pesker. Bardeen ('00) says:

> It is only comparatively late in embryonic development that terminal nerve fibers or fibrils enter into intimate relation with tissue elements. I have succeeded in staining them with methylene blue and gold chloride and the earliest unions I have seen have been in 6–7 centimeter pig embryos. I believe, however, the union of the nerve fibers and muscle cells occurs considerably earlier.

In a later article ('02) he says:

> The final union of the growing tip of the nerve with muscle cells seem in the rectus muscle of the pig to begin in embryos of 8 cm. but definite endings are few until considerably later. The formation of nerve-endings cannot be satisfactorily followed in mammalian embryos, owing to the great number and small size of the cells. . . . . Union of nerve fiber and muscle cells does not begin to be common until this embryo has reached a length of 15 cm. and it continues for a considerable time after this period. . . . . The development of the sensory and vaso-motor apparatus can only be followed with satisfaction when better methods for differentially staining developing nerves have been devised. Methylene blue, which is so good for adult tissues seems to act much less specifically on developing nerve fibers.

Bardeen's work was done with gold chloride preparations. Kölliker ('05) in contradiction to the much earlier doctrine of Schultze ('85) that the endings arise from 'cell nets' advanced the now generally accepted view that both sensory and motor ending can each be traced back to a single cell. Schultze had been misled by the nuclei of the sheath cells of Schwann which grow out along the axones—prior to medullation—and beyond it on to the naked fibers of the endings. Felix ('89) has made an extended study of the muscle spindle as a whole, in the human embryo, under the mistaken idea that they represented specific areas for the production of new muscle cells after such production by multiplication of myoblasts had ceased. He did not, however, work out the relation of the nerve to muscle. London and Pesker ('06) give four lines to their own work on the subject: "The development of the spiral sensory endings is in this way—the end fibers branch profusely and surround spirally the muscle fibers which are likewise increasing in size." In their article they give two figures of sensory endings, ten figures of nerve outgrowths and one figure of motor endings. They call them all muscle spindles. Twelve of them are taken from mouse embryos 1.2–1.6 cm. long, one from a mouse two weeks old. The drawings are rather unsatisfactory. The paper is chiefly a discussion of literature upon various points of controversy.

It was largely on account of the wealth of material that was at hand, together with the fact that I had previously done some

work upon the adult neuro-muscular spindles, that I began the present line of research. I had in mind at the time more the idea of merely completing the picture already two-thirds done than of adding anything of real value, realizing, however, that as in other investigations where embryological study has been the first to throw light on the complex adult picture, so here too in the simplicity of the early development I might be able to expose important structures later obscured by the intricate form of the fully-grown spindle.

The first things that require consideration in work of this character are the selection of material and the methods best adapted for solving your particular problems. I was fortunate in not having any difficulty about getting material, as we receive at the laboratory a daily supply of embryo pigs in utero from the slaughter-house near by, so warm that frequently the hearts are still beating. In this problem of the development of muscle-spindles, an abundance of material is the most essential thing, for the value of any conclusions varies directly with the number of observations one is able to make. Then too, the early development of the pig corresponds very closely to that of the human in the many points where comparisons have been made. With any method for staining nerve endings there are many possible pitfalls. I found that it was not only necessary to work up one set of specimens, from each stage of development, but to repeat these observations time and again upon new material. My procedure was to take whatever length pigs came each day, using the larger sizes only at first, however; work them up and file away my sketches and observations under that particular size without regard to any previous findings in the pigs of that length. In this way I accumulated in time a rather complete series ranging at about 5 to 10 mm. intervals from 1 to 20 cm. In addition, I had numerous observations on each length, made at various times upon pigs from different litters. As a result, I feel that I have been able to eliminate to a great extent personal errors in observation and judgment.

When I began this work I used gold chloride preparations of the muscle to bring out the nerve endings, following out the

Ranvier lemon-juice method. This method is essentially that used by Bardeen ('02) in his work on the cerebro-spinal nerves. The fresh tissue is placed for five to ten minutes in freshly expressed and filtered lemon-juice, until translucent. It is taken out and washed rapidly in distilled water; then transferred to a 1 per cent gold chloride solution for fifteen to thirty minutes, depending upon the size of the tissue. After this it is again washed in water and placed in $33\frac{1}{3}$ per cent formic acid (Kahlbaum) in the dark for twenty-four hours. The tissue is now placed directly in glycerine and examined for nerve endings.

While this method is fairly satisfactory with adult tissue, it is much less so with embryonic muscle. I soon abandoned it because I found it practically impossible so to regulate the various factors in fixation as to secure any constant results. At best the method seemed to be very capricious, with great odds on the side of failure. Next, I decided to adapt for my use in embryo pigs, if that were possible, the method of intravitam staining with methylene blue, which has impressed me as being the method of choice at the present time in work upon nerve endings. Most of the best work upon adult neuro muscular spindles has been done with this method. Besides, Wilson in his article in The Anatomical Record ('10) has described so completely even the finest details of the method, together with the possible sources of error, that there is little excuse for not securing constant results. Indeed, it was the simplest thing to make a few alterations and so adapt it to use with the embryo pigs. I realize that there are dangers in confining one's efforts to a single method in any study upon the structure of the nervous system. The use of several methods would be valuable as a check upon each other. I feel, however, that with access to sufficient material one can, especially with the methylene blue method, keep away from errors of this sort, because one can control the accuracy of his findings by the constancy with which they can be demonstrated. The constant findings of a single method are perfectly valid whether they be verified by other methods or not. The fact that certain details seen with one method do not show up with another does not prove their non-existence but rather the specific-

ity of that particular method for the demonstration of the structure in question.

I aimed to follow Wilson's directions for the methylene blue method as closely as possible with the few modifications I found it advisable to make in using it with embryos. Instead of the previous erratic results with the gold chloride technique, I soon found that the nerve endings could be demonstrated in practically every case. The method briefly is this: Keep on hand a stock of 0.5 per cent methylene blue (Ehrlich) in distilled water. From this a fresh solution is made each day for injection by diluting one part of the stock solution with nine parts of physiological salt solution. The injection flask filled with the fluid is kept in the thermostat at 40°C. It was found best to keep it at this higher temperature since it is bound to drop a few degrees while being used. While a variation of a few degrees does not seem to matter, it is best to aim to have the solution reach the embryo at about 37°C. In order to keep the embryos warm it is well to have the room very warm, and besides, either keep the uterus in warm water or wrapped in warm towels. While injecting the fluid it is advisable to have the flask arranged so that it can be readily raised or lowered. A rope and pulley is as good a way as any. In this way the pressure can be regulated very easily. When everything is ready, put a glass cannula in the umbilical vein, after previously laying a loose ligature—ready to be tied quickly—around the umbilical arteries. Then connect the cannula to the flask with glass tubing, using rubber tubing only when absolutely necessary for connections, and then taking pains to bring the glass ends as close together as possible. Now cut across the umbilical arteries and start the methylene blue at a low pressure (18 inches)—to wash out all the blood. When the fluid coming from the arteries is bright blue, the ligatures already in place are tightened up to stop the flow from the vessels. Continue to inject—raising the pressure gradually until the tissues are so distended that the thin skin of the embryo is just ready to break. With the small sizes it is necessary to inject them while the embryo still lies in the amniotic fluid, with the amnion intact, for if the embryo is handled the skin is bound to break in places,

which makes it impossible to distend the tissues sufficiently. In these I use one of the placental vessels, ligating all the others so as to direct the solution into the embryo. For the smallest sizes I had to use an hypodermic needle instead of the glass cannula. When enough of the solution is injected, all the umbilical vessels are clamped off with a small hemostat. Then the embryo is placed in the thermostat for five to ten minutes, varying according to the size of the specimen. For the dissection of the eyes it is necessary to use instruments which have been kept in normal salt solution. In removing the eyes, dissect away the skull bones and cartilages, brain and sphenoid bone, so as to keep all the muscles attached to the eyeball. Then fasten the eye down to paraffin at the bottom of a pan with enough salt solution to cover the eye. The muscles are carefully removed and examined in salt solution on a clean slide. This allows the dye, which has become reduced and thus colorless in its union with the nerves, to oxidize and become blue. If the endings do not appear at once, place the muscle in the thermostat and examine it every few minutes. I found that it never took more than ten minutes for the endings to appear. The study was confined entirely to the extrinsic eye-muscles—except the checking of the results on the limb muscles—for several reasons. Many of the related problems had been thoroughly worked out upon the eye-muscle in pigs, such as their development, the time of connection with the cranial nerves and the structure of the adult sensory endings. Besides these, there is this advantage; in the earlier stages the muscles are so small that one can examine the entire muscle in a single preparation.

As soon as the nerves appear the tissue is ready to be fixed. There are two ways of fixation. One is to place the tissue in a large amount of 8 per cent ammonium molybdate (Merck or Kahlbaum) overnight in an ice-chest. This entire method is best carried out with the reagents kept on ice. Then wash the tissue in ice-water one-half to two hours. Remove it from the water and pass it through several changes of 96 per cent alcohol at a low temperature for one-half to two hours, then through several changes of absolute alcohol for one-half hour. It can

now be put in fresh absolute alcohol in a warm room. It is hurried on through xylol into paraffin. This is the only permanent fixation of the methylene blue stain.

The other method is to place the tissue in filtered saturated ammonium picrate overnight; clear it in equal parts of this picrate solution and glycerine; mount in pure glycerine. This method is not permanent but the endings remain distinct for about a month. While I studied some of my material by the ammonium molybdate fixation (as Wilson gives this as the most reliable method) I found that it had few advantages over the ammonium picrate preparations, except that it is permanent, nicer to work with, and the thin sections give a better picture of the individual muscle fibers—which can be counterstained. The advantages of the glycerine mounts are these: one can examine much larger pieces of tissues—even an entire muscle of the smaller embryos—under a single cover-slip. The nerve plexus, the individual nerves and their relation to the spindles can be studied without having to follow them through several sections. Above all, the spindles can be teased out and examined separately. With this fixative the nerves stain violet and the muscle yellow. The muscle striations and nuclei can be seen without difficulty after one becomes accustomed to working with the material.

It was my habit to make sketches of and study the fresh as well as the fixed material. Of course it was impossible to study a fresh specimen and then fix it, as they fade so rapidly, and for good fixation it is necessary to place the tissue in the fixing-fluids before the endings show up very sharply, otherwise many details are lost, as the fixatives have the tendency to overoxidize and dissolve out some of the stain.

The methylene blue is not absolutely specific for nerve tissues and quite a little practice was necessary before being absolutely sure of my ability to say what was nerve and what was not. One of the best clues in the identification of a nerve is the densely staining nodules that appear at short intervals along the non-medullated nerve. The medullated nerves are easily recognized by the deeply staining nodes of Ranvier. There is no difficulty,

except in the very earliest stages, in distinguishing the sensory from the motor endings, as the sensory endings are from the very first much more intricate. This can readily be seen in the figures. Then too, the development of the motor endings has been very thoroughly worked out by both Boecke ('10) and Mays ('92). My findings of the motor endings were essentially the same as theirs.

Fig. 1 Three stages in the development of the motor end plate. The upper drawing is from a 55 mm. pig embryo; the middle one from a 100 mm.; the lower from a 250 mm. embryo. The outlines of the individual muscle fibers are indicated by straight lines. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

There is one disadvantage in the methylene blue method, especially with the ammonium picrate fixative. It does not give as distinct a picture of the muscle as one would like. However, with the work of Bardeen ('00) on the development of the muscle fiber in pig embryos, as a basis, I soon experienced little difficulty in distinguishing the various types of muscle cells prior to

the appearance of striations. As soon as the striations appeared there could be no further trouble.

In the development of the eye muscle (Lewis) the premuscle mass is first detected in the 7 mm. human embryo. At this stage the third cranial (oculomotor) nerve enters the anterior end of the premuscle mass, dorsal to the optic stalk. At 9 mm. the muscle mass is somewhat enlarged. It begins to split into the different muscles, each with its respective nerve. The oculomotor, trochlear and abducens nerves have all now entered the muscle, which as yet is not attached either to sclera or to the precartilage but is still directly continuous with the mesenchyme. At 11 mm. the muscle is to be found dorsal and caudal to the optic stalk and mostly medial to the eyeball, partly continuous with the primitive sclera and with the precartilage about the optic nerve. At 14 mm. all the orbital muscles can be distinguished and have nearly the adult relations to the bulbus oculi. The early development is identical in both the human and early pig embryos.

So far, the muscles are composed entirely of myoblasts with no definite muscle bundles (Bardeen '00). At 18 mm. some of the cells have passed from the round myoblast state to the spindle-shaped muscle cell, in which the myofibrils begin to be laid down. At the 26 mm. stage, the entire periphery of the cell is filled with the striated myofibrils. Here some of the cells are now of muscle fiber type—very long spindle cells. These are divided into bundles with the older fibers centrally arranged. From now on the individual fibers increase in length and thickness, fibrils fill the fiber and are added peripherally. At 120 to 130 mm. the muscle nucleus, which up to this time was centrally located, comes to lie at the periphery of the fiber. By the 180 mm. stage all the nuclei are at the periphery and the sarcolemma has become distinct. Striated fibers are not produced after the first half of embryonic life in the human (Macallum '98) and only for a short time after birth in the pig (Bardeen). Many of the fibers first formed degenerate during embryonic life—perhaps because they fail to form a union with a nerve fiber; I have never found a degenerating fiber with a nerve ending.

With this preliminary summary of the development of the muscle, which I have taken largely from Lewis (Keibel and Mall) and Bardeen, we are ready to go ahead with a description of the sensory nerve endings in the muscle. My own actual method of procedure at first was, in general, to start with the adult endings in adult muscle and work backward—gradually using smaller and smaller embryos—as this kept me from falling into the possible error of mistaking other structures for nerve endings, or, what is more likely, other types of nerve endings for early neuro-muscular spindles. Thus I was working from known to unknown. Besides, this method had the advantage of proceeding from the easier to the more difficult work, since the mechanical difficulties of demonstrating the endings increases as the pigs get smaller because of the smaller vessels to insert the cannula into and the difficulty in isolating the eye muscles from the surrounding tissue. Still, for purposes of description it is more logical to begin with the first appearance of the nerve in the muscle and follow it along in its development toward the adult type. So I have selected certain stages at more or less regular intervals, which illustrate rather completely all the stages from the earliest to the adult picture. In between these are many which have been omitted that simply fill in the gaps and make the whole one gradual process of growth. Besides, I have included one figure for each stage which seems to show best the main points. One specimen in this way is very inadequate, for every spindle is different in pattern and size. The only way to get a really good idea is to see many of them.

The 12 mm. embryo is the earliest in which I was able to demonstrate the nerves and feel certain that the nerve was in the muscle and not simply nerve in the surrounding mesenchyme. Here the muscle is composed chiefly of the large round myoblasts, among which the nerve fibers run here and there. The fibers are delicate and wiry. Scattered along the axones at short intervals are the densely staining nodules—perhaps the sites of the future nodes of Ranvier—which give them the appearance of knotted cords. These fibers (fig. 2) do not form a plexus but run more or less separately, without regard to direction, in and

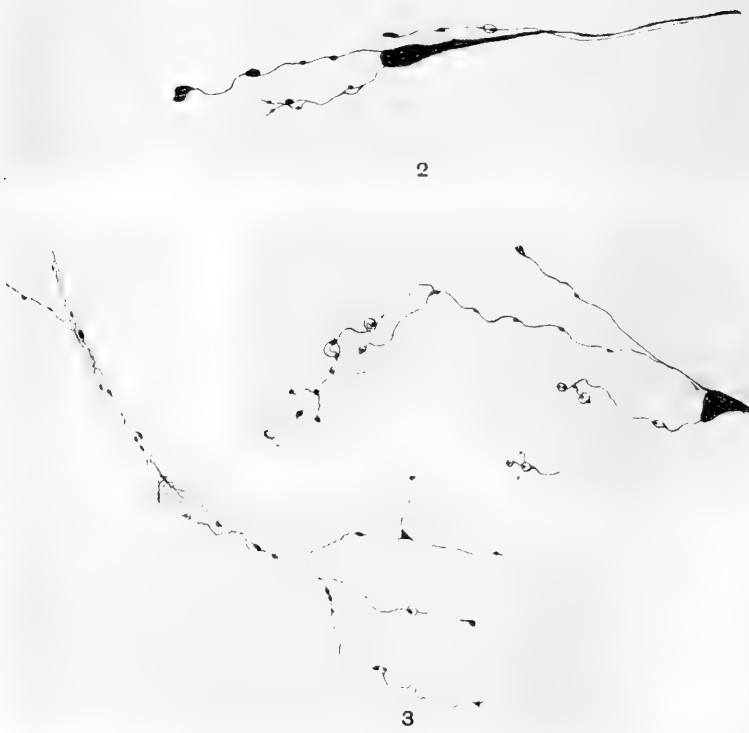

Fig. 2 The growing tip of a nerve, probably sensory, in a 12 mm. pig embryo. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

Fig. 3 Nerve ends from a 15 mm. pig embryo. The nerve in the upper right hand corner shows terminal fibrils in union with two myoblasts which are represented only in outline. The other nerve shows an inclusion of two connective tissue cells that are not to be confused with myoblasts. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

out among the myoblasts, terminating abruptly in bulbous ends, from which several more delicate fibrils extend out into the tissues for a short distance. These fibrils end in similar though smaller bulb-like tips, which have no apparent connection with any cells. This is seen best in the fresh specimens by producing currents in the salt solution under the cover-slip by means of a bit of blotting-paper placed at one side and a few extra drops of solu-

tion at the other edge. This gives a false circulation of fluid through the tissues which simulates rather closely what is seen when one examines a bit of tissue with the blood still moving in the capillaries. One sees the constant shifting of the cells, flattening out and becoming rounded again as they are jostled against each other by the moving fluids—the whole, a seething restless mass of cells. At this stage the nerve ends are seen to shift their positions among the cells, leaving old ones and coming to lie between new ones. Later, when a union is established between the nerve and myoblasts, this does not occur but they simply move back and forth with the cells to which they are attached. These preparations give one some idea of the continual motion of all the cells during life, which is lost altogether in studying fixed tissue only. From this it is an easy step of the imagination to picture the amoeboid growing tips of blood capillaries, lymphatics, and nerves working their way in and out among the tissue cells.

The first change of consequence from this very primitive picture is in the 15 mm. stage. Here, while the essential picture is the same as in a 12 mm. embryo, still one can see a decided advance. The nerve fibers are beginning to form a loose and very wide-meshed plexus throughout the muscle mass; the terminal fibrils are more numerous. These delicate tendril-like ends have come into connection with some of the myoblasts (fig. 3). This connection is a rather simple affair. The terminal branches either wrap themselves around the cell or widen out into small circular nets upon the surface as they cross the cell. As yet, not all of the terminal fibrils have united with myoblasts. So far I cannot say absolutely whether the endings are sensory or motor. Even here, however, there is a certain amount of difference in the type of terminal network from that in the motor endings. The spindle is already more complex than even later stages in the development of the motor plates. In the embryos that are longer than 15 mm. there is no difficulty whatever as can be seen readily by comparing the spindles with the figures of the motor endings, which I have included for this purpose.

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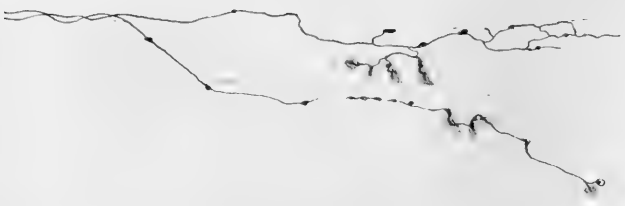

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Fig. 4 Sensory endings in a 17 mm. pig embryo. Numerous placques can be seen on the myoblasts. They are shown in gray, intimately connected to the neurofibrils. (Obj. Leitz, 1/12 oil immersion; ocular 4).

Fig. 5 A sensory ending in a 25 mm. pig embryo including three muscle fibers. A single myoblast, some of which still remain, is shown in outline to the right. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

In the 17 mm. embryos the endings show a definite change. Here for the first time we get, in addition to the nerve upon the myoblast, a placque (which I have drawn in gray; fig. 4) that extends along the fibril upon the surface of the muscle cell. In the fixed tissue it stains a dull violet in contrast with the greenish-yellow muscle cell and is paler than the rich violet color of the neurofibrils, which can be made out distinctly upon the placque, as plainly as in the figures. In the fresh muscle, where the nerve is bright blue, the placque is not so well seen, since there is not so much contrast between the two shades of blue

as there is between the violets. In some few cases it takes the stain very deeply, which also lessens the contrast of colors. This is especially true in the later stages as we approach the adult type. Here, however, it is obscured by the thickening of the neurofibrils plexus upon it, which if overstained will be a solid mass inseparable from the branching axone. I shall have more to say later as to the exact structure of this placque, which I regard as an intermediate substance between the neuro- and myofibril.

By the time the embryo is 25 mm. long, the endings have taken on a little more definite form. They are more numerous than earlier. Instead of the endings being bunched upon small round cells—as the myoblasts have grown into spindle cells—they are now spread out to a greater extent (fig. 5). The axone forms a very delicate plexus enclosing a part of the cell in a complete net. While the placques still lie more or less at random upon the muscle cell, they show a tendency to lie at right angles to the long axis of the cell. The number of muscle cells included in a single ending varies from one to four or five. More cells are added, from time to time, as the embryo develops. There does not seem to be any definite rule in this regard. Even in the adult neuro-muscular spindles the number of intrafusal fibers varies between wide limits. Adult spindles with only one or two fibers, however, are rare.

By the 55 mm. stage there remain few, if any, free nerve fibrils. All that I followed out led to either sensory or motor endings in the muscle. The axones intertwine among the muscle fibers, forming a very intricate plexus so arranged as to bring almost all of the muscle fibers into contact with a nerve. The nerves run along rather loosely in the smaller meshes of the plexus, leaving it abruptly from time to time, to extend off and end upon some nearby muscle fibers. The axones in the ending wind around the intrafusal fibers in a loose network. Some end upon definite placques, which usually occupy the middle section of the spindle, while others extend along the fibers for some distance and end in flower-spray ramifications. Within the spindles the muscle nuclei are more numerous than

elsewhere, though this is not an infallible rule. While the endings are, in the majority of instances, somewhere near the middle of the fibers, I have seen, at various times, endings in which the terminal net bound together two muscle fibers where they came together, end on. I think the nerve can attach itself at any point along the fiber. When union takes place in the myoblastic stage, as the cell grows the ending is left near the middle of the fiber. The spindles, which enclose the ends of muscle cells, have most likely included these fibers later within the terminal plexus. Not all the intrafusal fibers became a part of the spindle in the myoblastic stage. This seems to be the case in figure 8, where the ending upon the outer fiber is of the very simplest type. But no more muscle cells are added after the 200 mm. stage, for by this time the entire ending is enclosed in a definite connective tissue sheath. At this stage, where a few myoblasts still persist (in fact, they do not disappear altogether until 170 mm.—Bardeen) it is not uncommon to see them included in an ending with the more advanced muscle fibers (fig. 6). These cells do not all grow at the same rate. Just what is the determining factor is not known. The future history of the muscle cell seems to some extent to be dependent upon the neural connection. Bardeen ('02) says:

> It seems probable the sarcolemma is formed through the action of surface nuclei of the muscle fiber, in response, probably, to stimuli arising from the union of the nerve with muscle fiber; and it is so formed as to enclose that portion of the nerve which is spread out over the protoplasm of the muscle.

This is also suggested by the degenerative changes in many of the fibers later on in embryonic life. In any of the embryos longer than 75 mm., many muscle fibers can be seen which seem to be breaking up into irregular masses of protoplasm and nuclei. They also show an increase in the amount of intracellular pigment. With the methylene blue stain they are filled with many droplets which take up the blue stain. Bardeen ('00) regards this as a 'retrograde metamorphosis.' Perhaps the cells develop slowly up to a certain point and then, if there is no nerve connection established, they degenerate. If a nerve connection is necessary

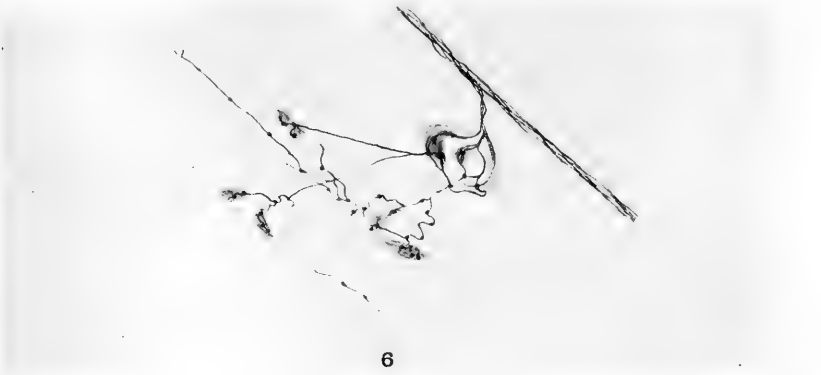

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Fig. 6 Neuro-muscular spindle from a 55 mm. pig embryo, showing both muscle fibers and a myoblast included in the same ending. (Obj., Leitz, 1/12 oil immersion; ocular 4.)

Fig. 7 A sensory ending composed of only two muscle fibers from a 65 mm. pig embryo. The branch to the spindle is seen leaving the nerve. The neurofibrils end both as free bulbous tips on the muscle fibers and with delicate branching upon the gray placques, which have nothing to do with muscle nuclei. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

for the muscle development it must be the sensory ending, for several anomalies which have been described (Leonowa '93; Frazier '95) show a fetus delivered at term in which the spinal cord with the ventral roots of the spinal nerves are absent while the muscles are normally developed with their sensory endings and peripheral nerves up to and including the posterior root ganglia. I know of no such cases, however, where the muscles were present and the peripheral sensory system lacking—unless some cases of amyotonia congenita fall into this class. But even in

these it cannot be said whether the absence of the sensory endings is a congenital defect or a late intrauterine degeneration. On the other hand, the congenital absence of single muscles may be due to their failure to receive a sensory nerve.

Harrison's work on tadpoles has proven conclusively that a nervous connection is not essential for the initial development of the muscle fibers. His experiments, however, do not go far enough to show that the life of a muscle fiber can continue sufficiently without a nervous connection, to give the picture found at birth. It is at this point that I would suggest that the sensory connection is of fundamental importance. It is impossible in view of Harrison's work to agree with Herbst ('01) that the sensory nerves are responsible for the initial development.

In the 65 mm. stage (fig. 7) we see some increase in the size and complexity of the endings. The placques are likewise increased in numbers. In all these stages a few of the spindles are farther advanced, others less so, than the ones I have drawn. It is almost impossible to pick out any one and say it is typical for that particular size. However, by taking what I consider the average, stages at 10 to 20 mm. intervals show rather definite progress toward the adult type. In figure 8, taken from a 75 mm. embryo, is shown an ending which is equally as numerous in both the 65 mm. and 75 mm. sizes, as the one shown for the 65 mm. stage. Here the plexus is spread out a little more but the placques are rather infrequent.

In figure 9, from a 100 mm. pig, I have represented the nature of the plexus which the axones make among the muscle fibers at this stage with its relation to one of the nerve endings. In every case I have omitted in my sketches, as far as possible, the structure of the surrounding muscle as it would simply complicate the picture without bringing out any important point. This spindle shows the most extensive plexus thus far, with numerous placques. The axones (neurofibrils) after branching upon these placques pass on in most cases to end in delicate tree-like branching along the muscle fiber.

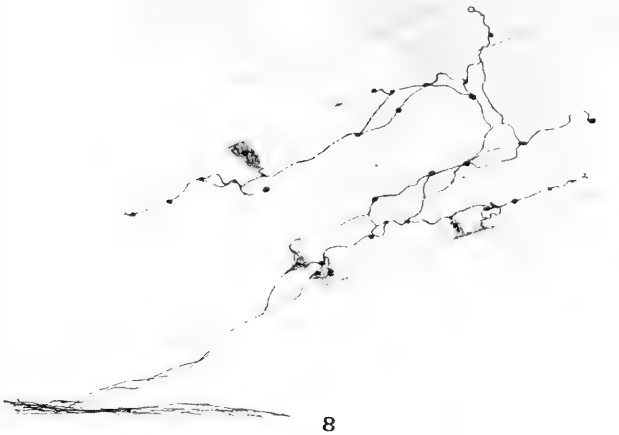

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Fig. 8 A neuro-muscular spindle from a 75 mm. pig embryo. (Obj., Leitz, 1/12 oil immersion; ocular 4.)

Fig. 9 The intermuscular nerve plexus showing its relation to one of the sensory endings, in a 100 mm. pig embryo. (Obj., Zeiss D; ocular 4.)

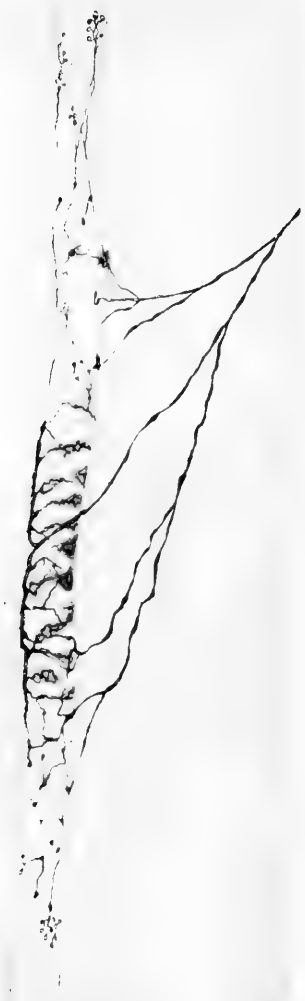

Fig. 10 A neuro-muscular spindle showing the annular arrangement of the placques with regard to the muscle fiber, from a 150 mm. pig embryo. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

In the 150 mm. stage, figure 10 shows how the nerve at times divides into several smaller branches as it approaches a fiber, some of them going to one end, some to the other, but all reuniting in a common network upon the muscle fiber. Now the placques are nearly all arranged perpendicular to the long axis of the fiber. All of them are the basis of the most delicate ramifications with the usual deeply staining nodules, along the fibrils and at their points of branching, only here much smaller than those on the axones themselves. These placques are applied closely to the under side of the sarcolemma, which is beginning to be formed around the muscle cell at this stage of development. They usually extend about one-half to two-thirds of the way around the muscle fiber, rarely encircling it completely. They appear to be made up of very fine granules with very high magnifications. In the later stages (fig. 11) from 175 mm. embryos on up to the full-term pig, they undergo a decided change in appearance. Instead of being the large structures with the delicate neurofibril network upon them which at once catch the eye, they become rather inconspicuous. The axones have thickened to a marked extent and in some cases have straightened out considerably, forming a close spiral around the fiber, still connected, however, by the rest of the original delicate plexus. At intervals along these thickened axones, though considerably hidden, are these same placques. In other cases where the spiral is not so prominent the light granulation of the placques is obscured by what seems to be a continuation or spreading out of the axone, undoubtedly due to an increase in the neurofibri net upon the placques to such a point that the methylene blue no longer differentiates them. These correspond with the type seen in the adult endings in tendons. Dogiel in his article on "The fibrillar structure of tendon spindles," refers to them thus:

> Some of the fine branches spread out as three or many cornered pieces and from these corners other delicate branches extend. The presence of these larger pieces gives the nerve apparatus an especially characteristic appearance. They have the form of placques which seem to grasp the fibers. The fine branches contain neurofibrils which in the placques widen out into a net from the corners of which they then

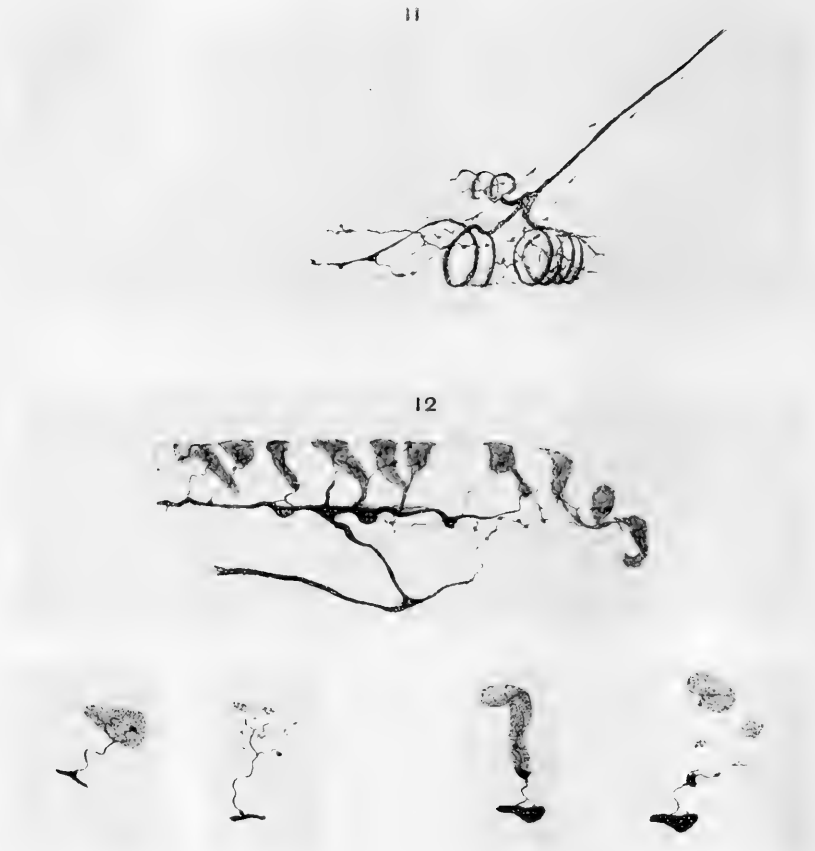

Fig. 11 A spindle in a 175 mm. pig embryo. Here the placques are thinned out along the annular nerve fiber, the whole of which is connected to a delicate network of neurofibrils that surrounds the entire muscle fiber. (Obj. Leitz, 1/12 oil immersion; ocular 4.)

Fig. 12 Fragments of a neuro-muscular spindle from a 150 mm. pig embryo. Above is part of a muscle fiber that has been teased out of a spindle. One of the placques, still retaining its nervous connection, hangs free from the right end of the muscle fiber. The lower drawings are of two of these placques, isolated from the muscle fiber, before and after crushing. They show the granular nature of the placques, and also how they can be separated from the wiry nerve filaments. (Obj. Leitz, 1/12 oil immersion; ocular 8.)

stretch over to the next placque, where we get a similar appearance. There is a great similarity between these and the true muscle spindles. The two may at times be continuous.

I am strongly impressed that the early placque which I have described is the basis upon which the neurofibrillar net is stimulated to form and in the later stages is obscured by it. I am sure that it differs in composition from both the muscle and nerve and that it is not a very fine neurofibril net. It stains rather characteristically and is constant in nature. It looks coarsely granular under high magnifications. By teasing out one of the muscle fibers from a spindle one will find at times that a placque will separate from the sarcolemma (fig. 12). If the fiber is now crushed in glycerine between a cover-slip and slide, other placques can be removed in a similar way, with the nerve plexus still intact upon them. This would show that they are distinct from the muscle fiber. In a number of instances I was then able to crush these placques further so that they separated sharply from the rather wiry little nerve filament (fig. 12). If it had simply been a delicate plexus of the nerve filament, the latter, after being torn loose, should have shown a few torn branches, but instead it was perfectly smooth throughout. Then too, when the placque did go to pieces its consistency did not impress one as that of a delicate network of fibers. I am rather inclined to look upon it as an intermediate substance between the neurofibrils and myofibrils.

That an intermediate substance does occur in the motor endings, most neurologists are agreed; this is based upon the action of curare, the South American arrow poison. We also know from Langley's work that this substance does not participate in the degeneration of the motor nerves. Langley holds the view that the receptive substances are radicles of the contractile molecule, and that those at the nerve ending are special developments of those present throughout the muscle fiber. As yet there is no anatomical basis for this substance. Boecke in his work upon the development of the motor endings reaches the conclusion that the neurofibrils and myofibrils are continuous in the motor plate. However, I think here the physiological evidence

will outweigh the appearance of high magnifications of neurofibrils coated with a precipitation of silver. If an intermediate substance stands between the passage of the impulse from nerve to muscle in the one case, the chances are that a similar, though most likely differently arranged, substance stands between muscle and nerve in the other case.

In order to see if this placque degenerated with the sensory nerve, I performed a series of experiments upon rabbits. Following Huber's ('00) work upon the motor endings, I cut the posterior tibial nerve of one leg and examined the plantar interossei muscles after the nerves had degenerated. Using the normal leg as a control, I was unable to stain anything that resembled the placques after the nerve and nerve endings had degenerated. This staining is not as satisfactory as a pharmacological test such as with curare and nicotine on the motor end plates—and I am unwilling to draw any conclusions from a negative finding one way or the other. It appears, however, that whatever the nature of the placque, it loses its staining properties with the degeneration of the nerve ending.

Beyond this 175 mm. stage there is little more to describe except the gradual growth in the size of the ending, which is already approximately identical with the adult spindle. Two changes, however, are worth mentioning; the intricate plexus of small nerves disappears gradually until we simply have the large branching nerves which have now become medullated, running here and there throughout the muscle. The other point is that the intrafusal muscle fibers, which in the stages already described are striated throughout their entire length, when they get beyond the 200 mm. length begin to lose their striations within the spindle sheath. At the 200 mm. stage the average spindle consists of a heap of nuclei of both connective tissue and muscle origin, in addition to the several muscle fibers with the intricate annular and flower-spray endings. The entire mass is enclosed in a connective tissue sheath, which becomes distinct at about this stage of development. Some of the spindles include as many as fifteen fibers. This is essentially the same picture that we find in the adult eye muscles of the pig.

SUMMARY

The nerve endings arise early in embryonic life. The axones grow out from the cells of the sensory ganglia into the premuscle mass. Here they form an intricate plexus, so as to bring the muscle cells in contact with the end of the nerve. The axone becomes attached to the myoblast by the simplest kind of a neurofibrillar net. With the development of the myoblast into the adult muscle fiber, this net becomes more and more complex. In addition to this net there is formed a placque which I am inclined, on morphological grounds, to regard as an intermediary structure, which future work may identify as a receptor substance similar to that occurring in motor endings.

I wish to take occasion here to thank Dr. Florence R. Sabin for the help which she so willingly gave me at various times during this work.

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THE DEVELOPMENT OF THE VENOUS SINUSES OF THE DURA MATER IN THE HUMAN EMBRYO

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SEVENTEEN FIGURES

CONTENTS

INTRODUCTION

In the course of a recent study of the structure and topography of the endolymphatic sac in older human embryos it was found necessary to determine in greater detail than is furnished in the present literature, the relations of the blood vessels of this region, and particularly of the large dural veins that eventually form the dural sinuses of the adult. The abundant material in this laboratory that was found available for this purpose has made it possible to fill out the essential stages in the development of these veins with considerable completeness. The work has been much facilitated by the proximity and advice of Professor Mall who, it will be remembered, was the first to give a comprehensive description of these blood vessels in the human embryo and, in a way, this paper forms a supplement to his original publication.

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Acknowledgment should furthermore be made to Professor Sabin and Professor Evans whose valuable experience in the study of the vascular system and whose unique preparations were generously placed at the writer's disposal. Under these conditions the opportunity has been especially favorable for clearing up some of the obscure factors in this interesting field. It is also believed that, in addition to any interest the reader may have in the morphological details of the veins themselves, his attention will be attracted by the bearing they have on the broader principles involved in the establishment of vascular drainage. On following through the successive stages, which are to be outlined in the following paper, it will be noted that they afford striking examples of embryonic change and repeated adjustment of the drainage channels consequent upon the alterations in the form and condition of the particular area drained. Nowhere do we find a clearer picture of these adjustment phenomena than in the region of the brain. Its marked change in form and especially the prolonged relative growth of the cerebral hemispheres make a continuous series of alterations of the veins necessary that extend far into the late embryonic stages.

MATERIAL AND METHODS

The material on which this study is based consists chiefly of serial sections of human embryos taken from the Mall Collection, which now forms the nucleus of the Department of Embryology of the Carnegie Institution. In many of the specimens the blood vessels had been previously injected with India ink or Berlin blue or both. In one instance, No. 458, 54 mm. long, a total preparation of an injected specimen was made and cleared in oil and drawings were made directly from the specimen with the camera lucida. The principal stages, however, were usually based on profile reconstructions prepared from serial sections. These will be specified under their individual descriptions. In some cases portions of the veins were modeled after the Born reconstruction method. The oldest stage examined was in an embryo (No. 234 a) 80 mm. crown-rump length. The youngest stage was an embryo (No. 588) 4 mm. greatest length, at which

time the otic vesicle has differentiated far enough to make it possible to recognize the situation of the endolymphatic appendage. Before this time certain important phases in the development of the blood vessels of the head have already occurred. These will now be briefly reviewed as an introduction to the subsequent conditions.

PRIMARY VASCULARIZATION OF HEAD

The most complete account we have of the vascularization of the brain and its earliest drainage is based on the chick and the pig. For descriptions of these we are largely indebted to Evans ('09, '12). According to this author, the first blood vessels to the brain take the form of a capillary plexus that sprouts out from the aortic arch. These sprouts form a vascular web that closely invests the neural tube, beginning in the region just caudal to the optic stalk and spreading from there up over the adjacent fore- and mid-brains. By the coalescence of other capillary sprouts from the dorsal aorta a continuous channel is established along the side of the hind-brain that drains these capillaries of the head caudalward toward the venous end of the heart.

A study of the cardinal veins in the chick has recently been made by Professor Sabin ('14, '15) in this laboratory, and she has kindly demonstrated her specimens to me. From these specimens the development of the drainage of the primary brain plexus can be plainly made out. In the head region, proper, capillary sprouts from the dorsal aorta fuse into a continuous channel that drains the brain plexus caudalward to the vagus region. At this point it joins a small capillary plexus in which the anterior cardinal vein is to form. This latter plexus is one that develops between the aorta and the vitelline vein. It has been demonstrated by Professor Sabin that it differs from the head plexus in two essential particulars: (1) The sprouts from the aorta from which it is formed bear a definite relation to the cervical myotomes; (2) These sprouts also bear a definite relation to the nephrotomes and thus identify themselves as belonging to the cardinal system. Shortly after the time that this plexus is joined

by the head vein its direct connection with the aorta is broken and a circulation is thus established from the primary brain plexus backward through the cardinal plexus to the venous end of the heart. In the formation of this circulation we are thus dealing with two capillary groups; the cephalic one, belonging intrinsically to the head, and the caudal one, belonging to the cardinal system.

Very soon after the establishment of this circulation in the head it can be seen that the single vessel derived from the fusion of dorsal sprouts of the aorta, which we have described as developing caudalward alongside of the brain, has become the middle segment of a prominent longitudinal channel that extends all the way from the optic stalk to the duct of Cuvier. The cephalic segment of this channel and its tributaries are formed in the more superficial loops of the primary brain plexus; its caudal segment forms in the cardinal plexus and constitutes the anterior cardinal vein, eventually the internal jugular vein. The whole channel is designated as the '*primary head vein*' and with its establishment we may regard the primitive arrangement of the drainage of the brain as completed. Essentially, it consists of a sheet of capillaries that nearly everywhere surrounds the brain tube, and this capillary sheet is drained by many irregularly placed anastomosing loops into the more superficially placed primary head vein, which runs along the side of the hind-brain and empties finally in the duct of Cuvier at the venous end of the heart.

STAGES OF DEVELOPMENT OF THE DURAL VEINS

1. Human embryos 4 mm. long

It is this primary arrangement of the drainage of the head, which we have just described, that exists in 4 mm. human embryos, and this is the earliest stage examined in connection with the present study. In figure 1 is shown a profile reconstruction of such an embryo (No. 588, 4 mm. long, Carnegie Collection). This is slightly younger than the stage shown by Mall ('05) in his figure 3. The conditions in the two, however, are very similar and the relations of the main vein are the same. In our figure the

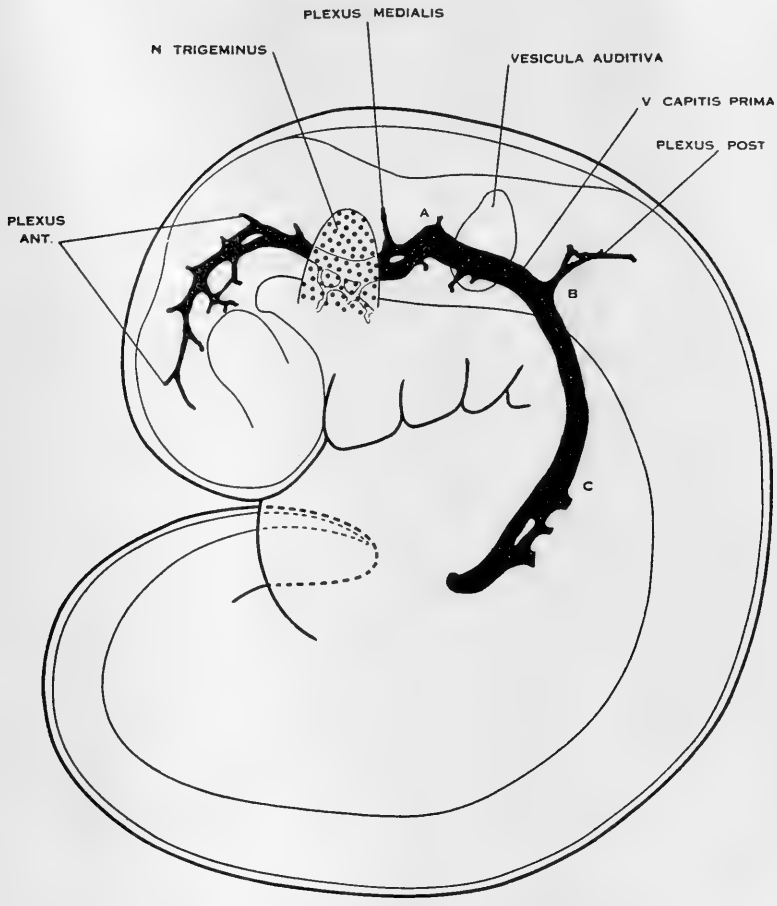


Fig. 1 Profile reconstruction of the primary head vein and its tributaries in a human embryo 4 mm. long (No. 588, Carnegie Collection). At the point marked 'A' the vein is bent out of its direct course by the facial and acoustic nerve-mass. The segment included between 'B' and 'C' represents the cardinal portion of the primary head vein, which eventually becomes the internal jugular vein. Enlarged about 33 diameters.

outlines of the neural tube, the semilunar ganglion and the otic vesicle, are indicated and their relation to the venous system of the head is shown. It happens that this embryo is bent transversally in its longitudinal axis, so that its left profile presents a convex surface and its right profile a concave surface. Thus,

on account of the oblique position of the head, the large vein of the head in the profile reconstruction shown in figure 1 necessarily assumes a position that is more dorsal than it would be in a true profile. In representing the veins only the principal channels are drawn in; no attempt was made to trace the small venules and their connection with the capillary plexus. Examination of the sections, however, shows that a capillary plexus exists and closely invests the neural tube and the adjacent nerves and sense organs. Minute anastomosing vessels can be seen connecting the plexus with the larger venous channels.

We thus have a simple system of drainage. The main channel constitutes the primary head vein, a vessel consisting of a single layer of endothelial cells. Its tributaries begin to unite in the region of the diencephalon. In the region of the semilunar ganglion they have coalesced into a main channel that passes median to the ganglion. Further caudal it is bent out of its course to pass lateral and dorsal to the acustico-facial complex. It passes lateral to the otic vesicle and the glossopharyngeal ganglion and then bends inward to become median, and finally dorsal, to the ganglion nodosum of the vagus nerve, whence it passes down to empty into the duct of Cuvier. The primary head vein receives everywhere many tributaries, chiefly ventral and dorsal. The ventral ones are more numerous near the optic stalk and in the neighborhood of the nerve-ganglion masses. The dorsal ones may be classified in three groups; (1) an anterior group from the region of the diencephalon and mesencephalon; (2) a middle or cerebellar group in the region between the trigeminal nerve and the acustico-facial complex; and (3) a posterior or occipital group from the neighborhood of the vagus rootlets. Especial attention is directed to these three dorsal tributary plexuses, as their arrangement is significant for all the later stages, which will presently be seen.

As we pass to older stages, where the dura mater and the arachnoid spaces are forming, we find that many of the anastomosing channels between the capillaries of the brain and the primary head vein close off, and there is a general separation or cleavage of the more superficial primary head vein and its tribu-

taries from the deeper veins, arising from and draining the capillary sheet that immediately surrounds the brain tube. This deeper system, however, continues to drain into the former at certain restricted places. We can thus distinguish between veins of the dura mater and the cerebral veins. It is the former that are chiefly concerned in the formation of the venous sinuses and with which we are chiefly interested in the present study.

2. Human embryos 14 mm. long

In figure 2 is shown an embryo in which the veins of the dura mater are already separated to a considerable extent from the cerebral veins. The veins of the head of this embryo (No. 940, 13.8 mm. long, Carnegie Collection) were distended with a natural blood injection and at the same time the surrounding tissues were quite transparent, so it was possible from a surface examination to determine their arrangement with considerable detail. The specimen was photographed and a print made, which was then elaborated with the details that could be seen in the specimen with the aid of a binocular microscope. Comparisons were also made with serial sections of other embryos of about the same age and in which the blood vessels had been injected with a colored mass. In the collection No. 544 is a particularly good series of that kind, showing about this same arrangement of the drainage of the head. Mall ('05) has pictured about the same stage in his figure 9. This stage is also pictured by Markowski ('11) in his figure 1. In the main points all three figures correspond rather closely. A large venous channel is formed in the region lateral to the diencephalon and passes backward, median to the trigeminal nerve and lateral to the otic capsule, through the region of the future middle ear, where it bends sharply downward in the neck region finally to empty into the duct of Cuvier. All the veins of the cranial region drain into this main channel. This constitutes the primary head vein, with which we are already familiar. It was described by different writers as the 'anterior cardinal vein' until Grosser ('07) showed that only the caudal portion of it—the part that is found in the region of the somites

and later forms the internal jugular vein—could be properly spoken of as the anterior cardinal. The portion in the pr segmental region was designated by Salzer ('95) in the guinea-pig as 'vena capitis medialis' and 'vena capitis lateralis,' depending on whether it was found median or lateral to the cranial nerve trunks. The more cephalic portion, in the trigeminal region, is always found median to the nerve and hence is always vena capitis medialis. Caudal to the trigeminal nerve Salzer describes it as at first coursing medial to the facial, glossopharyngeal and vagus nerves, and subsequently, by a process of 'island formation,' migrating lateral to these same nerves, that is, changing from vena capitis medialis to vena capitis lateralis. These terms were advocated on the basis of an homology with similar veins in the lower vertebrates. The importance of vascular homologies practically disappears on the acceptance of the Aeby-Thoma conception of the adaptive capillary formation of blood vessels, which has been so clearly established by the brilliant chick injections of Evans ('09) and therefore in this paper the terms vena capitis medialis and vena capitis lateralis will not be used. It is felt that the term 'primary head vein,' covering both of them, will be less confusing and will be entirely adequate from the youngest stages up to embryos about 20 mm. long. The composite origin of this vein, however, should not be forgotten. It has already been pointed out that it belongs in part to the trunk (the anterior cardinal vein) and in part is intrinsic to the head. As we shall presently see, it is the trunk portion, or anterior cardinal, that forms the internal jugular vein, whereas the intrinsic head portion in its more anterior segment becomes the cavernous sinus, the posterior portion (the so-called vena capitis lateralis) disappearing entirely and being replaced by a more dorsally situated channel.

The tributaries draining into the primary head vein are arranged in three plexiform groups (fig. 2), as was pointed out by Mall ('05), the first group emptying into the main channel in front of the semilunar ganglion, the second group between the semilunar and the acustico-facial ganglia, and the third group caudal to the otic capsule. These were designated respectively

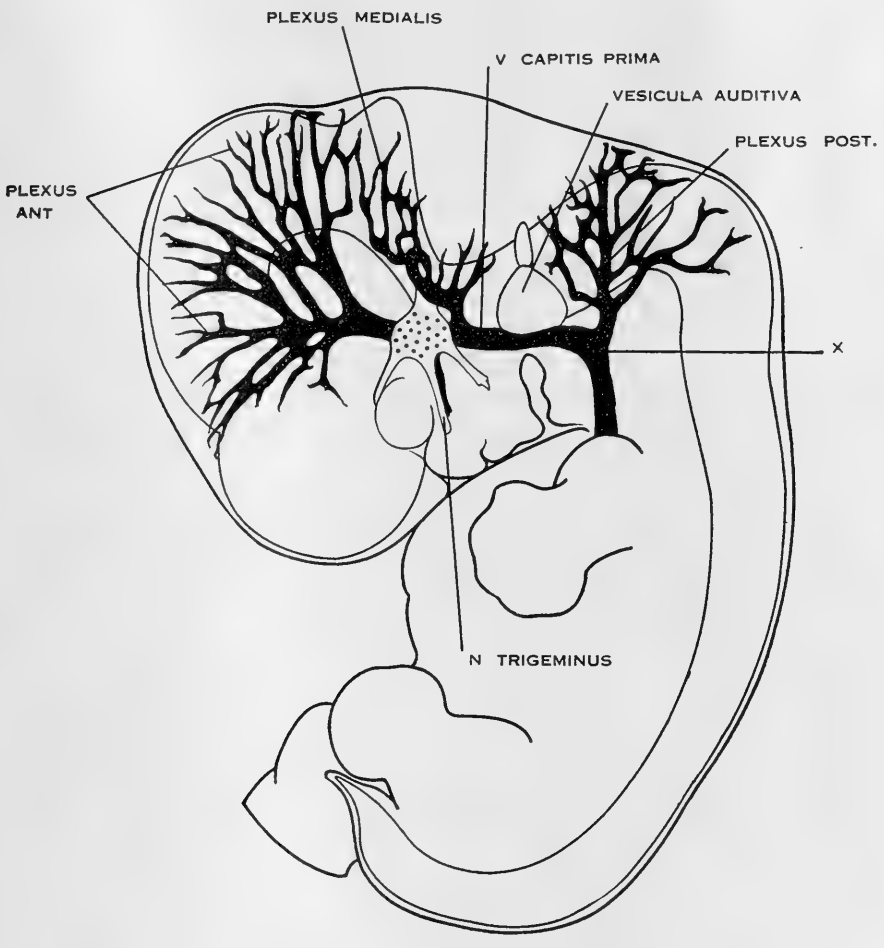


Fig. 2 Drawing of the primary head vein and its tributaries in a human embryo 13.8 mm. long (No. 940, Carnegie Collection). The point marked 'X' is the junction of the intrinsic head-portion of the primary head vein with the cardinal portion or internal jugular vein. Enlarged about 7 diameters.

the '*anterior*,' '*middle*' and '*posterior cerebral veins*.' The latter two each empty into the main channel through a single trunk, but the anterior cerebral vein maintains the character of the original plexus and has multiple openings into the primary head vein. Furthermore, the veins forming these three groups belong chiefly to

the dura mater and the tissues forming the membranous cranium. There is therefore an advantage in adopting a terminology something like that of Markowski ('11). In doing so, a distinction between the lateral and mesial portions will not be made; on the other hand, the three groups as given by Mall will be retained. We will thus speak of the '*anterior*,' '*middle*' and '*posterior dural plexuses*,' or more formally, '*plexus durae matris anterior*,' '*plexus durae matris medialis*' and '*plexus durae matris posterior*,' as they are indicated in figure 2. In this figure only the larger channels of the plexus are shown and it is to be understood that an intervening smaller venous mesh connects them more or less completely.

In the ventral portion the dural plexuses are more or less completely separated from the deeper-lying plexus belonging to the wall of the neural tube, from which are developed the cerebral veins. In tracing the plexus dorsalward toward the median line we find an increasing frequency of communication between the two, and near the median line they are so intimately connected that it is impossible to distinguish between them; in other words, in this region the cleavage between these two layers is not yet established. It is interesting to note that there seem to be favorable places for the larger channels to cross dorsally over the median line; one of these is the caudal end of the roof of the fourth ventricle, another at the junction of the mid-brain and hind-brain, and a third over the diencephalon along the caudal margin of the cerebral hemisphere. Where these vessels cross the median line they are usually bilaterally asymmetrical but may anastomose with the plexus of the opposite side. Other than at these three regions, the larger channels as a rule do not reach the median line.

In addition to the three dural plexuses mentioned above, the primary head vein receives a large ventral tributary that lies median to the maxillary division of the trigeminal nerve and also other small veins in that region. These veins drain the tissues that in part are to form the orbit and represent the future ophthalmic veins.

3. Human embryos 18 mm. long

On examining different series of about the 14 mm. period of development and a little older one can see that the primary head vein maintains the same course and relations, but the pattern of the dural plexuses is constantly changing. In embryos about 18 mm. long an important change occurs by which the blood from the region of the middle dural plexus drains caudalward into the posterior dural plexus through an anastomosing channel that becomes established between these two plexuses, passing dorsal to the otic capsule and just lateral to the endolymphatic sac. This can be seen in figure 3, which shows a graphic reconstruction of a human embryo, 18 mm. long (No. 144, Carnegie Collection, crown-rump length 18 mm. formalin; 14 mm. on slide). This is the same embryo shown in Mall's figure 11 and is about the same age as the embryo pictured in figure 2 of Markowski. In some respects the reconstruction referred to differs from both of these. From Mall it differs in that the greater part of the mid- and forebrain is still drained by the primary head vein. From Markowski it differs in that there is not yet a single large channel passing backward from the anterior and middle dural plexuses, but instead this region still shows an extensive anastomosing network not differing much from the pattern we have already seen in figure 2.

An interesting feature in connection with the dural plexuses presents itself in that the trunk that originally drained the middle dural plexus into the primary head vein nearly disappears, owing to the fact that the blood that it heretofore carried (i.e., from the cerebellar region and the posterior part of the mid-brain) adopts the new channel that is established dorsal to the otic capsule and is thus drained into the posterior dural plexus. As a result of this the original trunk that connected the middle plexus with the primary head vein becomes relatively small and partially breaks up into a small plexus. We shall see later, however, that with the next change in the head vein this trunk will open up again as an important channel.

In taking up the question of terminology for figure 3, it is found that most of the terms used in figure 2 are still applicable.

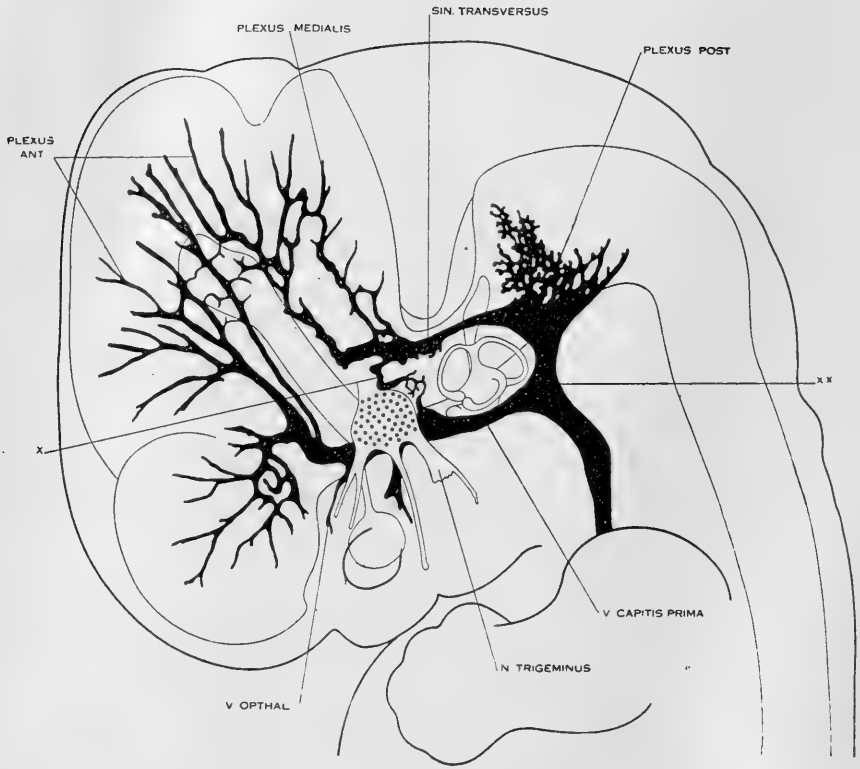


Fig. 3 Profile reconstruction of the veins of the dura mater in a human embryo 18 mm. long (No. 144, Carnegie Collection). The 'x' indicates the original trunk of the middle dural plexus; it corresponds to the superior petrosal sinus. The 'xx' marks the upper end of the internal jugular vein. Enlarged about 12 diameters.

There are the three dural plexuses draining into the primary head vein and also the opthalmic veins. The anterior dural plexus, however, can be seen to be reshaping itself so as to come into closer anastomosis with the middle dural plexus. The middle dural plexus by draining, as it does, over the otic capsule presents the first stage in the formation of the transverse sinus, that is, the sigmoid portion of it. The posterior dural plexus shows less change in its form and connections than any other group of the head veins and this is true also in the later stages. There are some minor alterations in its pattern, but otherwise it simply

extends to become the occipital sinus of the adult and at the same time, together with its fellow, develops drainage channels that empty into the plexus of the tentorium. The primary head vein can be subdivided into: the trigeminal portion that is to form the cavernous sinus; the otic portion which passes lateral to the otic capsule accompanying the seventh nerve; and lastly, the cervical portion or internal jugular vein, the boundary of which is indicated in figure 3. The otic portion already shows a diminution in volume as a result of the establishment of the new drainage channel dorsal to the otic capsule. Dorsal to the otic capsule there is sufficient free space for the development of a vascular channel, whereas the region ventrolateral to the otic capsule becomes crowded by the development of the cochlea and middle ear. This constitutes a mechanical factor that doubtless has a determining influence upon the change in the course of this blood channel.

4. Human embryos 20 mm. long

In embryos about 21 mm. long the veins of the head have an arrangement that is intermediate between the embryonic type and the adult type. The veins in the basal portion of the skull closely resemble the adult, while the dorsal veins still have many embryonic features. In figure 4 is shown a graphic reconstruction of the head of such an embryo (No. 460, 21 mm. long, Carnegie Collection). The reconstruction of the veins in this case was greatly facilitated by the work that had already been done on the head of this embryo by Professor Lewis, who kindly put all his tracings and photographs at my disposal. The outlines of the central nervous system are taken directly from a model prepared by him. The study of the veins was facilitated through the fact that the blood vessels had been injected through the umbilical vein with India ink by Professor Sabin, while the heart was still beating, so that there is a beautiful injection of the entire vascular system. Before the embryo was cut sketches and photographs of the vessels that could be seen from the surface were made by Professor Evans. There is enough material at hand in relation to this specimen to make a very complete study of its whole vascular system. The writer's attention, however,

was confined to the veins that are under discussion. For the sake of comparison, another embryo slightly older (No. 632, 24 mm. Carnegie Collection) was studied and a profile reconstruction of it is shown in figure 5.

On examination of figure 4 it will be seen that the primary head vein is now separated into its adult parts. In the trigeminal nerve region we can speak of it as the cavernous sinus, receiving as tributaries the ophthalmic veins and a large cerebral vein draining the lateral wall of the diencephalon. This vein belongs to the cerebral vein-system and runs for the most of its course through the pia-arachnoid membranes. It penetrates the dura and runs a short dural course before joining the cavernous sinus. It may be regarded as one of the diminishing number of channels that drain the cerebral venous system into the dural system. Besides these there are smaller tributaries from a network in the region of the semilunar ganglion. No tributaries were detected flowing into the cavernous sinus from the cerebral hemisphere, such as were found up to this time; all this blood now flows in the opposite direction, caudalward into the developing transverse sinus.

Tracing the cavernous sinus backward, it can be seen that the interruption between it and the internal jugular vein is complete, though there is still a remnant of that connection, which extends as a blind channel a short way along the facial nerve. It is interesting to note that we occasionally find in the adult skull a persistent foramen, the 'foramen jugulare spurium' of Luschka, which corresponds to the exit of this decadent channel. The vein itself, however, has never been described as persisting, although it exists normally in lower forms as a drainage for the anterior part of the brain, passing through this extracranial course to empty into the internal jugular vein. In the stage we are studying the drainage of the cavernous sinus is upward over the semilunar ganglion into what may now be recognized as the transverse sinus. This communication is through a short channel that approximately represents the original trunk of the middle dural plexus, and constitutes the superior petrosal sinus. This channel is designated by Markowski ('11) as the 'vena prootica,' and he

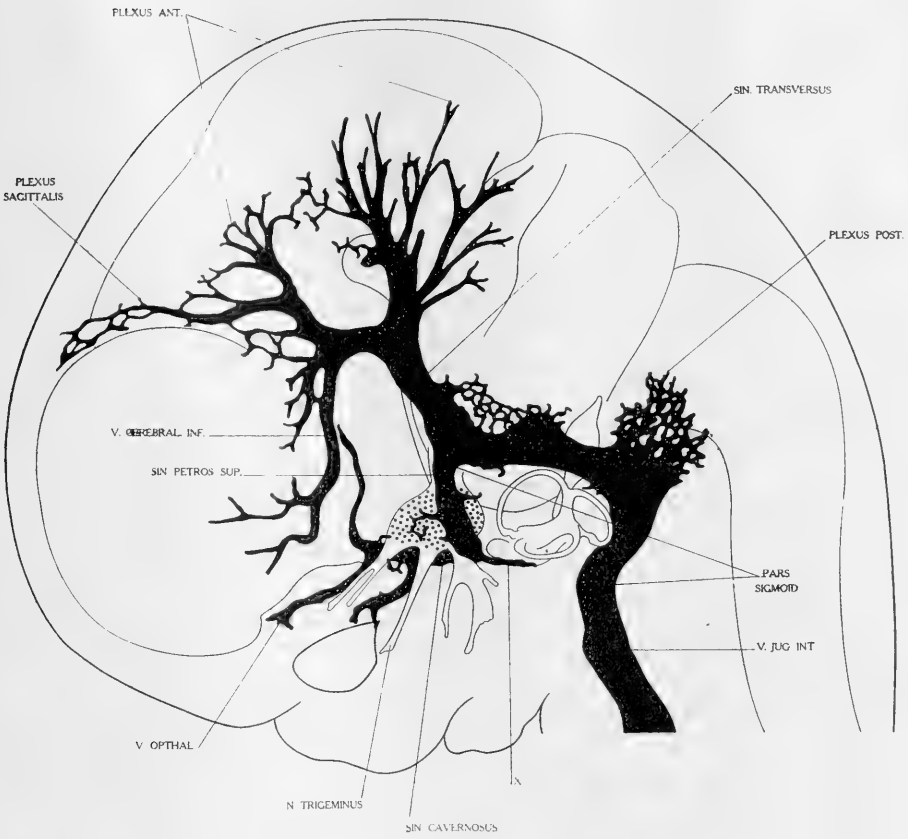


Fig. 4 Profile reconstruction of the veins of the dura mater in a human embryo 21 mm. long (No. 460, Carnegie Collection). The vein marked 'x' is the remnant of the otic portion of the primary head vein which originally connected the cavernous region with the internal jugular vein. The outlines of the central nervous system in this model were taken from a model made by Professor Lewis. Enlarged 10 diameters.

gives a different origin for the superior petrosal sinus. According to him (p. 599), it takes its origin from a small cerebral vein derived from the basal surface of the hind-brain which empties into the v. prootica. In the further development, the opening of the vein migrates by anastomoses along the v. prootica toward the sinus transversus and either empties into that sinus or into the v. prootica near it. According to him, the superior petrosal sinus

has little connection with the cavernous sinus and morphologically represents a metencephalic vein. Regarding the eventual fate of the vena prootica he has apparently made no observations, though he pictures it as a large channel in an embryo 46.5 mm. long. From the specimens I have examined I cannot confirm Markowski's description of the superior petrosal sinus and I feel convinced that his vena prootica and the superior petrosal sinus are one and the same thing, and that which he regards as the superior petrosal sinus is, instead, one of its tributaries. Mall ('05, p. 17) also described the superior petrosal sinus as the adult form of the 'vena cerebralis media,' which, it will be remembered, is the same as the trunk of our middle dural plexus.

With the alterations in the primary head vein, the anterior, middle and posterior dural plexuses are drained by means of the new dorsal channel, which empties through the jugular foramen into the internal jugular vein. This channel can at once be recognized as the transverse sinus and the sigmoid portion of it presents relations that are much the same as is found in the adult. The three dural plexuses are still of the embryonic type. The posterior plexus is practically the same as was seen in 18 mm. embryos. Only its coarser meshes are shown in figure 4. A finer plexus extends from this toward the median line and the region of the tentorium.

The whole dural area lying between the cerebral hemispheres and the margin of the cerebellum constitutes the tentorium cerebelli. It is very broad dorsally and is more constricted ventrally, thus in profile it is wedge-shaped. In the loose tissue composing it are found the meshes of the dural plexus. As this region becomes more compressed, consequent upon the growth of the cerebrum and cerebellum, there is a continual adjustment of the contained venous channels and repeated alterations in the pattern of the meshes. In general we find the larger channels radiating upward toward the mid-brain region, and as we approach the median line the plexus becomes finer and there is an intimate anastomosis with the subjacent plexus belonging to the brain wall.

On comparing embryos 21 mm. long with those 18 mm. long there are seen two characteristic changes that occur in the pattern

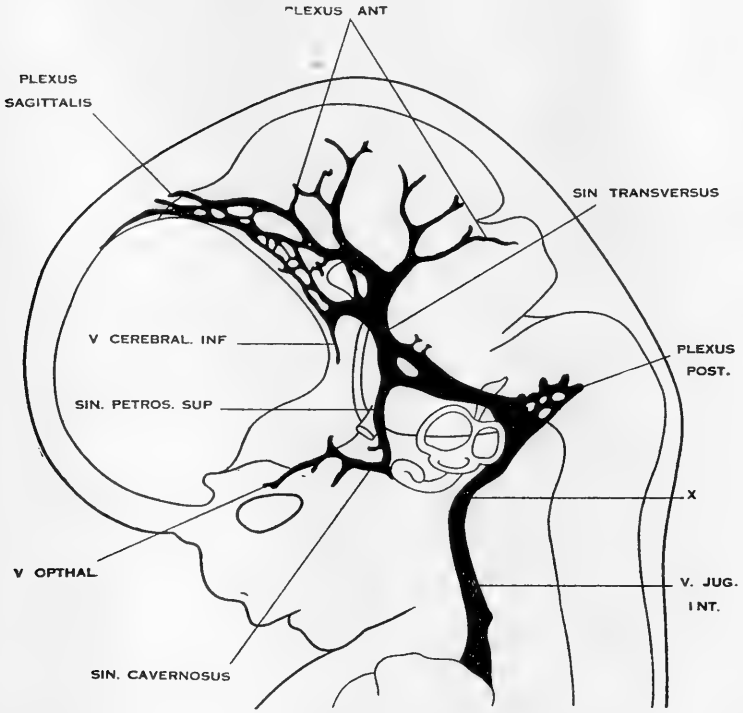


Fig. 5 Profile reconstruction of the dural veins in a human embryo 24 mm. long (No. 632, Carnegie Collection). The point marked 'x' is the junction of the sigmoid portion of the transverse sinus with the internal jugular vein. Enlarged about 4 diameters.

of the anterior dural plexus at this time (compare figs. 4 and 5 with figure 3). In the first place, the anterior dural plexus annexes itself to the middle dural plexus and drains backward through this into the newly established channel dorsal to the otic capsule. We will therefore, from now on, refer to the combined anterior and middle dural plexuses as the anterior dural plexus, on the basis that the middle plexus has now lost its identity. In the second place, there is differentiated along the margin of the cerebrum and between the hemispheres, a subdivision of the anterior dural plexus that is eventually to constitute the superior sagittal sinus (marked 'plexus sagittalis' in figs. 4–5).

Examination of photographs and sketches of embryos of about this age shows that there is a tendency to the formation of a

larger channel along the anterior margin of the anterior dural plexus, that is, along the caudal margin of the cerebrum. This was designated by Markowski ('11) as the 'anterior marginal vein' (vordere Grenzvene) and the large tributary, draining the lateral surface of the cerebrum, that empties into it he calls the 'lateral telencephalic vein,' of which there may be several. Markowski describes the anterior marginal veins of the two sides as extending forward and toward the median line and uniting in the formation of a plexus out of which is to be derived eventually the superior sagittal sinus. From examination of figures 3, 4 and 5 it can be seen that there is no sharp line between the sagittal plexus as described by us and the more ventral loops of the anterior dural plexus of which it is a part. The 'anterior marginal vein' of Markowski is a part of both of them. The discussion regarding the formation of the superior sagittal sinus will be reserved for a subsequent part of this paper. We may point out at this time, however, that the anterior marginal vein of Markowski apparently is not a definite vein but rather a constantly changing channel. What we find is that the more anterior loops of the anterior dural plexus are constantly dropping out and are replaced by the development of the more caudal channels. By comparing figures 4 and 5 we can see this change occurring. Our interpretation of the condition found in figure 5 is that what had been a larger channel along the cerebral margin of the dural plexus is now dwindling into a small mesh, while the main blood stream forms for itself a new course in a more caudal loop of the plexus. In this connection it is well to remember that migration of veins may occur in two ways. There may be a passive change in position or direction of the endothelial tube itself, due to mechanical causes arising from alterations in its environment. This is illustrated by the sigmoid portion of the transverse sinus and its change in form in the later stages (embryos more than 20 mm. long). On the other hand, a vein may change its position by forming or adopting a new endothelial channel and at the same time relinquishing its original endothelial channel. The embryonic plexiform character of the veins in the region of the tentorium is especially favorable for this procedure and we find

this type of alteration in the blood channels repeatedly illustrated in this region. In other words, under migration of veins we are to distinguish between *passive migration*, where there is a change in position due to some flexion or traction on the vein wall itself, and *spontaneous migration*, in which there is a change in position of the blood stream only, where by a process of what might be called circumfluent anastomosis or anastomotic progression, the blood stream develops a new channel in the adjacent loops of the plexus with a corresponding dwindling of the previously used loop.

The lateral telencephalic veins of Markowski apparently correspond to the inferior cerebral veins of the adult, so we shall label them in that way. Though emptying into the dural system they develop their course through the intradural membranes and become typical cerebral veins. It is interesting to note that in the 21 mm. embryo certain definite topographical points in the transverse sinus are already determined, namely, the jugular foramen, the location of the endolymphatic sac, the point of entry of the superior petrosal sinus and of the inferior cerebral veins. Thus we see that more than half the sinus is already established and that it is the terminal or jugular portion that is established first. The remainder of the sinus is relatively late in assuming a permanent form, which is doubtless the result of the prolonged period of growth of the cerebrum, making a continued adjustment of the tentorial plexus necessary. Even in embryos 50 mm. long which we shall now proceed to examine, the proximal end of this sinus is still in the formative stage.

5. Human embryos 50 mm. long

To cover the period of embryos about 50 mm. long the writer examined three series belonging to the Carnegie Collection: No. 84, 50 mm. transverse; No. 96, 50 mm. sagittal; and No. 448, 52 mm. sagittal-injected. There was also an embryo of about the same age (No. 458, 54 mm.) that had been injected with India ink. The head was removed and partially dissected, and then cleared after the Spalteholz method. This gave excellent total views of

the blood vessels. The profile reconstruction shown in figure 6 is based on series No. 96 and was made by preparing tracings on transparent paper which were then superimposed and a composite tracing made of the whole series. This is about the same stage that is shown by Markowski in his figure 4.

By this time the venous drainage of the cranium is established along channels that correspond fairly well to those found in the adult. It is clearly subdivided into three separate systems: (1) The superficial system draining the integument and soft parts; (2) the dural system lying between the dura and bone; and (3) the cerebral system. All three are originally outgrowths of the same capillary plexus. The separation of the dural veins and the cerebral veins we have traced through, step by step. The superficial veins in embryos 20 mm. long are already separated off from the dural system by the membranous and cartilaginous cranium. They appear first in the lower parts of the head, where they were originally separated off from the deep veins, and form a plexus that gradually spreads upward over the vault. They maintain a few anastomoses with the dural system, which constitute the so-called emissary veins; one of these is shown in figure 6. Aside from the channel maintained through the orbit the chief drainage from the superficial system is through the external jugular vein, which is pictured by Salzer ('95) as already present in guinea-pig embryos 20 mm. long.

On examining the dura in embryos 50 mm. long it will be seen that for the greater part it closely invests the interior of the developing cranium and is relatively poor in blood vessels. This is true especially in those portions where the cartilaginous and bony cranium is more advanced in its differentiation, as in the base of the skull and in the frontal, temporal and lower occipital regions. In other regions the dura projects within the cranial cavity, being separated from the future bony skull by a layer of areolar tissue, in the meshes of which are found the large blood channels and their tributaries. The largest area of this kind is found situated over the mid-brain and extending from the caudal margin of the cerebral hemispheres to the cerebellum. This area extends laterally down to the base of the skull, narrowing as it

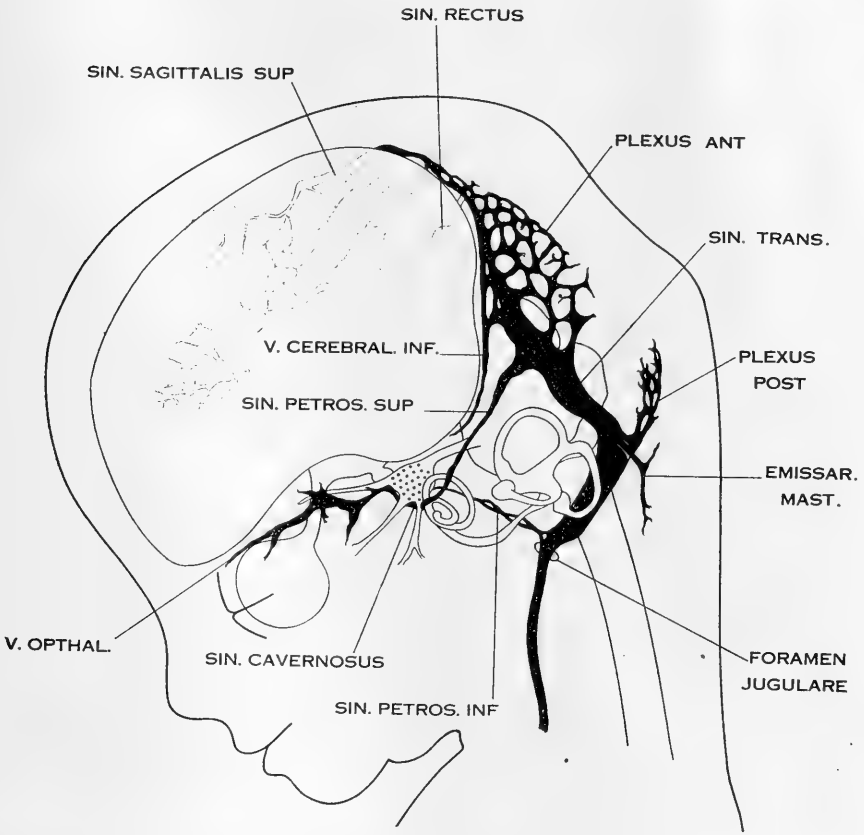


Fig. 6 Profile reconstruction of the dural veins in a human embryo 50 mm. long (No. 96, Carnegie Collection). The outlines of the veins of the falx cerebri can be seen through the cerebral hemisphere. Enlarged about 4 diameters.

does so. It constitutes what is known later as the 'tentorium cerebelli' and in it is included the greater part of the dural venous system. A basal extension of the tentorium widens out in the region of the semilunar ganglion and in its meshes is formed the cavernous sinus. A thinner area of the same tissue extends caudalward from the cavernous sinus, medial to the otic capsule, to join the jugular region. The slender plexus of veins extending through this constitutes the inferior petrosal sinus. Along all the sinuses we find this same areolar meshwork. It is not

to be confused with the developing arachnoid tissue, from which it is everywhere separated by the dura. Blood vessels supplying and draining the brain are also found in the arachnoid at this time and they are quite numerous in some regions, such as the region of the Sylvian fissure and along the more ventral parts of the mid- and hind-brain. These cerebral vessels are everywhere separated and distinct from the dural blood channels, with the exception of the few points where they empty into the big dural channels, as occurs in the adult. The connection between the dural system and the cerebral system is no longer by a multiple anastomosis of small vessels, but instead, by isolated larger veins.

Examination of figure 6 shows that we have here an arrangement of the dural venous system that in most respects follows the adult arrangement. The cavernous sinus has as yet a simpler character than is found in the adult. It is situated median and ventral to the semilunar ganglion and has the large ophthalmic tributaries in front. Caudally it communicates with the main blood stream by means of the superior and inferior petrosal sinuses. The superior petrosal sinus is a long slender channel that passes over the cochlear part of the otic capsule and empties above into the transverse sinus. The inferior petrosal sinus consists of a plexus of veins that passes median to the otic capsule to empty at the point of origin of the internal jugular vein.

As regards the transverse sinus, it has been pointed out that the terminal or jugular portion of it is established first. In figure 6 it can be seen that from the point of entry of the superior petrosal sinus to the jugular fossa—in other words, the sigmoid portion—it consists of a single large channel, and has the same tributaries and the same general relations that are found in the adult. The remainder or proximal portion of the transverse sinus is less well established and the large capillary meshwork found along its dorsal margin shows that the blood channels here are still in the formative stage and must still be spoken of as the remainder of the anterior dural plexus. The main channel is forming along the anterior margin of this plexus, into which the inferior cerebral vein empties. It can be seen how this channel

migrates backward in adjustment to the growth of the hemisphere and thus comes to assume a more and more horizontal course. This change in direction, together with growth in length and diameter of the main channel at the expense of the formative meshwork, remains to be completed before the adult condition can be considered as established. The variations found in the adult in the region of the confluens sinuum can be readily understood as variations in channel-selection through this tentorial meshwork.

In the region of the fore-brain a fold of dura is interposed between the two hemispheres and is compressed into a flattened sheet which is to constitute the falx cerebri. This, and the vascular meshwork belonging to it, is directly continuous with the tentorium. Like the tentorium it passes through a prolonged adjustment period. In embryos 50 mm. long two of its permanent channels, which are to belong to the dural sinus system, can be readily recognized. These are the superior sagittal sinus and the straight sinus. In figure 6 the superior sagittal sinus is quite irregular in outline, which is possibly a result of shrinkage of the specimen. Very likely in a normal state, as seen in profile, it would pass evenly along the margin of the cerebrum. The details regarding the vessels belonging to the falx cerebri and the drainage of the chorioidal masses will not be taken up in the present paper, but certain features in the formation of the superior sagittal sinus will now be referred to.

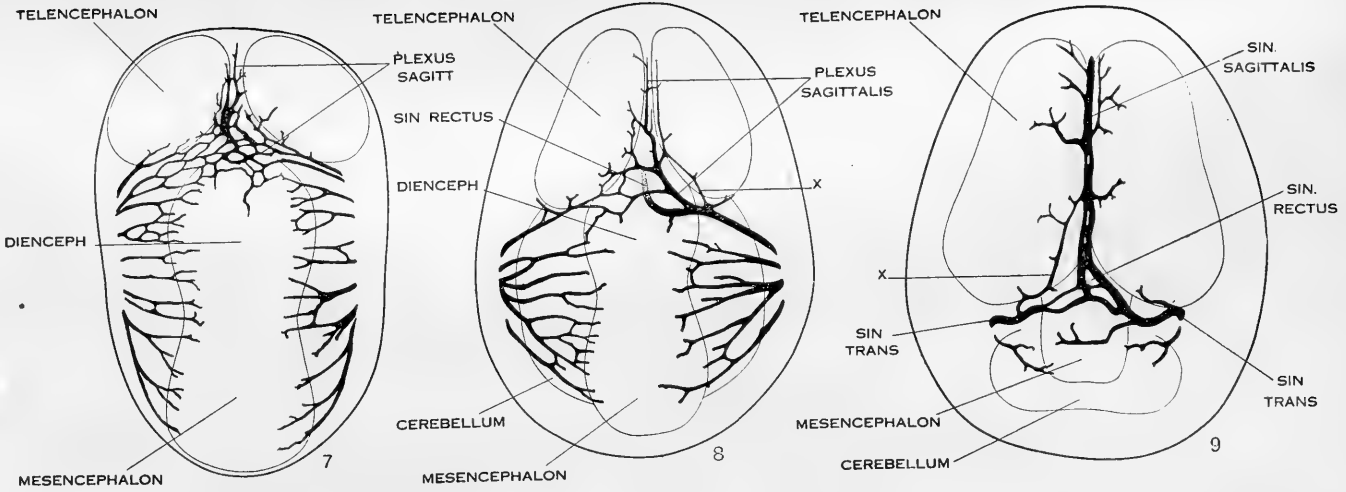
SINUS SAGITTALIS SUPERIOR

Under the description of embryos 21 mm. long, reference was made to the formation of a 'plexus sagittalis' as a subdivision of the anterior dural plexus. At that stage the plexus is clearly differentiated from the remainder of the anterior dural plexus, as can be seen in the dorsal view of an embryo of about that age shown in figure 8. Earlier than this (in embryos about 14 mm. long; figure 7) the plexus can be recognized, though here it is not so clearly separated from the general plexus. In such embryos it can be seen that the larger tributaries of the anterior and middle dural plexuses stop short of the median line—with the exception of anteriorly—where they merge into a longitudinal plexus that dips

in between the developing hemispheres. It is in the meshes of this plexus that we find the beginning of the superior sagittal sinus; and the principal steps in its transformation can be seen by comparing figures 7, 8 and 9. Sketches like these necessarily have to be simplified, and on examining them it should be remembered that only the larger channels are shown and in between there is everywhere a fine anastomosing network. Also, the channels do not lie all in the same plane. Furthermore, it is to be noted that there exists in embryos of the same age a considerable variation in the pattern formed by these channels. The three specimens selected, however, may be regarded as illustrating fairly definite stages in this transformation.

In figure 7 is shown a dorsal view of the head of the same embryo previously shown in figure 2 (No. 940, 13.8 mm. long, Carnegie Collection). Here we find the sagittal plexus represented in its simplest form. It will be noted that it possesses two characteristic features: In the first place, there is a tendency to an enlargement of certain portions of the plexus, irrespective of a continuous channel; we thus have a series of small lakelets connected by narrow channels; a definite single superior sagittal sinus cannot yet be said to exist. In the second place, the plexus is distinctly asymmetrical and shows a tendency to drain more freely to one side than the other—in this case, to the right.

A more definite and simpler channel system is found in 20 mm. embryos, an example of which is shown in figure 8 (No. 349, 20 mm. long, Carnegie Collection). Here one might possibly speak of a superior sagittal sinus. The channels, however, are still in the form of a plexus and hence the term 'plexus sagittalis' is retained. This view regarding the early identity of the superior sagittal sinus differs from that given by Evans, who pictures the primitive capillary plexus creeping up on each side of the forebrain in 8 mm. pig embryos. A portion of the dorsal margin of this plexus he labels as the 'primitive superior sagittal sinus' (Evans '09, fig. 15 b; Evans '12, figs. 399–400). According to him, it is thus originally paired and bilaterally symmetrical. According to the present writer, it is not until later that we can speak of a superior sagittal sinus. It is not until the plexuses,



Figs. 7, 8 and 9 Three stages in the formation of the sagittal plexus, showing its asymmetrical character and its conversion into the superior sagittal sinus. Draining into it from below is the drainage channel from the chorioidal bodies which becomes the straight sinus. The channels marked 'x' are interpreted as undergoing retrogression, being replaced by more caudal channels. Figure 7 is a vertex view of a human embryo 13.8 mm. long (No. 940, Carnegie Collection). Figure 8 is a vertex view of an embryo 20 mm. long (No. 349, Carnegie Collection). Figure 9 is a drawing of an injected and cleared specimen 54 mm. long (No. 458, Carnegie Collection).

described by Evans, have fused across the middle line and have formed a longitudinal network, in the meshes of which an asymmetrical channel is finally established, that we can speak of a superior sagittal sinus.

Owing to the growth of the cerebral hemispheres in 20 mm. embryos, the dural tissue lying between them begins to take on the form of the falx cerebri. It is in this loose dural tissue that the meshes of the sagittal plexus are found. At this time it can be seen that a larger channel is opening along the dorsal mid-line that will form the superior sagittal sinus, and connected with it by anastomosing loops, is a more ventrally situated large channel that constitutes the 'sinus rectus.' This latter extends forward and drains the lower part of the falx. It has two converging limbs in front that drain the chorioidal masses of the hemispheres. This and the more superficial channels in this embryo drain chiefly to the right side. Smaller anastomosing loops connect also with the plexus in the region of the left transverse sinus.

On coming to embryos 50 mm. long (fig. 9, No. 458, 54 mm. Carnegie Collection) we find that here the superior sagittal sinus is established, at least in part. In its cephalic portion there is a large characteristic channel, lacking only the dural connective tissue investment to make it an adult type. In its more caudal portion it still exhibits a plexiform character that indicates its transitional state. Upon comparison of a number of series the writer is led to interpret the formation of a single channel as the outcome of more than one process; in some segments there seems to be the selection of a favorable loop of the plexus which enlarges and becomes the main channel, and in other segments there is apparently an enlargement of two or more collateral loops which subsequently fuse into a more or less common channel. Both processes are apparently represented in figure 9. It is to be expected that we shall find a considerable variation in this in different brains.

No attempt was made to study the histological changes that occur in the completion of the superior sagittal sinus, nor of the cavernous sinus. These involve details with which the present

paper is not concerned. The caudalward growth, however, of the superior sagittal sinus in adjustment to the corresponding growth of the hemispheres is of interest in our general problem. By comparing figures 7, 8 and 9 it can at once be seen that this caudal development is accomplished at the expense of the meshes of the anterior dural plexus, in which process the transverse and straight sinuses also take part. These channels gradually obtain a more caudal course by what we have already described as 'spontaneous migration.' The channel repeatedly shifts into a more caudal loop of the plexus, the new loop enlarging and the old loop dwindling. The veins marked X in figures 8 and 9 may thus be interpreted as discarded channels. The eventual 'confluens sinuum' (torcular herophili) represents the point at which this caudal development reaches its completion. It usually retains a trace of the plexiform character that is found throughout the embryonic stages.

It is interesting to note that the asymmetry of the superior sagittal sinus expresses itself in the embryo, as well as in the adult, by a tendency to drain more to one side of the head than to the other. This becomes established by the time the embryo is 20 mm. long. The drainage is preponderantly toward the right side. It happens that in figure 9 the main drainage was in reality toward the left side. In reproducing the sketch the figure was reversed, right for left, in order to facilitate its comparison with figures 7 and 8. In table 1 is given a list of embryos which were examined as to this point and it will be seen that of eighteen specimens all but two drained predominantly toward the right side, that is, about 89 per cent. In order that account should be taken of the artificial element introduced in those specimens where the vascular system had been injected with coloring matter, such specimens are indicated in the table by an asterisk. No explanation has thus far been reached to explain this interesting asymmetry. The drainage of the straight sinus could not be determined as well in the younger stages and there were not enough of the older stages upon which to base an average. If we may judge from the adult, it would generally drain to the opposite side, that is, the left.

TABLE 1

Superior sagittal sinus

NO.	CROWN-RUMP LENGTH IN MM.	DRAINAGE OUTLET
296	17	Right transverse sinus
350	19	Right transverse sinus
128	20	Right transverse sinus
349*	20	Right transverse sinus
460*	21	Right transverse sinus
966	21	Right transverse sinus
382*	23	Right transverse sinus
632*	24	Right transverse sinus
145	33	Right transverse sinus
199	35	Right transverse sinus
449*	36	Right transverse sinus
224	40	Right transverse sinus
886	43	Right transverse sinus
95	46	Left transverse sinus
84	50	Right transverse sinus
96	50	Right transverse sinus
458*	54	Left transverse sinus
613	80	Right transverse sinus

* Injected specimens.

SUMMARY

If now we look back and bring together the essential features in the development of the venous sinuses of the dura mater, we will find their development may be analyzed somewhat as follows: (1) There is first the establishment of the primary arrangement for the drainage of the head; (2) this is followed by a separation of the veins of the head into two and finally three separate layers or strata, of which the middle layer constitutes the dural veins; (3) certain adjustments of the dural channels are made necessary by the environmental changes in the region of the middle and internal ear; (4) similar and still greater adjustments of the dural channels follow the marked growth and change in form of the brain; (5) and finally, there are the late histological changes in the vein walls that convert them into the adult sinuses. This last or histological feature is not taken up in the present paper. The other four features, however, will now be outlined.

Simplified sketches of the successive stages have been assembled on one page, as figures 10 to 17, and it is hoped that this will facilitate the identification of the steps in this process. It hardly needs to be pointed out that these steps overlap one another, and also that embryos of apparently the same age exhibit a considerable variation in the pattern of their venous plexuses.

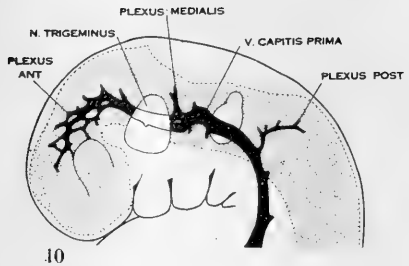
The primary arrangement for the drainage of the capillaries of the head (figs. 10–11) consists of a 'primary head vein', which starts in the region of the mid-brain and runs caudalward alongside of the brain tube and terminates at the duct of Cuvier. The primary head vein is composite in origin. That portion of it oral to the vagus nerve is an intrinsic vein of the head; the remaining caudal portion is in reality a neck vein and constitutes the anterior cardinal vein—eventually the internal jugular vein. Together these portions form a continuous channel, the primary head vein, into which the blood from the capillary sheet that immediately invests the brain tube is drained by means of anastomosing venous loops. These loops gradually become arranged more or less in the form of three plexuses, the 'anterior dural plexus,' 'middle dural plexus,' and 'posterior dural plexus.' Other small tributaries empty into the primary head vein which drain the structures ventral and lateral to the brain tube, such as the nerve ganglion masses. The largest of these come from the eye region and these eventually form the ophthalmic vein.

While the three head plexuses are forming (figs 11–12) the outlines of the dura mater and the arachnoid spaces make their appearance, and first of all in the ventral parts. This results in a general separation or cleavage of the more superficial primary head vein and its three tributary-plexuses from the subjacent vessels that arise from and drain the capillary sheet directly investing the brain tube. This deeper system, however, continues to drain into the former at certain places, notably in the more dorsal parts. The primary head vein and its three tributary plexuses thus become established as a true dural system as distinguished from the deeper 'cerebral veins' belonging to the arachnoid-pial membrane. The diploic veins are a later subdivision of the dural system. The superficial veins of the head

are separated off in the more ventral regions and from there spread upward over the head independently of the dural system. We then have for the head three separate venous systems: (1) the superficial layer belonging to the integument and soft parts; (2) the middle layer belonging to the dura and diploe; and (3) the deep layer of cerebral veins belonging to the brain. It is the middle layer, or dural system, that is exclusively concerned in the formation of the dural sinuses, and whose changes in form and position will now receive our attention.

The changes in form of the dural veins that occur after the establishment of the primary arrangement for the drainage of the head (figs. 12–17) are largely due to the mechanical factors involved in the changes of their environment, the two most conspicuous elements being the changes in the region of the cartilaginous capsule of the labyrinth and the changes involved in the growth and alteration in form of the brain. The changes thus produced include the reduction of the plexuses into simple channels, and the total obliteration or change in position of the channels themselves. Under this latter phenomenon we recognize a *passive migration*, where there is a change in the position of the vein wall itself, due to some flexion—or traction—force acting upon it. We also recognize a *spontaneous migration* where there is a change in position of the blood stream only, where by a circumfluent anastomosis the blood stream develops a new channel in the adjacent loops of the plexus with a corresponding dwindling of the previously used channel. An anastomosing plexus is essential in spontaneous migration but is not essential in passive migration. Both of these types of migration are to be distinguished from the formation of *replacement channels* which is another way, deserving mention, in which the venous channels are changed in position and direction in this process of adjust-

Figs. 10–17 Simplified profile drawings of the dural veins showing the principal stages in their development in human embryos from 4 mm. to birth. It is of particular interest to notice their adaptation to the growth and changes in form of the central nervous system. Figure 10, Embryo No. 588, 4 mm.; figure 11, Embryo No. 940, 14 mm.; figure 12, Embryo No. 144, 18 mm.; figure 13, Embryo No. 460, 21 mm.; figure 14, Embryo No. 199, 35 mm.; figure 15, embryo No. 96, 50 mm. crown-rump length; figure 16, Embryo No. 234 a, 80 mm. crown-rump length; figure 17, adult.



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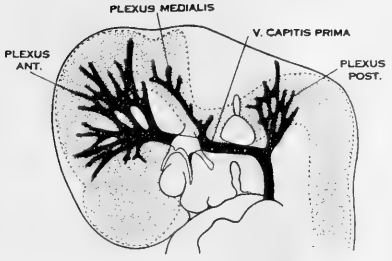

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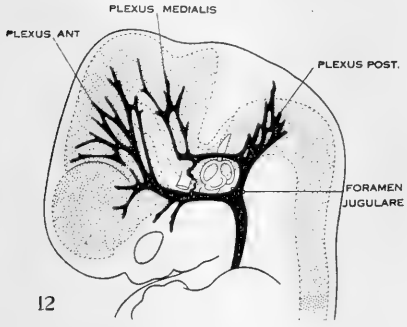

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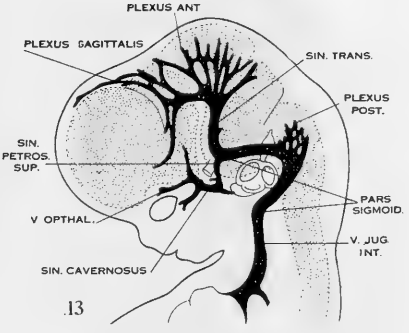

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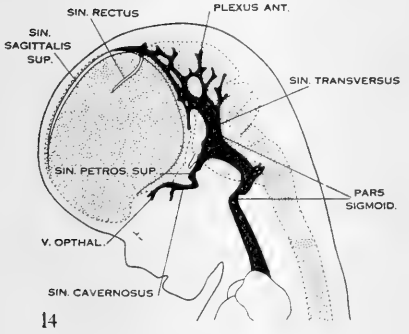

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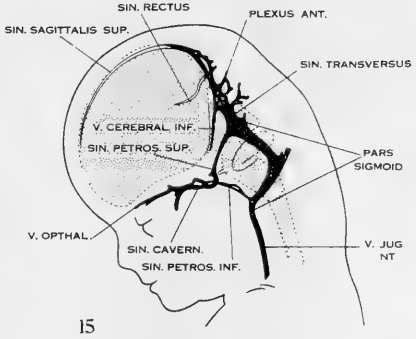

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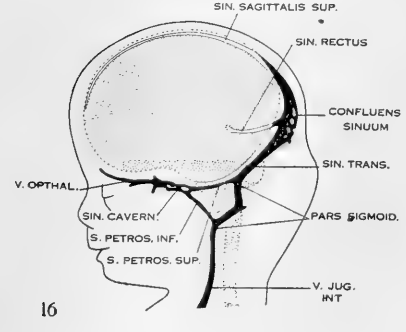

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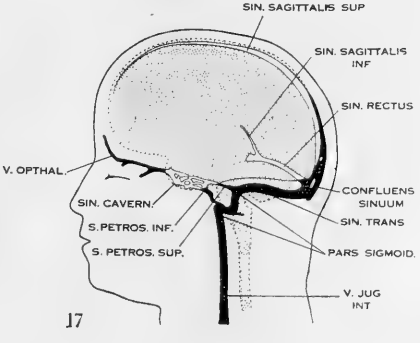

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ment. In the replacement channel there is the formation of a new channel and the obliteration of an old one, as in migration. It however differs from migration in that it is not a gradual and progressive change in position, but an abrupt and immediately complete one. Furthermore, the new channel lacks the morphological characteristics of the old one. This process is illustrated by the transverse sinus which replaces part of the primary head vein, as will be presently described.

Adjustment of the dural channels occur early in the region of the middle ear. In the same way that the facial nerve is bent out of its original course by the development of the membranous labyrinth and the middle ear, so the dural veins are definitely influenced by the same structures. Owing to the growth of these the course of the primary head vein, ventro-lateral to the otic capsule, becomes an unfavorable one and this segment of it becomes obliterated. To make the necessary adjustment two things happen (figs. 11, 12 and 13). First, an anastomosis is established above the otic capsule through which the middle plexus drains caudally into the posterior plexus. Secondly, the anterior plexus, which originally drained into the primary head vein, fuses with the middle plexus and drains caudally through this and through the newly established channel, dorsal to the otic capsule. This makes a complete trunk for the drainage of the head which is everywhere dorsal to the primary head vein as far as the jugular foramen, where it is continuous with the internal jugular vein. Of the primary head vein there is left, in addition to the cardinal portion of it or internal jugular vein, only that part in the region of the trigeminal nerve. This may now be called the 'cavernous sinus.' Into it drain the veins from the orbit and it, in turn, drains upward through the original trunk of the middle plexus, which is now the 'superior petrosal sinus,' into the newly established dorsal channel. By comparing with later stages (figs. 14–17) it will be seen at once that this dorsal channel is the 'transverse sinus' of which that part between the superior petrosal sinus and the jugular foramen forms its 'sigmoid portion.' Thus in the 21 mm. embryo the dural channels in the region of the temporal bone have acquired essentially all their permanent con-

nections, with the exception of the inferior petrosal sinus which appears a little later (fig. 15). Otherwise, there remains to complete the adult condition only a certain amount of passive migration in accommodation to the changes in the adjacent parts.

The adjustment in the dural channels rendered necessary by the protracted growth of the hemispheres extend much later in fetal life. A large part of this adjustment is accomplished by spontaneous migration of the principal channels and for this a venous plexus is essential. We thus find a continuous persistence in the neighborhood of the advancing occipital pole of the hemispheres of the transitory anterior dural plexus from which are evolved all the veins of the falx cerebri and of the tentorium cerebelli.

An anterior subdivision of the plexus extends forward in the median line as the 'plexus sagittalis' which is interposed as a vertical curtain between the hemispheres. Among its dorsal meshes is developed an asymmetrical longitudinal channel which we know as the 'superior sagittal sinus.' In its early stages this channel is made up of several collateral anastomosing veins. The eventual single channel is formed in the anterior portions by the selection and enlargement of the most favorable vein with a corresponding disappearance of the others. In the posterior portions there is apparently some coalescence of adjacent veins. The anterior part of the sinus is completed first. As the hemispheres extend backward the sinus correspondingly elongates itself by incorporating the more caudal loops of the plexus. Transverse sections through this part of the sinus in older fetuses thus usually reveal incomplete coalescence of the separate loops. The sagittal plexus very early exhibits a tendency to drain more to one side of the head than to the other and usually toward the right side. As the superior sagittal sinus becomes established we thus find it continuous caudalward usually with the ventral main channel of the right anterior plexus which eventually forms part of the right transverse sinus. The 'straight sinus' is formed in the ventral part of the sagittal plexus and its caudal adjustment is essentially like that of the superior sagittal sinus. It may drain chiefly toward the right or left anterior plexus or equally toward both.

In embryos between 35 and 50 mm. long (figs. 14–15) we can recognize a main channel of the anterior dural plexus, that is, to become the 'transverse sinus.' If we disregard the sigmoid portion of it, it forms a fairly straight line with the internal jugular vein. In the interval between the 50 mm. embryo and the adult, the transverse sinus bends backward until it comes to lie at an angle of 90° with the internal jugular. This marked change in position is accomplished in large part by spontaneous migration, by the repeated shifting back of the main blood current into more caudal loops of the plexus with subsequent dwindling of the discarded anterior loops. As the sinus becomes more definitely established the dural plexus becomes relatively smaller (fig. 16) and the final change in position is completed by passive migration, that is, actual traction on the vein wall by its environment. In this change in position of the transverse sinus the superior sagittal sinus and the straight sinus participate and we find in the adult, at the point where they meet, an anastomosis, the 'confluens sinuum,' which is usually plexiform in character and represents the last trace of the anterior dural plexus.

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THE ORIGIN OF THE RENAL ARTERY IN MAMMALS AND ITS ANOMALIES

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TEN FIGURES

An embryological explanation of any anomaly should show that from some pre-existing embryological condition both the normal and the abnormal results may be derived; the agents which may cause the anomaly should be simple in themselves, as pressure, or the blocking of a vessel, or the relative overgrowth or arrest of development of certain parts, though the ultimate cause of these agents will usually remain a mystery. Not infrequently other vertebrates may develop normally in ways which for man would be abnormal, and the citation of such instances strengthens the explanation of any human anomaly.

Certain of the anomalies of the renal arteries have been already satisfactorily explained. Organs which make extensive migrations during growth from one position to another may either retain vessels from their original location, as in the case of the testis, or receive and incorporate new vessels of the region invaded, as does the thyroid gland. The instances of accessory renal arteries arising from the iliac arteries, from the middle sacral and inferior mesenteric arteries are to be considered as a persistance of the original renal vessels, normally lost, derived from the plexus between the vessels mentioned, lying directly in the path of the renal outgrowth from the Wolffian duct. This plexus has been injected in the pig embryo of 14.0 mm. by Jeidell (1), and its influence commented on by her and by Evans (2); it is easily traceable in embryos of other mammals of similar age. Since it extends from side to side, and since the inferior mesenteric artery at this time arises by several roots from the aorta,

one can readily understand the rare cases in which the right and left accessory renal arteries both spring from the inferior mesenteric artery, or both arise from a common stem from the aorta below that vessel. Such a case has been reported and figured recently by Harvey (3).

This class of anomaly may be considered thus separately because it has no relation to the question of the origin of the normal adult renal artery. The plexus described does, however, give a valuable clue to other anomalous conditions. What then is the origin of the permanent renal artery?

Broman (4) and others have stated that in man the renal artery is the trunk of a former mesonephric vessel, utilized secondarily as the inferior suprarenal artery, and finally as the renal artery, with the suprarenal as a branch. On the other hand, Hochstetter (5), Hill (6), and others have maintained that in mammals other than man, the renal artery is normally a new branch from the aorta at about the time when the kidney has reached its permanent position. Hill places this at 28.0 mm. in the pig, at which age he succeeded in injecting this vessel, but states in another paper that in man the vascularization occurs between 22.0 and 24.0 mm. No explanation has been offered for the peculiarity of man in this respect, and in fact very little corroborative work has been done on this subject. This is all the more strange because the conception that a new vessel can sprout from a well established trunk, as stated by Hochstetter and Hill, is contrary to the modern ideas of vessel growth. Yet other writers also, notably Kolster (7), at a loss to explain certain renal anomalies, have fallen back on the supposition of a 'late branch' from some neighboring vessel.

The aorta of pig embryos much smaller than 28.0 mm. is provided with a well differentiated coat of modified mesenchyma, the future tunica media, through which the endothelium of a new branch would have to force its way. Now endothelium may and does sprout abundantly, but only in the looser, undifferentiated mesenchyma, or in its derivative, loose connective tissue. A slight condensation of the mesenchyma, as in the incipient somites, makes the ingrowth of endothelial sprouts impossible, and

causes the segmental arrangement of certain vessels. Equally, the condensation of mesenchyma around a vessel to form its coat should prevent any further outgrowth of endothelium to form new branches. Such a mesodermal wall, in a layer three or four cells thick, is found in human embryos at 10.0 mm. and in the other mammals examined at about the same stage. The renal arteries, then, should be present before this stage, or, if later, must arise from the smaller and as yet uncoated aortic branches.

Following further this idea of the inability of endothelial sprouts to pierce condensed mesenchyma, we must recognize that the presence of a very slightly differentiated capsule around an organ will protect it from future ingrowth of blood vessels. Many vessels may become incorporated in the further development of the capsule as vessels to the capsule, not supplying the organ itself, but the fact that a vessel enters the organ proper, whether at the hilus or at some point on the periphery, indicates that the vessel was present and actively in use before the capsule was differentiated, and that the differentiation took place around the vessel. Wilson (8), in his study of hypernephromata, states that the renal capsule is distinct in man at 13.0 mm., though not completely formed until much later, and my own observations agree with his on this subject. From the point of view of the ability of the kidney to receive, as well as of the aorta to send out new branches, we must, then, look for the renal vessels earlier than has been usually supposed.

We should not be deceived by the generally accepted idea of the great migration of the kidney and its relatively late arrival at its adult position. If we use the dorsal segmental arteries as a guide and the bifurcation of the aorta as a fixed point, and realize that the adult renal arteries arise opposite the third or fourth pair of dorsal arteries above this point (that is, the second or first pair of lumbar arteries in man), we find that the kidney has completed its migration with respect to its blood supply very early, at about 15.0 mm. in man and pig, and at corresponding ages in the other mammals examined. Moreover, the permanent renal arteries are found often to enter at first the anterior pole of the organ, and secondarily to be moved by its actual

enlargement to a position on its mesial border. The growth of the kidney itself and the rearrangement and growth of the surrounding parts complete the so-called migration and bring about the adult relations.

The problem thus resolves itself into the question, "What thin-walled arterial vessels are present in the embryo of roughly 10.0 to 15.0 mm. in the area invaded by the kidney?"

Though the mesodermal wall of the aorta or of other vessels forms a barrier to new endothelial sprouts, it will naturally develop around any branches, however small, which are still in use. These smaller branches seem to have been overlooked heretofore, but, as I hope to show, are numerous and important in the present study. The aorta is usually considered to have certain sets of branches, called ventral, dorsal, and lateral or ventro-lateral; but some confusion exists in the literature as to the distribution of these groups. The ventral vessels, originally bilateral, are derived from the vitelline plexus, and go to the intestinal tract; the paired dorsal branches, which may arise by a single trunk, supply the spinal cord, and usually also the body-wall; the lateral branches, originally body-wall vessels, are said to join the dorsal segmental arteries, and to be replaced by new lateral branches to the mesonephros, irregularly arranged in several vertical rows and not segmental. Yet later, other lateral branches arise in certain regions destined for the body-wall, represented by the subclavian artery. Dorso-lateral vessels may, in the thoracic region, supply the mesonephros, while in the abdominal region of the same embryo similarly placed arteries go to the body-wall, and the mesonephric vessels become ventro-lateral. Moreover, the arrangement varies at different ages and in different classes of mammals at any one age.

Such a lack of regularity implies, first, the absence of any specificity in the vessels and, second, some means whereby the vessels may change their position on the aortic circumference, or join with neighboring aortic branches. That vessels are not specific, do not go to the Wolffian body because they are ventro-lateral or dorso-lateral, but because they are best placed mechanically to supply the mesonephros, is shown strikingly in the reconstruc-

tion of a part of the aorta in a sheep embryo of 5.6 mm., in which branches from the left aorta have become right mesonephric arteries (fig. 1). The two aortae, right and left, are still partially separated by one of the bars or septa of mesoderm covered by endothelium frequently found where two vessels of irregular calibre are in the process of fusing. The left aorta at this point is much the larger, so that the bar is far to the right of the median line, as indicated by the position of the notochord and the root of the mesentery. Intestinal branches are lacking at this level, and we are left in doubt as to their relations; but two vessels, one large with many branches, the other much smaller, spring distinctly from the left aorta and pass to the right mesonephric glomeruli. If we consider the double aorta as a whole, these vessels are ventro-lateral, and thus normal in their distribution; but for the left aorta itself, since these branches are the nearest to the aorta of the opposite side, they are actually in the position of median ventral branches, ordinarily destined for the intestine. How frequently such irregularly placed vessels occur it is impossible to say, as all trace of their irregularity is lost with the absorption of the bars. It is evident, however, that aortic branches from the medial ventral angle of each aorta are not necessarily predestined to supply the intestine, but are governed by mechanical convenience; and also that, at this early stage, new sprouts may grow from the aortic endothelium at various points.

These new sprouts, not heretofore definitely recognized except as new mesonephric arteries or subclavian arteries, are much more numerous than has been supposed and afford to the already present vessels the means of changing their position on the aortic wall and of forming new connections with other vessels. They are sent out by the aortic endothelium between the existing vessels, as the circumference and length of the aorta increase, and like all endothelial sprouts tend to branch and anastomose, both vertically and horizontally, with each other and with the existing aortic branches. The intervals between the aortic roots, old and new, of the resulting plexus, and the size of its mesh are roughly the same as those figured by Clark (9) in his studies

on growing lymphatic vessels, and as those found by injections of blood vessels by Evans (2) and others. It is my idea that endothelium normally sends out sprouts at right angles to the main stem at fairly regular intervals, in the same way that the main stem of some trees sends out branches, and that in consequence the endothelial mesh throughout its growing portion is originally of a definite size. As this size is increased by the general growth of the body, carrying the capillaries further and further apart, new branches are given off between the old ones in order to regain the normal mesh size. This applies equally to the aorta, the circumference of which is, with growth, successively large enough to accomodate more and more branches with the normal interval between them, until, at the age when the mesodermal coat develops and stops further outgrowths, there are perhaps twelve or fourteen such branches, equally spaced, around the circumference. In the vertical plane there may be two or three sets of branches to each segment before the advent of the mesodermal coat. The later growth of the aorta, which may be greater in one part than in another, would separate these branches further from each other, but no new intervening vessels could be formed.

An anastomosing periaortic net having many connections with the aorta and linking the earlier aortic branches would result. Portions of such a net can be found occasionally, as certain of the accompanying figures show; part has already been injected by Jeidell, as already mentioned. Normally, the greater part of it degenerates very early. That it has not been more fully injected I attribute to the fact, as already suggested by me (10), that new endothelial vessels may be solid cords, even while connecting with hollow vessels or vesicles. Tracing such cords in serial sections and amongst mesenchymal cells is extremely difficult, though portions of the plexus, but not its connections, are recognizable in almost all the young embryos I have examined. Even the point of origin of the smaller roots from the aorta is hard to find, and I imagine that in many cases they may be absent in the prepared specimen because they have retracted from faulty preservation or under the influence of the

fixing fluid, leaving only slight irregularities in the endothelial wall. In many stained and sectioned embryos the endothelium of the aorta is in festoons with few points of contact with the mesodermal layers; such points of contact correspond to the positions of the roots of this plexus, as found in better preserved specimens.

The aortic wall of condensed mesenchyma will, as has been said, naturally form around such of the vessels of this plexus as are still in use, and thus frequently portions of the horizontal and vertical vessels are buried deep in the wall. The parts of the net not in use will retract or otherwise disintegrate, leaving perhaps no trace of their former position and connections. Figure 2 shows several of these smaller aortic branches, two between the dorsal segmental artery and the mesonephric artery, others ventral to the latter. A portion of the net remains as anastomoses between some of the more ventral, and together with the roots is partially buried in the mesodermal coat of the aorta. The more dorsal of the roots have been carried further apart by the growth of the aortic wall, have lost their connections with the net, and one of them has become practically obliterated, leaving only a point of attachment of the endothelium to mark its position. The tips of two of the roots lie in the loose connective tissue and can send out further sprouts. Figures 3 and 4 show a portion of the periaortic net in an older embryo; the persisting vessels in this case are the anastomoses near the aorta, and the capillaries from them peripherally. Though the intermediate roots from the aorta are absent, the position of these peripheral capillaries indicates, to my mind, that the roots were originally three in number between the dorsal segmental artery and the mesonephric artery. Figure 5 shows the wealth of partly buried vessels found in the dorsal region of the aorta, representing anastomoses, both vertical and horizontal, between dorsal segmental and lateral aortic branches, and indicating that two sets of roots per segment in the vertical direction is the general arrangement in this case.

These are only a few of the many instances in which I have found remains of this periaortic plexus. Similar connections between mesonephric arteries and ventral arteries are very com-

mon (figs. 8 and 10). Also anastomoses between paired dorsal segmental arteries are frequently found, and account for their later single trunk without the supposition that the two vessels fuse. The paired aortae, in the cervical region, may even send vessels toward the median line to form a plexus, especially well developed in the cat.

Through such a plexus any one of several paths may be chosen for a permanent vessel, and until the actual degeneration of the roots has taken place such a path may be moved from one position on the aortic wall to another. The mesonephric arteries of adult selachians are said by Dohrn (11) to arise normally from the lumbar arteries; a glance at figure 4 will show how this could come about. Even the extreme case of Bdellostoma, in which the adult mesenteric arteries arise from the dorsal wall of the aorta in common with the dorsal segmental arteries, is explicable.

With the general growth of the area around the aorta, or with the special lengthening of any of its main branches, the anastomotic vessels of the plexus may come to lie outside the aortic wall, and the regular meshes of the net become thus distorted.

Fig. 1 Sheep embryo, 5.6 mm. H.E.C. No. 1332, Sect. 220–235. Reconstruction of part of the abdominal aorta to show right mesonephric arteries from left aorta. *nch.*, notochord; *mes.*, root of mesentery; *m.art.*, mesonephric arteries. × 100 diam.

Figs. 3 and 4. Pig embryo, 24.0 mm., H.E.C. No. 1846, Sect. 1585–1610. Lateral view and semi-diagrammatic transverse section of a reconstruction of a portion of the abdominal aorta and periaortic net. The common trunk of the dorsal segmental arteries, *d.art.*, is connected within the aortic wall with a mesonephric artery, *m.art.* An intervening branch, *x*, from the aortic cavity has lost its connection. The lateral branches of the plexus suggest at least one more intervening branch to complete the net (cf. fig. 2). × 40 diam.

Fig. 5 Pig embryo, 24.0 mm., H.E.C. No. 62, Sect. 1375–1525. Reconstruction of the dorsal aspect of abdominal aorta, to show anastomoses within and without the aortic wall between the dorsal segmental arteries and lateral aortic branches, one of which, *k*, arises as an intercalated dorsal vessel, runs to a lateral position, and emerges laterally to reach the kidney. There are two sets of dorsal branches per segment. *d.art.*, dorsal segmental vessels, some with common trunk, some paired though connected. × 40 diam.

Fig. 10 Man, 16.4 mm., H.E.C. No. 1707, Sect. 774–858. Explanation in text. *coe.a.*, coeliac axis; *s.m.art.*, superior mesenteric artery; *supr.art.*, suprarenal artery; *r.art.*, renal artery. × 60 diam.


Fig. 5
d.art
k
d.art.
d.art
d.art.

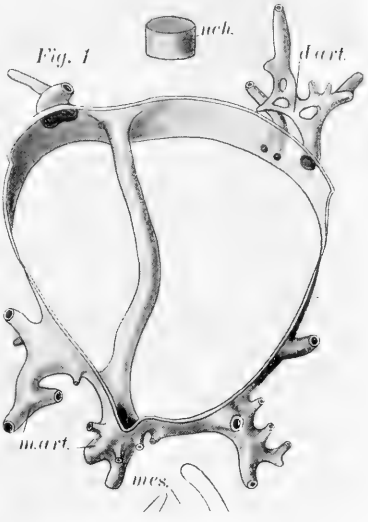
Fig. 1
sch.
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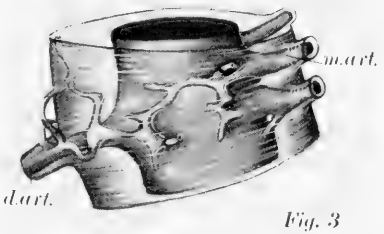
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Fig. 3

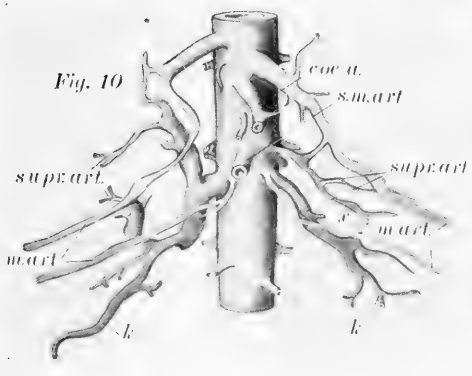
Fig. 10
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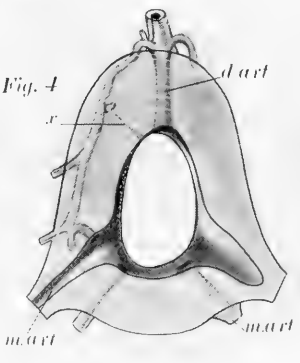


Fig. 4
d art
x
m.art
m.art

If this takes place after the main aortic branches are coated, no new sprouts will arise from them to form new anastomoses, and an area near the aorta will be left which receives no blood supply from the larger aortic branches. Into this area grow sprouts from some of the discarded roots of the original plexus, already protruding beyond the aortic wall, but not strictly to be considered new aortic branches, because they were present before the aortic coat developed. Such rejuvenated vessels become the small arteries to the lymph glands and sympathetic ganglia near the aorta, the bronchial and oesophageal arteries, and by recurrent branches the vasa vasorum of the aorta itself. They, or the plexus of which they were a part, also send twigs to the organs developing in or migrating into the area immediately lateral to the aorta, namely the suprarenal gland and the kidney.

Which part of the plexus between the mesonephric arteries and the dorsal segmental arteries will remain, and what its connections will be, depends apparently on mechanical forces brought into play by idiosyncrasies of development in the different classes of mammals. Of the embryos examined in this study,

Fig. 2 Rabbit embryo, 10.0 mm., 14 days, H.E.C. No. 155, Sect. 457. To show small aortic branches between the larger ones, and part of the periaortic plexus. The mesodermal aortic wall is present, and has partly buried the smaller branches. At 'x' an obliterated branch. *d.art.*, dorsal segmental artery; *m.art.*, mesonephric artery. × 165 diam.

Fig. 8 Cat embryo, 10.7 mm., H.E.C. No. 474, Sect. 434–452. Reconstruction of the aorta at second lumbar segment; lateral plexus, *pl.*, with mesodermal coat connected, at an acute angle, with a mesonephric artery, *m.art.*, by a thick walled vessel, *con.*, and with the aorta by three slender vessels of endothelium only, arranged in two vertical rows. Branches to body-wall and to kidney, *k.* Note portions of ventral plexus toward superior mesenteric artery, *s.m.art.* The left side of the drawing is incomplete (cf. figs. 6 and 9). × 130 diam.

Fig. 9 Cat embryo, 11.6 mm., H.E.C. No. 1979, Sect. 308. Drawing of the aorta and the left permanent renal artery, *r.art.*, just enlarged and with simple endothelial wall, from the aorta to the plexus, *pl.* The plexus is coated; its connection, *con.*, with the mesonephric artery, *m.art.*, is obliterated and can be followed no further in neighboring sections. The renal branch of the plexus, *r.br.*, enters the kidney, *k.*, at a lower level. On the right side is seen a lateral branch, *lat.br.*, not now continued to the right plexus, and dorsal to it the remains of another. *ve.*, veins; *sym.*, sympathetic nerves; *b.w.*, branch to body-wall. × 165 diam.

Fig. 8

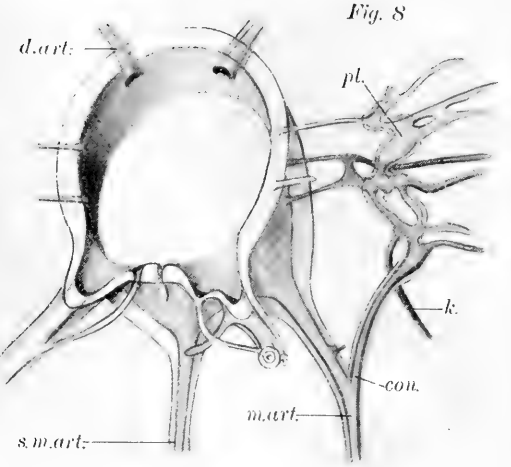


Fig. 2

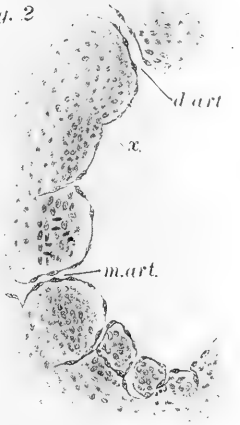
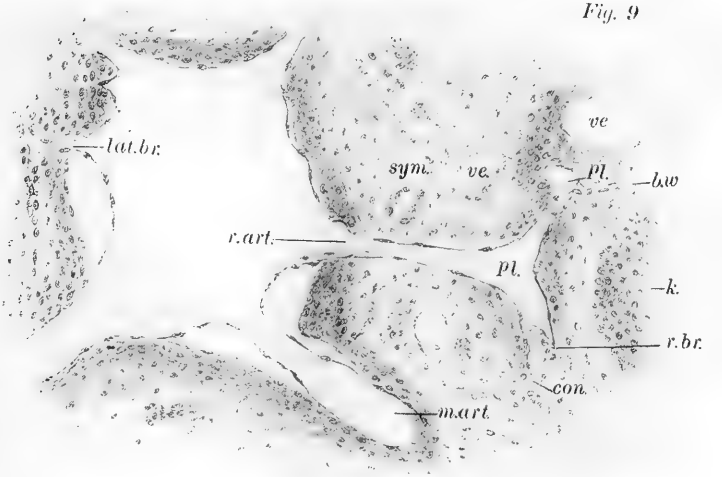


Fig. 9



namely pig, rabbit, sheep, cat, and man, the two former have relatively a large Wolffian body, both in length and thickness, from a very early embryonic period. The increasing bulk of Wolffian body itself is enhanced by the consequent increase in the subcardinal veins, and these two factors cause a great protrusion of the organ into the coelom. The glomeruli, occupying the ventro-mesial border of the organ, are thus moved from their original position lateral to the aorta to one far ventral; and the mesonephric arteries, running to the glomeruli, are lengthened and arise from the ventro-lateral surface of the aorta, instead of from the lateral. This is true, to some extent, in all mammals, but occurs earlier and is exaggerated in the pig and the rabbit. The horizontal anastomoses of the plexus leading dorsally from the mesonephric arteries either do not form, or if already present are strained until they become obliterated, leaving the mesonephric arteries unconnected with the plexus, and far ventral to the area in which the kidney is developing (see diagram, fig. 7).

The fact that the spermatic artery is originally a branch from one of the arteries to the glomeruli of the Wolffian body, or as stated for the pig by Hill (6), a new vessel of the same type and with a similar course, gives us a check on the origin of the renal artery, for, if the renal artery is connected in any way with the mesonephric plexus, it will be common to find, as an anomaly, the spermatic artery as its branch. Such a connection I have not found described for either rabbit or pig.

A second factor which distinguishes different types of embryos is the greater or lesser curvature of the rump region. A comparison of the profile drawings of cat and rabbit embryos of about 10.0 mm. or younger with those of man, sheep, or pig of the same age shows in the former a strikingly increased curvature of the back just anterior to the pelvic limb buds (fig. 6). A study of sections of these embryos shows that the aorta does not follow this curve closely, but takes a shorter path, and so lies here further from the spinal cord than in the thoracic region. This causes the dorsal segmental arteries of the lumbar aorta, which run to the spinal cord, to assume a course almost di-

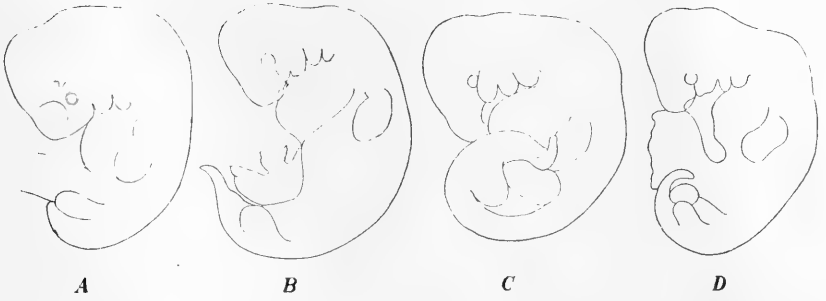


Fig. 6 Profile drawings of embryos to show the difference in the curvature of the rump region. A, man, 8.0 mm., H.E.C. No. 817; B, pig, 9.0 mm., H.E.C. No. 52; C, rabbit, 6.6 mm., H.E.C. No. 460; D, cat, 9.7 mm., H.E.C. No. 446.

rectly dorsal, instead of lateral or dorso-lateral, from the aorta. In this case the dorsal portion of the periaortic net will be distorted and obliterated, as was the ventral portion in animals with a large Wolffian body, and in the cat and the rabbit the dorsal segmental arteries may be expected to take no part in the development of the renal or suprarenal arteries (see diagram, fig. 7).

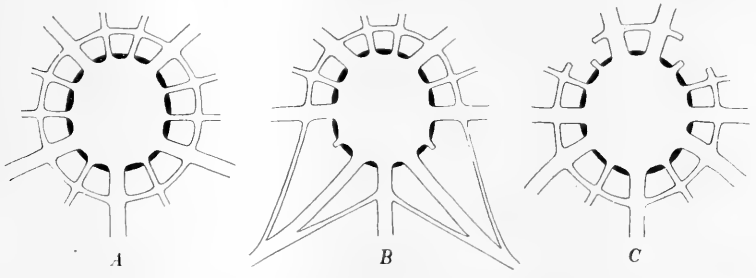


Fig. 7 Diagram to show, A, roots from the aorta between the dorsal segmental, mesonephric, and mesenteric arteries, and the periaortic plexus; B, result of large Wolffian body, carrying forward the mesonephric arteries; C, result of greater curvature of back, lengthening the dorsal arteries. In both cases lateral branches enlarge to supply the fields left vacant.

It will be noted that the rabbit is mentioned in both of these classes, and should therefore be restricted to a lateral origin of these arteries, unconnected with either mesonephric or dorsal segmental vessels; anomalies should be very rare. Sheep and

man, on the other hand, have neither large Wolffian body nor great curvature; less marked variations or the accidental blocking of one or the other of the vessels might be expected to determine the adult relations, and anomalies should be frequent and very variable.

A secondary result of the increased curvature of the rump region is the formation of the a. ilio-lumbalis and a. adreno-lumbalis in the cat, and of similar vessels in the rabbit. These vessels, situated in the lumbar region, are derived from the extension of the periaortic plexus into the area lateral to the aorta, left vacant by the separation of the aorta and the spinal cord. In the thoracic region of these animals and in all regions of other animals this area is supplied by the ventral branches of the dorsal segmental arteries, which are prolonged into the lateral body-wall. But the increasingly dorsal direction of the dorsal arteries, following the increased curvature of the body, causes a sharp angle where the ventral branches leave their dorsal segmental trunks, and thus puts the ventral or body-wall branches at a disadvantage, and makes their territory of distribution fall an easy prey to the rapidly growing branches of the plexus with its shorter and more direct lateral roots from the aorta. The resulting vessels, as described in the cat by Reighard and Jennings (12), pass in front of the psoas muscles, and after anastomosing with each other (the longitudinal strands of the plexus) send branches to the ventral body muscles. In this region the usual ventral branches of the lumbar arteries are lacking.

Such vessels to the ventral and lateral body muscles should not be mistaken for the normal lumbar arteries of the adult, which are the dorsal segmental arteries of the embryo. The latter, or true lumbar arteries, pass behind the psoas muscles near to the vertebrae, to which they send branches, and then supply the spinal cord and dorsal muscles on their way to the body-wall. The newer vessels pass in front of the psoas group and are without dorsal branches. Because of the connections of both with the body-wall, I think their differences have often been overlooked in the descriptions of the anomalous origin in man of the renal artery from a 'lumbar artery.'

The actual origin of the renal artery is found to correspond in the different groups of animals with the principles just stated. In cat embryos of 6.0 to 7.0 mm. a well developed net exists, opposite the four lumbar segments, beside the aorta, connecting the mesonephric vessels, which have an almost lateral course, with small lateral sprouts from the aorta, and those again with the dorsal segmental vessels. With the increased curvature of the body these dorsal connections are lost, new sprouts from the lateral portion of the plexus extend toward the lateral wall, and a main channel, from a mesonephric artery of the first or second lumbar segment, is formed and becomes important enough to assume a mesodermal coat. The lateral connections of the plexus with the aorta remain small and uncoated. In a 10.7 mm. cat embryo (H.E.C. No. 474) other branches from the lower end of this plexus have entered the superior pole of the kidney, which is still without a capsule (fig. 8). The renal blood supply may thus be said to be derived temporarily from the mesonephric artery, since the plexus receives most of its blood from this source. Meanwhile the growth of the Wolffian body, aided perhaps by the advent of the kidney dorsal to it, has forced the glomeruli to a more ventral position and lengthened the mesonephric arteries, among them the one which sends off the main coated afferent of the plexus. The result is that a sharp angle is formed between the mesonephric artery and its branch to the plexus, and that the smaller, lateral roots of the plexus become by far the most direct pathways to the plexus and hence to the kidney. One or more of these becomes enlarged, as is shown on the left side in an embryo of 11.6 mm. (H.E.C. No. 1979). The lateral aortic branch is of large caliber (fig. 9), and connects the coated aorta with the coated plexus, yet is itself provided with only an endothelial wall, showing its sudden rise to importance. The older main channel to the plexus from the mesonephric artery has become discontinuous in this case, and the other lateral branches from the aorta to the plexus, not seen in this section, have also lost their connections and remain as vessels to the immediate periaortic tissues. On the right side of this section one of these small vessels may be seen.

The plexus here is still connected with the mesonephric arteries, and no enlargement of the lateral aortic branches is found, so that the right side of this embryo is in the same condition as both sides of the embryo of 10.7 mm.

The permanent renal artery in the cat is thus formed partly from a suddenly enlarged but long present lateral branch of the aorta, partly from the longitudinal meshes of the periaortic plexus, and partly from branches from the lower end of this plexus to the upper pole of the kidney; the connections are present before 10.7 mm.

Anomalies to be looked for in the cat, easily explicable by the history as thus shown, are, first, double or multiple renal arteries from the aorta, since there are several lateral connections from aorta to plexus, and, second, a spermatic or ovarian branch of the renal artery. This latter would require merely the persistence of the original mesonephric union with the plexus, and a reversal of the blood current through it, so that the mesonephric plexus from which the spermatic artery sprouts would receive its blood through the renal artery and the lateral plexus; the mesonephric trunks might then all disintegrate. Spermatic branches of the renal arteries are mentioned as occasionally present in the cat by Reighard and Jennings.

The renal artery in the rabbit has been figured by Lewis (13) in reconstructions of embryos of 11.0 mm., 14 days (H.E.C. No. No. 1327), and 14.5 mm., 14 days, 18 hours. (H.E.C. No. 143), in his paper on the development of the vena cava inferior. Though hardly referring to this artery in the text, he thus correctly represented it in embryos younger than those in which it had then been reported. It is shown as a longitudinal vessel, reaching the kidney at its caudal end, attached near the middle of its length, in the 11.0 mm. embryo, by a vessel springing from the aorta midway between the dorsal segmental arteries and the mesonephric arteries. With more detailed study of rabbit embryos with this point of view, it is possible to find the periaortic plexus present very early, but it is partially lost in embryos of 8.0 mm. to 9.0 mm., leaving only the lateral sprouts from the aorta (cf. fig. 2). There are many of these in the lumbar region,

connected by longitudinal anastomoses, but unconnected with the mesonephric or dorsal segmental arteries for reasons stated above. From the longitudinal strands of the plexus branches pass to the body-wall, which by 10.0 mm. (H.E.C. No. 155) have become long vessels. In this same embryo branches from the lower end of the plexus have entered the kidney. The longitudinal anastomosis and one of its aortic connections are the vessels figured by Lewis. The lateral branches to the body-wall are not shown by him, but nevertheless form a permanent connection; one at least remains as a normal branch of the renal artery in adult rabbits. This is mentioned by Gerhardt (14) in the following description, p. 245: "From each renal artery, near its root springs a small (the 12th) intercostal artery." This is obviously not a true intercostal with dorsal connections, but must run in front of the psoas muscles and thus be comparable with the 'false lumbar' arteries already referred to. Spermatic branches of the renal artery are not described in the rabbit, to my knowledge, and are not to be expected because of the early separation of the plexus from the mesonephric arteries.

I think the detailed description of the origin of the renal artery in the cat and the rabbit is sufficient to show how the variations in the different classes arise. The pig makes use of a lateral aortic branch, or, as is shown in figure 5, of a branch of the dorsal segmental artery. In the sheep a lateral aortic branch is commonly enlarged. In both the connection of the plexus with the kidney is made early, and originally at the superior pole of the organ.

It should be noted that in all classes of mammals the early position of the kidney, before it has escaped from the crotch of the lower bifurcation of the aorta by migrating upward and backward, as described by Pohlman (15) and by Lewis and Papez (16), is nearer to the mesonephric arteries, because more ventral, than after this escape. My observations, contrary to those of Broman (4) in his paper on the renal portal system, show frequent early mesonephric branches to the kidney, some in use, some partially obliterated, in all the animals examined. When no larger branches are present, certain of the glomerular efferent

vessels of the Wolffian body can be traced toward the renal blastema. At no time does the kidney seem to be without arterial blood, from one source or another. The persistence of any of these mesonephric connections would make possible, as explained above, the presence of a spermatic branch of the renal artery; but an anomaly from this source must necessarily be extremely rare in animals with large Wolffian body, because it would depend upon the blocking of the more direct channels.

In man there is neither an excessive curvature of the body nor a large Wolffian body, so that theoretically the renal artery could be derived from the mesonephric, lateral, or dorsal segmental roots of the plexus; yet normally it is not directly derived from any of these. The reason for this is found in the precocious development of the suprarenal gland in man.

Goormaghtigh (17) finds the first growth of the mesothelial cords which form the cortex of the suprarenal gland at about ten days in the white mouse, about twenty days in the guinea-pig, but states that these cords do not separate from the mesothelium to form a recognizable organ until much later. Substituting the white rat for the white mouse, of which no specimens were available, I find that the gland is first seen as a large organ at about 14 days, 8.0 mm.; in the guinea-pig it occurs in embryos of about the same length. In the cat the suprarenal cortex can be recognized as such at about 11.0 mm., in the pig at 15.0 and at corresponding ages in the rabbit and sheep. In all of these animals, the organ occupies at this time the upper abdominal region, so that there is a long distance, representing five or six segments, between the upper pole of the kidney and the lower limit of the suprarenal gland.

The suprarenal arteries are also derived from the perioartic plexus or, if this has partially broken down, from its aortic roots. Thus in a cat embryo of 15.6 mm. (H.E.C. No. 1983) an upper suprarenal artery springs from a loop between a dorsal segmental vessel and the coeliac axis. The mechanical forces which influence the choice between the aortic roots are similar to those explained for the renal artery, but may differ in the upper and lower levels of an organ which becomes as long as does the supra-

renal gland. The rabbit utilizes as an inferior suprarenal artery one of the upper lateral branches of the plexus, from the lower end of which the renal sprouts develop, so that the suprarenal artery, as well as the 12th intercostal artery, is normally a branch of the renal, as stated by Gerhardt. Other animals may or may not have such connections between renal and suprarenal arteries, but in all cases the renal is the older vessel.

In contrast to the other mammals examined the early development and rapid growth of the suprarenal gland in man is very striking. At 8.0 mm. (H.E.C. No. 877) while the kidney is still a pelvic organ, the group of cortical cords has already grown down to the level of the first lumbar arteries, or in other words is occupying the region later to be invaded by the kidney. It would be interesting to speculate, in connection with the work of Cannon (18), on the activity of this gland in various emotions, whether man is given an advantage over other mammals by its precocious development; but that is at present beside the question. The result, so far as the arteries are concerned, is that the suprarenal gland primarily receives the vessels which in other mammals go to the kidney. These arteries are at first numerous, but only three survive, as was pointed out by Broman (4); and it is only the most caudal of the three which is immediately interesting. Broman states that the suprarenal arteries are branches of the mesonephric vessels, but I must disagree partially with this statement, for I have seldom seen such a connection in the embryos examined. Normally all of the suprarenal arteries are the lateral aortic roots of the plexus, similar to the renal arteries already described in many mammals. They never, in my specimens, extend to the mesonephric glomeruli, nor are they branches of vessels which do, though they are not infrequently connected by the anastomoses of the plexus with the mesonephric arteries. Exceptionally a mesonephric artery does send a small branch to the suprarenal gland but in the few cases I have seen, such branches are much smaller than the normal arteries, though probably capable of persisting under certain conditions.

The mesonephric arteries themselves in man differ from those of other animals in the younger stages in that they are much distorted and bent from their usual direct course by the growth amongst them of the precocious suprarenal gland. Some are deflected ventrally, some dorsally, so that they arise in two more or less distinct vertical rows from the ventro-lateral and dorso-lateral surfaces of the aorta, respectively. The suprarenal vessels are the aortic roots between these two rows. The infrequent branches of mesonephric arteries to the suprarenal gland may approach it from behind or from in front.

An ascending branch from the upper suprarenal artery, or from the upper end of the plexus, frequently becomes the inferior phrenic artery; one or both of these vessels may be connected with the coeliac axis or with a lumbar artery (cf. cat 15.6 mm., mentioned on p. 196). A descending branch from the lower suprarenal artery, or from the lower end of the plexus becomes the permanent renal artery. This vessel can be traced to the kidney in a human embryo of 16.0 mm. (H.E.C. No. 1322), and in one of 16.4 mm. (H.E.C. No. 1707). Terminal branches can be seen at the upper pole of the kidney in an embryo of 13.6 mm. (H.E.C. No. 839), but I am unable to trace with certainty the course of the vessel in this specimen.

In the 16.4 mm. embryo (fig. 10), the more ventral mesonephric arteries sweep ventrally in front of the suprarenal gland, and then turn laterally to reach the glomeruli. Dorsal to these, but connected with them by occasional anastomoses, a set of branches, represented by two roots on the right side of the figure, by one large root on the other, passes to the suprarenal gland. The two on the right have apparently just lost an anastomotic connection at 'x,' while on the left side the plexus has been reduced to two large longitudinal vessels, each sending sprouts to the gland. This left side of the figure resembles the reconstructions of rabbit embryos made by Lewis, the longitudinal vessel representing his 'renal artery.' At this level there are no mesonephric arteries passing dorsal to the suprarenal gland. From the bottom of the longitudinal trunk on the one side, from the lower end of the lower suprarenal artery on the other, single vessels pass to the

kidney, which lies immediately below the level of the reconstruction. From the upper end of the longitudinal vessel, on the left of the figure, a twig passes upward, possibly the future inferior phrenic artery. On the right side of the figure a similar vessel comes from a mesonephric artery, which also has two small suprarenal branches, thus showing an unusual condition. This same mesonephric artery is connected not only with a suprarenal trunk but also with the coeliac axis. Smaller aortic branches are shown, one ventral between the coeliac axis and the superior mesenteric artery, others lateral.

The permanent renal artery would have included the main trunk from aorta to kidney, arising normally in this case at the level of the second lumbar arteries, and would have had suprarenal branches. A spermatic branch would have been impossible in this embryo on the right side of the figure, highly improbable on the other, though a roundabout connection between kidney and mesonephric artery does exist. One can readily see, however, that if more of the plexus had been retained, spermatic branches would have been easily possible. The origin of the renal artery from the coeliac axis can also be imagined in this case, and similar anastomoses between lateral or mesonephric vessels and the superior mesenteric artery would account for a renal branch of the latter vessel. Dorsal connections of the suprarenal arteries with the body-wall or with true dorsal segmental arteries can be conjectured as a persistence or extension of the plexus. The permanency of all such connections, however, must depend on the occlusion of more favorable channels.

SUMMARY

1. The anomalies of the renal artery depend on vessels present in the embryo before the aorta and its larger branches develop mesodermal coats (10–15 mm. embryos). There are no 'late branches.'

2. Certain anomalies are due to the persistence of the early renal blood supply most frequently seen with pelvic kidneys; i.e., the renal artery as a branch of the iliac, inferior mesenteric or middle sacral arteries, or from the aorta below the inferior

mesenteric artery. Also a spermatic branch of the renal artery may be due to an early connection of a mesonephric artery with the kidney, normally lost. The kidney is probable never without arterial blood.

3. A periaortic plexus with many roots from the aorta exists in part in the mammals studied, affording an opportunity for the change of position of main aortic branches, and supplying smaller branches for possible future use.

4. The renal artery is derived from this plexus or from such parts of it as exist just before the kidney receives its capsule.

5. The channel for the renal artery is selected mechanically, and differs in different animals with the size of the Wolffian body, the greater or lesser curvature of the rump region, and probably other physical peculiarities.

6. Channels not so convenient may be utilized if the usual channel is occluded; hence the renal variations and anomalies.

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SOME CHARACTERISTICS OF THE EXTERNAL EAR OF AMERICAN WHITES, AMERICAN INDIANS, AMERICAN NEGROES, ALASKAN ESQUIMOS, AND FILIPINOS

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EIGHTEEN FIGURES (THREE PLATES)

The study of the external ear of man was begun by me in 1905, although I had been making casual observations even before this. While in the Philippines from 1907 to 1910 I segregated many forms of ears, grouping them under three main heads which I called Primitive, Australoid and Iberian for various reasons then set forth (1). Other considerations prevailed and I changed the names from Primitive to Hypo-onto-morph, from Australoid to Meso-onto-morph, and from Iberian to Hyper-onto-morph (2).

The present study is a continuation of those made previously and is more detailed and specific than former studies. It corroborates them in general and in particular, and adds racial distinctions to type differences.

MATERIALS

Morgue subjects

86 ears of New Orleans whites
200 ears of New Orleans negroes
94 ears of Manila Filipinos

Living subjects

103 ears of New Orleans students (white)
94 ears of New Orleans negroes
182 ears of American 'old whites' (three or more generations in America)
222 ears of Washington, D. C., negroes
73 ears of American Indians
171 ears of Alaskan Eskimos
68 ears of fetuses, newborn, and young infants (dead)

This paper is divided into two parts: "Ears of the morgue subjects," and "Ears of the living subjects."

EARS OF THE MORGUE SUBJECTS

The ears of the morgue subjects were measured in 1914 after having been preserved in formalin for variable periods of time from a few weeks to several years.

Each ear was measured as prescribed by Schwalbe, Ranke, Martin and others (13, 15, 16). Seven measurements of each ear were made, the total length, total breadth, cartilage length, ear base, Schwalbe's true ear length (15), the concha length and concha breadth. The concha breadth and length are measurements devised by me to determine the concha index. The concha length is measured from the point where the inferior crus of the concha emerges from the ascending helix, to the inferior border of the intertragic incisure. The concha breadth is measured from the supra-tragic incisure to the point at the greatest distance from this on the inside of the concha opposite.

Helix. Measurements and indices

The average total length and breadth of the ear in millimeters follows:

TABLE 1

Total length of the ear, average in millimeters

NO. OF EARS	RACE; SEX	RIGHT EAR	LEFT EAR	BOTH EARS
67.....	White men	64.95	63.20	64.18
120.....	Negro men	58.81	58.33	58.58
31.....	Negro women	57.67	59.31	58.32
80.....	Filipino men	59.69	58.43	58.80
14.....	Filipino women	57.86	57.00	57.43
19.....	White men	65.20		
41.....	Negro men	59.30		
8.....	Negro women	57.90		

TABLE 2

Total breadth, average, in millimeters

NO. OF EARS	RACE; SEX	RIGHT EAR	LEFT EAR	BOTH EARS
67.....	White men	37.65	36.85	37.19
120.....	Negro men	37.78	37.05	37.43
31.....	Negro women	35.61	35.69	35.63
80.....	Filipino men	33.71	33.43	33.57
14.....	Filipino women	33.30	32.57	32.94
19.....	White men	38.60		
41.....	Negro men	37.40		
8.....	Negro women	37.10		

Physiognomic index of Topinard (16) is the breadth divided by the length:

TABLE 3

Physiognomic index

67 White men	58.0
120 Negro men	64.0
27 Negro men	60.8
80 Filipino men	56.8
14 Filipino women	57.5

According to Martin (13) the lowest index of any race so far measured is that of the Aino, 52.8, and the highest is that of the Mawamba pigmies, 66.2. Europeans, as a rule, have a low index, below 60.0, although Alsatians (Schwalbe) have an index of 60.5 (15) and the negroes as a rule have a high index, above 60.0, although the Hottentots have an index of 58.8 [Karutz (12)]. The index of the new born is high, 73.7 for males and 73.1 for females, and it sinks to 58.7 at 70 years of age [Daffner (6)].

Modulus: The ear modulus is obtained by adding the total length of the ear to the total breadth and dividing by two. This gives the ear size in a factor of convenient form. The average module of 67 ears of white men is 50.69, of 120 negro men is 48.0 and of 27 negro women is 46.86. The module for 80 Filipino men is 46.19, for 14 Flipino women 45.19.

Résumé of the total ear dimensions: The ears of the white men are longer than those of the negro men and of about the same breadth. The ears of the negro women are about the same length as those of the negro men but not so broad. The ears of the white men are long and narrow, the ears of the negro men are relatively short and broad, the ears of the negro women are relatively short and narrow and the ears of the Filipinos are short and narrow, relatively narrower than any of the others. The ears of the white men are large, the ears of the negro men and women are small, those of the negro women smaller than those of the negro men, and the ears of the Filipinos are smaller than any of the others, the ears of the Filipino women are the smallest of all.

Morphologic index: Schwalbe (15) introduced an index of the ear which is obtained by dividing the ear base by the true ear length. Taking a series of mammals up to man this index increases from 27.6 in the antelope through the kangaroo 33.0, the lemur 76.0, the monkey 84.0–93.0, the chimpanzee 105.0, the orang 122.0, the gorilla 125.0, the negro women of New Orleans 172.67, the negro men of New Orleans 177.9, to the white men of New Orleans 180.2. The Filipino men have an index of 188.9 and the Filipino women 193.96. The index of the Bavarians is 122.0, Great Russians 139.8, Kalmucks 140.6, and Ainos 171.5 (13). The morphologic index is vitiated by the difficulty of finding Darwin's tubercle in a large number of ears. Martin (13) considers the index of doubtful value. The variability is very great, 83.7 to 195.5 in Alsatian men, 97.3 to 189.5 in Alsatian women, 110.0 to 223.0 among the Ainos, and 133.0 to 255.0 among the negro men of New Orleans.

Concha. Measurements and indices

Length: The length of the concha is 24.79 mm. in 56 ears of the white men, 24.25 mm. in 115 ears of the negro men, 24.04 mm. in 29 ears of the negro women, 24.62 in 80 ears of the Filipino men and 23.57 mm. in 14 ears of the Filipino women.

Breadth: The breadth of the concha is 19.14 mm. in 56 ears of the white men, 20.01 mm. in 115 ears of the negro men, 19.24

mm. in 29 ears of the negro women, 17.0 in 80 ears of the Filipino men and 16.93 in 14 ears of the Filipino women.

Index: The index of the concha, as instituted here by me for the first time, is obtained by dividing the length into 100 times the breadth. The index represents the breadth of the concha in terms of the length, the latter always 100. If the index is high, the concha is relatively broad, if the index is low the concha is relatively narrow. The index is 77.21 for the ears of the white men, 82.52 for the ears of the negro men, 80.03 for the ears of the negro women, 69.0 for the ears of the Filipino men and 72.0 for the ears of the Filipino women.

The concha index is more variable among the negroes than among the whites and it is more variable among the negroes than either the physiognomic or morphologic index. The great variations of the concha index need not vitiate its importance, but may add to its significance by indicating the degrees of mixture of negro and white.

The ears of the negro men with a concha index of 81 and over are more like the ears of the negro, because the average total ear length of the ears in this group is 5.6 mm., and the average age total breadth is 3.7 mm., with a physiognomic index of 66.1; whereas the average total ear length of the ears of the negro men with a concha index of 80 and less is 6.1 mm., the average total breadth is 3.8 mm., and the physiognomic index is 62.3, and the ears are therefore like the ears of the white. The concha index is thus proven useful as a racial differentiator of a mixed group.

There is a close correlation between the concha index, the morphologic index, and the physiognomic index.

Descriptive characters (plates 1 to 3)

True negro ear: In addition to the measurable characters of the negro ear there are descriptive characters which are as distinctive as the measurable ones and they may enable the observer to obtain a clearer picture of the true negro ear and its parts (12). The ear is small, almost flat, close to the head, and the helix is broad as if much folded over. The upper part of

the helix is almost horizontal and passes almost directly backward from the upper end of the ear base to join the vertical dorsal portion of the helix at a right or acute angle in a rounded point at the upper outer extremity of the ear. The superior and dorsal borders of the helix are separated by a depression above Darwin's tubercle where the helix is thin or absent. The dorsal border passes downward and turns forward at an obtuse angle to form the inferior border of the ear which enters the cheek almost at right angles, with no lobule, or a very small one which is nearly flat (figs. 1–12).

The satyr tubercle is well marked and Darwin's tubercle is small or absent. The skin lines formed by the infolding of the helix are less distinct on the true negro ear than on the white, and they usually converge on the true negro ear over Darwin's tubercle. The concha, like the ear as a whole, is wide and short. The true negro ear, is not seen in great numbers among American negroes. It occurred 245 times among 1478 New Orleans negroes (16.6 per cent), men, women and children, chiefly of the laboring classes.

Involuted ear: There is another form of ear that is found chiefly among the negroes, but it is also found not rarely among other peoples, even among the whites, and I have called this '*the involuted ear*' because it seems to represent an advanced stage in regressive evolution. It is the ear with a broad helix that is much rolled in and frequently has a gnarled or crumpled appearance, as if the ear had been burned around the border and had contracted irregularly in healing, leaving a thick, distorted helix. This ear type in its crumpled condition was at first thought to be due to accidental causes, but the presence of the skin lines of the ear tip in regular order proved the ear to be a true type. This ear form has been described by Fischer as the Hottentot ear (7). It was found 601 times in 1478 New Orleans negroes (40.7 per cent) and 52 times among 857 New Orleans whites (6.1 per cent). The individual characters of the ears of both whites and negroes will next be presented in detail.

Darwin's tubercle: There are three prominent parts of the inner or ventral border of the helix called the Satyr tubercle,

Darwin's tubercle and one below this that I will name here for the first time the 'inferior tubercle' of the helix. This tubercle is illustrated by Münch (14) in his study of the development of the ear cartilage. Between these there are two depressions, one at the upper outer corner of the ear between the satyr tubercle and Darwin's tubercle, and one on the dorsal border of the helix between Darwin's tubercle and the inferior tubercle. The three tubercles and the two depressions are made by the unequal turning in of the helix of the ear in the fetus.

Darwin's tubercle is small or absent in the negro, and large in the Filipino, although it is more often absent among them than among the others. It is also small in the white although there are many ears with large tubercles among the whites.

Skin lines on the helix (plates 1 and 2)

The position of the skin lines on the helix caused by the infolding of the tip were first seen in 1914 when I was making the drawings of the ears in figures 1 to 12. These lines have never before been described although they may be seen on practically all adult ears. They converge from the outer to the inner border of the helix beneath the prominence of Darwin's tubercle, or adjacent to it, although they may be seen rarely on the dorsal side of the helix. By their usual position they seem to indicate that the skin has been pulled forward under Darwin's tubercle or has been held there while the adjacent parts of the helix develop more rapidly, leaving lines that represent foldings of the skin.

The lines are more often absent or obscure on the ears of the negroes and Filipinos than on the ears of the whites. The skin lines are more scattered on the ears of the whites and more often occur about Darwin's tubercle on the ears of the others. The lines are also higher up on the helix of the white ears than on the helix of the others, and often appear over the satyr tubercle of the white ear. This may be due to the more regular form of the white ear and to the fact that the satyr tubercle is larger in the negro than in the white, and is turned over from above more in the former than in the latter. This rolling over of the satyr

tubercle in the negro ear may prevent the lines from forming above Darwin's tubercle as the latter undegoes retrograde metamorphosis.

The satyr tubercle is large in the negro ear, small in the white ear, and intermediate in the Filipino ear.

The helix of the negro ear is broad, the helix of the Filipino ear is narrow, and the helix of the white ear is intermediate. The broadness of the helix in the negro ear is especially well seen in the upper border in the region of the satyr tubercle, and this border of the ear is horizontal and at right angles to the ear base. There is evidence of greater inrolling of the negro ear in this part, which accounts for the shape of the negro ear, quadrangular, or pentagonoid, and also for its small size, and its greater breadth and less height or length, its short, broad concha, its small base and long true ear diameter.

The lobule is absent or small more frequently from the ears of the negroes and Filipinos than from the ears of the whites, and it is absent or small more frequently from the ears of the women than of the men. Keith (13) gives an increasing size of the lobule in the following order: Orang, chimpanzee, gorilla, negro, British. According to Martin the lobule is absent oftenest in the negroes, less in the Asiatics and least in the Europeans. It is also absent more frequently among the defectives than among normal individual (Bertillon).

The helix and other parts of the ear may be examined for their relative projection from the head, and as this determines in part the Hypo-, Meso and Hyper types of the ear it will lead up naturally to a consideration of these three types.

The negro men have projecting helices, the white men have projecting anthelices, the Filipino men resemble both the white and negro men, and the negro and Filipino women resemble the white men more than they do the negro men.

The lobule and the inferior border of the helix: This part of the ear is turned from the head in the negro men, and in toward the head in the white men, and the negro women resemble the white men in this respect.

The posterior auricular sulcus: The sulcus turns into the concha in the negro men, out over the helix or lobule in the white men, and in the Filipino men it does both. The negro and Filipino women are like the white men in this respect.

Tragus and antitragus: The negro men's ears have depressed or intermediate antitragus and tragus, and the white men's ears have prominent ones, in which the ears of the Filipino men and women and the negro women resemble the white men.

Asymmetry: (8) The ears of the negro men are asymmetrical in 33.3 per cent, and asymmetrical in 66.7 per cent; the ears of the negro women are asymmetrical in 30.0 per cent, and symmetrical in 70 per cent, and the ears of the white men are asymmetrical in 25 per cent and symmetrical in 75 per cent. This does not include slight differences, but only such as an oval ear on one side and a pentagonoid ear on the other, or the absence of lobule on one side and its presence on the other, etc. The ears of the two sides are almost invariably asymmetrical in a slightly different arrangement of the skin lines, different curves of the helix, depths of the concha, etc.

Résumé: The details of the ears of the negro and white are different as follows: The negro ears are glabrous, the white ears are hirsute; the satyr tubercle is large in the negro ear, small in the white; Darwin's tubercle is more difficult to find in the negro ear than in the white; the skin lines converge about Darwin's tubercle in the negro ear, and between Darwin's tubercle and the satyr tubercle in the white; the helix is broad in the negro ear, narrow in the white; the anthelix is more prominent in the white ear than in the negro; the posterior auricular sulcus is deeper in the negro ear than in the white, and in the negro ear the sulcus dips into the concha, whereas in the white it turns out over the helix or lobule. Also in the white ear the helix and lobule are turned towards the head and are not prominent, and the tragus and antitragus are turned away from the head and are prominent, whereas in the negro the reverse is true. The white ear is also more symmetrical than the negro, and the negro ear is involuted, whereas the white ear is not. All of these characters are mixed

in the Filipino ears, evidence of Negrito and European stock as their basis. The true negro ear and the involuted ear are characteristic negro ears, and they both represent great regression in evolution. This regression is represented by the involution of the helix, which is turned in and is broader in the negro than in the white. The involution is present on the few fetal negro ears I have examined as early as the third or fourth month at which time other negro characteristics are present. For this reason it seems to be rather a condition of regression in evolution than of retrograde metamorphosis in development. The small size of the negro ear, the great breadth and irregularity of the helix and the absence of hair all point to the negro ear as more advanced in regressive evolution than the white, and further removed from the apes.

Three fundamental ear types (plates 1 to 3)

It should be plain from the foregoing that the ears of the Filipinos, negroes and whites are different, and the form of the true negro ear, and the involuted ear should be clear, but there are other forms than these among the ears examined, forms that are characteristic of three types of white ears, but that may and do appear among all peoples of the world. The three types I have called the Hypo-, Meso-, and Hyper- types, and each of the three types may be subdivided into onto-morph and -phylo-morph forms, the phylo, the primordial form and the onto, the derived form. At birth the white child is a Hypo-phylo-morph, and as the child develops its passes consecutively through the stages of the Hypo-onto-morph, Meso-phylo-morph, Meso-onto- morph, Hyper-phylo-morph and Hyper-onto-morph, unless development stops at or between one or the other of the types. This is a process of differentiation and not of growth, because the Hyper-onto-morph, which is apparently the most highly differentiated type, is the smallest when the adult state is reached. The Hyper-onto-morph, the Meso-onto-morph, the Hyper-phylo-morph and very rarely the Hypo-onto-morph are European, or white, types in the adult; whereas the Hypo-phylo-morph, the Meso-phylo-morph, and rarely the Hyper-

phylo-morph are types of the negroes, Filipinos, and other primitive peoples.

It may be clearer to omit these distinctions at this time and to confine the grouping of the three types, Hypo, Meso, and Hyper, which are readily distinguishable. When this study was begun and the observations made, first the ear type was written down on the card for the ear to be described, then each part of the ear was described in detail, the skin lines, hair, Satyr tubercle, Darwin's tubercle, helix, anthelix, posterior sulcus, tragus, antitragus, lobule, etc. From the data thus obtained the following facts have been collected:

Hypo (plate 3): The helix is prominent, the lobule turns away from the head, the anthelix is intermediate, and the tragus and antitragus are depressed in the Hypo ear, therefore the ear is bowl-shaped or trumpet-like. The flaring ear of the other types must not be mistaken for the Hypo ear, because the flaring ear is due to a wide dorsal wall in the concha, which makes the ear stand out, whereas the Hypo type of ear is due to a greater rolling over of the helix all around to the lobule which forms a shelf. This is not the extreme and disordered rolling over of the helix as in the true negro and involuted ears, but the helix presents the form of a round roll like a thickened and turned in edge of a bowl or trumpet.

Hyper (plate 3): The helix and lobule turn towards the head, the anthelix is prominent and the tragus and antitragus project from their surroundings in the Hyper ear. The helix is thin all around, and has turned in very little except at the upper part, and even this is not rolled in to a great extent. None of the tubercles of this type of ear are large, due to the slight development of the helix. It looks as if the helix had stopped developing early and the other parts of the ear had continued to develop which results in a contracted helix, and as the helix had not rolled in enough to contract forward, it turns backward more or less behind the ear. The edge of the helix has a semispiral or italic f shape, or the shape of the old English letter *s*, when the ear is viewed from behind. This type of ear is the most distinctive of the three and the type of individual called the Hyper-onto-

morph is the most distinctive of all individuals. It is that type which is small, thin, wiry, and nervous, with long narrow head, long narrow nose, long narrow face, with pointed chin—the 'hatchet face,' as the Australians call the Englishmen in derision. This type has been found by me to have a very short intestine, sometimes only twelve feet in length, and it has also been found to have a low pylorus and duodenum, a low hepatic and splenic flexure of the colon, and a low transverse colon. The suggestion has therefore been advanced by Goldthwait, Brown (4) and others that the Hyper-onto-morph is immature, an under-developed individual morphologically. Also that it is the carnivorous type of Treves and others. There can be no doubt that the type is the end product of a hyperactive thyroid gland. The individuals are precocious in the development of the teeth, nose, face, head, and general body form (3). Their development may be so rapid that such structures as the ears, stomach, small and large intestine fail to finish their growth and transformation. Other evidences of thyroid activity are that the individuals are nervous, high-strung, and very active mentally, and there is a low grade of exophthalmos, the eyes are wide open and often present the wild look of a surprised animal, a condition that has been noted especially among the Indo-European women, and is considered to be a mark of beauty. They are susceptible to diseases of the alimentary canal, nervous system and lungs (epitheliopaths), as well as exophthalmic goiter. They have small bones and there is evidence of calcium insufficiency due to the thyroid secretion.

The Hyper type I have called an epitheliopath, and the Meso a mesotheliopath, because of the susceptibility of the epithelial tissues to disease in the Hyper type and the susceptibility of the mesothelial tissues to disease in the Meso type. Goldthwait of Boston has carried my work further and has demonstrated that a large majority of the inmates of insane asylums are of the Hyper type and that this type is susceptible to joint affections. Percy Brown of Boston has demonstrated associated alimentary diseases with the Hyper type (4), and Bryant of Boston has utilized the Hyper and Meso type in a successful rational

diet, feeding the Hyper on concentrated nutrition, meat, eggs, and milk, because the intestine is short, and feeding the Meso on coarse bulky foods because the intestine is long. I may add the results of measuring the intestine in the dissecting room during the past two years (2).

Fourteen subjects of the Hyper type have an average length of 17.2 feet for the small intestine, the shortest 10 feet and the longest 27; eleven subjects of the Meso type had an average length of 23.3 feet for the small intestine, the shortest 16.3 feet and the longest 29.5; and 27 subjects of the Meso-Hyper type have an average length of 20.8 feet for the small intestine, the shortest 16 feet, the longest 29 feet. Only one subject of the Hypo type had the small intestine measured, and the length in that one was 16.9 feet. The lengths are only approximate because of difficulties in measuring, but the condition is evident and the correlation positive.

The Hyper type of ear and the Hyper type of person may be looked upon as the result of rapid transformation with slow growth, and the Hypo type of ear and the Hypo type of person may be looked upon as the result of slow transformation with rapid growth. We are reminded of the work of Gudernatsch (10) and his feeding experiments with tadpoles, in which he obtained the Hyper type of tadpole by feeding tyhroid gland, and the Hypo type by feeding thymus gland. Reasoning from this we may suppose that the Hypo type of man is due to excessive secretion of the thymus gland during development, and that the Hyper type of man is due to excessive secretion of the thyroid gland during development.

In connection with conditions of Hypo-morphism and Hypermorphism, I would like to present observations made from time to time on two children, Mary, aged 7; Helen, aged 2. Mary was the first child, Helen the third. During the first pregnancy there was evidence of increased activity of the thyroid gland in the mother; during the third there was evidence of less activity of the thyroid gland in the mother. Mary was prematurely born about 1 month. Helen was delayed several weeks. Mary has been premature in everything except size, has metamorphosed

rapidly but grown slowly. Helen has been delayed in everything except size, has metamorphosed slowly but has grown rapidly. Mary had a Hyper ear at birth, Helen had a Hypo ear at birth. Mary's nose was long at birth and rapidly approached the Hyper form, Helen's was short at birth and has retained the Hyper form. Mary's head elongated rapidly. Helen's elongated slowly. Mary crawled, climbed, stood alone, walked and talked earlier than Helen. Mary's teeth are small and apparently immature, Helen's are large and perfect. Mary is Hyper with rapid metamorphosis and slow growth, and Helen is Hypo with slow metamorphosis and rapid growth. I shall watch with interest their future development.

The Meso ear has a prominent or intermediate helix, lobule and anthelix, and a depressed or intermediate tragus and antitragus. The ear is nearly flat, the bowl is shallow, and the helix is not so much rolled in as in the Hypo ear. The Meso ear type is somewhat intermediate between the Hypo and Hyper types and it may be considered as the fundamental or generalized type of ear from which the Hypo and Hyper type have evolved in different directions.

The Meso ear is usually large and thick and heavy. The helix and anthelix being equally prominent a double roll is formed near the dorsal margin of the ear. The lobule and lower helix turn out from the head in the form of a shelf, and not to the same extent as in the Hypo ear, and the shelf, instead of being horizontal as in the Hypo ear, has a gentle slope forward or may be precipitous. At times the ear is almost flat, and quadrangular in shape.

Hyper-phylo-morph: An ear that is more or less intermediate between the Hyper and Meso types occurs frequently. The Hyper characteristics predominate although in each ear some Meso characteristics may be seen. This is a male ear type because very few appear among the women.

It may be well to determine the relation of the form of nose, head and face to the type of ear.

Nasal index and ear type: The noses of the persons with Hypo ears are relatively broader than those with Hyper ears

and the noses of the negroes are broader than those of the whites. The nose of those negroes with involuted ears is about the same as those of the Hyper-phylo-morph type.

Cephalic index and ear type: There is not the same regularity of difference here as in the nasal index and ear type although the negro woman shows the same regularity. In the latter the head is relatively broad in the Hypo and relatively narrow in the Hyper. The heads of the negroes with involuted ears are relatively long and narrow, and as this is characteristic of the negro head in general, we may attribute this to the fact that involuted ears are characteristic of the negro also.

Face index and ear type: The face index is better than the cephalic index as a differentiator, but it not so good as the nasal index. The face of the Hyper is relatively longer and narrower than that of the Hypo or Meso. The face of the white man is relatively longer and narrower than that of the negro man or woman.

EARS OF THE LIVING SUBJECTS

The data for the ears of the Eskimos, American Indians, negroes of Washington, D. C., and 'old whites' (3rd generation or over, in America) came to me through Dr. Hrdlicka, and a part at least has not before been published. The measurements were made of the left ear only, of the Eskimo, by Dr. Riley D. Moore, and of the others by Dr. Hrdlicka. Only the total length and total breadth were taken.

The Eskimo has the longest ears, the Indian is next, the 'old white' third, the New Orleans students fourth and the negro has the shortest ear. Comparing these with the morgue subjects, the latter have slightly shorter ears (white and negro) and the Filipinos have the shortest ears of all. The difference in length between the 'old whites' and the New Orleans students may be due to difference in age as the students are younger and ear length increases with age.

The Eskimo has the broadest ear, the Indian next, the 'old white' next, the student next and the negro has the narrowest ear. Compared with the morgue subjects the living have nar-

rower ears (white and negro) and the Filipinos have the narrowest ears of all.

The Indians have the largest ears, the Eskimos next, the 'old whites' next, the students next, and the negroes the smallest. Compared with the morgue subjects, black and white, the ears of the living are larger, and the ears of the Filipinos are the smallest of all. The small size of the Filipino ears is probably due to the small size of the Filipino people and to the negro (Negrito) mixture. The small size of the negro ear is a racial trait, and is not due to the size of the American negro, who is not a pigmy but almost as large as the American white or American Indian, and larger than the Eskimo.

The negroes have the highest physiognomic index, the students next, the 'old whites' next, the Eskimos next, and the Indians have the lowest index. Compared with the morgue subjects, black and white, the index of the living is less. The Filipino's index is greater than any of the living except the students and negroes, although it is less than the morgue white. By relative physiognomic index the Filipino would be classed with the Eskimo and Indian, and the white in each case is between these and the negro. The Filipino has a large portion of Negrito, which is evident because of the presence of negro and involuted ears in large numbers among them, but the physiognomic index does not show this.

The Filipino, Eskimo and Indian are related to the Mongolian in the physiognomic index of the ear and this relationship is apparently so strong that it is shown in the ear form, in spite of the large share of negro (Negrito) mixture in the Filipino.

The ear length increases with age to 70 years and beyond, and this increase is more appreciable in the white men than in the white women. It is also a little greater in the white men than in the Indian, and about the same as in the Eskimo. The white female and the negro male increase about the same.

The ear breadth increases with age very little and in the negro apparently none at all, but not enough negro ears were measured to establish this. The increase is greatest in the white men.

The physiognomic index decreases with age except in the Eskimo female, the white female decreases less than the white male and the Indian male decreases more than any. The decrease in the index is due to the greater increase in length than breadth of the ear with increasing age.

CHANGES IN THE EAR DURING GROWTH

Fetal stage: The ears of 44 negro fetuses and 22 white fetuses were measured and the total fetal length taken. It is seen that there is a decrease in the physiognomic ear index from 75 to 67 in the white ears, with an increase in total fetal length from 30 cm. and less, to 60 cm., but the negro ears are the same in physiognomic index in all the fetal stages, except from 30 to 39 cm. The index of the negroes is greater than that of the whites in fetal life, and thus early the racial characters are evident. This was also determined by inspection of fetal white and negro ears.

The female fetal ear is longer than the male, and the breadth is about the same as the male, which is what was found in the adult.

Stature and ear dimensions, negro children: It is seen that the length of the male negro ear increases from 49.3 mm. when the stature is 90 to 99 cm., to 56.0 mm. when the stature is 140 to 149 cm., and to 59.1 mm. in the adult. Likewise, the female negro ear increases from 51.8 to 54.7, and to 57.8 mm. in the same periods. The female negro ear is longer than that of the male negro ear until the adult state is reached, after which it is shorter. Likewise, the breadth for the periods mentioned is 33.0, 36.0 and 37.6 mm. for the male negro, and 32.5, 34.3 and 36.3 for the female negro. The female negro ear is narrower than the male and this difference is greater in the adult. Likewise, the physiognomic index for the four periods is 67.4, 64.0, and 63.8 mm. for the male negro, and 62.8, 62.8, and 60.8 for the female negro. The index decreases with growth more in the female from the earliest fetal stages up to the adult, and in this way the negro female approaches the white. The decrease in the index continues throughout life. Daffner (6) gives it in white males

as 73.7 mm. at birth, and 58.7 at 70 years of age; and I found it to be 68.1 in the 22 white fetuses and 55.0 at 60 to 69 years of age in the 'old white' American males, and 69.1 in the 44 negro fetuses and 60.3 at 50 to 59 years in the American negro males.

Stature and ear dimensions, adults: It is seen that the length and breadth of the ear increase with increase of stature, the length relatively more than the breadth, therefore the physiognomic index of the ear decreases with increase of stature, except among the negroes where the reverse is true. The breadth of the ear increases more than the length with increase of stature in the negro males, therefore the physiognomic index increases with increase of stature. Thus the negro racial characteristics of the ear are emphasized with increase in stature, purity of ear type goes with tallness in the negro males.

If we take the same stature in each group, for instance the stature of 160 to 169 cm., we find that the ear length, breadth and index are not the same in all the groups. Racial differences appear. The Eskimo and Indian ears are the longest, the negro ears are the shortest, the white are in between in length. The Eskimo and Indian ears are the broadest also, and the white and negro ears are equal to each other in breadth. The result is that the index of the Eskimo and Indian ears is low and that of the negro is high, with the white in between.

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The drawings in plates 1 and 2 were made from the original ears by using the projectoscope with the same focal distance for each drawing. The relative size of the ears and their parts are exact. The ears are reversed, what appears to be the right ear is the left, and vice versa.

PLATE I

EXPLANATION OF FIGURES

1 Right and left ears of a white man, Hyper-phylo-morph, or Hyper-meso, as seen from in front (center figures) and from the side (right and left figures). The same positions of the ears are given in each figure from 1 to 12. Note the skin lines on the helix converge over the folded auricular tubercle (Darwin). Compare these ears with the negro ears of figure 2.

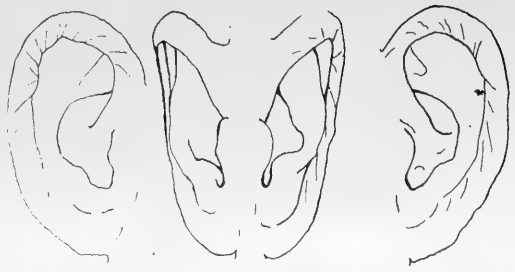
2 Ears of a negro man; the right ear is a true negro ear, the left is involuted. Note the distorted helix, with almost horizontal top, and the skin lines converging over the folded auricular tubercle. Compare these ears with the white ears above and below (figs. 1 and 3).

3 Ears of a white man, Hyper-onto-morph. Note the skin lines and compare the ears with the negro ears above and below (figs. 2 and 4).

4 Ears of a negro man, Hyper-meso. Note the skin lines, and compare the ears with the white ears above (fig. 3).

5 Ears of a white man; the hyper-phylo-morph type. Note the skin lines converging above the auricular tubercle; compare these ears with those of the negro man in figure 6.

6 Ears of a negro man of the Hypo-phylo-morph type. Note the skin lines, with absence of the auricular tubercle; compare these ears with the ears of the white men above and below (figs. 5 and 7).



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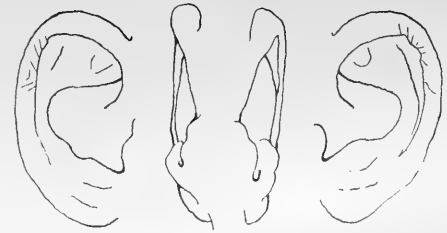

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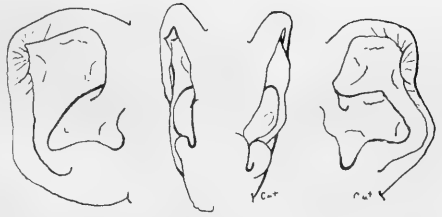

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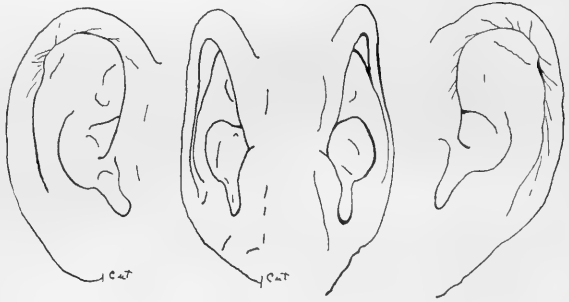

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PLATE 2

EXPLANATION OF FIGURES

7 Ears of a white man of the Meso-onto-morph type. Note the skin lines, with absence of the auricular tubercle; ears asymmetrical; skin lines converge high and low; skin tip is on the lateral border of the right ear. Compare these ears with those of the negroes above and below (figs. 6 and 8).

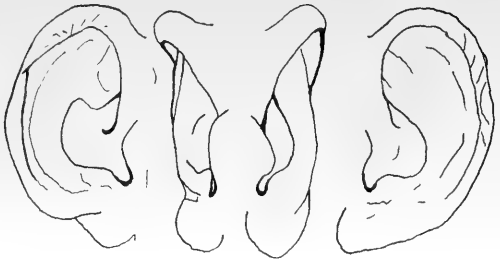
8 Ears of a negro man, involuted ear form; skin lines scant; auricular tubercle absent. Compare these ears with those of the white man above (fig. 7).

9 Ears of a negro man; involuted ear form. Note the skin lines over the auricular tubercle and the large irregular helix.

10 Ears of a negro man of the Hypo-phylo-morph type. On the left ear the satyr tubercle is very large; the skin lines are scant.

11 Ears of a negro woman of the Hypo-phylo-morph type. Note the horizontal 'top' of the ear, the broad helix and tubercle and the skin lines.

12 Ears of a negro woman; involuted ear form; the satyr tubercle is large on the left ear and on both ears the skin tip is on the dorsal margin of the helix.



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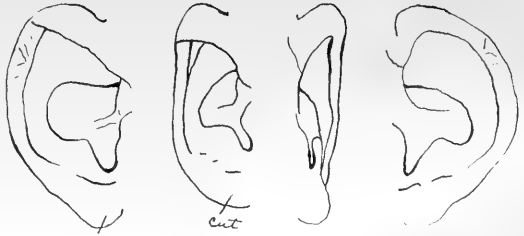

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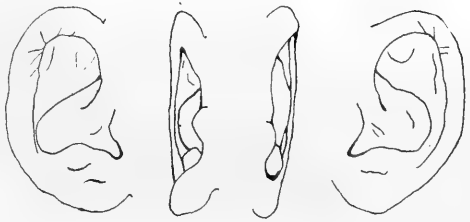

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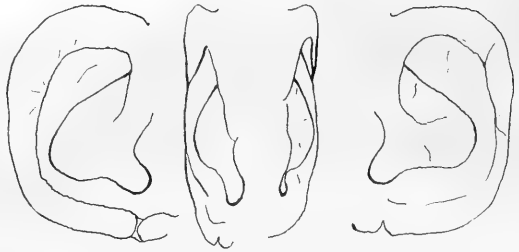

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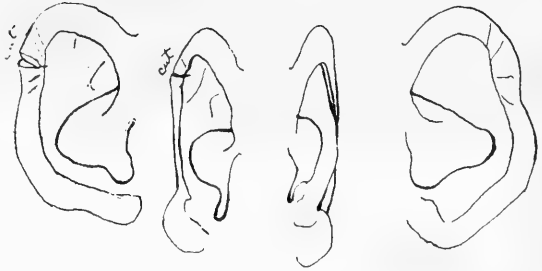

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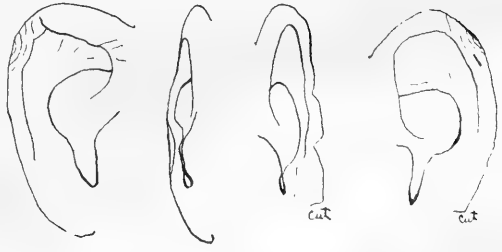

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PLATE 3

EXPLANATION OF FIGURES

13 Ears of a Filipino woman of the Hyper type; see also figure 16 for the front and back views of the same ear. Note the turning back of the helix, the prominence of the anthelix, tragus and antitragus, which are characteristics of the Hyper type. The skin lines are somewhat irregular.

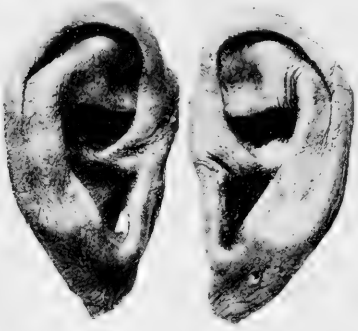
14 Ears of a Filipino man of the Meso type; see also figure 17 for the front and back views of the same ear. Note the large helix, semi-bowl shape, and sloping shelf lobule, which are characteristics of the Meso type. The skin lines are distinct on the left ear, but indistinct on the right, and converge about the auricular tubercle in each ear.

15 Ears of a Russian of the Hypo type; see also figure 18 for the front and back views of another Hypo ear (Filipino). Note the very large helix, much rolled in, forming a bowl-shaped ear, with shelf lobule, depressed anthelix, tragus and antitragus. These ears are both involuted; the skin lines are obscure.

16 Front and back views of the ear shown in figure 13.

17 Front and back views of the ear shown in figure 14.

18 Front and back views of another Hypo ear (Filipino); see figure 15.



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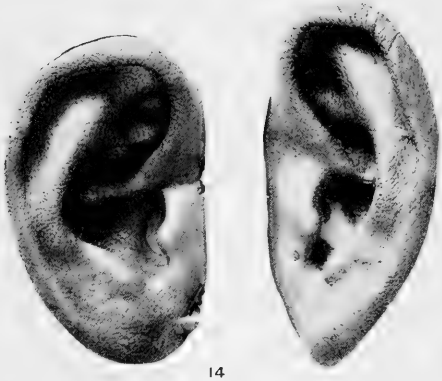

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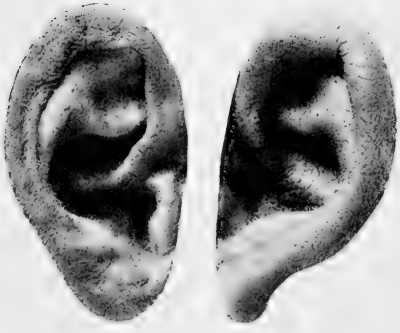

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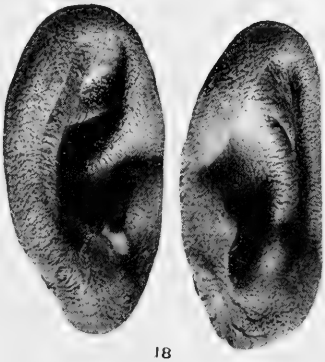

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THE ORIGIN OF BLOOD AND VASCULAR ENDOTHELIUM IN EMBRYOS WITHOUT A CIRCULATION OF THE BLOOD AND IN THE NORMAL EMBRYO

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FORTY-NINE FIGURES

CONTENTS

INTRODUCTION

The origin of blood presents an almost unique problem in embryology. First, on account of the fact that the initial blood anlage in many animals is contributed to by wandering cells. Second, owing to the establishment of an early flow or circulation of embryonic fluids before the blood corpuscles have arisen.

Soon after the cells and corpuscles are formed they are swept into this circulating current and carried to all parts of the body. In this way the blood cells become associated and mixed with numerous other types of cells, and it is difficult, if not impossible, to establish their true relationship with their surroundings. For the above reasons one is often ready to believe that many of the even careful and long thought out contributions to the development of blood are, after all, largely a matter of the author's own interpretation rather than a record of the actual processes.

The general current of opinion at the present time would seem to indicate that all blood cells arise from a mesenchymal type of cell. A number of very competent workers have described the change of this mesenchymal cell into a stem cell or mother cell. On one side from this mother cell are developed various leucocytes, which it is important to note always occur in an interstitial position, while on the other hand, this same general type of mother cell gives rise to other cells which later differentiate into typical erythroblasts, and finally erythrocytes which are always found to be located within the vessels. These so-called indifferent mesenchymal cells probably, from the evidence contained in the literature, do form blood cells, but to the discriminating reader the evidence is not at all convincing that both white blood cells and red blood cells really arise from one common mother cell or common embryonic anlage.

The possibility, and even probability, is certainly present that these so-called stem mother cells may in reality not all belong to one type, but are different and may already be destined to form either red cells or white cells. Yet on account of their wandering capacities as well as on account of the fact that the earliest blood cells are swept around in the circulating current

they have become so mixed and confused that it is almost impossible to separate the cell groups or differentiate between them. One must in this connection, remember the fact that almost all authors have concluded that only red blood cells are formed in the blood islands of the yolk-sac in most vertebrates. All authors who have studied the development of the blood in Teleosts have invariably described only red blood cells as arising in the intermediate cell mass. No one has ever mentioned the presence of white blood cells in the true blood forming anlage of the Teleosts.

The monophyletic school really goes still further and not only claims that all types of blood cells arise from a common mother mesenchymal cell, but also that the vascular endothelial cell is likewise capable of giving rise to the various types of blood cells and is originally a cell of the same type as the stem mother cell. There are numerous descriptions and illustrations of the origin of blood cells from the vessel linings in the literature of the past twenty-five years, since Schmidt in 1892 described the transformation of individual endothelial cells into white and red blood corpuscles. Yet again, I believe that the really skeptical reader will not be at all convinced that such a thing ever takes place from the evidence presented in the literature, certainly not from any of the illustrations that have been made of this process.

No real vascular endothelial cell has been actually observed to metamorphose into a blood cell or to divide off another cell which forms a blood cell, and until such a direct observation is forthcoming one can only question the accuracy of the interpretation of the various observations up to now recorded.

The mesenchyme is a very generalized embryonic tissue and from it arise the various kinds of blood cells, endothelial cells, connective tissue cells, etc. There can be no doubt of the great genetic difference between blood cells and connective tissue cells, yet their parent cells are with our present methods indistinguishable. We may with equal justification go further and hold likewise that the cells from which the vascular endothelium, red blood cells and white blood cells arise are mesenchymal

cells really differing in nature according to whether they will give rise to one or the other of the three cell types. Yet they may not differ from one another in any way by which we can at present distinguish them. If this proposition be true, or even if the weight of evidence lean in this direction, it is scarcely more justifiable to derive these completely different cells from a common mother cell than it would be to derive connective tissue and blood cells from a common mother cell.

Of course, we are only considering the mesenchymal cell just before its differentiation is to begin. Carried back further, no doubt, all the cells become more and more alike and possess more and more complex potentialities as is so thoroughly demonstrated by the numerous studies of cell lineage. In the beginning, of course, all cells arose from one single egg cell capable of giving rise to every tissue of the body, but after tendencies in differentiation have proceeded sufficiently far in the various cells some then form real mesenchymal cells. Later individual mesenchymal cells incline in certain directions and finally become incapable of giving rise to any other than the definite type of tissue or cells towards which their particular tendencies have directed just as certain endodermal cells become specialized to form the liver while others near by and at first indistinguishable from these give rise to the ducts and acini of the pancreas.

All of the vertebrate classes present these many questions of blood origin, etc., but the forms upon which this investigation has been conducted, the Teleosts, possess in addition many extremely interesting special problems. In all other meroblastic embryos the majority of the earliest blood cells arise in yolk-sac blood islands. Yet in many of the Teleosts there are apparently no early blood islands on the yolk, but all of the blood forming cells are contained within the embryonic body.

This intra-embryonal blood anlage has been frequently described by many authors as the "intermediate cell mass." The intermediate cell mass as has been suggested by Marcus ('05), Mollier ('06), and others, is really the homologue of the blood forming yolk-sac mesoderm in the other meroblastic types.

The bony fish is important as an object of study on account of the fact that so many of its organs and tissues arise in a way peculiar to the group and differing from the other vertebrate classes. The solid gastrular invagination described by Sumner ('00), the original solid condition of the central nervous system, the solid optic knob which changes into the optic vesicle, and in the present connection, the very particularly interesting solid cord of cells, the intermediate cell mass, which is to give rise to the red blood corpuscles of the individual make the Teleosts a group of great embryological interest.

The complexity of the problem concerning the origin of the various types of blood cells is then largely due to the migration and mixture of the cells involved. It is strange that up to now no investigator has attempted in an experimental way to analyze the situation. It would seem to be one of the most favorable problems for an experimental analysis, and in the end it is certainly an analytical problem.

If it were possible by any means to separate the anlage of the red blood cells from that of the white blood cells and prevent the flow of fluid in the embryonic body so that these cells would not frequently become intermixed, then it would seem possible to determine clearly the entire genesis of the various type cells. If all the types of blood corpuscles did arise from a common mother mesenchymal cell they should then be found in intimate association throughout all blood forming regions. Further, if the vascular endothelium really has blood forming power, it should be found that blood cells arise in any region of the embryo which possesses vessels lined by such endothelium.

There have been various experiments performed which have interfered more or less with the circulation of the body fluids of the embryo, but none of these experiments where aimed at a solution of the genesis of blood cells or have been used for such a purpose. Knower ('07) removed the heart anlage from early frog embryos and they continued to develop in some cases with almost no circulation. In other specimens there was a very feeble sluggish circulation due to the pulsation of the lymph

hearts or of remnants of the heart which remained after the operation. The embryos were not particularly adapted for the study of the blood questions since some circulation always took place, and this no doubt was sufficient to contaminate the original sources of blood cells and so confuse the situation. Loeb ('12) has reported experiments on bony fish hybrids and embryos treated with certain chemicals in which there was a heart beat but no circulation. These embryos were, however, not studied for either blood or vascular genesis.

The first demonstration of the fact that the embryo could develop without the circulation of the blood was given by Loeb in 1893. He showed that Fundulus eggs developing in solutions of KCl had no heart beat and no circulation of the blood, yet some vessels formed. In 1906 the writer repeated this experiment and confirmed Loeb's results entirely, but found that the vascular system and general development of the embryo was extremely abnormal and was hardly reliable for conclusive studies on the origin of special tissues.

With these experiments in mind, and appreciating the problems indicated above regarding the origin of blood as well as vascular endothelium, I have undertaken an extensive experimental analysis of this subject in conjunction with a careful systematic study of the histogenesis of the blood and vessels in normal embryos. The results of the experimental study which has been carefully followed during the past three years are presented in the following pages of this paper.

METHODS OF EXPERIMENT AND MATERIAL

Six years ago, while studying the influence of alcohol and various anaesthetics on the development of Fundalus embryos, I noticed that many of these embryos had a feeble heart beat, but no circulation of the blood. At that time, particular attention was given to a study of the defects of the central nervous system and of the organs of special sense and no attempt was made to investigate thoroughly the conditions present in other tissues and organs of the body.

During the last few years, special attention has been devoted to the study of these embryos without a circulation of their body fluids with the main object of analyzing as completely as possible, the origin and subsequent development of the heart, vessels and blood. All of the experiments have been repeated through three summers in the Marine Biological Laboratory at Woods Hole. It has been possible to produce embryos that were almost normal in all particulars yet in which the blood failed to circulate on account of the fact that the heart was blind at one or both ends or disconnected at the venous end or finally was a completely solid cord of tissue.

The embryos were studied in life and the particular individuals which had been so observed were fixed and finally studied in microscopic sections. The observations made on the experimental embryos have in all instances been completely checked and controlled by careful detailed study of the blood and vessels in normal embryos.

The experiments have been performed on two species of Fundulus, heteroclitus and majalis, and the results are practically identical for both. The eggs were stripped from the female into small dishes containing no water and fertilized by milt pressed from the male. About fifteen minutes after the application of the sperm water was added to the eggs. In this way one gets a very high percentage of fertilized eggs, while fertilizing the eggs under water or adding sperm to a dish of water gives a much poorer result. The fertilized eggs are then divided into groups so that the experiment and control are all from similar sources. The eggs just before dividing into the two-cell stage were introduced into solutions of alcohol.

The solutions which gave the most favorable results were prepared in the following way: 50 cc. of sea-water was placed in each dish and to this was added respectively, 1.5 cc., 2 cc. 2.3 cc., 2.6 cc., 2.8 cc., 3 cc., 3.5 cc., of 95 per cent commercial alcohol. The eggs remained in these solutions for twenty-four hours, after which time the solution was renewed. After forty-eight hours the eggs were removed from the alcohol solutions and placed in pure sea-water.

To give a general idea of the way in which the embryos developed in these different grade solutions of alcohol, I may cite some of the details of one experiment. After forty-eight hours many eggs are dead in all the solutions. The dead eggs are thrown out. When seventy-two hours old many others are dead in the solutions with 1.5 cc., 2 cc., 2.3 cc., and 2.6 cc., while all but one individual had died in 2.8 cc. It should be added here that about seventy-five eggs are placed in each dish. From the 1.5 cc. solution sixteen are alive at seventy-two hours, several with various eye defects, the hearts are beating but contain colorless fluid and the only trace of red blood color is in the intermediate cell mass and caudal vein. Several others of this lot have a full circulation with corpuscles in the current. From the 2 cc. solution one has a feeble heart beat but no blood is visible and there is no circulation; some embryos have no circulation but blood is present in the posterior end of the body, while many have blood circulating with a strong heart beat.

This brief reference to the notes of the experiment show that such doses are just on the border line of effectiveness since some individuals in the solutions are not able to develop a circulation, while others with a higher degree of resistence do develop a more or less normal circulation. It is very important in such experiments to use these threshold doses since they are the least injurious possible to give the desired result. In this way one gets individuals which have no circulation of the blood, and, therefore, in which the blood anlage develops and remains in its permanent position, without having any serious defects or abnormalities in the general body tissues of the embryo.

From a study of such specimens carefully controlled by a study of normal individuals, one is fully justified, I believe, in drawing final conclusions as to the significance of the developmental processes taking place. It cannot be argued so far as the blood anlage is concerned that the conditions recorded are pathological or other than those which would occur in a normal genesis of the blood except that it never circulates.

Embryos which are intended for microscopic study have been prepared in the following manner: The eggs are placed

in picro-acetic (saturated aqueous solution of picric acid and 5 per cent glacial acetic) from thirty to forty minutes, then put into 70 per cent alcohol. This is frequently changed in order to wash out the picric acid. After they have been about one-half hour in the 70 per cent alcohol, the egg membrane is removed with fine dissecting needles. This is the most favorable time for removing the membrane. If the eggs have been left for a long time in the alcohol, the membrane is more difficult to remove and the embryo is brittle and more liable to injury.

After removing the entire membrane, the yolk-sac is then punctured at its ventral pole and the yolk mass very slowly and cautiously removed from the sac. To remove the yolk mass, requires a great deal of practice and extreme care in every case. It should be done with the use of a binocular microscope so that the operator can be certain not to tear or destroy the yolk-sac or injure the delicate heart lying close above the yolk. After a great deal of practice it is possible to remove the yolk from a number of embryos and leave the yolk-sac in perfect condition with the heart and pericardium practically undisturbed. In the great majority of cases, however, it is generally impossible to completely remove the yolk. It is usually necessary to remove the yolk on account of the fact that when the eggs are imbedded in either paraffin or celloidin, the yolk becomes so hard that it often breaks the sections and makes it very difficult to get a complete or perfect series. When the yolk-sac is punctured one-half hour after having been in the 70 per cent alcohol, the yolk material is in a gummy or viscid condition and is more easily removed than at any other period tried.

After having removed the egg membrane and the yolk, the embryos are then allowed to stand twenty-four hours in 70 per cent alcohol when they are changed to 80 per cent to be kept until the time of imbedding. The embryos are imbedded in paraffin and cut in serial sections from five to ten micra thick. They are stained in hematoxylin and eosin and extracted or carefully differentiated so as to bring out a clear stain of the blood cells and tissues. A complete series of these embryos have been made from a time before the appearance of blood up to

sixteen days old, the normal embryo hatches and becomes free swimming at about twelve days.

As mentioned above, a similar series of normal embryos have been prepared and used for comparison with these non-circulating individuals.

In order to be certain of the final developmental product of the blood cells of the fish, numerous smears have been made from various tissues and from heart blood taken from the adult Fundulus. In these smears one finds the various types of white blood cells and the ordinary red blood corpuscles of Teleosts.

THE STUDY OF LIVING EMBRYOS WITH AND WITHOUT THE CIRCULATION OF THE BLOOD

1. Normal development up to the establishment of a circulation

The rate of development of Fundulus embryos is very variable, differing at different periods of the breeding season, and also differing in groups of eggs from different individuals.

When twenty-four hours old, the germ ring has descended almost to the equator in the most rapidly developing individuals. In others the ring is only one-third way over the yolk sphere. The embryonic shield and the first line indicating the position of the embryo's body is now to be made out. At forty-eight hours, the yolk sphere is completely covered by the ectoderm, the embryonic body is well shown, with the optic knobs projecting prominently and several somites easily distinguishable. The heart has not begun to pulsate and no blood cells or blood anlage are distinguishable in the living specimen. Very soon after this time, or at least by sixty-eight hours, there are about ten somites present and collections of cells on the yolk-sac are the first indication of blood islands. No pigment cells have formed up to sixty hours.

At seventy-one hours pigment cells are recognizable but the blood islands are not yet colored and are sparsely arranged over all the yolk region except the anterior half. Near the lateral borders of the embryo and on the posterior yolk surface the islands are most abundant. At this time there is still no visible

heart-beat or circulation to be seen with the high power microscope. At seventy-five hours the pigment particles are just beginning to show in the chromatophores. A well formed vesicle is clearly seen at the posterior end of the embryo from forty-eight to seventy-two hours and older. This is the so-called Kupffer's vesicle, and it, like the pericardium, becomes greatly distended by an accumulation of plasma in those individuals which have no circulation.

Embryos with fourteen somites may still have no heart beat and no circulation of the blood. Any later than this, however, all normal embryos establish a heart-beat and a circulation of colorless fluid, there being no blood cells present in the initial circulating medium. Very soon after the circulation is established at first a few but quickly many blood cells are added to the stream. This is merely an abbreviated summary of the development of the Fundulus embryo up to the time of the establishment of the heart-beat and circulation, but as stated above, the rate is variable and it often happens that an embryo of seventy-two hours has already established a vigorous circulation and the plasma is loaded with well formed blood cells.

2. History of experimental embryos to the time when a circulation should begin

In the experimental embryos development proceeds more slowly than in the normal. The plasma which should circulate in the vessels accumulates in the sinuses over the yolk and finally seems to collect in great amount in the pericardium, the lateral coelomic cavities and in Kupffer's vesicle, so that these spaces become hugely distended and appear as great sacs or vesicles of colorless watery fluid. The excessive presence of this fluid in the pericardium seems to exert a mechanical effect which tends to separate the head of the embryo an unusually great distance from the yolk mass and thus stretches the heart out into a long string-like cord, passing from the embryo to the surface of the yolk.

This pushing away of the head from the yolk is very well indicated in figures 15 to 20, which show various types of hearts found in these embryos during later stages. The stretching or

pulling out of the heart may possibly be the cause of the failure to develop its proper connections with the veins, or in some cases to establish and maintain its lumen. On account of these mechanical deficiencies in the heart, we find that it is incapable of propelling the body fluids and establishing the circulation of the blood. The fluids thus accumulate in the large sinuses or spaces, in most cases the coelomic spaces and in the case of Kupffer's vesicle in an endodermic or endo-mesodermic cavity.

The red blood cells become evident after the fluid accumulation has partially taken place. They are always seen to originate in definite localities and are never found in any place very distantly removed from these localized regions unless a partial circulation or accident of some kind has occurred. Although red blood cells in many places arise from wandering cells the blood cells themselves have little capcity to wander.

3. Early formation of blood cells in living embryos

a. Intra-embryonic blood cells: The chief place of blood cell formation is the intermediate cell mass which extends from about the level of the anterior portion of the kidney back posteriorly to behind the anus and well into the tail of the embryo. This is the principal blood mass, but in addition to this, there are present in all of these non-circulating individuals small blood islands over the posterior and ventral yolk regions. These blood islands are also present on the yolk of normal individuals but in these the islands are swept away when the circulation begins.

b. Yolk-sac blood islands. A number of observations were made on the embryos which failed to develop a circulation and also on normal embryos to determine the significance and relationships of the blood islands on the yolk as far as was possible in the living individuals.

In one experiment when the embryos were seventy-two hours old, or just about the time that the heart-beat was beginning in many, it was found that although the plasma was circulating blood islands were present on the posterior yolk region. Ten embryos were selected which showed these posterior yolk blood

islands and isolated to determine whether the blood islands would subsequently enter the circulation or what their fate would be. Ten other embryos with a feeble heart-beat but with no circulation yet also showing small posterior yolk-sac blood islands were isolated for comparison.

On the following day, nine out of ten individuals which had established a vigorous circulation had no blood islands remaining on the yolk. All of the islands had been taken into the circulation or vascularized, so that instead of blood islands there was now a network of vessels over that portion of the yolk and the blood cells had entered the current. One of the ten embryos exhibited an abnormal arrangement of the blood vessels that was particularly instructive. On one side there was a large vein running from the embryo out onto the yolk and on this side all of the blood islands had disappeared forming a network of vessels which conducted the blood to the venous end of the heart. On the other side of the specimen there seemed to be a suppression in the development of vessels near the embryo. The islands were still in the same condition they had been on the previous day except that the cells composing them had become much redder so that there was now no doubt whatever that they contained erythroblasts and early erythrocytes.

The ten embryos which had no circulation on the previous day were now found to present the following conditions: Two had established a perfectly free circulation and all of the blood islands had been swept away. In two other individuals circulations were established in an abnormal manner so that many blood islands still remained and presented a bright red appearance. Six of the ten specimens had no circulation whatever and the entire arrangement of blood islands over the yolk was in exactly the same condition as on the day previous, except that the blood cells were now much redder in color.

During the course of the experiments similar tests and observations have been repeated four times. In each instance isolated groups of embryos showing yolk-sac blood island were selected and examined in order to ascertain on the following day the fate of these islands. In every case my experience was identi-

cally similar to that just described. The islands on the yolk-sac are often very far distant from the embryonic body as is readily seen by reference to figures 21 to 24. There is no doubt that wandering cells migrate out on to the yolk surface at a very early period and here give rise to blood islands comparable to those formed in other meroblastic embryos. It must be recognized, as I shall bring out in a consideration of the microscopic study of these embryos, that the yolk-sac of the fish is not entirely comparable to that of all other vertebrate types, yet there are many observations on the early living embryos which have convinced me that mesenchymal cells do wander from the embryo to various parts of the yolk-sac. These cells occupy a position between the ectoderm and the periblast (periblastic endoderm?) just as the peripheral mesoderm would in other yolk-sacs. Some of the wandering cells are future pigment cells of either the red or black variety to be mentioned later, others future endothelial cells, but many at least are to give rise to future blood cells.

Therefore, in embryos at about the beginning of the circulation one finds two distinct blood regions: The major region and most evident is the intermediate cell mass of former investigators, and the second position in which the blood cells are seen is the yolk-sac blood islands. The earliest yolk-sac blood islands are very easily overlooked. The writer had examined these embryos in great numbers and studied them for sometimes before finally discovering the existence of the early islands. With a high power single objective binocular, however, after their location is known the observer is readily able to see the nuclei of these cells in the posterior region of the yolk-sac, and they may then be followed from time to time. After these observations there is finally no doubt that blood islands do form on the yolk-sac of the Teleost embryo but these islands are probably to be regarded as disconnected portions of the intermediate cell mass or blood anlage.

It is freely admitted that this yolk-sac island blood formation may not take place in all Teleosts. The early wandering cells that here give rise to blood islands may in reality be compared

to the growing out onto the yolk of masses of cells from the intermediate cell mass as described by Swaen and Brachet ('99, '01). These cells in the species here studied may wander earlier and more freely than in the trout.

At any rate, as shall be brought out later, the ventral mesoderm of the yolk-sac in other vertebrates and the intermediate cell mass of the Teleosts are very closely related homologous portions of the mesoderm, if not one and the same thing.

4. The five-day embryos

The conditions which the embryos have attained five days after fertilization are illustrated in figures 1 to 4. In figure 1 a normal individual of this age is shown. The heart is slightly twisted but still more or less tubular. The vascular network on the yolk-sac is well established. The pigment cells are numerous but not yet fully developed and have not assumed an alignment along the blood vessels or taken on the usual embryonic pattern. The heart, of course, is pulsating vigorously and the blood current is easily seen both within the embryo and on the yolk-sac.

The three other figures in this group show different conditions of arrested development in individuals without a blood circulation. In figure 2 the pericardium is seen to be hugely distended so that the head is pushed or raised away from the yolk surface and the heart is stretched out into a long narrow tube extending from the ventral surface of the head to the sheer anterior surface of the yolk. This heart pulsates feebly and can be seen to contain a small amount of fluid which is churned up and down by the pulsations. None of this fluid, however, is ever pumped away from the heart. The pigment is much less plentiful than in the normal embryo of the same age, and the individual chromatophores are smaller in size than those of the normal embryo and have not sent out processes of any great length. No blood vessels at all are seen within the yolk-sac but very small scarcely noticeable blood islands are present on the posterior yolk region though not indicated in the sketch.

Figure 3 illustrates a more or less similar condition seen from a dorsal view. A small portion of the heart slightly projects beyond the anterior end of the head. If this egg were placed in lateral view, as was the case in figure 2, then the heart would be seen to show a similar condition, since it also was stretched out into a long narrow tube.

Figure 4 represents a very defective embryo. Specimens similar to this occur in great numbers in the stronger alcohol solutions. The bodies are very short since the descent of the germ ring over the yolk sphere is slow and at times incomplete, and the tail end of the embryo is often bifid or split giving a condition of cauda-bifida. At the anterior end, the upper right side of the figure, is shown the distended pericardial vesicle, and at the posterior end another large distended vesicle is in most cases the Kupffer's vesicle, but in some instances this is possibly distended spaces in the yolk mass just below the Kupffer's vesicle. These two sacs or spaces at the opposite ends of the body seem to be the places in which the non-circulating plasma most often accumulates to a great degree, in fact the accumulation of plasma is the actual cause of the exagerated condition of the spaces. In the individual illustrated by figure 4 the chromatophores are extremely small, but have arranged themselves to some extend so that they are very abundantly accumulated around the periphery of the Kupffer's vesicle while others have collected in the region of the pericardium. In the lateral yolk regions there are scarcely any pigmented cells present. All of these chromatophores, however, are small and contracted with very few processes of any extent.

In Fundulus embryos there are readily seen two distinct types of chromatophores. The one is a large dense perfectly black body with short broad processes. While the second is of a reddish color at first small and without processes, but later sending out very long graceful radiations which grow at the expense of the central mass until finally the whole chromatophore assume a moss-like branched structure, figure 6 shows both types well expanded.

Loeb ('93) at one time pointed out that these chromatophores migrate to the blood vessel walls and thus map the circulation

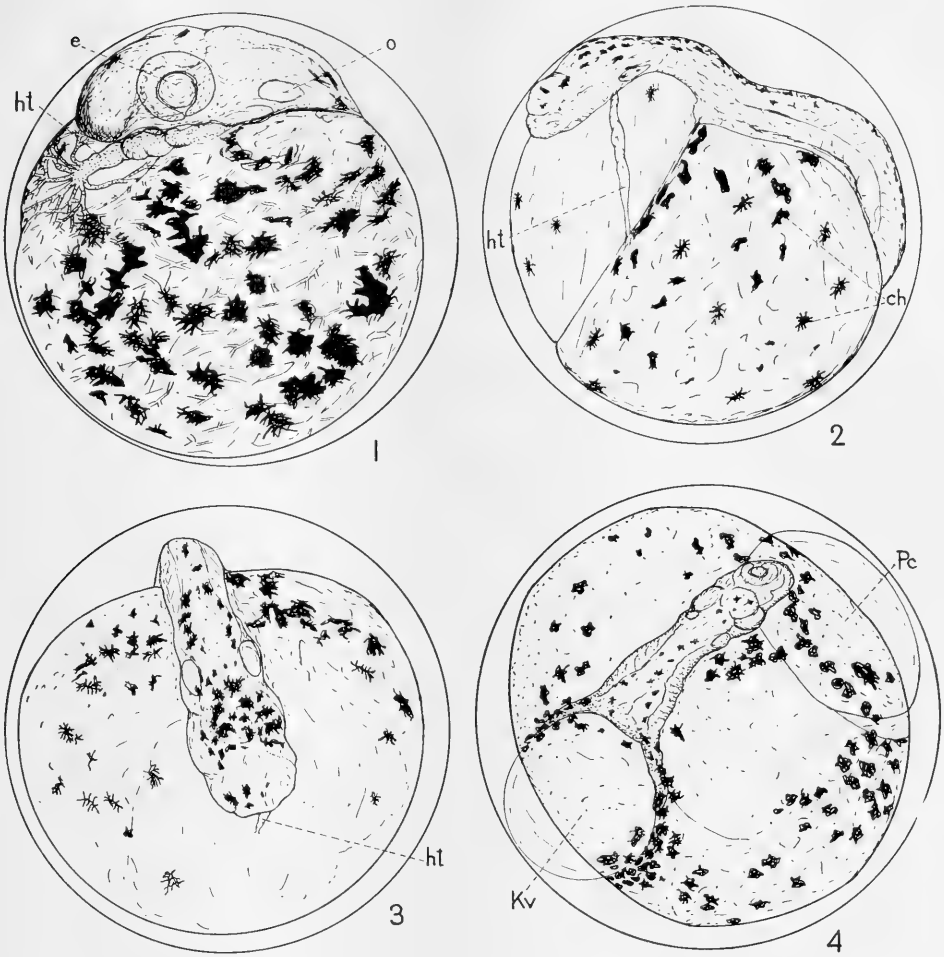


Fig. 1 The head end of a normal Fundulus heteroclitus embryo from life, five days old; *ht*, the heart, showing its S-shape; *e*, eye; *o*, ear.

Fig. 2 From a living embryo of the same age, developed in a weak solution of alcohol; the blood does not circulate and the body is small; *ht*, the heart stretched into a straight tube surrounded by a much dilated pericardium; *ch*, small and unexpanded chromatophores.

Fig. 3 The head end of a five-day embryo without a circulation, from a weak alcohol solution; *ht*, the heart slightly projecting from beneath the head.

Fig. 4 A five-day embryo from a stronger alcohol solution; the eye is cyclopean, the posterior end of the body split, cauda-bifida, and no blood circulation. Pigment cells are collecting about the sinuses; *Pc*, distended pericardium; *Kv*, Kupffer's vesicle, also hugely distended.

on the yolk-sac of a normal embryo. While in embryos treated with KCl in which there was no circulation, the pigment cells failed to asume any definite pattern. They remained more or less indefinitely scattered over the surface of the yolk and the body of the embryo in no way tending to align themselves along the vessel walls. From this fact, Loeb concluded that it was probably due to some chemotactic reaction that the pigment cells lined up along the blood vessels when the blood began to circulate and the attracting substance was possibly the oxygen contained within the blood corpuscles. Observing the various individuals without a circulation which we shall here consider, it will be seen that the pigment cells have a strong tendency to migrate to any cavity filled with plasma or fluid, and it is not probable that this plasma or fluid contains any more oxygen than is present in the other portions of the yolk-sac or body. It would, therefore, seem more likely that some constituent of the plasma itself and not the oxygen contained within the blood cells was the stimulating principle which caused the migration of the pigment cells to the vessel walls.

5. The eight- and ten-day embryos

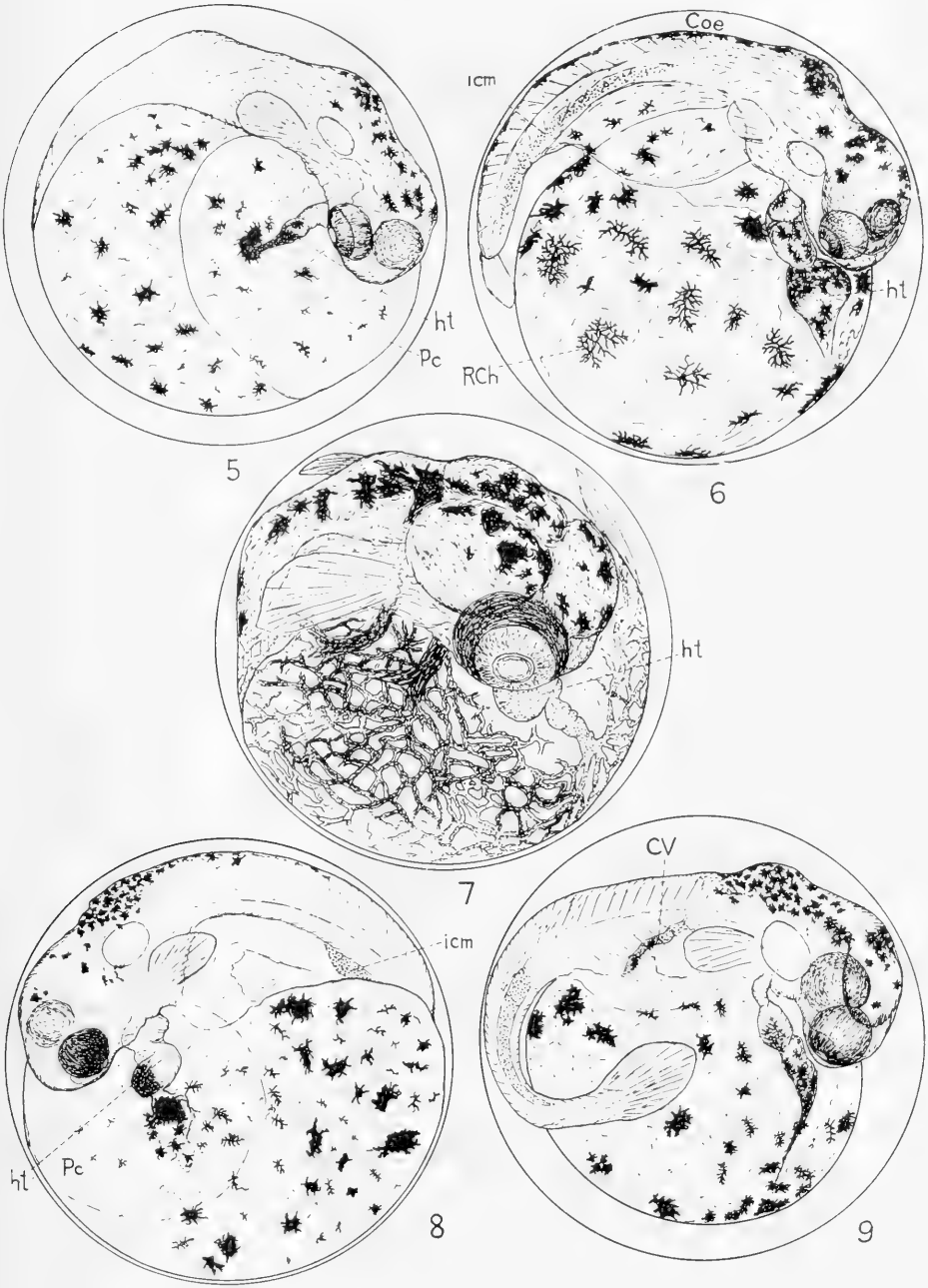
The next group of figures illustrates the advanced condition which the embryo has reached by the eighth day. Figure 7 represents a normal Fundulus embryo of this age. The body

Fig. 5 An embryo eight days old, without a circulation; the heart, *ht*, poorly developed, beats feebly twenty-eight times per minute, about one-quarter the usual rate; *Pc*, pericardium greatly distended with fluid; from a "1.5 cc. alcohol solution."

Fig. 6 Eight-day embryo without a circulation, *ht*; the heart dilated with plasma pulsates ninety-five times per minute; *RCh*, the red chromatophores beautifully expanded, but no vessels present on the yolk. *Coe*, the lateral coelomic cavity dilated with fluid; *icm*, the intermediate cell mass now a great string of red blood corpuscles.

Fig. 7 A normal eight-day embryo, the heart, *ht*; pulsating rapidly and the network of yolk vessels mapped out by the chromatophores.

Figs. 8 and 9 Two eight-day embryos without blood circulation; chromatophores unexpanded but collected on the heart, *ht;* the normal heart has no pigment cells on it; *Pc*, the dilated pericardium; *icm*, median mass of erythroblasts; *CV*, cardinal vein containing erythroblasts.


Coe
icm
ht
Pc
RCh
5
6
ht
7
CV
icm
ht
Pc
8
9

is seen to be well developed, the fins are already capable of movement, and the brain and spinal cord are well shown and covered with the black type of chromatophores. The heart is seen to be more twisted than in the younger embryos and now occupies a position further under the head of the specimen. The network of vessels on the yolk-sac is beautifully mapped out by the arrangement of the pigment cells, largely the red type of chromatophore. It is to be especially noticed that pigment cells are never present on the heart of the normal embryo.

The other four figures of this group show individuals in which there was no circulation of the blood, although the hearts pulsated in a more or less feeble manner. In figure 5 the greatly distended pericardium is again shown, the heart is stretched from the head to the anterior surface of the yolk, and the lower part of the heart is completely sheathed with pigment. All of the pigment cells, however, are small and unexpanded.

In figure 6, the heart is very greatly distended and filled with plasma, yet it is apparently closed at one end since the plasma is churned up and down and never pumped out of the heart. In this case, there were several cells or particles suspended in the plasma contained within the heart, and these particles could be watched for long periods of time constantly moving up and down but never going out of their confined position. The pigment cells in this individual are greatly expanded, the red type chromatophores showing beautiful mossy processes. The lateral body cavities, *Coe*, or the coelomic spaces formed between the layers of the lateral plates of the mesoderm are greatly distended with plasma. A condition particularly noticeable in many such individuals. Red blood corpuscles are distinctly seen throughout the entire extent of the intermediate cell mass as indicated in the figure by the stippling in the posterior region of the body and the tail. The heart of this specimen is also richly covered with pigment and thus presents a striking contrast to the normal heart in figure 7.

In figure 8 much the same condition is presented except that here again the pigment cells are still contracted. The pericardium, however, is distended and the heart is covered with pig-

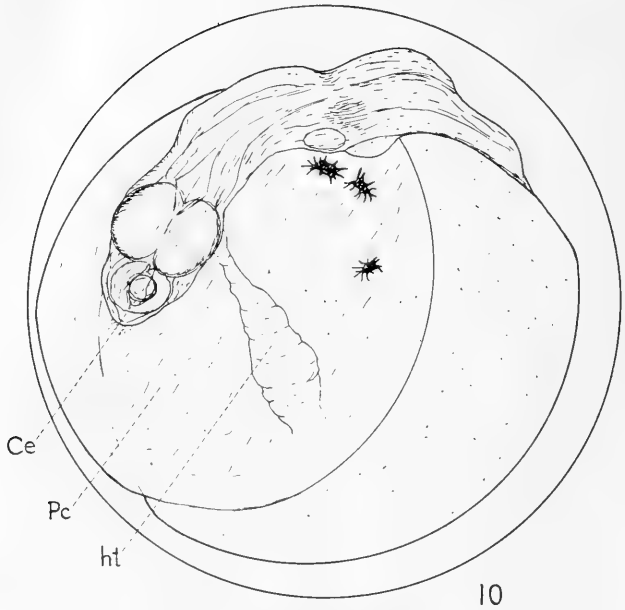
ment. The anterior end of the intermediate cell mass showing erythroblasts is just seen where the body of the embryo turns over the yolk sphere.

Figure 9 illustrates a lateral view of a small embryo. In all of these embryos in which the blood fails to circulate the fins are much smaller and less well developed than in the control, the entire body of the embryo is smaller and the whole appearance is that of a general developmental arrest, the rate of development being behind the normal. Yet such individuals have rather perfectly formed bodies, are capable of movement and seem in general to be very well developed, their only defect, so far as can be determined in many cases, is the absence of the circulation of the blood.

In figure 9 the heart is again sheathed with pigment cells, the blood cells in the intermediate cell mass are very distinctly present in the posterior body region, and in this individual a lateral vein in the position of the posterior cardinal also contains blood corpuscles. This appearance is seen in a number of individuals and may merely result from the fact that in these the intermediate cell mass is bilateral or split rather than entirely median in position. Such an explanation will seem probable, I think, after a consideration of the embryos in section.

Figures 12, 13 and 14 show three individuals of ten days old. These happen to be more or less abnormal. Figure 12 has very small eyes but the general body structure and shape are fairly normal. The pericardium is dilated, and the heart is small and pulsating feebly with a little pigment towards its aortic end. There is a great accumulation of pigment cells around the posterior region of the yolk sphere and near the distended Kupffer's vesicle in this embryo. Here again the cardinal veins are seen to be loaded with blood and only in the posterior body region do the two lateral masses come to unite into a median cell mass. Figure 13 shows much the same conditions, the heart is a mere filament indicated by the chromatophore along it.

Figure 14 gives a dorsal view of an embryo of ten days. The pigment spots are very few in number and the embryo has a pale



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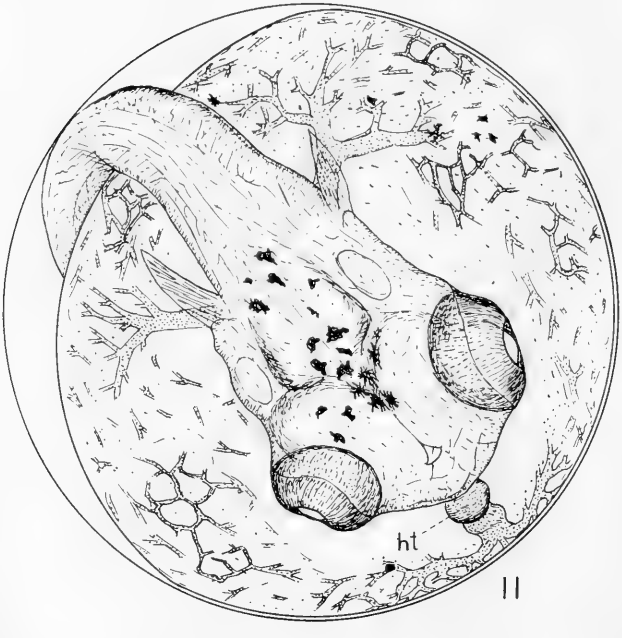

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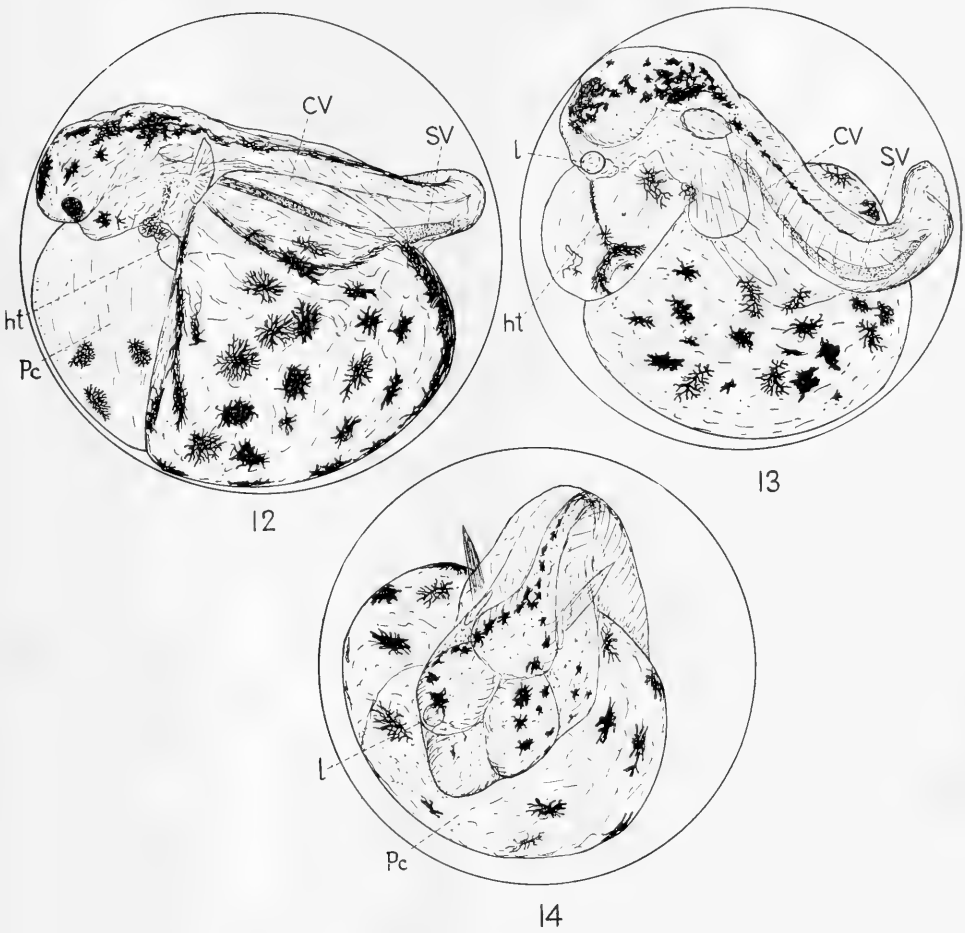


Fig. 10 An eight-day embryo of Fundulus majalis from 2 cc. alcohol solution, showing a condition similar to the heteroclitus embryos; *Ce*, cyclopean eye; *Pc*, distended pericardium; *ht*, straight heart and no circulation.

Fig. 11 A normal majalis embryo of eight days with S-shaped heart, *ht*, and yolk vessels forming a net. Same magnification as the smaller heteroclitus embryos.

Fig. 12 A ten-day heteroclitus embryo, no blood circulation, chromatophores expanded and accumulated on posterior yolk region; *ht*, heart; *Pc*, distended pericardium; *CV*, cardinal vein filled with erythroblasts; *SV*, stem vein also full of red blood corpuscles.

Fig. 13 Embryo ten days old; *ht*, the heart a mere string covered with pigment; *l*, an independent crystalline lens; other lettering as in figure 12.

Fig. 14 View of head of ten-day embryo, no circulation, distended pericarddium, *PC; l*, free lens.

appearance when compared with a normal individual, such as the one of eight days shown in figure 7.

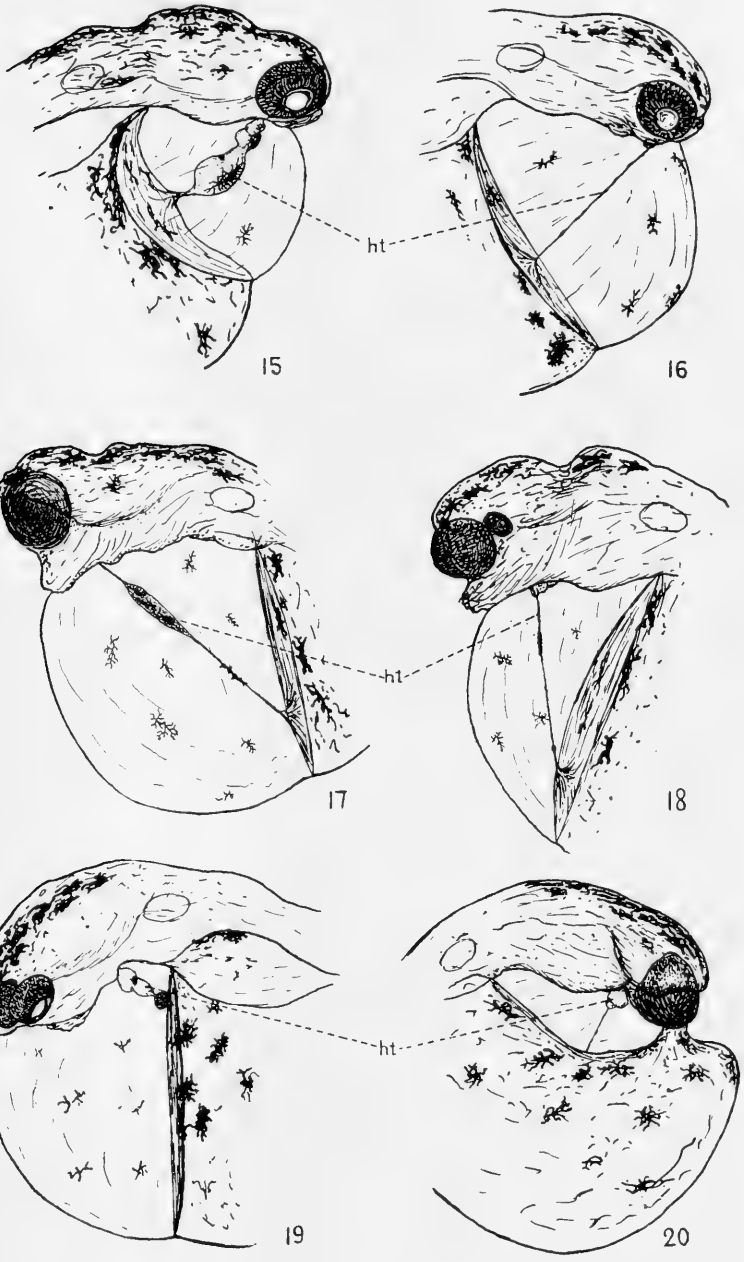
Figures 10 and 11 illustrates two specimens of Fundulus majalis drawn to the same scale as the previous figures. The egg of this species is considerably larger than that of heteroclitus, but its response to the experimental treatment is the same.

Figure 11 represents a normal embryo eight days old. It is seen not to be comparatively so far advanced at this period as is the heteroclitus, since its development is much slower and it requires from five to ten days longer to hatch. Very little pigment is present, yet the vessel net is well formed on the yolk-sac and the heart is distinctly seen to be more or less S-shaped.

Figure 10 is an embryo of the same age that had been subjected for forty-eight hours to a solution of 2 cc. of 95 per cent alcohol in 50 cc. of sea-water. Scarcely any pigment is present, the pericardium is typically distended and the heart is stretched into a long conical shape. No vessels are seen upon the yolk. The posterior end of the embryo is not shown in the figure, but in it could be seen early blood cells in the intermediate cell mass while a few small blood islands were present on the yolk-sac near the posterior end of the embryo.

After these eight- or ten-day stages very few changes of interest take place. The normal embryos hatch at from eleven to fourteen days as a rule, and become free swimming. The individuals without a circulation of the blood never succeed in breaking out of the egg membrane but may remain alive for twenty-five or thirty days in some cases, and almost all of them will live at least sixteen to twenty days. The red blood corpuscles are very distinctly noticeable in these older individuals and can be seen to remain permanently in their original places of origin. The intermediate cell mass may cease to be distinguishable, as was evident in the old specimens. This, however, is not due

Figs. 15 to 20 Living embryos sixteen days old, without blood circulation, showing variations in the pericardial distension, the position of the heads, and the perculiar heart conditions. These hearts, *ht*, all pulsate feebly and in several figures the slight lifting of the anterior yolk membrane is shown; this small membranous cone is rythmically raised with the pulsations.


ht
15
16
ht
17
18
ht
19
20

to the blood cells having wandered away from the mass since they have largely degenerated *in situ* probably on account of lack of aeration. The blood islands on the yolk-sac maintain their red color for a much longer period of time, and they continue to present a pattern closely identical with that seen in the same individual during its earlier stages.

6. Condition of the heart in old embryos without a circulation

The conditions of the heart in some of these old embryos is well shown in the series, figures 15 to 20. These figures are from embryos of sixteen days old. The control specimens at this time would as a rule have hatched. In the sketches the peculiarly distended pericardium is strikingly shown. This great distention of the pericardium seems to have exerted pressure in such a way as to have straightened the anterior end of the embryo and lifted it well away from the yolk surface. The mechanical pull caused by the separation of the head from the yolk would seem to be largely responsible for the fact that the heart becomes stretched into a very much attenuated tube or string.

In the upper left hand figure 15, the heart is not so greatly stretched and the pericardium in this case is not distended so much as in the others. The upper right hand figure 16, and the two central figures, 17 and 18, show the pericardium distended to its utmost, and in these specimens the heart is pulled out into a mere string. Pigment cells seem invariably to wander along these string-like hearts, and they, therefore, stand out in the embryos as a black cord just as is indicated in the figures. The venous end of the heart which is connected with the yolk-sac is seen at each pulsation to lift slightly the yolk membrane in a cone-like projection from the surface of the yolk. As these hearts pulsate in their feeble fashion, one thus observes the yolk membrane as it is pulled up and down.

The two lower figures, 19 and 20, show other somewhat different conditions of the heart. The hearts are small and do not reach so far towards the ventral portion of the yolk.

There is an almost limitless variety of peculiarly abnormal hearts in these embryos and the six figures convey but a slight idea of the many very strange conditions which are presented.

7. Development of the yolk-sac blood islands in life

The blood islands in the living embryos, as was mentioned before, are quite difficult to see in the early stages. But a few hours before the heart begins to pulsate and the circulation be-becomes established they are very evident in the posterior ventral yolk regions. The arrangement of the blood islands display various patterns in different individuals in some being inconspicuous while in others an extensive network is present. These blood islands probably arise largely from wandering mesenchymal cells since the yolk-sac of the Fundulus embryo consists at first only of the yolk periblast with the ectoderm immediately above it. There is no true mesodermal layer to the yolk-sac and this mesenchymal blood formation on the yolk can, in all cases, be traced to the early wandering cells.

In figure 21 the posterior end of an embryo of ninety-six hours is shown. The Kupffer's vesicle, *Kv*, is dilated and pigment cells have accumulated around it. Immediately posterior to this are a number of blood islands indicated by stippling.

Figure 22 shows an embryo eight days old with the posterior ventral surface of the yolk well covered with blood cells. Erythrocytes are also seen in the intermediate cell mass. The blood in this embryo has never circulated and one can scarcely conceive that the blood islands on the extreme ventral surface of the yolk are due to the crowding out or pushing away of cells from the intermediate cell mass within the embryo. These cells are rather to be regarded as true yolk-sac blood islands which have arisen from early wandering mesenchymal cells probably in the beginning derived from same source as the intermediate cell mass.

Figures 23 and 24 show the caudal ends of two normal embryos of seventy-two hours. In these the heart has not begun to pulsate nor the blood to circulate, yet a distinct group of erythroblasts or early blood cells are seen already arranged on the yolk-sac in this posterior region.

In figure 24, it would look as though these cells had wandered out from and grouped themselves around the tail end of the embryo. At this period, seventy-two hours, the intermediate cell mass within the embryo is not visible in life.

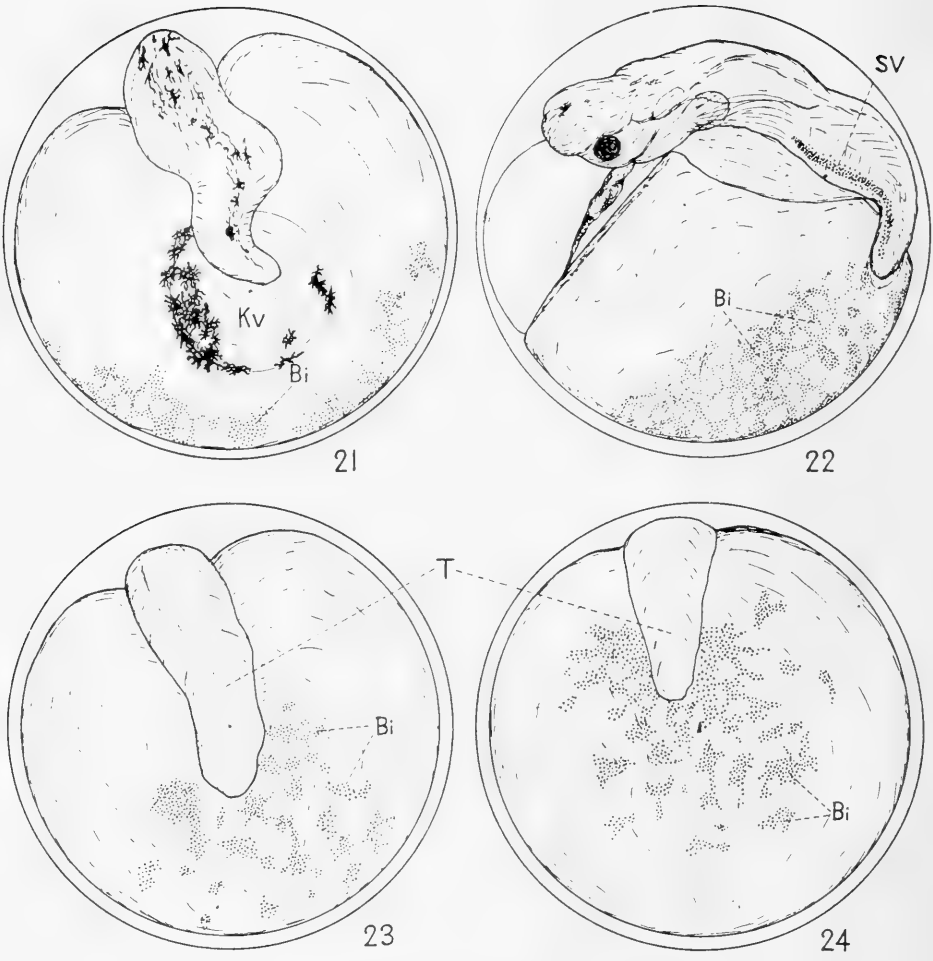


Fig. 21 Caudal end of an embryo of ninety-six hours, without a circulation; *Kv*, the distended Kupffer's vesicle with pigment cells collected around it. *Bi*, yolk-sac blood islands on the posterior yolk region.

Fig. 22 Lateral view of eight-day embryo, without a circulation, showing red blood corpuscles in the stem vein, *SV*, and also masses of blood islands, *Bi*, on the posterior ventral yolk regions.

Figs. 23 and 24 Posterior veiws of two seventy-two hour embryos, without blood circulation. Cells are seen wandering out from the tail, *T*, region into the position of the peripheral mesoderm in most meroblastic eggs; these cells collect into groups and form the blood islands, *Bi*.

It may then be concluded from a study of the living embryos with a circulation and others without a circulation, that in the normal ordinary individuals as well as in those having their blood flow prevented, the origin and formation of blood in the bony fish occurs as follows: The chief source of origin of the erythroblasts is that so fully described by previous investigators as the intermediate cell mass, la masse intermédiare. This mass, according to Felix ('97), Swaen and Brachet ('99, '01) and others, arises from the median portions of the two lateral mesodermal plates, primary seiten-platten. These bi-lateral masses migrate towards the middle line and there fuse to form the intermediate cell mass or blood string. In the living embryo this very important mass of blood cells is readily demonstrated. It is usually median in position, but in many cases, as illustrated above, it may be double or bi-lateral, at least in its anterior portion. This bilateral arrangement may possibly be the result of a failure of the blood forming portions of the two lateral plates to move to the middle line and fuse to form a Stammvene, in other words, a type of arrest.

The second seat of differentiation of red blood cells which is distinctly shown in living embryos is to be found on the yolk-sac in the posterior and ventral region where numerous typical blood islands form and develop. All recent investigators of the development of the blood in Teleosts have denied the development of blood on the yolk-sac. Most of their investigations have been on the eggs of the trout, and it may be that in this group there are no blood islands. But in Fundulus we seem to have a transitional condition in which the yolk-sac islands have not been firmly incorporated within the intermediate cell mass but still remain out or wander out upon the yolk. At any rate, we must conclude that there is a secondary seat of red blood formation in Fundulus embryos, and that in life it presents the typical appearance of yolk-sac blood islands.

From a study of the living embryos, it is apparently impossible to determine whether all cells of these blood islands are only erythroblasts or of mixed types. This is, however, readily ascertained by a careful study of sections.

THE ORIGIN AND HISTOGENESIS OF VASCULAR ENDOTHELIUM AND BLOOD CORPUSCLES AS DETERMINED BY STUDY OF MICROSCOPIC SECTIONS

1. The structure of the heart in embryos without a circulation

The hearts of the embryos in which there is no blood flow have been described in the living in the preceding consideration, but when they are studied in section an additional number of very instructive points are brought out.

In the first place, the heart wall is usually very thin and not well developed. This is particularly true, in the long string-like hearts that are present in those individuals in which the pericardium is so greatly distended. In the group of figures 25 to 28, one sees sections of these hearts taken through various regions.

Figures 27 and 28 show sections through a long narrow heart. In figure 28 the myocardium is seen to be practically one layer of cells, and within this the endothelial lining is distinctly formed. No noticeable structural difference beyond slight variations in shape can be determined between the nuclei of the mycocardium and those in the endocardium. The myocardial layer is a thick more or less structureless cell mass while the endothelium is well differentiated into a thin single cell layer lining. This condition is found in a non-circulating embryo of four days old. Tracing the series towards the conus end of the heart, we find the arrangement indicated in figure 27. The myocardium is here also a thick layer of cells enclosing a distinct endothelial tube.

Fig. 25 Section through the heart of a four-day embryo without a circulation; Experiment 11, 1912, Embryo 6. Heart wall poorly formed; large chromatophore, *Ch*, in wall; *ph*., pharynx.

Fig. 26 Section of a similar heart; Experiment 11, 1912, Embryo 2. The guide outline gives the general relationships of the heart. *MC*, myocardium; *EC*, endocardium; *Br*, brain; *pb*, periblast nuclei, and, *pbs*, periblastic material filling the heart cavity, *c*; red staining cell.

Figs. 27 and 28 Through the aortic end and figure 28 through the tube-like body of a similar heart; Experiment 11, 1912, Embryo 7. *Br*, brain; *ph*, pharynx; *EC*, definitely formed endocardium, endothelium; *MC*, myocardium. The nuclei of the endocardium and myocardium are indistinguishable except for slight differences in shape.

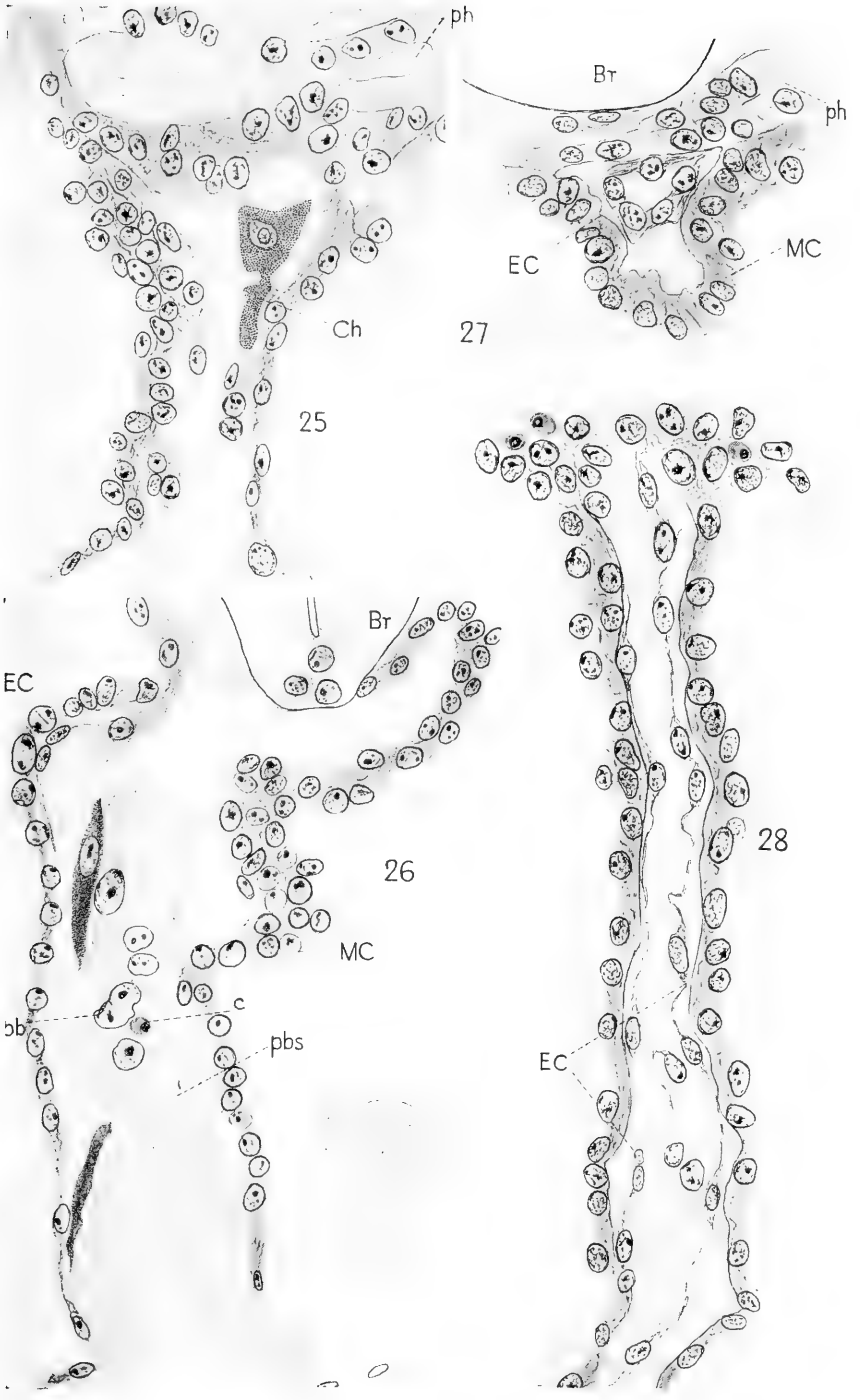

ph
Ch
25
Br
ph
EC
MC
27
EC
Br
26
MC
c
bb
pbs
28
EC

Figure 25 is a section through another heart of the same age. In this a huge pigment cell is shown within the heart cavity. It is recalled that pigment cells were frequently seen to lie along these abnormal hearts while chromatophores were never present on the normal heart. The endothelium in figure 25 is more or less broken and the general condition of the heart is poorly developed.

In figure 26, a section is illustrated through a heart as it leads into the aortic arches. Here also large pigment cells are present. The endothelium is indicated in several places and within the cavity of this heart is a mass of periblastic material. It would look as though the periblast had been sucked from the surface of the yolk into the heart cavity. Several large periblast nuclei, *pb*, are indicated and are easily recognized on account of their amorphous shape and huge size.

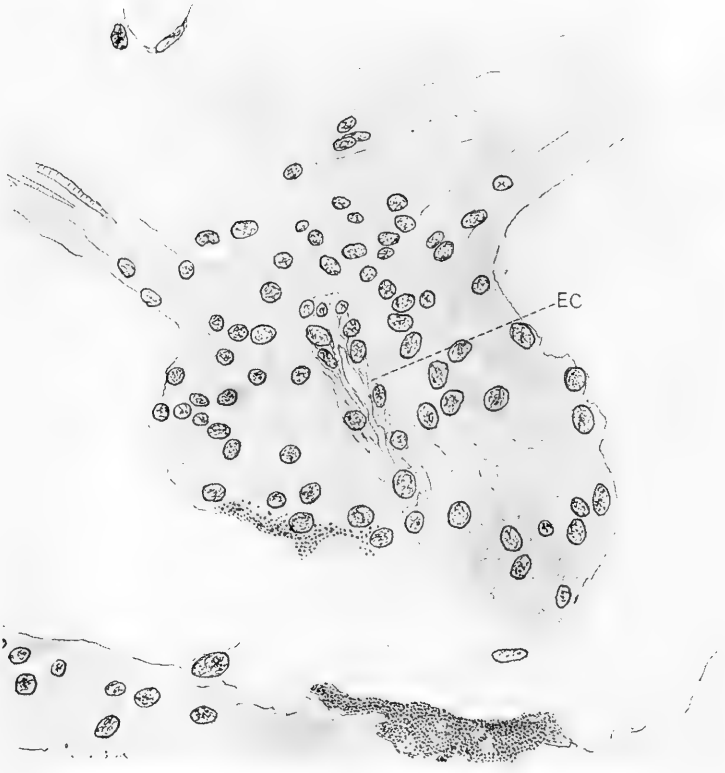
In figures 26 and 28 there are several cells, *c*, of a questionably degenerate type, the cytoplasm of which stains an extremely red color while the nucleus is small and pycnotic in appearance. These cells might in cases be looked upon as some type of wandering cell, but in most instances they are very degenerate in appearance.

It must be distinctly noticed that in none of the figures are erythroblasts shown. Throughout these heart regions at all stages the observer is impressed by the entire absence of any form of red blood cells in embryos that have absolutely had no circulation. One must constantly guard against the possibility of a slight circulation having existed for a short time and then having ceased. Another reason for blood movement may be the twisting or twitching reactions of the embryonic body. Conclusions regarding the permanent position of blood must

Figs. 29 and 30 Two sections through different parts of the heart in an embryo sixteen days old, without a blood circulation; Embryo 314.

Fig. 29 The aortic end of the heart, an almost solid mass with endocardial, EC, cells near the center.

Fig. 30 Through the string-like portion of the same heart; *pb*, periblastic nuclei and material completely fill the heart cavity; *EC*, endocardial cells; *Ch*, chromatophores surrounding the heart wall. The upper part of the section is cut slightly oblique.



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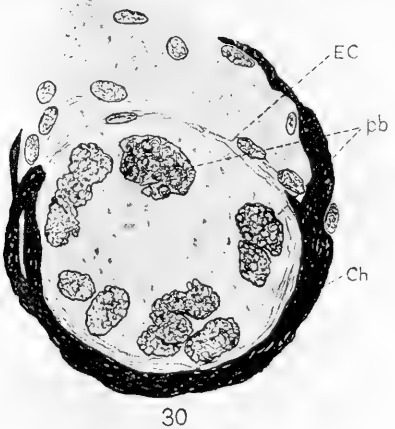

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be based only on embryos that have been carefully observed throughout their existence.

Figures 29 and 30 show sections through different parts of a solid heart string from an embryo of sixteen days old. In figure 29, the aortic end of the heart is shown to be almost a solid mass, and only near the center of the figure is a slight endothelial-like cavity or formation.

Figure 30 is a cross-section through the long string-like portion of this heart. It is seen to be completely solid, the central portion or core consisting of periblastic material containing large amorphous periblast nuclei, *pb*. Chromatophores have almost ensheathed the structure and present in the figure a dense black border. In one part of the section, however, a distinct endothelial-like formation is shown surrounding the periblastic core, and this heart again would seem to have sucked itself full of periblastic material from the surface of the yolk. As stated, this heart was from an embryo sixteen days old in which the blood had never circulated, and it is quite evident that at the time the embryo was fixed, it would have been impossible to have had a circulation of blood through such a solid heart. In this specimen, however, numerous blood islands on the yolk-sac and well formed blood cells in the intermediate cell mass were to be seen.

The endothelial lining of these hearts has certainly arisen *in loco*, and has emphatically not grown into the heart from the yolk-sac vessels since the heart is not connected with such vessels, and further, no typical vessels are present on the anterior portion of the yolk-sac. In all cases, the intra-embryonal vessels are much better developed than the vessels of the yolk-sac. A general survey of these embryos would quickly convince one that the vessels within the embryo are in no case derived from ingrowths. This fact is peculiarly emphasized in a study of bony fish embryos, and is so convincing that it led Sobotta ('02) to develop a theory of vascular outgrowth from intra-embryonic vessels in contrast to the older parablast notion of His ('75), but I must agree with Mollier ('06) in his view that both theories are equally untenable.

The hearts in these experimental embryos as a rule lead directly into a more or less well formed aorta which, in all cases shows an endothelial lining. The arches arising from this ventral aorta are very variable in the different embryos, yet in some cases are formed in an almost normal fashion. These arches also show a beautifully formed endothelial lining, but here again one is impressed by the absolute absence of erythroblasts in any stage of development within the neighborhood of the heart or aortic endothelia.

2. The "intermediate cell mass;" its origin, position and significance as an intra-embryonic blood anlage

On tracing the sections posteriorly one finds the intermediate cell mass to begin caudad of the pectoral fins and in the region of the anterior portion of the kidney duct. In studying a progressive series of very young stages forty-eight, sixty-six and seventy-two hours, the intermediate cell mass may readily be demonstrated to arise from the lateral mesodermic plates in the manner so clearly described by Swaen and Brachet ('01, '04). Felix ('97) previously pointed out that the primary lateral mesodermic plates extend away from the somite and later become divided into the following three parts. The median cells lying close to the somites separate away to form a continuous longitudinal string, the string from each side forming one lateral half of the future intermediate cell mass. The intermediate cells of the primary lateral plates just lateral to the above median group give rise to a second cord of cells which later forms the primary nephric duct. The remaining lateral layer of cells now constitutes the secondary lateral plates which split to form two lamellae.

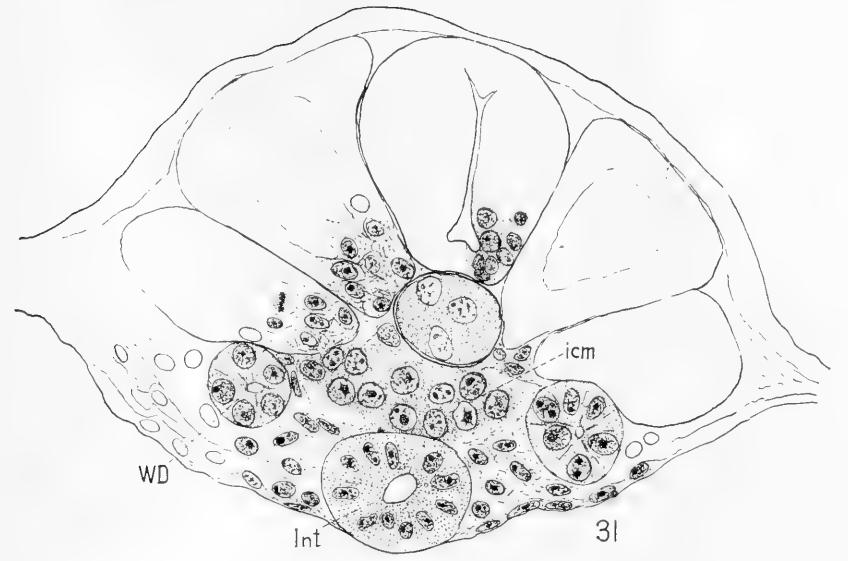
The primary lateral plate mesoderm thus gives rise to the intermediate cell mass, the primary nephric ducts and the somatic and splanchnic mesodermic layers of the lateral body wall. Between these two lateral mesodermic layers arises the portion of the coelomic cavity which we have seen in the living embryos without a circulation of the blood to be greatly distended with fluid.

The later development of the intermediate cell mass is found to proceed in almost exactly the manner described by Swaen and Brachet ('01, '04). This mesenchymal mass of cells is at first of an indefinite type lying between the notochord above and the intestine below and being flanked on either side by the primary nephric ducts. The first notable differentiation of the intermediate mass in the normal embryo begins shortly previous to the establishment of a heart beat. In an experimental embryo of seventy-two hours old, that is one in which the heart was just about ready to begin beating, figures 31 and 32 show the condition of the intermediate cell mass in cross section.

In figure 31, which is the extreme anterior end of the mass and, therefore, less well differentiated than the more posterior regions, the cells are seen to possess large round nuclei differing but slightly from the nuclei of the surrounding cells and those of the epithelium of the Wolffin ducts. The mass of cells is completely unsurrounded by endothelium, and I agree entirely with Swaen and Brachet that the central cells go to form the red blood corpuscles while the cells about the periphery of this mass form the vascular endothelium.

Figure 32 illustrates a section through a more posterior region of the same embryo, the intermediate mass is seen to be much further differentiated. The cells are here typical early erythroblasts and many are observed to be in active mitosis. The cells in the mass are becoming dissociated so that they are no longer so densely packed as in the section through the anterior region. This section is posterior to the ends of the Wolffian ducts, as well as the closed intestine and beneath the cell mass is shown the periblast over the yolk.

On tracing the series still further caudad, we find the indefinite cell mass end Knospe described by Marcus ('05), figure 33. This is a ventral cellular mass into which leads the notochord, intermediate cell mass and end of the endoderm. In other words, this mass would seem to represent the end bud at the dorsal blastopore lip, as if it were the point from which differentiation had taken place or from which the layers had grown forward.



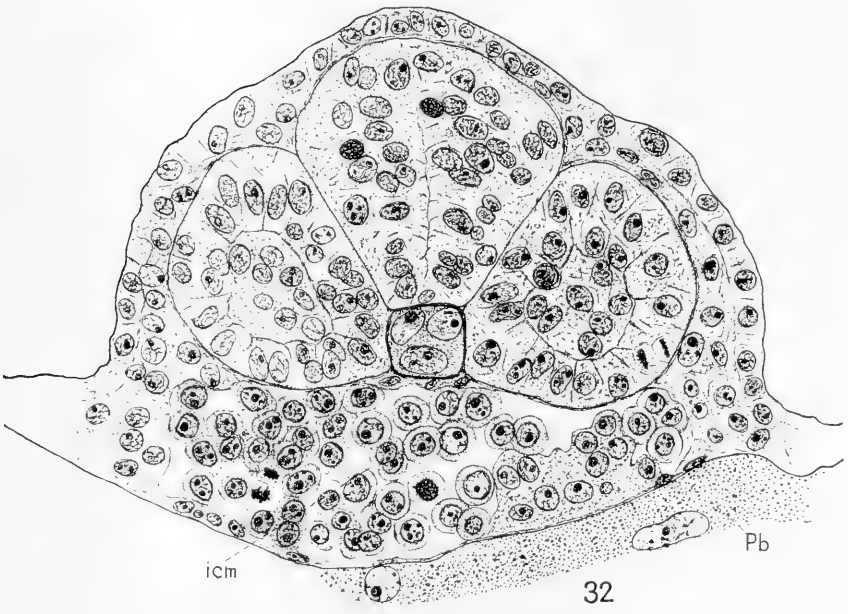


Fig. 31 Section through the trunk region of a seventy-two hour embryo without a circulation; Experiment A, 1913. The extreme anterior end of the intermediate cell mass, *icm*, is represented by deeper staining cells between the notocord and intestine, *Int;* the primary kidney ducts, *WD*, are lateral to the mass.

Fig. 32 Represents a more posterior section through the same embryo; in this region the intermediate cell mass, *icm*, is more extensive in cross-section and its cells are further differentiated than those in the more anterior region, *Pb*, periblastic material and large nuclei between the embryo and the yolk.

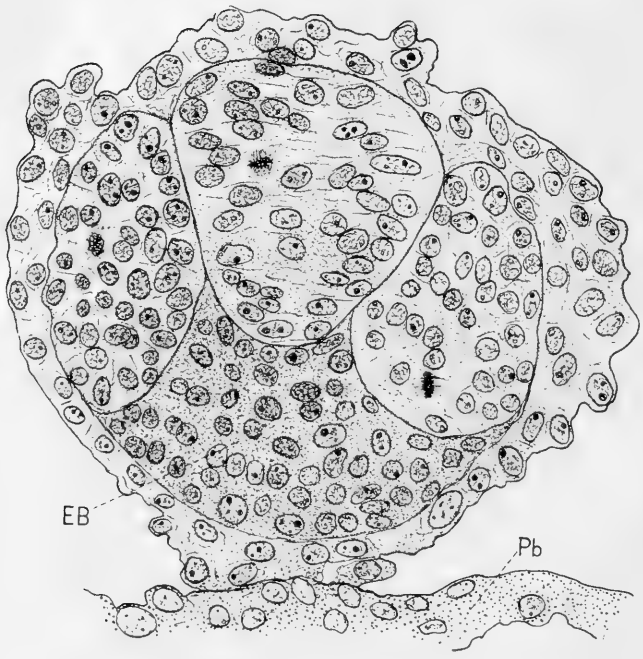


Fig. 33 A still more posterior section through the same seventy-two hour embryo as figures 31 and 32. This section is posterior to the place where the intermediate cell mass and gut endoderm fades out into the indifferent cell mass, *EB*, which may be considered to represent the end-bud mass; *Pb*, the yolk periblast.

The condition in the seventy-two hour embryo is, of course, quite early and the cells are not yet as a rule taken into the circulation. The appearance shown in these figures is exactly that of very slightly younger normal embryos in which a circulation would later be established. The figures, however, were made from sections of an embryo that had no heart beat at the time of its fixation, and, therefore, there is no chance that any of these cells could have become misplaced by having been circulated or carried about.

The most careful study with the highest power of the microscope has failed to reveal any type of cell in the intermediate cell mass other than the early erythroblast, and in later condi-

tions one finds here only red blood corpuscles. In other words, this chief stem blood anlage of the bony fish seems to be a specific red blood cell forming mass. This mass was first discovered by Oellacher in 1873 and has been shown by numerous investigators, Zeigler ('87), Winckebach ('86), Henneguy ('88), Sobotta ('94), Felix ('97), Swaen and Brachet ('99–'01) and others, to be peculiar to the Teleosts.

It is important to know that none of these investigators have yet recorded any type of blood cell arising from this mass other than the erythroblast. Of course, it may be argued that no special study was made of this particular point. Yet it is certainly true that if lymphocytes or leucocytes had been present to any extent, they should have been observed by many of these very capable and careful workers. It has recently been claimed by Maximow ('09), Dantschakoff ('07, '08) and others, that the popular opinion that leucocytes are very late in arising is erroneous since they actually arise just as early as the erythroblast. Then it seems all the more probable that if lymphocytes or leucocytes had been present in this intermediate cell mass such cells would have been discovered, since the mass has been carefully investigated right up to the moment at which it becomes swept into the circulating plasma.

Various investigators have differed as to the vascular products derived from the intermediate cell mass. Some have claimed that it forms only blood cells and no vascular endothelium, Sobotta ('02), while others have attributed the production of cardinal veins or venous endothelium as well as the blood cells to this mass, Felix ('97), and finally others, Swaen and Brachet ('01, '04) in particular, have considered this to be the source of both the aorta and cardinal veins as well as the red blood cells. I have spent considerable time in a study of this question and am inclined to believe that the endothelium of the cardinal veins and aorta arises from the mesenchymal cells surrounding the intermediate cell mass, which are different in nature from the cells actually constituting the mass. Yet it must be admitted that up to the present moment a complete demonstration of the origin of aortic endothelium from the cells about the periphery

of the intermediate cell mass has not been satisfactorily shown. This question will be more fully considered in another section.

In the embryos in which the red blood cells remain confined within the median region throughout life these cells develop in a normal manner and become completely differentiated into typical ichthyoid erythrocytes and exist as such for some time. Finally, however, for reasons at present impossible to state but likely associated in some way with an insufficient supply of oxygen, these erythrocytes begin to degenerate and in old embryos of sixteen to twenty days only a very few or in some cases none are left in the large intermediate vessel. Mesenchymal cells seem to wander into the mass of erythrocytes and may take part in their destruction.

Figure 41 is a section through the intermediate cell mass of a sixteen-day old embryo and presents this degenerate condition. The erythrocytes are all small and necrotic and many mesenchymal cells are scattered among them.

As we shall see below, the power of existence of the erythrocytes is very much stronger in the blood islands where aeration is no doubt considerably better than in the intermediate cell mass.

3. Blood islands of the yolk-sac, their origin and development

The question of origin of blood cells on the yolk-sac of the Teleostian embryo has been a much debated topic. Almost all of the earliest workers claimed that blood arose in the yolk-sac islands of the bony fish just as in other meroblastic eggs. The later workers, however, have denied this statement and hold that the bony fish forms an exception to the rule, and is the only type of meroblastic embryo in which blood cells do not occur in islands on the yolk-sac.

It has been frequently admitted by several recent workers that certain wandering mesenchymal cells do migrate to the yolk-sac from the embryo and there form isolated blood cells or small cell groups, but that this blood formation is insignificant in amount as compared with the great blood forming intermediate cell mass.

The yolk-sac of the bony fish is peculiar in this connection. In most meroblastic embryos there is a definite mesodermic layer or membrane between the ectoderm and entoderm of the yolk-sac, and it is in this mesodermal layer that the blood islands arise. When one examines the yolk-sac of the Teleost embryo, the mesodermic layer is found to be largely, if not entirely, absent. Thus, the ectoderm lies directly over the yolk periblast which may be considered to represent the primary entoderm. Between these two layers many long spindle-shaped mesenchymal cells are noticed on careful examination, but these cells in the specimens examined are never arranged in a definite continuous layer.

Goodall ('07) has recently stated that in the sheep embryo, the yolk-sac mesenchyme is not to be considered a continuous layer, but consists merely of diffusely scattered wandering mesenchymal cells. These mesenchymal cells in the sheep as in the fish finally collect into groups and such groups ultimately give rise to the blood islands. In the fish it would seem as though the entire ventral or yolk-sac mesoderm, the chief source of blood formation, had been in its phylogenetic development incorporated or drawn into the body of the embryo as the intermediate cell mass, and only a few cells lag behind or later wander out to form the collections of mesenchymal cells upon the yolk. Ontogenetically there is no longer any indication of a mechanical drawing-in process but the wandering out of cells may be readily observed. It is also easily conceivable that this condition probably differs in different species of Teleosts. Therefore, some species may really form no blood cells in the yolk-sac, while again others might have an almost complete mesenchymal layer in the sac and in such a case would probably give a typical blood island arrangement. Whereas, an intermediate condition would be well represented in the species of Fundulus here studied in which there are numerous disconnected wandering cells later grouping themselves to form the blood islands on the yolk-sac.

The appearance of the wandering cells as they radiate out from the caudal end of the embryo on to the yolk-sac is strikingly similar to that shown by the cells wandering away from the cen-

tral tissue mass in a living tissue culture. The cells are elongated spindle-form and all are moving straight away from their seat of central origin. This phenomenon is well illustrated by the numerous figures of tissues growing in culture media and I shall give illustrations of it in a special study of this subject now in preparation.

In all of the non-pelagic bony fish eggs investigated up to now, the chief blood forming cells are without exception the intra-embryonic intermediate cell mass, and this mass is claimed to form both vessels and blood. While in the pelagic type of bony fish egg the mass is usually concerned in the formation of vascular endothelium, and the blood cells only arise after the embryo is hatched and free swimming.

This peculiar specialization in intra-embryonic blood formation which seems typical for the bony fish has caused the yolk-sac formation of blood to be almost completely neglected or overlooked by recent investigators. Yet in the species upon which I have experimented there is no doubt whatever that blood islands do arise on the yolk and their origin is from the wandering mesenchymal cells. The wandering cells may be connected in some manner with the intermediate cell mass, yet the presence of the islands cannot be explained in the way Swaen and Brachet ('01) have attempted to account for the yolk-sac blood. They assume that the islands are pushed out laterally as branches or portions of the intermediate cell mass. In many cases no direct continuation of cells is traceable between the yolk islands and the intermediate cell mass and even in extremely young embryos yolk islands may appear on the ventral yolk surface at a great distance away from the intermediate cell mass.

The group of four figures, 36 to 39, indicate the progressive patterns assumed in life by these yolk islands. In the very early stage, figure 36 shows separate collections of cells here indicated by stippling. These groups then become confluent as in figure 37, then more or less net-like in appearance with certain nodes or portions thicker and darker than the general net. In these nodes cell proliferation or blood formation is more active. Finally a typical vascular network arises which goes to make up the capillary yolk circulation of the embryo.

These appearances, as stated above, are not readily distinguishable in the very young embryos, yet with a little experience and a high power microscope any one may convince himself that the blood island formation proceeds to a very definite and considerable degree in these embryos.

Figure 34 represents a cross-section through the yolk-sac of an embryo of seventy-two hours old. The ectoderm of the yolk-sac now becomes two-layered, this continues to thicken as age advances until finally in old embryos the yolk-sac ectoderm is many cells thick and often folded and complex in arrangement sometimes showing villus-like processes. Beneath the ectoderm a group of early erythroblasts or blood cells is illustrated. These cells lie immediately upon the yolk mass here indicated by the heavy dark granules. The appearance of the cells in this blood island anlage are closely similar to those shown in cross sections of the intermediate cell mass in figures 31 and 32. The cell nuclei and general cellular arrangements of the two tissues are seen to correspond in appearance, and the manner of differentiation followed in both cases is identical.

In figure 35, a group of five early erythroblasts are shown which were present in a neighboring blood island. They had loosened themselves from the general island mass and appear very much, if not exactly, similar to the early erythroblast seen separating themselves from the compact mass, the intermediate cell mass (fig. 32). The nuclei in all cases are typically those of early red blood cells and the cytoplasm just begins to stain a very pale pink color characteristic of the halo seen around the young erythroblast. All of the cells shown in these yolk islands, both in the earliest condition of the island and in the late old yolk vessels of embryos without a circulation are invariably of the erythroblast or erythrocyte type. In no case has any type of lymphocyte or leucocyte been present in these yolk islands except as late wandering cells.

Not all of the wandering cells which are found on the yolk-sac go to form blood cells since many of them are future chromatophores or future endothelial vessel cells. The types, however, are distinguishable in rather early stages and do not seem

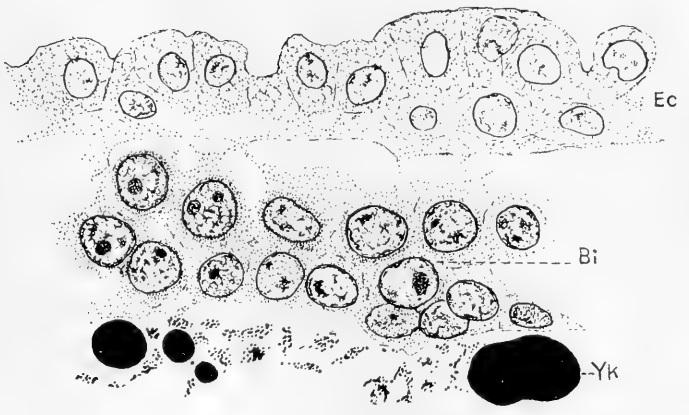
to be in any way related except that all are of mesenchymal origin. The chromatophores as before mentioned, often come to lie along the walls of the blood vessels.

The early yolk-sac is non-vascular, the blood masses being completely uncovered by endothelium. Later endothelial walls are formed around the blood cell masses and a vascular network is established in the yolk-sac of the normal embryo though poorly formed in the individuals without a circulation. All of these yolk vessels seem to arise by arrangement of wandering mesenchymal cells. Certain of these cells elongate and group themselves in such a way as to form vessel tubes. After the vessels are formed they may then be seen to send off buds and sprouts in the manner Clark ('09) has described in amphibians. The difference between the cells giving rise to the vascular endothelium and those forming the blood cells is not distinguishable in early stages. Yet after considerable study and careful observation, nothing has been observed that would indicate that these vascular endothelial cells possess the power to change into the blood cell type, nor is there any evidence to indicate that cells having once assumed even the earliest blood cell type are capable of metamorphosis to form endothelial cells. It is impossible to state emphatically that the vascular endothelium of the yolk-sac in all Teleosts arises in the same way as that described here for Fundulus embryos. But any one familiar with the very complex yolk circulation of the trout family, in the light of the above knowledge is scarcely justified in assuming, that this network of vessels is completely derived from outgrowths from the aorta and cardinal veins within the embryo as Sobotta

Fig. 34 Section through the yolk-sac of an embryo seventy-two hours old, without a blood circulation. A group of cells forming a blood island are distinguished by a slight condensation of cytoplasm about their nuclei; Experiment A, 1913; *Ec*, the ectoderm several cells thick; *Bi*, the cells of the blood island; *Yk*, granular yolk.

Fig. 35 Young erythroblasts just isolating themselves in another island on same yolk as figure 34. Compare the early blood cells with those of figures 31 and 32, in the intermediate cell mass.

Figs. 36 to 39 Illustrate the progressive steps in the development of the network of yolk-sac blood islands.



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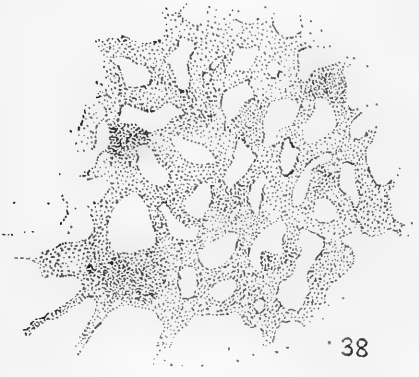

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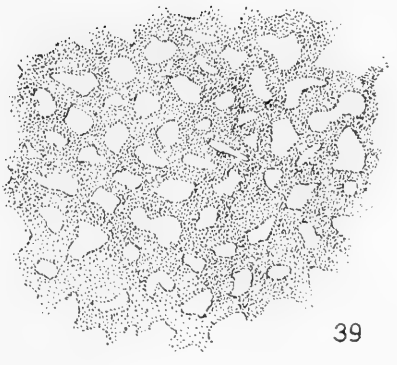

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('02) would have one believe. It is probably true that in the trout family also, wandering mesenchymal cells are of great importance in the formation of extra-embryonic vascular endothelium. There is a strong possibility, as admitted by Swaen and Brachet ('01) that some blood cells are also formed on the yolk-sac of the trout from wandering mesenchymal cells.

The blood cells in the yolk islands increase by mitotic division and soon become very prominent features in those embryos without a circulation, so that in old individuals of eight or ten days the entire posterior and ventral regions of the yolk are almost completely covered with red blood islands. The corpuscles in these blood islands persist in a more or less normal condition for a considerable length of time.

4. Fate of the blood corpuscles in embryos without a circulation

Figure 42 shows the corpuscles in a yolk-vessel of an embryo of sixteen days old in which the blood had never circulated. The vascular endothelium is well formed about the corpuscles and proliferation or multiplication of blood cells has completely ceased, the nuclei are very densely stained and somewhat pycnotic in appearance suggesting a more or less atrophic condition of these erythrocytes.

Figure 40 is a cross-section of a vessel from the yolk-sac of a normal embryo of seven days old. In this the vascular endothelium is also fully developed, large chromatophores have spread themselves along the vessel wall and the erythrocytes are in a vigorous physiological state. The nuclei are lightly staining alveolar structures quite different in appearance from those of the erythrocytes in the older embryo of sixteen days that has never had its blood to circulate. Yet the erythrocytes in the old non-circulating embryo are ladened with haemoglobin and certainly function to some degree.

Figure 41 in the same group is a section through the intermediate cell mass of a sixteen-day embryo without a blood circulation. This is the only intra-embryonic vessel which contains blood cells in this individual. The vascular endothelium

is completely disintegrated and has disappeared, and the very degenerate small erythrocytes are now intermixed with mesenchymal cells. In a slightly older embryo, all of these blood cells have disappeared within the tissue as if the invading mesenchymal cells had really assimilated or destroyed the old blood cells.

It is thus seen that in these non-circulating individuals, although red blood cells arise in a perfectly normal fashion and differentiate as completely as in the control embryos, yet they are not capable of maintaining their fully developed condition. Sooner or later they undergo degeneration and finally are completely absent from the body of the embryo.

It is noticed in all cases that very soon after the erythroblasts become completely surrounded by endothelium, they gradually lose their power of multiplication and then differentiate into typical erythrocytes. Before the vascular wall has completely enclosed the erythroblasts, all groups often show many cells in active mitosis, and as I shall bring out below those spaces in which blood cells multiply both in the embryo and in the adult are spaces not completely surrounded by vascular endothelium.

In examining figures 40 and 42, it may be of interest to note that the erythrocytes in figure 40 are the typical ichthyoid type of Minot ('11), while those in figure 42 are what Minot would describe or term, the sauroid type; that is, erythrocytes in which the nucleus has become slightly more degenerate or more densely staining than in the ichthyoid type and in which the cell body is smaller. This sauroid type of corpuscles Minot has designated as being characteristic of reptiles and amphibians, and the condition in these embryos without a circulation indicates the very artificial nature of the proposed classification of Minot. The cells are, of course, ichthyoid but are degenerate and, therefore, assume the 'sauroid type.'

It is difficult for one to believe that all of the functioning erythrocytes in the amphibians and reptiles really have a degenerate nucleus for the simple fact that mammals have blood corpuscles which have completely lost or discarded their nuclei. It must here be remembered that birds are as truly derived from

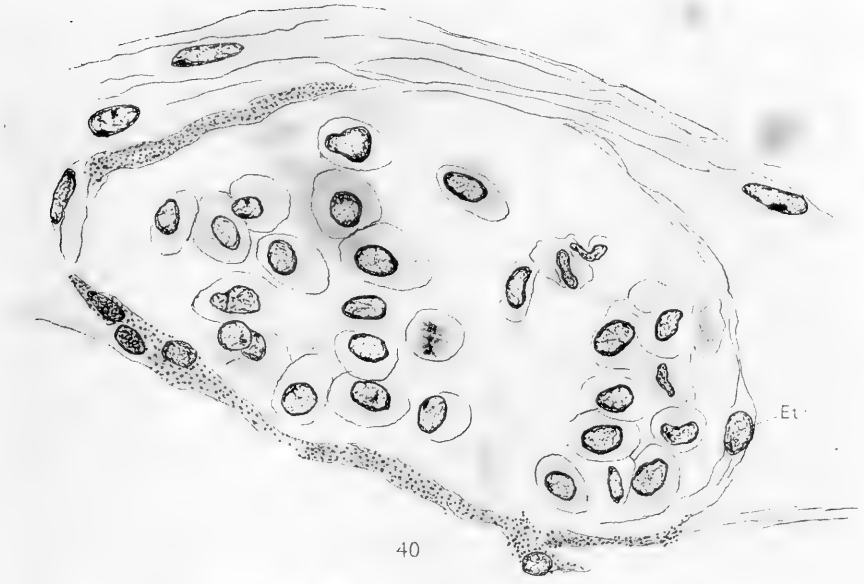
reptiles as are the mammals, in fact, the connection between the reptiles and birds is even closer. Yet the degenerating nuclei of the reptilian blood corpuscle is able to maintain itself in the corpuscles of the bird, although it is according to Minot, so far gone as to degenerate entirely in the corpuscles of the mammal. Such classifications are extremely misleading as they convey to the mind the impression that there is a continuous developmental or evolutionary chain of events illustrated in the blood cells of the different vertebrate groups and actually repeated in the development of the blood in the mammals. The "biogenetic law" is scarcely vigorous enough at present to be submitted to such a strain.

Finally in considering these yolk-sac blood-corpuscles, one must mention the possibility of origin from the yolk periblast or endoderm. It is often stated even in modern text-books and contributions that blood-cells may arise from endoderm and that the primary blood forming layer was actually the endoderm. It is very positively certain that none of the blood cells on the yolk islands of the fish arise from the periblast, but all are derived from wandering mesenchymal cells. The sharp distinction between endoderm and mesoderm is not a thing of any great or definite importance, since everyone recognizes the primary association and origin of mesoderm from the endoderm and the ectoderm. When the mesoderm is once formed, however, it contains within itself a blood forming anlage. It must be further remembered by speculators on the phylogeny of the vertebrate

Fig. 40 A highly magnified section through a yolk-sac vessel in a normal embryo of seven days; *Et*, the vascular endothelium with chromatophores along it. The large beautifully developed erythrocytes are seen in the lumen.

Fig. 41 An equally magnified section through the intermediate cell mass in an embryo without a circulation when sixteen days old; Embryo 413. This is the only intraembryonic blood present, the vascular endothelium, which probably at one time surrounded the erythrocytes, has broken down and mesenchymal cells, *Mcn*, are now intermixed with the small degenerate erythrocytes, *Ery*, which should be compared with the normal ones in figure 40.

Fig. 42 Shows erythrocytes in a yolk-sac vessel also in Embryo 413, at the same magnification as in the two preceding figures. These erythrocytes are in a better condition than the intra-embryonic ones, yet they are very degenerate as compared with those of figure 40; all, however, still contain haemoglobin.



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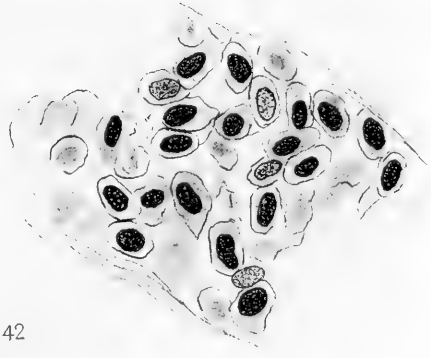

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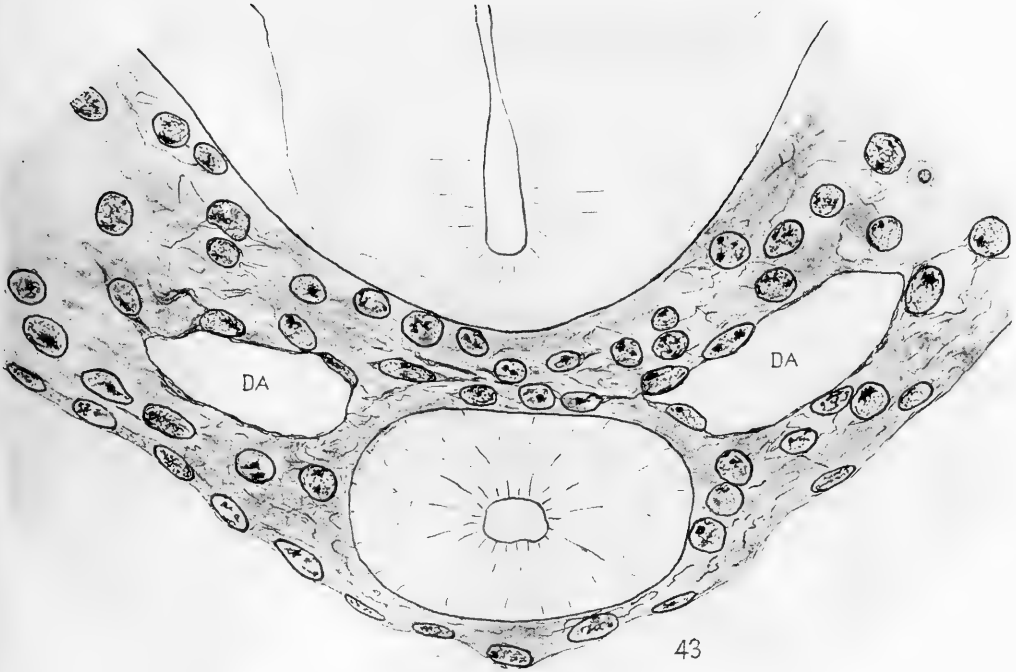
blood that the invertebrate animals, many of which possess highly functionating white blood cells, amoebocytes, as well as oxygen carrying corpuscles, are thought to derive these cells and the vascular endothelium from mesenchyme and not from endoderm.

5. Has vascular endothelium a haematopoetic power?

It has been mentioned in describing the origin of blood in various parts of these embryos that no observation could be interpreted to indicate that blood corpuscles ever arise from vascular endothelium. The endothelium of vessels containing blood never presents any cell in a transitional stage. These experiments, I think, furnish a crucial answer to persistent claims that vascular endothelium has the power to change into various types of blood corpuscles. If vascular endothelium had such a power, then one might expect that this power would show itself in cases where it was most needed, for example, in these embryos in which the blood has never circulated. The blood cells are confined entirely to the intermediate cell mass and to the blood islands on the yolk.

The heart and aorta and numerous vessels in the head and anterior portion of the body are lined with typical vascular endothelium, yet in no instance has it been found that one of these vessels contains a single red blood cell in any stage of development. From these experiments, one is warranted in making the bold assertion that the endothelial lining of the heart and aorta is perfectly incapable of giving rise to any type of blood cell. This fact has been mentioned in considering the endothelium of the heart. When we now refer to figure 43, a section through the anterior region of a four-day-old embryo without a circulation, two dorsal aortae are shown. These vessels are lined by typical embryonic endothelium but are completely empty so far as cellular elements are concerned. This is true of the dorsal aortae of all embryos from the earliest to the latest stages when the circulation of the blood has been prevented. Felix ('97) has also noted the fact that the aortae in early normal Teleost embryos are invariably free of blood cells.



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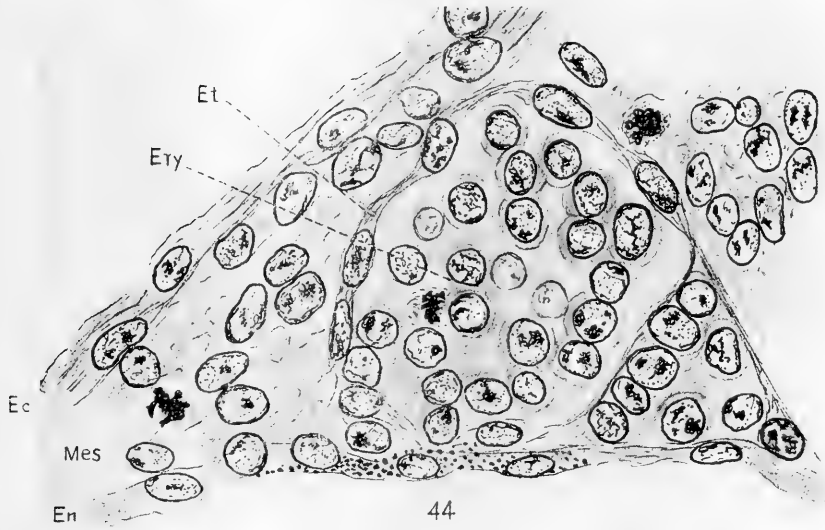

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Fig. 43 Section through an anterior body region of a four-day embryo without a circulation; Experiment 11, 1912, Embryo 6. The dorsal aortae, *DA*, are seen lined by typical embryonic endothelium, yet they are throughout completely empty, never containing blood cells.

Fig. 44 A section through the cardial vein of a similar four-day embryo without a circulation; Experiment 11, 1912, Embryo 4. Again the embryonic endothelium, *Et*, is well formed but the lumen of the vessel is packed with erythroblasts; *Ec*, ectoderm; *Mes*, mesenchyme; *En*, entoderm.

Figure 44 accompanying figure 43 shows a striking contrast in the contents of the cardinal vein. This section is through a more posterior region of the same embryo. The vascular endothelium is here also well differentiated and the vessel is completely packed with early erythroblast some of which are still dividing. None of these erythroblasts, however, have been derived from the vascular endothelium and were actually present before the endothelium was differentiated.

The only source of intra-embryonic blood is from the blood anlage which is contained within the intermediate cell mass, as a rule. But in the splitting away of this mass from between the lateral plate and the somites, it is, of course, conceivable that some future blood forming cells might be left either in the somitic portion or the lateral plate portion. In such cases all those organs arising from regions which had been in contact with the intermediate cell mass either medially or laterally might be contaminated with blood forming cells. If the separation of the blood cell anlage takes place in a clean and complete manner in the individual embryo, then I believe the statement is true that all the intraembryonic blood will be contained in the intermediate cell mass or cardial veins which amount to the same thing.

6. The origin of lymphocytes and leucocytes or so-called white blood corpuscles

We may now turn to a consideration of the origin of lymphocytes and leucocytes or cells other than red blood corpuscles. Many authors have claimed from observations on various embryos that these cells are entirely distinct in their origin from the origin of the red blood corpuscles. Both types, however, arise from the same germ layer or mesenchyme. It has been repeatedly pointed out and seems to be thoroughly substantiated by fact that the lymphocytes and leucocytes in their first appearance are always interstitial in position and are only later contained within the vessels. Whereas, the erythroblasts are invariably formed or divided off into the vessels. In other words, the red

blood cell formation tends to be towards or into the vessels and the formation of white blood cells seems to be extra-vascular or interstitial.

It is recently claimed by Goodall ('07) that in the haematopoetic organs of the sheep embryo such as the liver, there are definite groups of proliferating cells forming the various types of white blood corpuscles, and these are distinctly isolated from other groups of proliferating erythroblasts. In the bone marrow this same state of affairs has been described, and in a number of diseased conditions of human marrow I have observed that certain nests or groups of cells were giving rise to leucocytes while other separate groups consisted of erythrocytes. This observational evidence might seem to indicate that white and red blood cells were arising from different parent cells. Yet in normal embryos it is very difficult to obtain material which will conclusively establish such a position, since both types of cells are swept around by the circulation and are intimately inter-mixed in all of the haematopoetic organs.

It would seem that in these experimental specimens in which the blood was prevented from circulating that there might possibly be some way to distinguish completely the source of origin of the white blood cells from the red blood corpuscles if these sources were really different. Should the two types of cells arise from the same common stem cell or parent cell, then the white and red blood cells should be invariably found in association in all embryos. If the two types of cells had different origins they might be found to occur in separate regions of the body and the various sources could thus be readily differentiated.

As frequently stated, in the early intermediate cell mass and among the cells immediately developing out of this mass no leucocytes or lymphocytes are found. The yolk-sac blood islands also consist entirely of cells of the erythroblast type. These observations are in accord with those of all other investigators studying the development of the blood in the bony fish. They have invariably described the intermediate cell mass as being the source of red blood corpuscles and no one has ever recorded either lymphocytes or leucocytes as arising from this mass.

The only cells within the embryo which resemble lymphocytes or leucocytes in their general structure and staining capacities have been found in the anterior portions of the body and in the head region of the young embryos. In very young embryos of seventy-two hours, numerous isolated cells and occasionally small groups of cells are found within the mesenchyme which present a peculiar appearance. The nuclei are more or less dense, the cytoplasm very small in amount in many and in others very extensive, and staining with a color quite different from that of other cells in the embryo.

Figure 45 shows a section through the head just behind the optic stalk of an embryo of seventy-two hours. In this section there is seen a nest of the above-mentioned cells, several are polynuclear and present various peculiar appearances. The mesenchyme within this region is in active mitosis.

Figure 46 is also taken from the anterior end of an embryo and shows two large mesenchymal nuclei with numerous small leucocyte-like cells within the mesenchyme. Numerous pigment granules are also present in these mesenchyme cells. Some of the cells present nuclei of the polymorphonuclear type.

Figure 47 shows an enlarged binuclear cell and indicates the fine granular nature of the cytoplasm. Such cells resemble very closely the embryonic white blood cells.

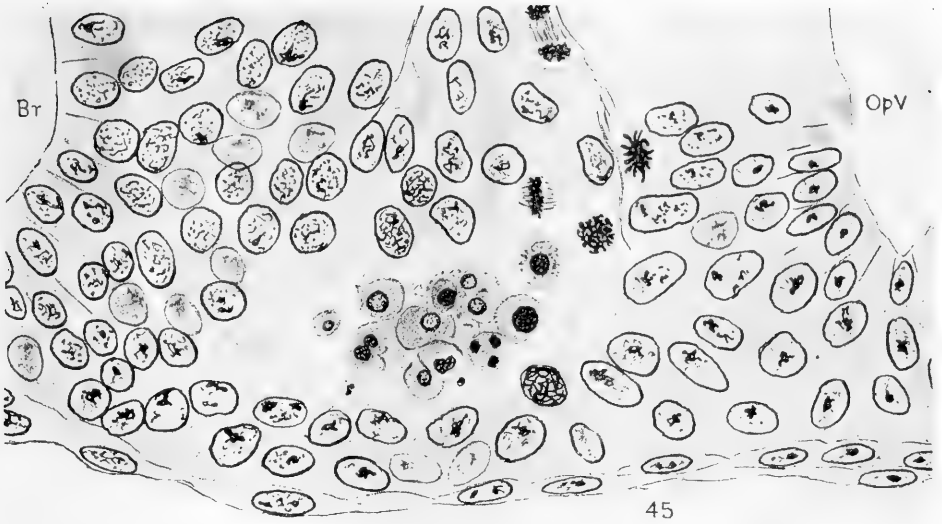
Figure 48 represents a section through the anterior end of an embryo, and shows an endothelial artery which is entirely empty of blood cells. Within the mesenchyme, near the vessel, are two of the leucocyte-like cells. Other cells in these embryos resemble very closely ordinary lymphocytes and these

Fig. 45 A section immediately posterior to the optic stalk in an embryo without a circulation when seventy-two hours old; Experiment A, 1913. A nest of peculiar finely granular cells lies in the mesenchyme which contains many dividing cells; *Br*, brain; *Opv*, optic vesicle.

Fig. 46 Cells from a four-day embryo; Experiment 11, 1912; *Mcn*, mesenchyme nucleus; small leucocyte-like cells are grouped in the neighborhood of chromatophores.

Fig. 47 A binuclear leucocyte from a sixteen-day-old embryo.

Fig. 48 Section through one of the dorsal aortae in a four-day embryo; Experiment 11, Embryo 6; embryonic leucocytes in the mesenchyme.



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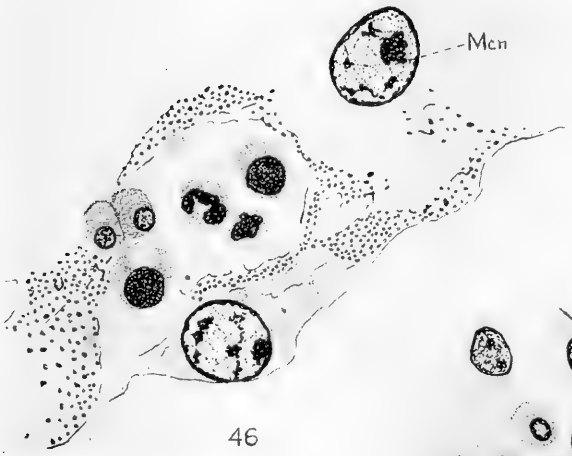

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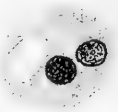

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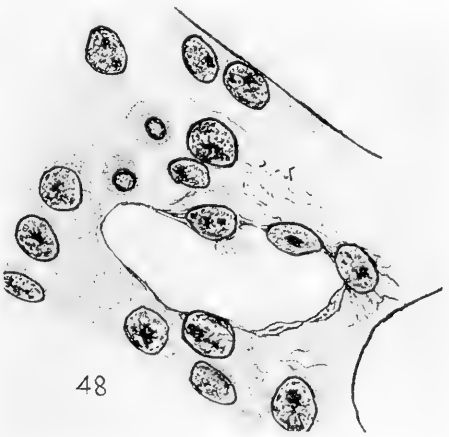

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also are within the tissue spaces and not in the vessels. None of these cells are found in associations with the early red blood cells.

I have examined a number of smears of heart blood, spleen pulp, bone marrow and peritoneal fluid from adult Funduli and have found within these specimens numerous coarse granular leucocytes, lymphocytes and various types of wandering cells. The embryonic cells above described all show a more or less degenerate appearance, but if they can be classed as any type of white blood cell their origin is definitely removed and entirely distinct from that of the red blood cell. In appearance they are as closely similar to embryonic leucocytes as are the cells designated by other investigators to be of that nature. It seems to me that the only possible method of differentiating between the origins of white corpuscles and red blood cells is to prevent their association in the circulating body fluids. These experiments along with numerous observations do show that the red blood cells arise in a region distinctly separated from those localities in which the white blood corpuscles are formed.

When one examines a specimen such as those in which Maximow, Dantschakoff and others have described the origin of white and red cells from a common stem cell, it is impossible to be absolutely certain that the two types of cells do arise from the same individual stem cell. The stem mother cell is shown within the mesenchyme, near this on the one side are various early lymphocytes or leucocyte-like cells, and on the other side are the various stages in erythroblast development. Each type of cell graduates directly back to the stem mother cell or to a mesenchymal cell. This much may be freely admitted, but to say more is merely a matter of guess or interpretation. Since it is absolutely impossible on fixed material to make the definite statement that both of these two types of cells have arisen from the one individual stem mother cell. The observer must actually witness the stem mother cell divide into two cells, and then observe one of these two cells either by differentiation or continued division give rise to white corpuscles, and the other either by differentiation or continued division give rise to erythro-

blasts before the existence of a common mother cell is proven. This very necessary observation has never been made and all of the evidence in the present literature seems insufficient to warrant the conclusion that such a thing does actually take place, whereas there is considerable evidence to indicate that white and red blood cells probably arise from two different mesenchymal cells. Of course, these two parent mesenchymal cells may be, so far as our powers of observation go, indistinguishable. Yet this would not indicate that they were not different in their potentialities. One of the two mesenchymal cells might be capable of giving rise to the various types of white blood cells depending upon the conditions of differentiation and function, while the other apparently similar mesenchymal cell could on account of its internal difference give rise to erythroblasts. It is a little strange at least that the white blood cells arise far interstitially, while the erythroblasts have such a decided tendency to proliferate into the sinusoids or vascular spaces if they both arise from a common stem cell. The two environments in which they develop could scarcely account for the differences between red and white corpuscles, since in the body of an embryo in which the blood circulates there are several places where the two types of cells develop side by side, as Maximow and others have described. The reasons for the differences are the internal differences between the mesenchymal cells from which the two types of corpuscles arise.

The white blood cells and red blood cells, although both are derived from mesenchyme, arise from mesenchymal cells which have already differentiated sufficienctly far not to be interchangeable. This statement is probably true also of the vascular endothelium in its relationship to blood forming mesenchyme. The embryonic mesenchymal cell if taken in an early enough stage could no doubt give rise to other mesenchymal cells which would later form any of these different type cells. When, however, differentiation has proceeded to some degree, sufficiently far to form what is termed by embryologists an organ anlage, and yet not far enough to make it possible to distinguish between the appearance of various mesenchymal cells, they are then, nevertheless, different in their potentialities.

The mesenchymal cell with the power of forming vascular endothelium is probably very diffusely distributed throughout the embryonic body as well as the yolk-sac. Numerous investigators have supplied evidence indicating this fact. On the other hand, the mesenchymal cells which are to form the erythroblasts are in the bony fish very definitely localized. The latter cells are chiefly confined to the intermediate cell mass, but in addition other erythroblast forming cells wander out probably from the same source of origin, the primary intermediate cell mass, to become distributed on the yolk-sac.

Finally, the mesenchymal cells which are to give rise to lymphocytes and leucocytes of various types seem in Fundulus embryos to be more or less localized in the head and anterior region of the body and do not seem to be particularly associated with vessels. For this reason in early embryos the first apparent lymphocytes and leucocytes are found in the head and anterior body regions, and even in older individuals such cells are more abundant here than in other portions of the body. Yet these cells are doubtless of a roving or wandering type and may finally become scattered throughout the embryo's body. While the non-migrating red blood corpuscles rarely if ever leave their original sites of differentiation.

7. Environmental conditions necessary for blood cell multiplication and differentiation

The above facts and interpretations lead us to a consideration of the later conditions of cell multiplication and differentiation. Are the so-called haematopoetic organs of the embryo and even the adult actually haematopoetic, or are they merely favorable localized environments in which various types of blood cells may multiply or reproduce themselves throughout the life of the embryo or individual? There is little doubt, from the recent suggestive studies on the cultivation of tissues in artificial media out of the body of the organism, that certain environmental surroundings are conducive to cell proliferation and growth while other environments inhibit these processes and tend to favor differentiation and functional activity.

Many points mentioned in the previous pages indicate that blood cells change their mode of behavior as the conditions of the embryonic vessels and body are changed during development.

A careful consideration of various embryos as well as the different regions of the same embryo suggests that erythroblasts only multiply in spaces unlined by endothelium, whether these be on the yolk-sac, or within the embryonic liver, spleen or bone marrow. The unlined spaces thus afford an environment in which for physical or chemical reasons the erythroblasts are able to multiply and reproduce themselves. When, however, such spaces or channels become converted into endothelial lined tubes then the erythroblasts tend to differentiate into erythrocytes and very soon cease to reproduce themselves any further in this location. In the intermediate cell mass of the fish embryo for instance, one notices in early stages many dividing erythroblasts. Just about the time that this mass becomes completely enclosed by vascular endothelium, this division process slows down and finally ceases although the confined erythrocytes may never be able to leave the vessel. Soon after being surrounded by vascular endothelium the erythrocytes assume a passive non-productive state and remain in this condition throughout their existence.

One of the earliest places of blood cell proliferation or haematopoesis is the sinuses on the yolk-sac. Almost as soon, however, as these sinuses become converted into the yolk vessels, blood cell production ceases in the yolk-sac and only blood circulation takes place.

The haematopoetic processes are then transferred to the embryonic liver and this in most vertebrate embryos is an important seat of blood cell multiplication. The multiplying erythroblasts are never enclosed by endothelium. In other words, they are not within the vessels but their final products are invariably budded or divided off into the sinusoids. Finally, the spaces in which blood cells multiply in the liver become obliterated or converted into endothelial lined vessels and spaces and very soon after this takes place the haematopoetic processes cease in this organ. The liver cells themselves or the interstitial tissues of

the liver are not blood forming cells, the only blood formed within the liver arises from existing blood cells which are carried there in the circulating current.

In this manner the multiplication of blood cells is shifted from place to place and becomes more and more localized until ultimately in higher animals the red bone marrow is the only body tissue in which these open spaces furnishing the required environment exist. It is, therefore, the only body tissue in which erythroblasts live and continue to multiply and give rise to the entire stock of red blood corpuscles which circulate throughout the body.

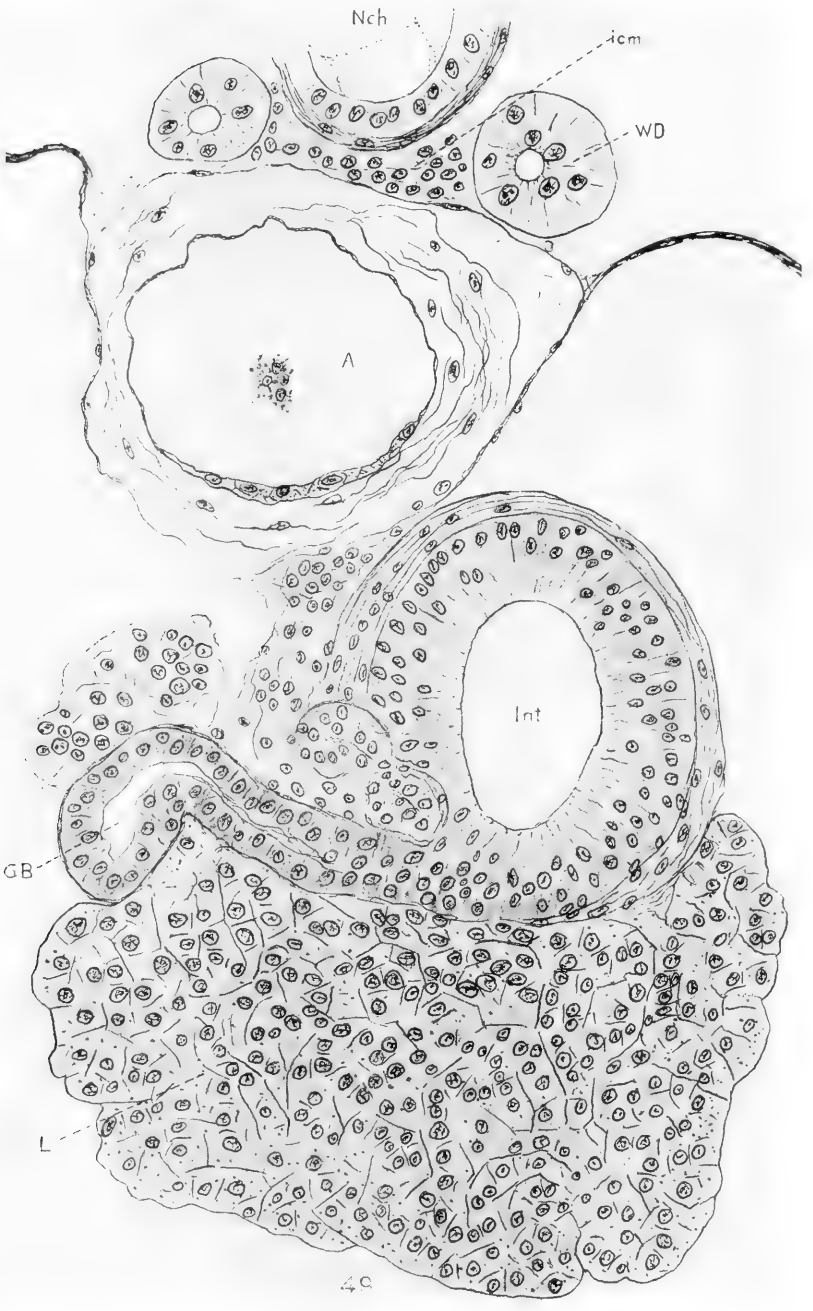
Blood cells always multiply in the unlined spaces but normally never multiply to any extent within closed vessels. It might be possible that certain abnormal growth tendencies on the part of the endothelium of the vessels and sinusoids in the bone marrow might cause an inclusion or vascularization of the spaces in which blood cells multiply and this growth might indirectly result in the cessation of the production of red blood corpuscles. This might be experimentally tested should some method be devised by which the growth of vascular endothelium could be so stimulated as to close the spaces of the bone marrow.

8. Question of haematopoetic organs

In the fish embryo the haematopoetic function of the liver is not of great importance. Yet in the liver of normal individuals numbers of blood cells are always found and numerous dividing blood cells are present. In the non-circulating embryos the blood is unable to reach the liver and in such cases there are no blood cells of any type to be seen in this organ.

Figure 49 represents a section through the gall bladder, bile duct and body of the liver in a sixteen-day old embryo. In

Fig. 49 A section through the liver of a sixteen-day-old embryo, without a circulation; Embryo 413, 1913. The gall-bladder, *GB*, and bile duct are seen connected with the intestine, *Int;* the liver, *L*, is a compact mass containing neither vessels nor any type of blood cell. *A*, the well developed dorsal aorta is lined by endothelium but its lumen is completely empty except for a slight coagulum near the center; *icm*, if followed posteriorly leads into the remains of the intermediate cell mass; *WD*., nephric duct; *Nch*, notocord.


Nch
icm
WD
A
Int
GB
L
4c

this individual the heart is a solid string and the blood had never circulated. The liver presents a dense appearance, no blood vessels are seen and blood corpuscles are entirely absent. The general differentiation and condition of the tissues are, however, fairly normal and not at all degenerate. The intestinal epithelium is typical in structure. Above the intestine the well differentiated dorsal aorta is shown with connective tissue fibers abundantly present in its wall and a definite endothelial lining. The lumen of this aorta, however, has never contained any type of blood cells and the only solid particles within it are a slight coagulum near the center of the vessel. Above the dorsal aorta are the two Wolffian ducts and between them under the notochord are a few mesenchymal cells which represent more posteriorly the remains of the intermediate cell mass. Almost all of the erythrocytes in this mass have completely degenerated or have been destroyed by mesenchymal cells.

The embryos without a circulation thus furnish a definite means of establishing the actual haematopoetic value of any organ. They demonstrate that unless the blood current reaches the organ and thereby introduces embryonic blood cells into it the organ iself is incapable of giving rise to blood cells.

This experiment also demonstrates with equal force the inability of vascular endothelium to form blood cells in the fish. I can see no reason if vascular endothelium possesses a blood forming power why the aorta and other interior vessels of these embryos are invariably empty and never contain any type of blood cell. It cannot reasonably be claimed that this inability is due to the abnormal condition of the embryo having taken away the power of the endothelium to form blood cells, since it is so absolutely demonstrated that real blood forming material in other portions of the embryo possesses its perfectly normal capacity to produce blood and does produce it in a very abundant fashion.

These embryos furnish no evidence to indicate that there is any connection or association between the mesenchymal cells which are to form the connective tissue and those destined to form blood cells. There is no instance of a tendency for connective tissue cells to change into blood cells or of blood cells to give rise to any type of connective tissue cells.

Finally, one may conclude that the blood cells like many other specific tissues and organs have a definite localized specific anlage and that this anlage is distinct and separate in most cases from that of the vessel linings. In some cases, however, the blood and endothelial anlagen may come into intimate association, yet even here the two are probably of different mesenchymal origins.

A CONSIDERATION OF THE EXPERIMENTAL STUDY ON THE ORIGIN OF BLOOD IN TELEOSTS IN RELATION TO THE MORE RECENT STUDIES ON THE ORIGIN AND DEVELOPMENT OF VESSELS AND BLOOD CELLS

1. Introduction

This experimental study of the origin and development of blood and vessels relates itself to three more or less separate fields of investigation.

In the first place, the manner in which the blood anlage in Teleosts has separated itself as a unique intermediate cell mass has caused it to be studied as a special subject somewhat isolated from the more general literature on the development of blood in other vertebrates. Yet one very soon appreciates the mistake of this isolation since contributions such as those of Felix ('97) and Swaen and Brachet ('99, '01, '04), in particular, on the Teleosts are of more general importance than most investigations dealing with the broad subject of blood development in the vertebrates. The very fact that in this group the blood anlage is so peculiarly localized in the embryo lends itself as a great aid to the solutions of many questions of haematopoesis or blood genesis.

Secondly, a consideration of the origin and formation of the heart lining or endocardium and the vascular endothelium, in these embryos which have developed without having had plasma or fluids to circulate within their vessels, may furnish much important data towards a final solution of the origin and significance of endothelial lining cells, and the manner of spread and distribution of such cells through the embryonic body and the yolk-sac.

Lastly, such an experimental study bears closely upon the general questions of relationship between different blood cell types. The time and place of origin of the different cells, and the developmental relationship and powers of transmutability existing between various sorts of blood corpuscles as well as the endothelial lining cells of the vessel walls are all problems upon which the experimental results discussed above may throw light.

Each of these three divisions of the problem embraces an extensive and often cumbersome literature which it would be quite out of place to consider in detail at the present time. We shall, therefore, only consider the bearing of the facts recorded in the previous pages upon the opinions and positions maintained by the more recent investigators of the origin and development of blood and endothelium.

2. The specific problems of blood and vessel formation in the bony fish

It may be well to review first the special problems and questions involved in the development of the blood in Teleosts as a group. According to Swaen and Brachet ('01), the mesoblast in the middle and posterior regions of the trout embryo is arranged in two parts, a median primary somite portion and an outer primary lateral plate part. The lateral plate in the mid-body region then divides off a portion immediately adjacent to the somites to constitute the intermediate cell mass. Lateral to this a second part of the lateral plate is separated off to form the primary nephric duct. In the mid-region of the body the somites become separated from the primary lateral plate and the lateral plate pushes or grows towards the median plane and gives off a keel shaped mass between the somites and hypoblast. This mass unites in the median plane with a similar mass from the other side and here forms a large cell group triangular in cross-section, the intermediate cell mass. In the posterior region of the embryo a similar mass pinches away from the primary lateral plate and becomes the posterior continuation of the intermediate cell mass.

Anterior of the first somite in the unsegmented mesoblast of the head this division or pinching away also takes place. Thus the intermediate cell mass of the body becomes continuous with a definite lamella of the head. This well definied topographical portion of the embryonic mesoblast, the intermediate cell mass and cell lamella, is, according to Swaen and Brachet, the only material which gives rise to the heart, the chief vessels and the blood in the embryo. This description by Swaen and Brachet ('01) agrees very closely with that formerly given by Felix ('97), except that Felix disagrees in not deriving the aorta from the intermediate cell mass but from the sclerotoms.

The observations made upon the intermediate cell mass in Fundulus are in close accord with this summary. But no attempt has been made to solve the detailed question as to whether the aorta is derived from the intermediate mass or from the sclerotoms. It would seem that this vessel might arise from either source and still be formed from practically identical cells. Since in the separation of the primary lateral plate from the somite it is easily conceivable that some cells which generally accompany the primary lateral plate might be left as part of the lateral portion of the somite. This lateral portion of the somite is the part which later separates as the sclerotom so that the cells destined to form the aortic endothelium might occur equally well within the intermediate cell mass or within the sclerotom. Their location might vary among different species or even among individuals, and yet these aortic cells would be derived from the same genetic source.

Swaen and Brachet also indicate the head mesoblast as separated into three portions: the intermediate cell mass close to the top of the pharynx, the lateral plate split into two lamellae and the general head mesoblast close around the brain. The intermediate cell mass is more intimately connected with the splanchnic layer of the lateral plate. The pharynx widens in forming the gill pouches which continue to grow dorsally and finally separate the intermediate cell mass into two portions, one part thus comes to lie ventral of the pharynx and the other part dorsal. The ventral portions, at first solid masses below

either side of the pharynx, begin to migrate towards the middle line. The two masses fuse into one, spaces are developed in the mass and finally the endothelial lining of the heart is differentiated out of this group of cells. The lamellae of the side plate become separated and the space between them gives rise to the pericardial cavity. Oellacher ('73), Wenckebach ('86), Henneguy ('88), and Sobotta ('94) have all described the origin of the heart in Teleosts in much the same way.

Several of these investigators, Wenckebach, Swaen and Brachet and others, have called attention to a small mass of cells derived from the heart anlage which comes to lie beneath and outside the heart endothelium. This mass of cells has been claimed to wander away from below the pericardium and later to give rise to vessels and blood on the yolk-sac. In the non-circulating Fundulus embryos, however, neither vessels nor blood are formed on the extreme anterior portions of the yolk-sac. I have seen nothing in my studies which would indicate that any cells left over from the heart formation had wandered upon the yolk or given rise to blood cells or vascular endothelium.

Swaen and Brachet are alone in showing that the heart cells are definitely continuous with the intermediate cell mass of the the trunk mesoderm.

Many early workers on the fish embryo have claimed, as has been done for most vertebrae classes, that the heart lining arises from endoderm. The weight of evidence at the present time is so overwhelmingly against such a view that it warrants only a passing mention. Again, however, it must be realized that in the separation of the mesoderm from the endoderm it is possible that some future mesoderm cells may be left behind not cleanly separated. These cells might later isolate themselves from the endoderm to form vessels or blood. It nevertheless seems generally true that all blood forming cells are at one time in development contained within the mesodermal portion of the embryo. Gregory ('02) came to the conclusion that the endoderm and mesoderm could be traced to an indifferent cell mass mesentoderm in certain Teleosts, and according to his view, there is no way to say from which germ layer the heart endothe-

lium actually arises. A mixture of endoderm and mesoderm cells gave rise to endocardium.

The later development of the heart of the bony fish proceeds much as in the case of other vertebrates, as has been carefully described in detail by Senior ('09). The only point of interest in the present discussion is the origin and significance of its endothelial portions, and here Senior after a very thorough investigation confirms in all general points the previous findings of Swaen and Brachet.

In Fundulus as in other Teleosts the heart endothelium partially forms in loco but is also added to by wandering cells or ingrowths of mesenchymal cells adjacently located. The venous end of the heart leads directly down upon the yolk periblast, and as was shown in several figures, this periblastic material with huge amorphous nuclei may be at times drawn up into the cavity of the heart. This would indicate that the venous end was entirely free or not connected with any other vascular endothelium. This condition is, no doubt, due to the absence of the anterior yolk vessels which should in ordinary cases unite or fuse with the end of the heart so as to establish a closed circulation.

According to Swaen and Brachet in the region of the third somite the intermediate cell mass forms only the aorta, while caudad the aorta arises from the dorsal cells of the mass and the great part of the mass forms the red blood corpuscles and the venae cardinales. The endothelium of the cardinal veins finally surrounds the blood cells, but before these cells are fully developed or free, plasma has begun to flow in the aorta and other arteries.

In pelagic forms in which the egg is extremely small and develops very rapidly, the intermediate cell mass in the forward body sections is very small, sometimes only seen between the somites. This portion gives rise to the aorta. The cells are somewhat more numerous in the middle and posterior sections, but they never form a mass to the extent found in the larger demersal eggs. At the time of hatching the posterior cell strings form two lateral longitudinal vessels from the beginning of the mesonephros caudad to the anus. These two vessels, Swaen and Brachet consider to be homologous to the unpaired median stem vein

of the trout and this is thought to represent the conjoined cardinal veins. We have noticed that in Fundulus the intermediate cell mass is sometimes divided forming two lateral cardinals loaded with blood cells, while generally it exhibits a median unpaired condition. In the pelagic forms the vessels are all hollow at the time of hatching and the blood cells have not appeared.

Derjugin ('02) claims from a study of the pelagic egg of Lophius that the vessel cells of the aorta and cardinals are derived from the sclerotom. Felix ('97) like Ziegler ('87) differs with Swaen and Brachet ('01, '04) in that he derives the aorta not from the "Venenstrang" but from the sclerotom which under the notochord forms a mesenchymal aortic string. Felix states that no blood cells are to be seen in the aortic anlage, while the cardinal veins, of course, are loaded with the blood cells of the intermediate cell mass. Felix, therefore, derives the two chief vessels of the embryo from two different parts of the mesoderm, the somites or sclerotoms and the lateral plates.

Sobotta ('02) terms the intermediate cell mass "Blutstränge" and derives it from the lateral plate, though he had earlier claimed it to arise from the somites. He described it in the trout embryo in the region from the eighth to the thirty-third somite. The 'Blutstränge' at first paired, are naked cellular strings without a true vessel covering. This they receive later as the cardinal vein anlagen. The endothelial cells of the cardinal veins he derives from the same source which produces the aorta, namely, the sclerotoms.

Finally, then, Swaen and Brachet derive the blood and vascular endothelium of the aorta and venae cardinales from the intermediate cell mass which arises from the lateral plate. Felix derives only the blood and vascular endothelium of the cardinals from the intermediate cell mass which is separated originally from the lateral plate. The aortic endothelium arises from the sclerotoms. Sobotta considers the intermediate cell mass an exclusive blood forming material, while all vascular endothelium, including the heart, is derived from the sclerotoms which are budded off from the somite system. This disagreement, as we have pointed out before, is not of primary importance and

may result merely from the fact of the intimate connection of the sclerotom and intermediate cell mass before their original separation.

The question now arises whether all the blood of the Teleost embryo is exclusively derived from the intermediate 'Blutstränge.' Felix admits that the endothelium of the glomerular vessels of the mesonephros arise in loco and at the same time blood corpuscles often occur in this region. Sobotta claims that in the vascular network in the tail of the trout embryo some of the blood corpuscle anlage exists.

Both of these exotic positions of origin may be easily understood. In the first place, the nephric anlage is formed from cells in direct association with those constituting the early intermediate cell mass, and in the separation it probably happens that some future blood cells are held within the kidney anlage and these cells later develop in their proper fashion. The presence of blood corpuscles in the vascular network of the tail is due to the fact that the intermediate cell mass in many Teleosts, as Marcus ('05) has pointed out and as Senior ('09) particularly emphasized extends far back into the caudal region.

A similar consideration is the question of origin of vessels from material other than that of the intermediate cell mass and sclerotom. This is also important, and numerous observations would indicate that in the early bony fish embryo vessels unquestionably arise in loco and not solely as outgrowths or sprouts from a central vessel anlage. Sobotta ('02) on the contrary imagines a gradual growing away of the vascular system from its local origin, the sclerotom. The aorta is the primary vessel and, for example, the sub-intestinal vein arises from the aorta by vascular sprouts which grow around the gut, broaden out and fuse on its ventral side and finally give rise to the longitudinal vein. This theory of Sobotta is as unacceptable in the face of the great body of evidence to the contrary, Felix ('97), Rückert ('88), Hahn ('09) and many others, as is the opposite ingrowth parablast theory of His ('75).

The consideration has been confined so far to the intra-embryonic blood vessels. We may now briefly discuss the develop-

ment of vessels and blood upon the yolk. There are here two opposed or different views. The first derives the yolk vessels and blood directly from the yolk syncytium or periblast. The second derives blood cells exclusively from the intermediate cell mass in the embryo, but admits that cells may secondarily come to lie on the yolk by being pushed out from the intermediate cell mass with which, however, they maintain a definite continuity. The vascular endothelial cells are also derived from the embryo as mesoblastic wandering cells, but these are not to be compared directly with blood cells since their parent cells have a separate place of origin. Most of the earlier workers thought that the blood in the Teleosts arose on the yolk-sac, as it does in other meroblastic embryos. The more recent workers have gone to the other extreme and deny the presence of blood islands upon the yolk-sac as separate from the intermediate cell mass.

As mentioned in describing the heart formation, numerous investigators have recorded wandering mesenchymal cells upon the yolk-sac, but from a study of the literature no clear conception can be formed as to the origin of blood cells or the vascular endothelial cells upon the yolk from these wandering cells. Some authors claim that the majority of wandering cells become pigment cells, while the remainder form the yolk vessels. In Fundulus the pigment cells very soon present a different appearance from the mesenchymal cells which are to form the vascular endothelium. Both types of cells may be readily seen wandering over the yolk between the ectoderm and periblast. Before the yolk vessels are completely formed, the circulation of a cell free plasma has begun. The extent of the spaces in which this circulation takes place is very variable. The arrangement of the veins of the yolk circulation is also extremely different in the different groups of Teleosts. One must agree with Hochstetter ('93) in stating that the yolk circulation in different forms is so different from the start that it is not possible clearly to summarize the condition in order to give satisfactory comparisons with the same vessels in Selachians, and Amphibians.

When the plasma is flowing in a closed system within the embryo, it is still running as a wandering stream through lacunae and sinuses on the yolk. This probably explains why the blood cells reproduce for so long a time on the yolk-sac while no such reproduction is taking place in the well formed vessels of the embryo.

It is difficult to determine the exact moment, when, or place at which the first blood cells get into the circulation. This probably varies even among embryos of the same species. Ziegler ('88) thinks, however, that just beyond the lateral plates in the plasma filled spaces of the yolk-sac which lie between the periblast and ectoderm, the first blood cells project into the circulation. They are in the form of cell strings which later connect the cardinal veins with the vascular yolk net. Swaen and Brachet saw in trout embryos of eleven days in the region of the fourteenth somite and posterior that the intermediate cell mass spreads out laterally below the lateral plate and on to the yolk surface. The cells thus came to lie above the yolk syncytium and first attained their red color in this position. These authors thus claim that in the bony fish with a large yolk-sac the haemoglobin free early blood cells through continued contact with the yolk become transformed into erythrocytes.

The experimental embryos considered in the present paper demonstrate, however, that it is not at all necessary in such a Teleost to have the erythroblasts reach the yolk-sac in order to acquire their red haemoblobin condition. The tightly packed erythroblasts within the intermediate cell mass of the embryo develop perfectly and readily attain a normal red haemoglobin color.

Finally, comparing the processes of vessel and blood formation in Teleosts with these processes in other vertebrate embryos, we find no definite explanation for the formation of the intermediate cell mass. In other embryos the blood is largely formed upon the yolk. However, it must be recognized from recent contributions that the formation of intra-embryonal blood is much more extensive and important than has formerly been

supposed. The relation of the blood anlage to the cardinal vein and the position of the blood forming cells dorsal of the gut are unique in the Teleosts. The late formation of the yolk vessels and their type of origin from wandering mesenchymal cells is also of special interest.

It would seem as though the peripheral mesoblast which in other vertebrate types grows and develops outside the embryo, had in the Teleosts been peculiarly concentrated and drawn into the embryo during its phylogenetic history. Yet in this intra-embryonic position the peripheral mesoblast gives rise to the same cells which it would ordinarily produce on the yolk-sac. The different Teleosts probably show this drawing in of the peripheral mesoderm to various degrees so that in some cases only part of the mesoderm is incorporated in the intermediate cell mass, while the remaining part may still be outspread upon the yolk and there differentiates extra-embryonically. The intermediate cell mass is connected caudally with the end bud, just as the peripheral mesoblast of the Selachians is with the blastopore lip. In its genesis the intermediate cell mass is split off from the lateral plate and localized along its median border.

Marcus ('05) in his study on Gobius capito advanced the opinion that the intermediate cell mass in this embryo is comparable to the peripheral blood forming mesoderm of other meroblastic eggs. In an embryo of eleven somites, the intermediate cell mass passes without a break caudad to the end bud and there connects with both the ectoderm and entoderm, just as the peripheral mesoderm would meet the other two germ layers at the blastopore lip. He attempted to show by diagrams the relationship between the intermediate cell mass in Teleosts and the blood forming mesoderm of Selachians.

As the homologue of the peripheral mesoderm the intermediate cell mass has the power to form vessels and blood cells. Most authors admit this power and only Sobotta ('02) denies the vessel forming property, while others claim that only the cardinal veins arise from the intermediate cell mass, still others, as Swaen

and Brachet, would derive the endocardium and aorta also from this common source.

The important fact is that in the small pelagic embryos, where no blood formation takes place before hatching, the intermediate cell mass forms the aorta and the cardinal veins and is also derived from the lateral plate. The lateral plate thus contains cells capable of forming vascular endothelium, and this is the case in all vertebrates.

At an early time in evolution the extra-embryonic blood forming mesoderm has been included within the body of the Teleost embryo and lies over the gut as the intermediate cell mass representing the yolk vascular layer. Here it is important to note that the yolk-sac of the Teleosts contains no real mesodermal layer, only separate wandering mesenchymal cells are found between the ectoderm and periblast, and these wandering cells have migrated out from the embryo.

Finally, as Mollier ('06) states in his review of this subject, it is not a question of the formation of the intermediate cell mass in the individual bony fish, but the wider question of the behavior of the blood forming peripheral mesoderm in the bony fish. All of the results must be considered in this light in their application to other animal classes.

The intra-embryonic blood formation in the bony fish does not represent the primitive type for vertebrates as Sobotta ('02) claims, but this is, no doubt, a modified secondary condition accompanying the various other modified and special developmental processes which bony fish embryo so frequently presents.

Wilson ('91) states of the mesoderm of the Teleost that: "The ventral subvitelline mesoderm, having in this way losts it function in the Teleost, must be regarded as a rudimentary organ of the gastrula. It always remains very small, and does not form any special organ or set of organs in the embryo." The real fact is that the subvitelline mesoderm is misplaced, being within the embryo as the intermediate cell mass and here forms the blood of the individual and, therefore, the yolk-sac of the bony fish has no mesodermic layer.

3. Vascular endothelium, and vascular growth and development

Mollier ('06) concludes in his review regarding the origin of vessels as follows.

> As to the genesis of embryonal vessels we may pass the judgment that the theory of the local origin of the vascular endothelium is valuable. The notion of His ('75) and Vialleton ('92) that the vessel strands of the embryo grow in as sprouts from the extra-embryonal anlage (vascular anlage) is not nearly so probable as that the individual vessel cells arise in loco and thus form the vascular nets.

This statement agrees in every way with the contentions so fully presented by Huntington ('10, '14), McClure ('10, '12) and others, regarding the origin of lymph vessels. Lately it receives additional substantiation from the experimental results recorded by Miller and McWhorter ('14) on the origin of blood vessels in the chick embryo. Such a position is further strengthened by the still more recent experimental evidence, presented by Reagan ('15) which shows the origin in loco of vessels in isolated parts of chick embryos. All of these experiments confirm the earlier results of Hahn ('09) on the origin of vessels in the chick.

In the Teleost embryos studied during the present investigation there can be no doubt that the heart endothelium and aortae arise in loco within the embryo, and here there are no vessels, or even mesoderm, present on the yolk-sac in the anterior portion. Certain vessels do partially grow from the embryo out on to the yolk-sac and other smaller vessels arise in many separate regions of the yolk-sac as the products of wandering mesenchyme cells which become arranged to form the tubular vessels. All of these vessels after they have arisen may grow by budding or sprouting off new vessels or may increase in length by a forward growth so well described in living embryos by E. R. Clark ('09, '12) in his careful studies of this subject.

Felix ('97) describes the origin of the aorta as follows:

> The 'mesenchymaortenstrang' arises from the two lines of sclerotoms after they are finally pinched away from the somites. No fusion of cell material occurs between this and the 'venenstrang,' the intermediate cell mass. This 'mesenchymaortenstrang' comes from that

part of the somites that was immediately in contact with the intermediate cell mass portion of the primary seitenplatte. As the forward somites bud off sclerotoms, these also are added to the 'mesenchymaortenstrang.'

The median part of the 'strang' forms the aorta, 'aortenstrang,' the lateral the 'mesenchymgewebe' (mesenchymestrang). *The 'aortenstrang' is at first solid and does not obtain a continuous lumen to begin with, but here and there develops a space, and these spaces become confluent to form the tubes and build the paired aortae.* Certain portions of the strang remain solid much longer than others. The association of the paired aortae to form an unpaired single vessel soon follows. *While the aorta is being so formed, one never finds blood cells within its lumen.* Blood cells occur only in the 'venenstrang' and in certain vessels of the nephric glomeruli. Occasionally certain of the glomerular vessels contain blood corpuscles at a time when the blood circulation is not yet closed.

Felix ('97) cites the observation of P. Mayer ('94) on very young Selachian embryos in which the medulary tube was still open. It was found in such embryos that the aorta is segmental and derived from the somites and subsequently the longitudinal tube is formed by the fusion of these isolated points. Felix agrees with P. Meyer's observations from his study on the Teleost.

There has been great diversity of opinion regarding the germ layer from which the vascular endothelium and blood corpuscles arise. In the literature it may be found that certain competent investigators have in each vertebrate class claimed the vascular endothelium and blood cells to be derived from the endoderm, while other workers of equal authority have found the vessels and corpuscles to arise from the mesoderm. The consistency of the disagreement which one finds in a review of this literature is most peculiar. These disagreements have their foundation in the extreme difficulty of the problem on fixed material.

It is interesting to note that in no case has the same author derived the blood and vessel endothelium from different germ layers. Each author always takes the position that blood and vascular endothelium arise from either the mesoderm or the endoderm.

We have here much to do with wandering cells which become lost from their epithelial layer, and it is impossible to state their

origin. This is left to the imagination of the individual investigator and further possibilities of error are open.

Wenckebach's ('86) observations of living embryos are most important in this connection. He noted that not only the layers but that independent mesoblast cells with amoeboid processes wander out of the embryo and over the yolk. These wandering cells play a great part in the formation of the anlage of the heart endothelium and great vessels. In the Teleost embryo one may readily observe these wandering cells in the yolk-sac, and they doubtless give rise to the yolk vessels and blood islands as well as the pigment cells so abundantly present.

Ziegler ('87) has suggested that it may be that the blood anlage in phylogeny has been passed to the mesoderm from the endoderm, and for this reason the endodermal origin may sometimes occur in coenogenetic development. Goette ('90) also held that the endodermal origin of the blood was the more primitive one. This point of view overlooks the fact that in the invertebrates generally the blood and vessel walls are derived from the mesoderm.

In discussing the question of the place of origin of the vessels, Felix ('97) points out that Rückert ('88) claimed in Selachians, that the aorta arose *in loco*. P. Mayer and Strahl ('95), have also stated that the great vessels are late in appearing and arise *in loco* in the embryo's body. Felix states that the glomerulus of the bird mesonephros originates *in loco* independently of the aorta. Further that the stammvene, venenplexus of the mesonephros, certain vessels of the glomerulus, and also the mesenteric artery along with the aorta in the Salmoniden arise *in loco*. Regarding the anlage of the heart and vena sub-intestinales, Felix is not certain but thinks that these likewise arise *in loco*. All of these observations are directly opposed to the theory of ingrowth of vessels from the yolk-sac, the parablast theory of His ('75) as well as the outgrowth of vessels in the sense advocated by Sobotta ('02).

Ziegler ('89) and Felix ('97) have both speculated considerably as to the relationship of the cavity of the circulatory system with the primary body cavity and the coelom. Ziegler pointed out

that in the phylogenetic origin of the blood vascular system we have the following changes: The primitive condition is represented by the development of a space between the body wall, the ectoderm, and the gut wall, the endoderm, that is, the primary body cavity or protocoel. Embryologically the blastocoel of the blastular or after gastrulation, the space between the invaginated endoderm and the ectoderm, the schizocoel, represents the primitive vascular space. The body cavity in rotifers, nematodes, bryozoa and arthropods is a primary body cavity and is filled with a fluid, the haemolymph. In the arthropods on the dorsal side of the body is the pulsating heart which sets the fluid in circulation and this fluid contains corpuscles similar to the white blood corpuscles of vertebrates.

In the arthropods the vessels and heart are often highly developed but all communicate with lacunae and spaces between the gut wall and body wall. The heart is surrounded by a pericardial space (not truly coelomic) which is full of haemolymph, and as the heart pulsates this haemolymph is drawn in through ostia along its walls and then propelled out through the aorta and its arches to the vessels and spaces of the body. These body spaces, or the haemocoele, are thought by some to be a secondary or specialized cavity. Yet it is not coelomic and has no definite lining and resembles very closely the primary body cavity of the rotifers, nematodes, and other invertebrate forms which it most probably represents. In some of the higher Crustacea a secondary body cavity or coelomic space of limited extent is present enclosing the ophthalmic artery in Paelamonetes. The cavities surrounding the gonads are also coelomic, and since these are well developed species the coelomic space here probably represents a progressive rather than a regressive condition.

The second step in Ziegler's evolution of blood vessels is illustrated by the conditions in the molluscs. In these animals between the gut and body wall lacunae and interstitial spaces exist which occupy the position of the primary body cavity and these are filled with blood. Vessels lead into the lacunae and the cavities of these vessels as well as the cavity of the well formed heart are also considered to be part of the primary body

cavity with which they are continuous. The pericardial cavity in the molluscs is true coelom and not a part of the primary body cavity and contains no blood. In almost all of the molluscs the pericardium is in communication with the nephridia and the nephric duct usually leads from the pericardium to the outer body-wall. The pericardial cavity in contrast to the primary body cavity is designated as secondary body cavity or true coelom.

The final step in the phylogeny of the blood vascular system is characterized by an important expansion of the secondary body cavity or coelom as is the case in the echinoderms, annelids and vertebrates. As a result of the expansion of the secondary body cavity, the primary cavity is reduced merely to a system of channels or vessels and small interstitial lacunae. In the vertebrates, therefore, according to Ziegler, the blood and lymph vascular system represents the persistent part of the primary body cavity. Ziegler considers the blood vascular system and lymph vascular system to have had a common origin. The blood vessel endothelium is closely similar in all respects to the lymphatic endothelium. He thus agrees with Bütschli ('82) that in all metazoa the blood vascular system has its origin from the blastocoel.

Felix ('97) holds that his studies on the Salmoniden will not fit into Ziegler's scheme. He claims that the origin of the stammvene in the cranial portion is the same as that of the primary mesenephros in the caudal region, and is also of the same origin as that of the primary nephric duct. Cells of the splanchnic as well as cells of the parietal layer of the mesoderm enter into the structural material of the stammvene. The cavity of the venenstrang is the same as the cavity between the lamellae of the secondary lateral plate, that is, true coelomic cavity. The three structures referred to are all portions of the same base, the lateral plate mesoderm, the primary seitenplatte. Felix states, as there is little doubt that the cavity of the primary nephric duct is homologous with the secondary body cavity, so there is little doubt that the cavity of the venenstrang is also. The development of the aorta shows similar relations. It arises, according to Felix, from the sclerotomes which come from the

somites and contains both the somatic and splanchnic layers of mesoderm. The origin of the aorta from the 'mesenchymaortenstrang' is from the same cell material as the mesodermal layers. The cavity of the myotom is secondary body cavity, coelom, and so also is the aortic cavity. Neither is in any way primary body cavity. The formation of the aortic cavity is a similar process to the canalization of the stammvene. Felix in this way arrives at a conclusion diametrically opposed to Ziegler.

These conclusions he recognizes are not facts but are based on facts obtained from a study of Teleosts which are a side branch of the vertebrate stem, but from which one may still generalize to some extent. Felix calls attention to the fact that in the selachians Zeigler ('92), and in the reptiles Strahl ('83, '85), and in the birds Kölliker ('84) and Ziegler ('92), and in the mammals Kölliker ('84), all claim that the first vessel anlagen are found in the mesoderm and not between the mesoderm and endoderm. Only in the mesoderm the secondary body cavity arises by splitting, and since the solid vascular anlagen are formed within the mesoderm their cavities should not be considered primary body cavity. The writer is entirely unable to agree with such an analysis of the origin of vessels, particularly yolk vessels, as well as of the primary and secondary body cavities for reasons given below.

Felix ('97) now goes further and assumes that the lymph vessels arise in mesenchyme and their cavity is primary body cavity and their wall cells are modified connective tissue cells. This position is difficult to appreciate since it must be admitted that mesenchyme is a direct product of the mesoderm, and, according to Felix, any definitely formed cavity arising between such cells would seem to be coelom. I question, however, whether any other morphologist would put the same interpretation on all the spaces cited by Felix as being in the coelomic category. Felix states, for example, that the aorta arises from a mesenchymaortenstrang derived from the sclerotom. The sclerotom is more or less mesenchymal in nature and certainly contains many cells which will later give rise to types of con-

nective tissue. If the aorta did arise from this group of cells its cavity is scarcely of an origin comparable to that of the coelom. Its endothelial wall is certainly much the same as that of the lymph vessels.

The cavities of the nephric duct, ovarian duct, kidney tubules and other tubules derived from the mesoderm are not usually considered to be parts of the coelomic cavity. The blood and lymph vessels do arise from the mesoderm but not in such a way that their cavity can be readily homologized with the coelomic space originating between the lamellae of the mesoderm. The vessels on the yolk-sac of the Teleosts are formed from disconnected wandering mesenchyme cells which are easily demonstrated. The cavity of these vessels surely cannot be interpreted to arise between mesenchyme cells some of which are derived from the somatic and some from the splanchnic mesodermal layers. The yolk vessels in Teleosts arise by arrangement of mesenchyme cells and so apparently do other vessels within the embryo. Thus these blood vessels are similar in origin to the lymphatics according to Felix's notion of the mesenchymal origin of lymphatics. The numerous recent investigators of the origin of the lymphatics, athough to some extent divided into two schools, all treat the lymph vessels and blood vessels as being of the same general genetic type Sabin ('13) and Huntington ('14).

Finally, the most damaging evidence against Felix's notion that the blood vascular spaces are derived from the coelom, and that these spaces are actually now comparable to the coelomic space is the following: Before a true coelom, such as that to which Felix refers in the vertebrates, has arisen in the animal series blood vessels are already present and these vessels often communicate with or are actually a part of the primary body cavity. When the true coelom does arise in the invertebrate series blood vessels never open into its cavity or communicate with it. Felix has therefore derived an older and more generalized animal system from a newer or later formation. This of course is contrary to any principle of phylogenetic calculations.

The weight of evidence at the present time is then in favor of the earlier notion of Ziegler. The blood vascular system if it is associated with, or phylogenetically derived from any other body cavity, that cavity is really the primary body cavity or embryologically the blastocoel.

4. Haematopoesis, the monophyletic and polyphyletic views, etc.

The experiments recorded above are of particular value in the solution of that very complex problem, the origin and relationship of the different types of blood corpuscles. We may here then briefly consider the evidence they furnish in connection with the various theories and points of view recently advanced in explanation of the origin of blood cells.

The vertebrate animals present two entirely different types of cells floating in their blood fluid. The white blood corpuscles are cells of primitive type and are not only found within the vessels but they also wander through the interstitial spaces of all the tissues of the body. These wandering white blood cells, amoebocytes, are almost universally distributed throughout the animal kingdom being found in all the invertebrate groups above the one or two very lowest as well as in all the vertebrate classes. In no animal do these cells contain haemoglobin, haemocyanin or any compound that would particularly qualify them as oxygen carriers, or give to them any function as an organ of respiration. These white blood cells found outside of the blood currents as well as in the blood are to be looked upon as cells which are not particularly associated with any specific blood function. They merely find the blood current a ready or rapid means of being carried from place to place within the body.

The red blood corpuscles, erythrocytes, are in contrast to the white cells a very highly specialized type of cell and specifically a blood cell. In fact, this is one of the most specialized cells within the body. In mammals, for example, it is specialized to such a degree that its functional perfection is actually accompanied by the loss of its nucleus and necessarily, therefore, the loss of its own future existence after a short period of time.

Contrasted with the almost universal distribution of the white cells within the animal kingdom the erythrocyte is confined to the vertebrates phylum and to certain particular cases among the invertebrates. The respiratory function of the invertebrate blood is often claimed to be confined to the fluid or plasma mass, and only among certain members of the higher groups is a cell developed with the function of carrying oxygen to the body tissues and even this cell can not be said to possess the regular typical characters of the vertebrate erythrocyte.

The vertebrate erythrocyte along with the typical vertebrate mouth, the pharyngeal gills, the dorsal nerve cord, the notochord, and bony skeleton and the many other possessions characterizing the vertebrate group, separates it in gulf-like fashion from the invertebrates. The white blood cells bridge this gulf but the red blood corpuscle differs from that of the invertebrate in a way comparable to the difference between the vertebrate mouth and that of the invertebrate, both serve the same function but are structurally unlike. Just as the mouth and pharyngeal gills and vertebral column have no invertebrate forerunner, so no cell within the invertebrate animals can at the present moment be sought out or designated as the certain ancestor of the red blood cell.

The cells of the vascular walls are closely similar in both vertebrates and invertebrates, as pointed out above. In both animal divisions they probably arise and develop in the same fashion. The white blood corpuscles probably do also. Yet the red cells, although they too originate from the mesenchyme in the vertebrates, are not in any way certainly descended from the invertebrate oxygen carrying cell or the wandering leucoblast or amoebocyte.

There is certainly no phylogenetic or comparative morphological evidence to warrant one in deriving vascular endothelium, leucocytes, and erythrocytes from a common cell ancestry except, of course, they are all derived from the mesenchyme or same germ-layer.

The fundamental histological study of the early developmental stages of the blood elements in vertebrates was contributed by

Van der Stricht ('94). His studies were especially confined to the mammals. As has often been the case the conclusions reached from this pioneer study are largely correct in the light of recent investigations. Van der Stricht held that the first blood cells arising within the area vasculosa are entirely young red cells, erythroblasts. When one surveys the literature of this subject, it is found that all authors with three or four recent exceptions (Bryce ('05), Dantschakoff ('07) and Maximow '09), hold that the blood islands give rise exclusively to red, haemoglobin bearing corpuscles, erythroblasts or finally erythrocytes. This is true for the Fundulus embryos described in this paper and even though the cells are confined to their place of origin and never flow away, since there is no circulation, yet the groups always consistently contain only erythrocytes.

Van der Stricht holds that the leycoblasts and leucocytes are independent of the erythroblasts and arise extra-vascularly in the mesenchyme and later wander into the vessels.

Browning ('05) and Goodall ('07) have both recently claimed that the leucocytes have a different origin from the erythrocytes and arise at a later period. Goodall states:

> When leucocyte proliferation in the liver has begun, the islands of erythroblasts and leucoblasts are definitely separate in position, and the distinctness of their identity is obvious, and no transitions between them can be seen. These facts argue strongly against the view that the erythroblasts are derived from the primitive leucoblasts.

Jolly and Acuna ('05) have pointed out that in early stages only red cells are found in the blood. The first lymphocytes occur very late and still later the granulocytes, so that the guinea-pig embryo has attained a length of sixteen mm. before white blood corpuscles are present.

Again all authors with few exceptions seem entirely agreed that the leucoblasts arise much later than the erythroblast. All without exception also agree that the leucoblasts arise extra-vascularly while the erythroblasts arise partially within the sinuses, and that the island groups of erythroblasts soon become surrounded by vascular endothelium while no vessel walls have ever been described to form around the groups of leucoblasts. These facts are no doubt of much genetic importance.

The question involved is then: Which is correct, the monophyletic or polyphyletic theory of haematopoesis? It is recognized by all that both propositions are classed only as theory. It must further be recognized that both theories are based at the present time only upon the interpretations of various observers, these interpretations are not necessarily facts. I trust, therefore, that the experiments on Fundulus embryos may add a basis of unquestionable facts which may show the correctness of one or the other of these interpretations.

With this point of view, we may undertake a critical examination of the evidence so ably presented by Maximow ('09) in his study on the mammalian embryo. The observations he construes as strong argument in favor of the monophyletic origin of all types of blood cells and vascular endothelium. This contribution by Maximow ('09) has been accepted by many embryologists *à bras ouverts*, and has been largely incorporated into several recent chapters on the development of the blood, for example, by Schaefer ('12), and Minot ('12).

In the primitive streak stage of the rabbit embryo Maximow states that the peripheral mesoderm in which the blood islands will later occur has in no sense the character of a connected epithelial layer, but consists merely of local accumulations of cells of mesenchymatous type. The cells of this mass are of long thin spindle shape or with star-like processes. These cells are probably much of the same type as the wandering cells seen on the yolk-sac of the Fundulus embryos. In this peripheral mesenchymatous mesoblast the first blood islands arise in the caudal part of the area opaca, as originally described by Van der Stricht. The blood islands are formed from the spindle or branched mesenchyme cells which become associated into groups.

Maximow states that the first endothelial cells like the primary blood cells are also derived from the mesoblast-mesenchyme. With this one may fully agree and several other tissues could be included in the statement as derived from mesenchyme. Maximow, however, goes further and thinks this general source a common specific source. Thus the endothelial cells and blood cells are closely related and arise from a common stem cell in

the blood islands and may continue to arise from such a cell during later development.

Die ersten Endothelien und die ersten Blutzellen sind also beides Mesoblast- resp. Mesenchymzellen. In den Blutinseln sehen wir sie von unseren Augen aus einer gemeinsamen Quelle entstehen. Auch in der späteren Entwicklung werden wir oft Gelegenheit haben, die enge Verwandtschaft dieser beiden Arten von Mesenchymzellen zu beobachten.

This is merely a matter of interpretation and not at all a demonstrated fact. In reply to such a position we must call for an explanation of the demonstrated fact presented on previous pages showing that vascular endothelium forms in a perfectly normal fashion within the heart and head region of embryos without circulating blood, but in no case in early or late stages was the endothelial lining of the aorta or other vessels capable of giving rise to any type of blood corpuscles. Yet the power to form blood corpuscles was abundantly present in the same embryos as shown by the huge numbers of blood cells within the blood forming regions, the intermediate cell mass and yolk islands. Why do not the mesenchyme cells within the liver and all vascular endothelium form blood when no circulating blood reaches them? (If ever, there should then be the stimulus to give rise to its formation).

The red blood cell anlage is a definite mesenchyme cell or group of cells and only members of this cell group possess the blood forming power. To cite a parallel case, the liver cells are derived from the common endodermal cell stock yet not all early endodermal cells, in fact only a few, have the power to develop into a liver, or a pancreas or a lung as the case may be. The embryological argument is indeed rather loose that on account of the fact of vascular endothelium and blood cells arising from mesenchyme would assume, therefore, that these very different cells had a common stem mother cell and later actually possessed some powers of transmutability.

Maximow advances the interpretation that the first blood cells in the area vasculosa are not all erythroblast or future red blood corpuscles. These cells he designates as 'primitive blood

cells' since they may form either white or red corpuscles. Yet in the yolk islands of the Fundulus embryos without circulation only red blood cells, erythrocytes, are produced and they remain in this location to be observed throughout embryonic life. The evidence for Maximow's position seems to me somewhat insufficient.

During the summer of 1914, I had the privilege of examining Mme. Dantschakoff's preparations which both she and Maximow cite in support of the monophyletic theory of blood cell origin. One so inclined might interpret these specimens as showing that the red and white corpuscles do arise from the common stem mother cell. The youngest lymphocytes were invariably scattered among the mesenchymal cells while the erythroblasts were budded off into more or less well defined vessels. No one could emphatically state that the two classes of blood corpuscles had ever actually divided off from any one single mother cell.

The more or less constant separation of the early leucoblasts and erythroblasts, as is also shown in Maximow's figures and those of other workers, would seem to indicate their origins from two different mother cells. If one mother cell only forms or divides off cells which develop into lymphocytes or leucocytes and another mother cell gives rise to only erythroblasts, then there is no reason to say that the two mother cells were the same although they appeared to be two similar mesenchymal cells. They were potentially different, and this potential difference is all that the diphyletic notion of blood cell origin demands. A careful study of the embryos without a blood circulation will demonstrate the fact of this different origin of white and red corpuscles.

Maximow then advocates the last clause in the monophyletic code, and states that the intravascular primitive blood cells are not only increased by mitosis but are also added to by the production of the same kind of cells from the fixed endothelial wall of the primitive vessels. Endothelial cells may wander away into the mesenchyme or may wander into the vessel lumen. One often sees according to Maximow a cell project into the vessel, its body assumes a rounded form and its protoplasm

changes into that of an erythroblast. It must be distinctly remembered that these appearances are in dead stained specimens and many possibilities exist which might explain their occurrence.

Granting that such a phenomenon actually appears to occur there is one very probable explanation without assuming that true vascular endothelium may form blood corpuscles. Let it be supposed, for example, that in the formation of the vascular wall around the yolk-sac blood islands that some of the peripheral cells of the island might lag behind in their differentiation retaining their more or less mesenchymal type. Such a cell may come to be closely pressed against the vascular wall and really appear as though it were one of the vessel wall cells. This might readily happen, and probably does happen, and may account for the occasional appearance of 'vessel wall cell' forming a blood cell.

Why do not the endothelial cells in the experimental embryos possess the power to form blood cells when the vessel is totally empty of blood cells? Even though it is clearly shown that other cells of the embryo do possess the normal blood building power. These specimens are exactly such as should supply definite proof of blood cells arising from endothelium, but the evidence they furnish really disproves the proposition.

Schridde ('07, '08) according to Maximow has gone so far as to claim that in young human embryos endothelium can directly form primitive erythroblasts. Maximow does not agree with this since in his specimens the endothelium gives rise only to indifferent colorless cells. Shridde's claim is based upon misinterpretation and so, I believe, is any claim that blood cells arise from formed vascular endothelium.

Most authors find that in the very early embryonic blood there are no white corpuscles but only red cells present. Bryce ('05) however, describes in Lepidosiren the very early origin of leucoblasts from primitive blood cells, and later Dantschakoff and Maximow find lymphocytes not only in the vascular net of the area vasculosa but also, though at first very few, in the circulating blood. Maximow thinks that when these early

lymphocytes are not seen it is due to poor technique or defective material.

Maximow believes the red blood cells may finally arise from lymphoblasts as erythroblasts, then erythrocytes. This mode of development of the definite erythroblasts continues throughout life and is accomplished in the same manner in all erythropoetic organs. Wherever such indifferent mesenchyme cells, lymphoblasts, are found this locality is eo ipso a new place of origin of erythroblasts out of these colorless stem cells. If this be actually true, why then do not red cells, erythroblasts, finally form all through the body of non-circulating fish embryos, since the wandering lymphocytes surely have the power to reach many places other than the normal sites of erythroblasts formation, the intermediate cell mass and yolk islands?

Maximow claims that both types of blood cells red and white arise at one and the same period from one and the same source, the primitive blood cells in the area vasculosa. The experiments on Teleosts do not bear out such a position since the original or first blood cells from the intermediate cell mass are all erythroblasts and show a characteristic type at a very early time. The blood origin on the area vasculosa is not so extensive, but here also first form only erythroblasts.

Supporters of the polyphyletic origin of blood cells have been able to make equally strong observations in favor of their view on similar material to that studied by Maximow, Dantschakoff and other advocates of the monophyletic theory.

Maximow suggests that since the "primitive blood cell" has no haemoglobin it really stands nearer to the leucocyte than to the erythrocyte, and one might say that the leucocyte arises first in development and the haemoglobin cell later. This is most decidedly not the case in the Teleosts where the primitive mesenchymal blood cell passes directly into the erythroblast without ever showing a stage suggesting either lymphoblast or leucocyte.

With Weidenreich ('05), Maximow takes the position which he had earlier manitained that all non-granular leucocytes and also the wandering cells of the tissues constitute one great cell

group. The position determines the direction of their development and only in certain places does one find all types being formed, for example, in the embryonic liver and the adult bone marrow.

In reply to the extreme monophyletic position it may be asked: Why are only erythrocytes present in the old blood islands on the yolk of non-circulating specimens? Why is no cellular blood element present in the aorta and other endothelial lined vessels in the anterior region of similar embryos? Why are wandering "primitive blood cells" unable to form blood in the liver and other positions while blood forming power is present to a vigorous extent in certain regions of the same embryo but from a definite anlage? Wandering cells in the Teleost embryos have to do with yolk-sac blood origin, but these wandering cells are the equivalent of a part of the peripheral mesoderm and always wander out on to comparable regions of the yolk and never wander to other places within the embryo.

Maximow probably criticizes correctly the many artificial distinctions between various leucocytes which are pointed out by some advocates of the polyphyletic theory. My experiments do not bear on this point up to the present time.

Finally Maximow does not imply that a granular leucocyte or red corpuscle can change into anything else, they cannot undifferentiate. He states that before there is any development of granulation or haemoglobin in two different cells, there is really a difference in the cells though we are unable to distinguish it. This invisible difference determines the destiny of the cell to form either a leucocyte or erythrocyte. This is certainly true, but we cannot stop just at this point; these differences must be carried a step further or really back to their actual beginning. Then it is found that although two wandering mesenchyme cells on the yolk-sac of the fish embryo are indistinguishable so far as our powers of observation go, yet they are fundamentally different since one is destined to form only an erythroblast while the other possesses no such power and can form only an endothelial lining cell or pigment cell as the case may be.

This is all the diphyletic or polyphletic school would ask. That is, that certain definite mesenchymal cells are actually the red blood cell anlage and only from these particular mesenchymal cells do red blood cells arise. Here we logically stop, for this is what is conceived by embryologists to be an anlage, back of this we go to certain germ layers and still further back to the developmental potentialities of certain individual cells as followed in studies of cell-lineage and finally we reach the elementary proposition of deriving everything from the original egg cell. But to stop with the tissue anlage we find strong evidence to indicate that certain special mesenchymal cells are designated to form erythroblasts, others leucoblasts, and still others, and these are much more universally scattered throughout the embryo, give rise to vascular endothelium. These latter may really be admitted to form endothelium largely as a response to physical conditions.

SUMMARY AND CONCLUSIONS

The present contribution attempts an experimental analysis of the origin and development of blood cells and the endothelial lining cells of the vascular system. Studies on the origin of blood and endothelium in the normal embryo are rendered peculiarly difficult on account of the important rôle that wandering mesenchyme cells play in this process as well as the perplexing mixture of cells of different origin brought about by the early established circulation. The origin of no other tissue is so confused by mechanical and physical conditions.

The first difficulty has been met by a study of living Fundulus heteroclitus embryos with the high power binocular microscope. The wandering mesenchyme cells may in this way be followed to a great extent. The disadvantages due to the intermixture of cells in the blood current have been overcome by the investigation of embryos in which a circulation of blood is prevented from taking place.

When Fundulus eggs are treated during early developmental stages with weak solutions of alcohol the resulting embryos in

many cases never establish a blood circulation. In other respects these embryos may be very nearly normal and the development and differentiation of their tissues and organs often proceeds in the usual manner though at a somewhat slower rate. The heart and chief vessels are formed and the blood cells arise and develop in a vigorous fashion. The heart pulsates rythmically but is unable to propel the body fluid since its venous end does not connect with the yolk vessels and in many cases its lumen is partially or completely oblitered by periblastic material and nuclei which seem to be sucked into the heart cavity from the surface of the yolk.

In these embryos without a circulation of the blood one is enabled to study the complete development of the different types of blood corpuscles in the particular regions in which they originate. There is no contamination of the products of a given region through the introduction of foreign cells normally carried in the blood current.

The *actual* haematopoetic value of the different organs and tissues may be determined in the experimental embryos, and clearly distinguished from the ordinary reproduction or multiplication of blood cells which in the normal embryo would reach these organs through the circulation.

The debated question as to the production of blood cells from vascular endothelial cells may be conclusively answered, at least for the species here studied.

The results and conclusions derived from these experiments may be summarized as follows:

1. The Teleost embryo is capable of living and developing in an almost normal fashion without a circulation of its blood. Red blood cells may be seen to arise and differentiate in these living embryos in two definite localities, one within the posterior body region, and the other the blood islands on the yolk-sac.

The blood cells remain confined to their places of origin, yet they attain a typical red color and may persist in an apparently functional condition on the yolk-sac for as long as sixteen or twenty days. The normal embryo becomes free swimming at from twelve to fifteen days, but these individuals without a

circulation never hatch although they may often live for more than thirty days.

All recent investigators have claimed that there are no blood islands present on the Teleostean yolk-sac. Yet the presence of such islands is readily demonstrated in living Fundulus embryos, in normal specimens as well as in those with no circulation.

2. The plasma or fluid in the embryos failing to develop a circulation begins to collect at an early time in the body cavities. The pericardium becomes hugely distended with fluid as well as the lateral coelomic spaces and the Kupffer's vesicle at the posterior end of the embryo. The great distension of the pericardium due to this fluid accumulation pushes the head end of the embryo unusually far away from the surface of the yolk. The heart is thus stretched into a long straight tube or string leading from the ventral surface of the head through the great pericardial cavity to the anterior yolk surface (compare figures 15 to 20).

No blood vessels form on the extreme anterior portion of the yolk-sac, so that the venous end of the heart is never connected with veins, and does not draw fluid into its cavity to be pumped away through the aorta. When the heart cavity does contain fluid it is unable to escape and small floating particles may often be observed rising and falling with the feeble pulsations of the heart.

3. The hearts in embryos without a circulation are lined by a definite endocardium, but the myocardium is poorly developed, sometimes consisting of only a single cell layer. Chromatophores are not present in the wall of the normal heart but in the experimental hearts these large cells ladened with pigment granules are invariably found. The cavity in many of the hearts is almost if not entirely obliterated by the presence of periblastic material and large amorphous periblast nuclei.

The conus end of such hearts leads directly to a more or less closed ventral aorta, portions of the aortic arches are seen in the sections as open spaces, and dorsal aortae are almost invariably seen as typical spaces lined by characteristic embryonic endothelium.

A point of much importance is the fact that *neither these hearts with their endothelial linings nor any portion of the aortae at any stage of development have ever been seen to contain an erythroblast or an erythrocyte.* Cells of this type are completely absent from the anterior region of the embryo.

4. Pigment cells normally occur on the Fundulus yolk-sac and arrange themselves along the vascular net so as to map out the yolk-sac circulation in a striking manner. Loeb has thought that this arrangement along the vessel walls was due to the presence of oxygen carried by the corpuscles within the vessels. In the embryos without a yolk-sac circulation the pigment cells arise but rarely become fully expanded so that the usual long branched processes are represented only by short projections, the chromatophore consequently seems much smaller than usual.

The unexpanded pigment cells, however, wander over the yolk-sac and collect in numbers around the plasma filled spaces. The yolk surface of the pericardium and the periphery of the Kupffer's vesicle are often almost covered with pigment. The hearts are during early stages full of plasma and the pigment cells form a sheath around them, while pigment cells are never present on the normal hearts during the embryonic period.

These facts would seem to indicate that the plasma rather than the erythrocytes contain the substance which attracts the chromatophores and initiates their arrangement along the normal vascular net of the yolk-sac.

5. A definite mass of cells characteristic of the Teleost embryo is located in the posterior half of the body between the notochord and the gut and extends well into the tail region. This so-called 'intermediate cell mass' is the intra-embryonic red blood cell anlage in many of the species.

The peripheral cells of the mass as claimed by Swaen and Brachet or the mesenchyme about the mass, Sobotta, forms a vascular endothelium which encloses the central early erythroblasts.

In individuals without a circulation the erythroblasts arise in a normal manner in this centrally located position, and be-

come erythrocytes filled with haemoglobin. Typical vascular endothelium completely surrounds the erythrocytes which instead of being swept away as usual by the circulating current remain in their place of origin. All of the early blood forming cells of this intermediate mass give rise only to erythroblasts.

6. Contrary to the opinion of most recent observers on blood development in Teleosts, the Fundulus embryos both with and without a circulation possess blood islands on the posterior and ventral portions of the yolk-sac. These blood islands are formed by wandering mesenchymal cells which migrate out from the posterior region of the embryo. They represent all that remains of the peripheral yolk-sac mesoderm in the Teleosts and probably wander way from mesoderm related to that of the intermediate cell mass. The intermediate cell mass may possibly represent the bulk of the peripheral mesoderm which is here included within the embryonic body, while in other meroblastic eggs it is spread out over the yolk. The only mesodermal portion of the yolk-sac in Fundulus is made up of the disconnected wandering mesenchyme cells some of which group themselves to form the blood islands, while others give rise to the yolk vessel endothelium, and still other wandering cells develop into the chromatophores.

7. The non-circulating red-blood corpuscles within the embryo remain in a fully developed condition for eight or ten days and then undergo degeneration. In an old embryo of sixteen days it is sometimes found that very few of the corpuscles in the intermediate mass are still present and these are degenerate. The vascular endothelium has been lost and numerous mesenchymal cells have wandered in and lie among the corpuscles.

On the yolk-sac the corpuscles no doubt have a better oxygen supply and here they maintain their color longer but finally also present a degenerate appearance with small densely staining nuclei and cell bodies much reduced in size.

8. Vascular endothelium arises in loco in many parts of the embryonic body in which blood cell anlagen are not present. This endothelium is in all cases utterly incapable of giving rise to any type of blood cell. This incapacity cannot be attributed to the abnormal condition of the embryo as true blood cell anlagen in the same specimen produce blood corpuscles in abundance.

Vascular endothelium in the fish embryo has no haematopoetic function.

9. Neither lymphocytes nor leucocytes have been found to arise in the yolk-sac blood islands nor within the intermediate cell mass.

The embryonic white blood cells are most abundant in the anterior body and head regions, and these cells occupy extravascular positions usually lying among the mesenchymal cells.

The sources of origin of the white and red blood corpuscles in Fundulus embryos are distinct, and these two different types of cells cannot be considered to have a monophyletic origin except in so far as both arise from mesenchymal cells.

The adult blood of Fundulus contains lymphocytes and several varieties of granular leucocytes.

10. There is evidence to indicate that definite environmental conditions are necessary for blood cell proliferation or multiplication. Blood cells do not normally divide when completely enclosed by vascular endothelium. This is the key to the shifting series of so-called haematopoetic organs found during embryonic development.

Erythroblasts lying about spaces unenclosed by vascular endothelium proliferate steadily and give off their products into the spaces from which they find their way into the embryonic vessels. Should such an erythroblast be carried by the circulation to another unlined space it may become arrested there and again undergo a series of divisions giving rise to other erythroblasts. When, however, these spaces become lined by endothelium the blood cell reproduction stops.

It most embryos the earliest blood cell formation occurs in the yolk-sac blood islands. The cells in these islands continue to divide until they become surrounded by endothelium, then the yolk-sac blood islands lose their haematopoetic function and become a vascular net through which the blood circulates. The liver now takes up the rôle of harboring dividing blood cells within its tissue spaces, when these spaces become vascularized by endothelium, here again the blood cells no longer multiply but merely circulate.

Finally, in the mammalian embryo, one organ after another ceases to offer the necessary harbor for dividing blood cells until the red bone marrow is the only tissue presenting the proper relationship of spaces and vessels, and here alone the erythropoetic function exists to supply the red blood cells for the entire body circulation. The red blood corpuscles are always produced so as to be delivered into the vessels and thus very soon occupy an intra-vascular position, while the white blood cells arise and remain for some time among the mesenchymal tissue cells in an extra-vascular position.

11. Lymphocytes and leucocytes along with the invertebrate amoebocytes are all generalized more or less primitive wandering cells, and are almost universally distributed throughout the metazoa.

Erythrocytes are very highly specialized cells with a peculiar oxygen carrying function due to their haemoglobin content. In contrast to the universal distribution of the leucocytes the erythrocytes are only found in the vertebrate phylum, except for a few cases existing in some of the higher invertebrate groups. Yet even in these particular cases the oxygen carrying blood cell never presents the typically uniform appearance of the vertebrate erythrocyte. The oxygen carrying function in invertebrates is usually confined to the liquid plasma.

Typical vascular endothelium is widely distributed in the animal kingdom and appears to be formed from a simple slightly modified mesenchymal cell.

These three very different types of cells all seem to arise from mesoderm—the mesenchyme. Yet the present investigation would indicate that each arises from a distinctly different mesenchymal anlage.

The erythrocyte anlage is localized and perfectly consistent in the quality of its production.

The lymphocyte and leucocyte anlage is more diffusely arranged and not definitely localized in any particular cell group.

The vascular endothelium appears to be formed in loco in almost all parts of the embryonic body, and its formation is absolutely independent of a circulating fluid or the presence of blood cells.

The facts presented seem to indicate that vascular endothelium, erythrocytes and leucocytes although all arise from mesenchyme are really polyphyletic in origin: that is, each has a different mesenchymal anlage. To make the meaning absolutely clear, I consider the origin of the liver and pancreas cells a parallel case both arise from endoderm but each is formed by a distinctly different endodermal anlage, and if one of these two anlagen is destroyed, the other is powerless to replace its product.

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THE DEVELOPMENT OF THE HUMAN PHARYNX

I. THE PHARYNGEAL DERIVATIVES

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THIRTY-FOUR FIGURES (FIVE PLATES)

CONTENTS

INTRODUCTION

The following study began as an examination of the transformation of the second branchial or pharyngeal pouch.[1] Of the branchial pouches that appear in the development of the embryonic pharynx of man, the second, and the second alone,

[1] The problem was suggested to the writer by the late Dr. Charles S. Minot, and was begun in his laboratory during a sabbatic leave in 1911. It is a pleasure to acknowledge the generous help from that laboratory, and particularly the kindly interest and suggestions of Dr. Minot and Dr. F. T. Lewis.

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possesses rather negative characteristics. While the one cephalad of it, the first or hyomandibular, develops into the middle ear and eustachian tube (tuba auditiva) and those caudad of it have the very interesting derivatives—thymus, parathyreoids, so-called lateral thyreoid, etc.—the second pouch leaves no clear and characteristic record in the bodily structure, save perhaps the palatine tonsils, which appear to mark its site and to whose appearance this branchial pocket may possibly bear some causal relation.

It is true that the second pharyngeal pouch gives rise to a thymus body in anurous amphibia, among fishes, and according to de Meuron, ('86) Van Bemmeln ('86), and K. Peter ('01), in the lizard; and that a parathyreoid or epithelial body is said by Van Bemmeln to develop from it in snakes; while Maurer's claim from the conditions found by him in Echidna and Anura that the carotid body is, or represents, such an epithelial body II (parathyreoid II), is likewise a matter of record. Such findings in lower forms but naturally suggest the presence of at least potential thymus and parathyreoid elements in the second pouch as well as in the third (and fourth). Critical and careful examination of the abundant material in the way of human and higher mammal embryos in the past has failed to determine the presence of traces of such structures belonging to the second pouch (p. 360), and I may say at this point that careful examination by myself of the quite complete series of human embryos used in this study but confirms the negative findings of other workers.

It would be but natural, however, in spite of negative evidence, to consider that these structures, as branchiomeric organs, were nevertheless potentially present. Particularly since the classical study by Verdun ('98) these organs are frequently thought of as branchiomeric organs, there being a thymus and a parathyreoid (epithelial body) component belonging to each branchial pouch. Such a conception would of necessity assume that these structures, as such, were in some way intrinsic in the cells of the pharyngeal entoderm metamerically arranged. Quite a different interpretation, however, is possible. The second pouch, unlike the

first, third and fourth, early becomes 'spread out' or flattened out with but shallow depressions representing it (figures 18–21). This seems clearly to be due to the forward growth and widening of this portion of the pharynx associated with the growth of that portion of the head. It might therefore be possible that the 'negative' character of the second pouch is a result of its growth relations; that what became of a pouch or what came out of it was largely a factor of its position; that the determining factors for the 'branchiomeric' organs might be extrinsic rather than intrinsic. My study had not proceeded far before it became evident that, when ultimately interpreted, the entodermic pharynx and its derivatives would have to do considered in conjunction with the growth transformations of the entire region, taking full cognizance of the shiftings due to unequal growth.

One of the most alluring features of the region is its ancestral character, which is involved in the question of its interpretation and significance. The fact that the mammalian embryo possesses gill clefts, pouches and arches, though never possessing branchial respiration, has been known since the first observations of the mammalian embryo; while the occurrence has been prominently cited as evidence of descent, as one of the most striking illustrations of the 'Biogenetic law' of Haeckel, of vestigial organs, etc. The present-day attitude, it is true, is inclined to be one of scepticism as to so-called vestigial organs; the recapitulation theory is pronounced a failure in furnishing broad interpretations, while the validity of the 'law' is questioned.

Sedgwick ('09) in an interesting essay challenges the validity of the 'Recapitulation theory':

> The question at issue is: did the pharyngeal arches and clefts of mammalian embryos ever discharge a branchial function in an adult ancestor of the Mammalia? We cannot therefore without begging the question at issue in the grossest manner apply to them the term 'gill-arches' and 'gill-clefts.' That they are homologous with the 'gill-arches' and 'gill-clefts' of fishes is true; but there is no evidence to show that they ever discharged a branchial function. The recapitulation theory originated as a deduction from the evolution theory and as a deduction it still remains.

Karl Peter ('10) critically examines the biogenetic law and seems to reject it. He explains the development and persistence of the branchial pouches as due to the middle ear and the ductless glands that develop out of them, but in Lacerta, which he chooses as an example, he is compelled to confess that this point of view hardly suffices as an explanation of the development of the fourth pouch, fifth pouch, and the sixth pouch (his interpretation) on one side. Kranichfeld ('14) looks at the problem in a different way. His contention is that the embryonic pharynx is not a 'vestigial' structure but possesses throughout a function correlated with the embryo's nutrition (metabolism) and hence it is but to be expected that glands having important metabolic functions should develop from it since their function is but a continuation of a primary one possessed by the entire pharyngeal epithelium. These criticisms do not seem to me to weaken the general validity of the biogenetic law but rather to strengthen it, inasmuch as they must of very necessity concede that the pharyngeal pouches, clefts and arches, *do* exist and are homologous with the gill pouches, clefts and arches of lower forms. It becomes possibly a matter of definition, as is so often the case. If interpreted to mean that there are in the development of the individual definite stages corresponding to definite ancestral type forms, the 'law' does not of course hold (Keibel '98); but as the formulation of a fundamental element in the morphological pattern of development, which is only comprehensible in the light of descent, the biogenetic law cannot be escaped. It is, I believe, but a special aspect of a more fundamental principle of life processes, correlated with their cyclical character and including 'heredity,' which may be designated somewhat loosely and metaphorically perhaps as the 'Principle of ancestral resemblance.' It is not the intention to enter into a detailed discussion of the 'biogenetic law,' but as to disappointment that the biogenetic law has not afforded much in the way of explanation of biological facts, it may be stated that it stands for fact and is itself subject to explanation.

The fact remains that the morphology of a large part of the face and neck are comprehensible only on a clear comprehension

of the transformations of this region, whose morphology reflects its primitive character as a respiratory apparatus. For the skeletal elements of the region, the transformation history is nearly though not completely clear; for the nerves, largely so; for the musculature, less satisfactorily worked out. As to the epithelial elements and the vascular elements, which are of course the dominant tissues in the branchial respiratory apparatus in lower forms, our knowledge is exceedingly crude; it covers only the simplest morphology, while more recondite characteristics that the constituent elements may possess are unknown.

From the references to the views of Peter and Kranichfeld in the foregoing brief comment on the branchial pharynx and the 'biogenetic law,' two views of the relation of embryonic pharynx and 'pharyngeal derivative' are apparent—association in development and fundamental genetic unity. It has been intimated in the preceding paragraphs of this article that a different interpretation may be advanced. This will be considered subsequently. At this point, I will venture to state only that the biological significance and physiological effect of the pharyngeal derivatives—thyreoid, thymus, and parathyreoids—are wrapt up in the past history of the region and ultimately explicable only in the light of their origin. This postulate I conceive as applicable not merely to the structures in question, but to all organs and all development.

The general aspects of the problem of the embryonic pharynx as outlined above determined my attitude in the study of its developmental changes. I have furthermore, in attempting to follow the growth transformations and shiftings, endeavored to keep in mind, as far as possible, the original character of the region and the fact that as far as the branchial epithelium and vascular elements are concerned, it is a region whose primary adaptative value is lost.

While attention has been largely limited to the epithelium, it became apparent that the epithelium could not be interpreted entirely by itself; that its growth transformations were but a part of the growth changes in the region as a whole. It has ever been the aim to keep the ultimate explanation of the mor-

phological transformations in mind, even where they cannot be directly analyzed, since only through an analysis of the developmental factors will the morphogenesis be finally comprehensible. Accordingly, it is felt that the emphasis should be fixed differently from where it is ordinarily placed; not that organs develop in and from such and such places; but that organs owe their character to their origin. Expressed differently: that the body is not an aggregate of distinct organs, but that the body is a unit whose different portions are variously differentiated (as organs) the character of the differentiation depending upon the way the adaptation is met, determined by the past history.

Historical

So recently has the development of the human pharynx been considered in a general article (Grosser '11 b) that a review of the literature dealing with the subject is unnecessary. The main framework of morphologic fact was then quite complete, due to the studies of His ('80–'85), Verdun ('98), Hammar ('01), Sudler ('02), Tandler ('09), Grosser ('11 a) and others; and it has been added to since then (Hammar '11). Many details, however, remain undetermined and the interpretational aspect of the development is still largely a controversial field. In the last, the point of view is frequently a dominant factor, and hence the following study seemed quite appropriate. Confirmation of previous work has also a distinct value, and in the main my results are confirmatiory of those of Hammar and Grosser.

Nomenclature

There exists by no means uniformity in the terminology of the pharyngeal region. This perhaps is to be expected in view of the great transformations that the region undergoes and the differences in interpretation. Hammar ('13) has specifically dealt with the question of the nomenclature of the region and his suggested terms are excellent, although some of them are clearly unsatisfactory, the term ductus being an example. Most of the

terms here used are those employed by Grosser. Branchial is employed in preference to pharyngeal, pouch (sacculus) is used for the entodermic outpocketing; while cleft (fissura) is employed to designate the ectodermal insinking. Each pouch cleft, arch, parathyreoid, thymus, etc., in the series, is designated by number (I–V). The term 'Complex III' includes the structures appearing in the transformation of the third pouch, while Complex IV or 'caudal pharyngeal complex' denotes the structures associated with the fourth pouch. As the complex, in the process of growth, becomes separated from the pharynx, the attenuated connection is designated as 'ductus pharyngeobranchialis' (III, IV), while the corresponding diminishing connection with the ectoderm is termed 'ductus branchialis' (II, IV), the 'ductus cervicalis' being the connection of the cervical vesicle with the ectoderm. 'Parathyreoid' is employed in preference to 'epithelial body.'

Material and methods

The study was begun in 1911 in the Department of Comparative Anatomy of the Harvard Medical School and continued at Ithaca, New York, and is based upon the human embryos in the embryological collection at the Harvard Medical School, supplemented by those in the collection at Cornell University, many of which were placed at my disposal by Prof. S. H. Gage, whose generous assistance I am glad to acknowledge. My studies were futher greatly assisted by the willingness of Prof. Minot and Prof. Lewis to loan me from time to time embryological series from the Harvard collection. The list of embryos consulted is given in table 1. The ones more particularly studied include those modelled, namely, 3 mm., 5 mm., 7.5 mm., 9.4 mm., (2) 10.0 mm., 13.0 mm., 14.5 mm., 16.4 mm., 18.2 mm.

MORPHOLOGICAL PLAN OF THE PHARYNX

In considering the developmental changes in such a region as the pharynx, from the viewpoint of its ancestral character as a branchial chamber, it is of obvious importance to obtain

TABLE 1

MEASUREMENT IN MILLIMETERS		COLLECTION NO.	MEASUREMENT IN MILLIMETERS		COLLECTION NO.
3.0	Cornell	31	19.0	Cornell	3
4.0	Harvard	714	19.3	Harvard	1597
5.0	Cornell	5	19.7	Cornell	30
6.25	Harvard	1918	21.0	Harvard	744
7.5		256	22.0		851
8.3	Cornell	59	22.8		737
9.2	Harvard	734	22.8		871
8.0		817	23.0		181
9.4		529	23.0	Cornell	48
9.4		1005	25.0		29
9.6		1001	25.0		64
10.0		1000	25.6		12
10.0		1919	28.8	Harvard	1598
10.2		736	29.0		914
13.0	Cornell	26	30.0		913
11.5	Harvard	189	31.0		1706
12.0		816	31.5	Cornell	62
12.0	Cornell	34	32.0		63
13.5		52	35.0	Mall ✱3*	
13.6	Harvard	939	35.0	Cornell	44
14.5		1003	36.0		61
15.0	Cornell	53	37.0	Harvard	820
15.0		12	40.0		1917
16.0	Harvard	1322	41.0	Mall ✱1*	
16.0		1128	42.0	Cornell	58
16.4		1707	44.0		49
18.0	Cornell	57	44.0	Mall ✱2*	
18.2	Harvard	1913	44.3	Harvard	1611
19.0		819	48.0	Cornell	13
19.0		828			

*Three embryos that had been loaned to Prof. S. H. Gage by Prof. F. P. Mall were kindly placed at my disposal as well. They were examined for the stage of development of the thyreoid and number and position of the parathyreoids, and are therefore included in the above list.

a clear conception of the fundamental morphology of the region; the primitive and typical plan which is more or less modified and departed from in the particular form considered. In the case of the human pharynx the attempt at once carries the investigator into controverted ground and brings him face to face with the problem of primitive chordate types and the 'Ancestry

of the vertebrates.' In the search for the primitive type of pharynx one must pass over the higher forms and teleostomes to the elasmobranchs, cyclostomata (larval lamprey), Amphioxus and the ascidians, which for a number of reasons must be considered the more primitive of living chordate forms. There we find, in addition to the lateral branchial region with the gill pouches, well-definied hypobranchial and epibranchial (hyperbranchial) longitudinal zones, each with characteristic ciliated grooves—(or ridges)—the hypobranchial and epibranchial grooves (Acanthias, larval lamprey)—which again are comparable with the corresponding grooves of Amphioxus and the ascidians. I shall therefore consider the primitive pharynx as consisting morphologically of four zones: the hypobranchial region or zone, the 'floor'; the epi (hyper) branchial region or zone (the roof), and the lateral wall or branchial region proper. As to the number of branchial pockets which the primitive pharynx possessed, there is no way of estimating, nor is the point important in the present connection; there would seem to have been at least eight. While the terms 'hypobranchial' and 'epibranchial' are subject to criticism, particularly the latter, they nevertheless are defensible and the former has long been used—as, hypobranchial groove, hypobranchial skeleton, hypobranchial musculature, etc.

In considering the fundamental morphological relations of the region we encounter also considerable difficulty in their precise determination. In the first instance, this applies to an exact delimitation of the pharynx caudally, where the pharynx passes more or less insensibly into the esophagus. In the hypobranchial region the fundamental plan of the vascular relations is quite characteristic. While the heart appears in all cases to occupy a position caudad of the pharyngeal region (infrapharyngeal), the truncus aorticus is hypobranchial and its bifurcation well forward—as judged by the characteristic relation to the thryeoid gland, at the level of the second arch, although from comparative observations it is clear that the position of the bifurcation may vary considerably in the adult.

While the heart seems infrapharyngeal, the pericardial mesothelium is joined to the mesodermal cords of the branchial

arches, within which branchial coelomic cavities may exist in a number of vertebrates (e.g., elastobranchs, turtles) and may appear occasionally in mammals (Froriep, Zimmermann, myself). In some instances these branchial coelomic cavities are actually or potentially in communication with the pericardial coelom.

In man and mammals, a number of developmental features serve to modify the more primitive relations of the pharyngeal region. The precocious development of the lungs thus profoundly alters the developmental pattern by arising early in the development of the region. If not infrapharyngeal (postpharyngeal) from their mode of development in the amphibia, they represent the most caudal (7th ?) branchial pouches. Due to the precocity of their development, the 'intrusion' of the tracheo-pulmonary anlage within the floor of the pharynx is a most marked feature. The early and marked development of the heart correlated with the placental circulation may also be mentioned as altering seemingly in a mechanical way the simpler morphological relations of the region.

A third factor which modifies the plan of development of the mammalian pharynx is the tongue. Despite the recent statement by Lewis ('10) that the hypoglossal musculature develops from mesenchyme *in situ*, it seems highly probable although not yet actually demonstrated—that the myoblasts are directly derived from the first three (or four) myotomes and occupy secondarily the hypobranchial region; differently put, that the pharyngeal region is primarily cephalad of this musculature and in its developmental differentiation and expansion caudally there is a mutual shifting which brings about the characteristic intrusion of the hypoglossal musculature and the development of the tongue. The course and relations of the hypoglassal nerve have thus a marked morphological value. Inasmuch as a hypobranchial musculature is present throughout vertebrates, beginning with the elasmobranchs—the morphological relations in the cyclostomes being somewhat problematic—it is clear that in this respect other vertebrates as well as mammals illustrate a departure from the primitive pharynx morphology. How-

ever interpreted, the tongue early complicates the development of the pharynx.

Finally there must be mentioned the evident and marked modification of the dorsal region (epi-hyperbranchial) of the pharynx by the growth of that portion of the head as epitomized by the brain-tube, whose growth in length and bendings has clearly been accompanied by corresponding effects on the adjacent pharyngeal structures. The expansion dorsally, as contrasted with the ventral restriction of the pharyngeal region, is expressed in the arrangment and relations of the external gill clefts, as illustrated in any typical lateral view of a mammalian embryo.

DEVELOPMENTAL TRANSFORMATIONS OF THE HUMAN PHARYNX

From the above brief considerations it will be apparent that the pharynx of mammals (and man) departs widely from the primitive conditions and relations that the region must have presented in the vertebrate ancestor. In the examination of the growth transformations and shiftings it has thus been necessary to keep in mind the fundamental morphology of the region, since it constitutes the basis to which the growth transformations must be referred.

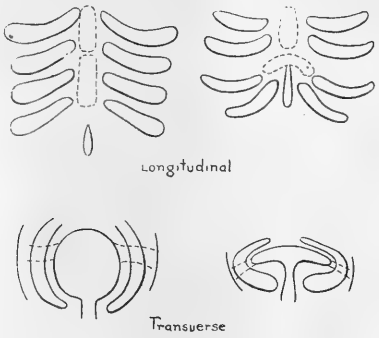
The different growth relations of the cephalic portion of the pharynx and the caudal portion of the pharynx (well illustrated in the figures of plates 1 to 4, particularly 14, 15 and 21), the former being characterized by its expansion and participation in the growth of the head, the latter by its concentration and ventro-caudal growth, soon differentiates the pharynx into two fairly well defined regions, for which Mayr ('12) has proposed the names of 'propharynx' and 'metapharynx' ('laryngopharynx') respectively. The investigation of the growth shiftings in the domain of the pharynx thus falls quite naturally into three parts: (1) that of the dorsal wall and cephalic portion, including the first (and second) branchial arches and the first and second pouches; (2) that of the tongue and the corresponding portions of the pharyngeal floor; (3) the caudal and ventral portions in-

cluding the third and fourth pouches and clefts and the second cleft, together with the thyreoid gland. It is mainly the last section of the pharynx development which is considered in the present paper. While it is the author's plan to consider at a subsequent time some of the growth changes in the territory first mentioned, no attempt is contemplated to add to the knowledge or interpretation of the tongue, inasmuch as it is felt that there is not a great deal to add to the published work of Kallius and others, save in the earliest transformations, material for which is not at hand or at present easily available.

Closely interwoven with the developmental changes which the lower portion of the pharynx undergoes, are (1) the development of the lungs (larynx and trachea) and (2) the development of the heart and aortic arches, mentioned above as of modifying influence in the development of the pharynx. To these should be added a third modificatory factor, the epibrancial placodes; while the neck-bend as an expression of cranial growth relationships and similarly, to a certain degree, the different postpharyngeal growth tendencies, affect the morphogenesis of the region.

The precocity in the development of the lungs has already been referred to. In a 2.5 mm. embryo (Robert Meyer, No. 300) Grosser ('11 a, '11 b) has described its first appearance as well caudad or the branchial region at a stage in which the laryngo-tracheal groove extends to the caudal limit of the 'mesobranchial field.' With the subsequent development of the glottideal opening and of the branchial region, the laryngo-tracheal groove is described by Grosser (p. 463) as "extending farther into the mesobranchial field between the medial ends of the fourth and (later) even the third branchial arches." This intrusion of the laryngeal opening into the branchial region of the pharynx might, I believe, be better described as essentially a 'telescoping' or intussusception, due to the expansive cephalocaudal differential growth of the branchial pharynx and the precocious development of the lungs (trachea and larynx), in the nature of a heterochronia. The attendant alteration in the morphology of the region may be illustrated by means of

the accompanying simple diagrams (schema A). Such an interpretation was essentially set forth by His ('85), as would appear from his well-known view of the origin of the so-called 'lateral thyreoid.' However one is inclined to interpret the origin of the lungs—as primarily developed out of and representing bran-



Schema A

chial pouches and hence primitively double in origin, or as arising caudad of the branchial region and appearing as a medial ventral outpocketing of the pharyngeal entoderm caudad of the branchial region—its morphological position is caudal to the branchial portion of the pharynx as developed in the higher vertebrates. The position of the laryngeal opening in the pharynx is in the nature of an intrusion, due in part, as has already been stated, to its precocious appearance, affecting thus markedly the essential morphology of the developing pharynx. It is not, I believe, going too far to interpret the 'furcula' described by His ('85) and shown in his well-known and often reproduced figures (40) as itself but a fold due to the interrelated growth of glottideal lips and branchial pharynx and in itself, therefore, of no intrinsic morphologic significance.

How early the tracheo-pulmonary outpocketing makes its appearance it is difficult to determine. Grosser's ('11) account is the most satisfactory, although clearly other embryos of about this stage (Robert Meyer embryo No. 300) and earlier should be examined. With the mid-ventral groove (ventral pharyn-

geal furrow by Grosser) figured by him before the definite appearance of the thyreoid outpocketing (in embryo Keb., N. T. No. 3) it apparently has nothing to do. This groove, which appears thus early extending caudally from the thyreoid whose early anlage it includes, may tentatively be regarded as a hypobranchial groove, in its cephalic part at least. Subsequently with the expansion of the pharynx and the growth of the neighboring heart and pericardium, it becomes obliterated.

With the expansive development of the heart within the pricardium, the branchial arches and truncus aorticus, begin the important and characteristic transformations of the pharynx, ending only with the assumption of its adult morphology. It is generally assumed that the branchial pouches possess ventral and dorsal extensions or diverticula. Ventral diverticula are shown for several of the pouches in figures 12, and 13, while small dorsal projections exist in the 7.5 mm. embryo (figure 14). Although these are usually considered as branchial extensions of intrinsic significance, careful study of their relations indicates that in their shape and extent they are also a partial expression of the growth relations of the region and are affected by the adjacent arches whose size and direction they in a degree indicate; this readily appears in models such as those represented in figures 12, 13 and 14, in which the impressions of the arches, as negative pictures, are shown in the molding of the epithelium.

In figures 12 and 13, which might be compared with similar figures, such as figure 22 of Grosser ('11 a) and figure 7 of Ingalls ('07) and figure 6 of Coulter ('09) for the cat, there is shown the 'thyreoidipetal' direction of the ventral branchial diverticula I, II, III and IV. This direction of the pockets of course is also in conformity with that of the arches. As important constituents of the arches are the branchial aortic arches, which arise from the truncus aorticus, so that the region where the axes of these ventral branchial diverticula center might be described with equal propriety as being that of the truncus aorticus or its bifurcation (cf. also figures 1, 2. 3). In front of (cephalad of) the bifurcatio trunci aortici and in close apposition to it is the thyreoid gland. In embryos of 5 to 7 mm. length

the branchial pharynx attains its most marked development. The transformations that speedily succeed are marked by the growth shiftings, whose most striking and central features center around about the descent of the heart[2] the influence of which on the subsequent development of the pharynx is striking.

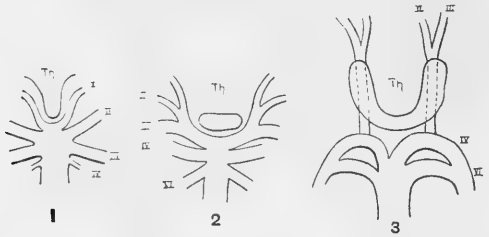
Thyreoid

In the 3 mm. embryo—the youngest of the series studied—the thyreoid gland is present as a median outpocketing, still broadly in communication with the caudal limb of the first branchial pouch and in immediate contact with the truncus aorticus. The first (mandibular) aortic arch is disappearing, the second and third continuous to the dorsal aorta, while the fourth (apparently) has not yet become completely developed. In the 5 mm. embryo the thyreoid is somewhat elongated transversely and is close to the aortic trunk bifurcation, which has 'moved' caudally somewhat in the process of growth. The bifurcation has now a somewhat different morphological value as compared with the earlier stage. The second aortic arch has 'moved out' along the third arch, while the first arch has apparently 'moved out' along the second, the first arch seemingly having lost its connection with the dorsal aorta. As at some points these vessels were collapsed, it was somewhat difficult to determine their exact extent. The condition appears very similar to that in the His embryo R (figure 120). The apparent migrations of the first and second arches are undoubtedly correlated with the growth of the region as a whole. The anterior portion of the pharynx, including the first and second arches, expands in a 'cephalic' direction, and the growth of these bilateral moieties carries the arches with them, forming thus the so-called ventral aortae. The median structures (thyreoid, truncus, heart, and the pericardial cavity) do not share in the forward growth, and there is thus instituted the 'descent of the heart.' The neck and thoracic wall, in completely burying the heart and blood

[2] In the following pages the term 'descent of the heart' is, for convenience, frequently used and represents a growth shifting that has, of course, many sides. It is used without the intention of giving this side of it undue emphasis.

vessels, gives to the 'descent' of the heart somewhat the character of a growth funnel or eddy. This analogy is not without its force in comprehending the growth transformations of the branchial region, as will be seen subsequently.

The vessels which have appeared in the process of growth that has carried the second arch forward may even at this stage be considered as the common carotid arteries, while the common trunk for the first and second arches is the external carotid. At this stage the common carotid artery springs rather directly from the aortic trunk forming the bifurcation. Diagrams 1 and 2 in the accompanying schema B may illustrate the general



Schema B

morphological relations of thyreoid and arches at this stage. Continuance of lateral growth, together with relative caudal displacement of the truncus, increases the length of the common carotid artery and causes it in its turn to move out along the fourth aortic arch to its permanent position.

The thyreoid gland in its expansion shares in the lateral 'upward' growth and median 'down sinking;' it then becomes transformed into a U, already marking out the lateral lobes and isthmus of the adult organ, the isthmic portion still being close to the aortic bifurcation, while the lateral lobes are molded about the common carotid arteries upon their medial and ventral sides, as they have plastically followed them in their lengthening (figs. 2, 4, 6, 7, 15, 18). With the lateral growth of the thyreoid the descent of the gland as a whole becomes arrested; it lags behind the heart and truncus, altering its relative position but little in subsequent development. The growth of the ventral neck

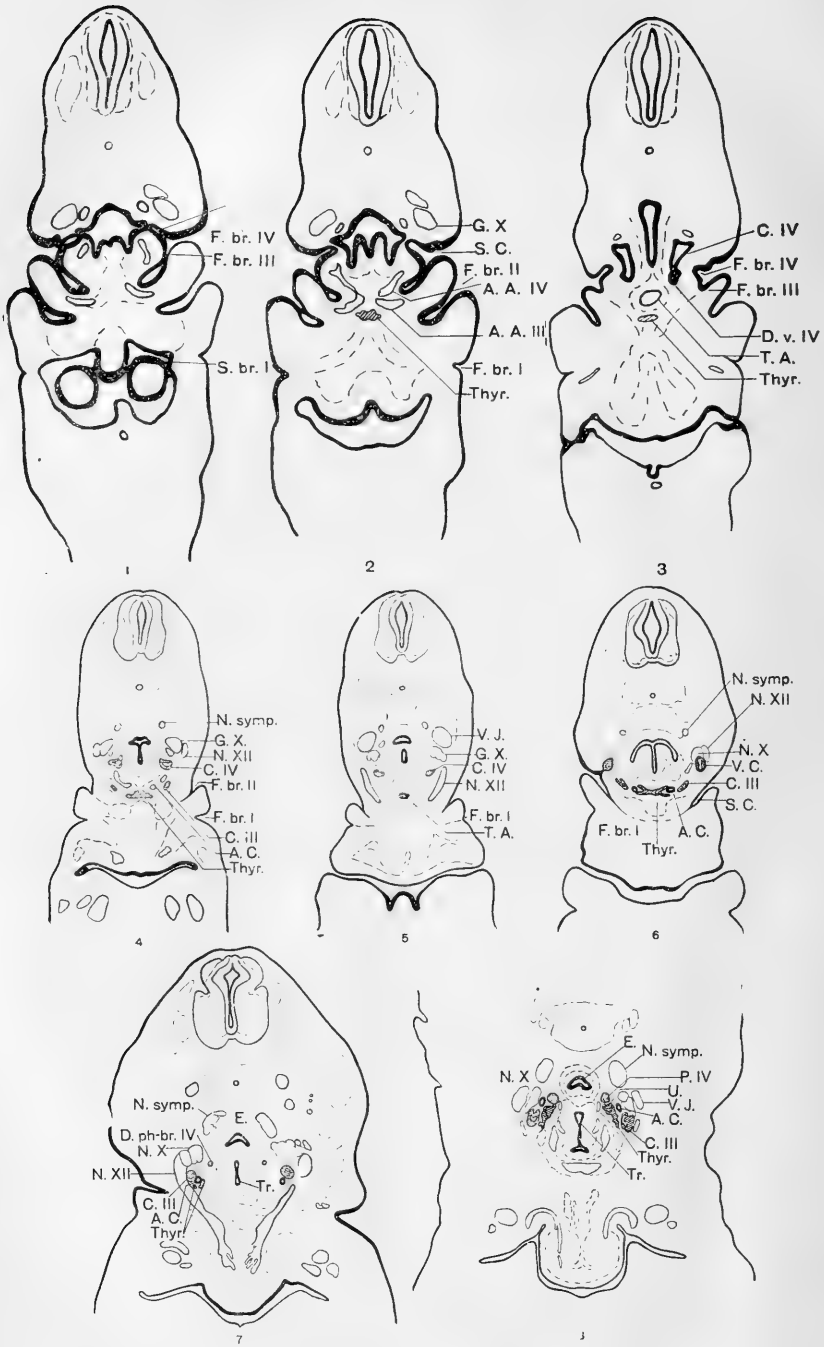
material, and of the larynx particularly, soon removes the thyreoid from the intimate contact with the carotid arteries (18.2 mm.). Its adult morphology is then speedily assumed. Diagram 3 of schema B may illustrate the third stage in its growth transformation, based on the conditions in the 13. mm embryo (fig. 20), and comparable with the well-known figures of Verdun ('98, pl. 1).

Complex III

The above paragraphs clearly show the influence of the unequal growth and growth movements summed up as the descent of the heart on the development of the thyroid, which from its first appearance follows the aortic bifurcation. The third branchial pouch is also drawn into the 'growth eddy' caused by the descent of the heart.

As has just been said, the branchial pouches are directed toward the aortic bifurcation (figs. 12–13), this arrangement being clearly due to the arrangement of the branchial arches. From their mode of appearance—each pouch following in development the one cephalad of it and therefore lagging behind it in extent and size—the more cephalic pouches and arches tend to overlap those more caudally located unless the relations are disturbed by unequal growth. This arrangement appears in figures 12, 13, and 1, 2. In the case of the first two pouches this relation is inappreciable, or speedily lost. In the growth of the head the propharynx including these pouches is carried forward away from the mid-ventral or cardiac region. The third pouch is thus not only morphologically cephalad of, but also laterad to, the fourth pouch. It also occupies a position of peculiar interest in the series of branchial pouches in its relation between the third and fourth arches, it is at first (morphologically) caudad to the common carotid artery. As the aortic truncus and the thyreoid descend the third pouch soon becomes lateral to these structures as they move by, and to the common carotid. This position is shown in figures 4, 7, 8, 15 and 20.

As the truncus descends the third pouch follows it in its growth movement. Drawn, so to speak, into the heart eddy, growing


F. br. IV
F. br. III
S. br. I
1
G. X
S. C.
F. br. II
A. A. IV
A. A. III
F. br. I
Thyr.
2
C. IV
F. br. IV
F. br. III
D. v. IV
T. A.
Thyr.
3
N. symp.
G. X.
N. XII
C. IV
F. br. II
F. br. I
C. III
A. C.
Thyr.
4
V. J.
G. X.
C. IV
N. XII
F. br. I
T. A.
5
N. symp.
N. XII
N. X
V. C.
C. III
S. C.
F. br. I
A. C.
Thyr.
6
N. symp.
E.
D. ph-br. IV
N. X
N. XII
C. III
A. C.
Thyr.
Tr.
7
E.
N. symp.
N. X
P. IV
U.
V. J.
A. C.
C. III
Thyr.
Tr.
8

as the heart descends, the epithelial tube representing the ventral diverticulum of the third pouch becomes more ventral in position, the material from the pouches of the two sides finally meeting upon the ventral side of the vascular funnel, where cephalad of the pericardium in the anterior mediastinum it becomes the thymus gland (so-called) which will be considered subsequently. For illustration of the above, figures 15, 18, 20 and 22 may be consulted, as well as figures 1, 2, 4, 6, 7 and 8, which will give the relative location of the third pouch territory in succeeding stages.

Hammar ('11) has already given an excellent description of the growth and morphological differentiation of the thymus and the Complex III, so that it is necessary to offer only a few comments upon the transformations of the pouch as a whole, in its connection with the growth shiftings accompanying the descent of the heart. As is well known from the work of Hammar and others, the growth of the third pouch that accompanies the descent does not keep pace with it, with the results: (a) that the complex as a whole moves down and the epithelial connection with the remainder of the pharyngeal epithelium becomes drawn into a tube or cord (ductus pharyngeo-branchialis III) and broken (in embryos of about 14 mm. length). The proximal (cephalic) end—the so-called thymus nodule or head, now com-

Figs. 1–8 Were drawn by means of an Edinger projection apparatus, x 40. The wall of arteries (e.g., carotid) is shown in heavy black line, pharyngeal epithelium and surface ectoderm in solid black, the pharyngeal derivatives in oblique cross line, the parathyreoid IV (fig. 8) being stippled. In some instances only are muscles and nerves outlined; 'territories' are frequently indicated by interrupted lines.

Figs. 1, 2 and 3 Outline drawings of sections 143, 147 and 155, respectively, from a 7.5 mm. human embryo (No. 256, Harvard collection). × 20.

Figs. 4 and 5 Outline drawings of sections №229 and №239, respectively, from the 9.4 mm. human embryo (No. 1005, Harvard collection). × 10.

Fig. 6 Outline drawing of section №288, 10 mm. human embryo (No. 1000, Harvard Collection). × 10.

Fig. 7 Outline drawing of section №228, 14.5 mm. human embryo (No. 1003, Harvard Collection). × 10.

Fig. 8 Outline drawing of section №457, 16.4 mm. human embryo, (No. 1707, Harvard Collection). × 10.

posed largely of parathyreoid III moves down, for a time, although more slowly, until the parathyreoid III has considerably passed the parathyreoid IV. (b) The intermediary epithelial cord (thymic cord of Hammar) joining the caudal portion (thymus) to the cephalic portion (parathyreoid III) becomes attenuated and ruptures (in embryos of about 35 mm. ± length). The parathyreoid III, as a result of small growth shiftings and largely through the expansion of the thyreoid, comes to occupy its adult position as the inferior parathyreoid.

The ventral pocket of the third pouch is from the beginning quite close to the pericardium and retains this intimate association throughout the descent (figs. 23–24) so that the statement of Hammar ('11, p. 217) that "after entering the thoracic cavity the thymus comes into relation with . . . the pericardium" hardly, I think, conveys the correct impression of the topographical relation of thymus to pericardium in the course of development. The small amount of mesenchyme between the branchial epithelium and the pericardial mesothelium does, in fact, become thinned out, doubtless due in part to the expansive growth of both structures.

Critical and careful examination of the series of sections of embryos of progressively advanced stages has convinced me that the mesenchyme surrounding the third pouch participates in the down-growth accompanying the heart's descent, so that it is not only the branchial epithelium but the material of the region as a whole that suffers the growth displacement which is so characteristic of it. What significance this may have in the later thymic transformation can only be inferred (vide subseq.). The parathyreoid III and the thymus, which remain as 'derivatives' of the transformation of the Complex III, will be discussed subsequently.

Complex IV

The transformations undergone by the fourth pouch and associated epithelium comprised under the name of 'caudal pharyngeal complex,' or Complex IV, have already been briefly

discussed by me (Kingsbury '14 b) from a somewhat different point of view, so that it is only necessary to supplement somewhat what was then said and with more particular reference to the growth transformations of the region as a whole.

The Complex IV from the method of its development occupies a position not only morphologically caudad to the third pouch, but also from its later development and growth is almost imediately medial and dorsal to it, the fourth arch intervening (figs. 1–3, 12, 13). It therefore is not so intimately involved in the descent of the heart as is the third pouch complex. Growth continuing after the establishment of the fourth pouch, there is formed the so-called ultimobranchial body, which, however, I do not find extending to or toward the ectoderm, and hence hardly to be given the value of a fifth or of a sixth pouch. The question of the significance of this body has already been discussed in the earlier paper. The so-called ultimobranchial body lies next the laryngeal mesoderm and between it and the fourth (fifth) arch. At this stage (5–7 mm.) the Complex IV consists of the so-called thymus IV—the ventral diverticulum of the pouch, the portion making contact with the ectoderm and the so-called ultimobranchial body. The form and position of the complex is shown, not quite satisfactorily, for the 5 mm. embryo in figure 12, while figures 13 and 14 show the corresponding structures in the 7.5 mm. embryo. Figure 3 is a section through the ventral diverticulum IV. These regions of the Complex IV speedily become lost in the transformations which it undergoes, and a fourth portion is differentiated—the parathyreoid IV—already well indicated at 8.3 mm. (figure 30) although not recognizable in the 7.5 mm. embryo.

In order to understand the part that the descent of the heart plays in these transformations and growth shiftings, it is essential to keep in mind the relative movements of the third and fourth arches. The third arch has become the common carotid artery, and in its 'migration' out upon the fourth arch it becomes relatively more dorsally located. The head of the third pouch complex, at first morphologically caudad to it, moves thus around to its ventral side, becoming soon more widely separated

from it (figs. 20, 21, 22). The parathyreoid III thus rotates from a lateral position upon the dorsal aspect of the pouch to a more ventral one (Hammar '11, p. 207). The dorsal position of the Complex III shares thus the ventral movement so pronounced in the growth of its ventral moiety. The Complex IV—which, as seen, is more deeply located—comes less under the 'influence' of the descent and grows and shifts caudally more slowly and to a less degree. The ventral diverticulum (IV), like the ventral pocket of the third pouch, at first as it lies between the fourth branchial aortic arch and the pulmonary arch is well marked and directed like that of the third pouch toward the bifurcation of the aortic trunk (figs. 12, 13, 3). It does not, however, like the third pouch, follow the caudal movement of the truncus but is soon withdrawn into the complex and is thenceforward indistinguishable.

A caudal movement of the complex as a whole also takes place, although more slowly than the fourth aortic arch. The broad communication of the Complex IV with the pharynx, on the one hand (ductus pharyngeo-branchialis IV), and its connection with the cervical sinus (ductus branchialis IV), on the other, are speedily stretched to slender cords or tubes and ruptured, the latter first in embryos of 10 to 13 mm. length, (figs. 15, 17, 20), the latter somewhat later, in embryos of ca. 14 mm. length (figs. 18, 21, 22). With the rupture of the ductus branchialis IV, in the 10 mm. embryo (fig. 17) already thinned and bowed, the fourth aortic arch passes it by in the descent upon the outer side. As this occurs the complex follows it by a rotation whereby the parathyreoid IV moves from a position upon the lateral aspect (fig. 15) in the dorsal portion (morphologically) of the complex to a dorsal one (figs. 20–21), rotating thus roughly through ninety degrees, as does the parathyreoid III, but in an opposite direction.

After the rupture of the ductus pharyngeo-branchialis IV, a somewhat more rapid movement of the caudal portion of the body of the complex causes the appearance of a more attenuated portion (neck) to appear, whereby it is joined to a head consisting mainly of parathyreoid IV (fig. 8). Subsequent rupture

and disappearance of the neck separates the two portions, leaving the parathyreoid—at this time near the upper pole of the thyreoid lobe—upon its dorsal side, in the interval between esophagus and trachea, therefore near the position which it typically occupies as the superior parathyreoid.

At the same time that the Complex IV is more slowly descending in a more dorsal position and more deeply placed, and the Complex III is descending by growth more rapidly in a more ventral and superficial position (figs. 20, 21) the thyreoid is rapidly extending in the intermediate interval between these materials, following the carotid artery as it shifts relatively more dorsally (figs. 4, 6, 7, 8). The body of the complex is thus enveloped by the expanding thyreoid lobe, is brought in contact with its medial dorsal edge and fuses with it (fig. 8). Nothing further relative to its fate can be added to the discussion already published.

Considerable variability characterizes the morphological transformations of the Complex IV, which is particulary apparent in the models from the 13.0 mm., 14.5 mm., and 18.2 mm. embryos, in which the two structures upon the two sides are markedly different. Differences between the two sides are evident in most of the embryos of about this size or larger. In the 18.2 mm. and 31.0 mm. (1) specimens, tubular epithelial prolongations from the complex occur in the mesenchyme upon the dorsolateral aspect of the thyreoid, while in the 32 mm. embryo a thymus IV is present outside the thyreoid. These variations in the form of the complex clearly indicate differences in the conditions that determine its growth and in which altered environment in undoubtedly a factor.

Aside from the early differentiated area that becomes the parathyreoid IV, it is impossible to distinguish and follow, in the transformations which the complex undergoes, any regions of specific structural value, such as a thymus IV, or a corpus ultimobranchiale as a definite organ of however 'vestigial' a character, and it may be asserted that the evidence of the existence of such structures as intrinsically branchiomeric organs is exceedingly meager. The occasional occurrence of thymus

bodies or persistent epithelioid structures developed from this complex is subject to a quite different interpretation, as has been previously stated, and this will be considered again subsequently.

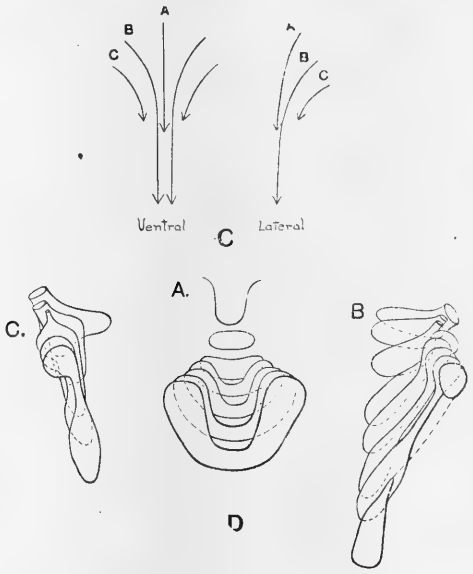
Recapitulation

Thus the downward movement of material in the shiftings as a result of unequal growth, usually summed up under the descent of the heart, involves the branchial region as well. The thyreoid follows the aortic trunk, but expanding laterally its descent is arrested, as has been described. The third pouch complex, from its peculiar position in relation to the forward growth of the propharynx and to the truncus and aortic arches, becomes peculiarly involved, being drawn in ventrally, the ventral prolongation (diverticulum) following by growth the pericardium throughout the descent. The entire pouch complex, however, moves down and becomes differentiated into a head (embodying the parathyreoid III), body (thymus) and intermediary portion, which in man becomes attenuated and ruptures. Likewise and to a less degree, the more deeply located caudal pharyngeal complex or Complex IV participates in the movement and undergoes a comparable differentiation, the initial complexity and the ultimate fusion with the thyreoid differentiating it from the Complex III. The following diagrams may crudely schematize the movement of these three pharyngeal componants—thyroid, Complex III and Complex IV. In schema C the arrows serve to indicate the general line of movement for (a) the thyroid, (b) Complex III, and (c) Complex IV, in horizontal and sagittal projection, while in figure D successive positions for each of these three pharyngeal derivatives are superimposed. Figure D is purely schematic, but figures 23 and 24 and the figures of plates 1 to 4 may be consulted in comparison, as also the text figures 1 to 8.

The descent movement is accompanied by rotation, most obviously shown in the case of the parathyreoids, as already described. As the ventral portion of Complex III is drawn into the 'heart eddy,' a rolling or rotation upon its long axis gives to

it a slight spiral twist (18.2 mm.) which is rendered obvious by the growth furrows in the beginning expansion, as well as by the shifting of the thicker epithelium of the tube from a dorsolateral position higher, to a ventrolateral position lower down. The latter of these features has been commented on by Hammar ('11).

The prevalent asymmetry in the adult relations seems clearly due to a corresponding asymmetry in the descent. Thus the right parathyreoid III is usually lower than the left, while the



Schema C-D

pyramidal lobe of the thyreoid, when present, is in the majority of cases asymmetrically placed, to the left. Inasmuch as the pyramidal lobe unquestionably, I believe, marks the line of descent of the thyreoid, both its more usual excentric position, as well as the generally lower position, of the right parathyreoid III indicates a more extensive descent tendency upon the right side, probably associated with the asymmetry in the aortic arches (IV), or is due to the asymmetrical position of the common carotid arteries upon the right and left sides, associated therewith. Decisive proof of this would be difficult to secure.

The epibranchial placodes

While the growth shiftings in the cephalo-caudal growth in head and neck produce the effects upon the branchial pharynx just described, these are modified and correspondingly influenced by the growth and expansion in the transverse dimensions. In this connection it will be necessary to call attention to the effects produced by the expansion of the branchial arches II, III and IV, particularly the second arch, which through its growth, together with that of the postbranchial and epibranchial regions, outlines a roughly triangular depression and determined its transformation into the sinus cervicalis, into which the second, third and fourth clefts open. It would be entirely superfluous to describe, in repetition of others, the further transformation of the sinus cervicalis into the ductus branchialis II and the vesicula cervicalis, save in so far as it is necessary to emphasize the fact that they are formed in the increase in thickness as a result of a growth whose relations, direction and degree they express, and to call attention to the influence which the epibranchial placodes have in their formation.

These last named structures have until recently received small recognition and little emphasis in texts of embryology when it is considered how long their occurrence has been described. Since von Wijhe ('82) described them in the elasmobranch, and Froriep ('85), under the name of 'Schlundspaltenorgane,' in the mammal, they have been described in elasmobranchs (Froriep '91), the lamprey (v. Kupffer '91), teleosts and ganoids (Landacre '12), amphibia (Landacre and McLellan '12; Drüner '04[3]), reptiles (Lucy W. Smith[4]), birds[4] (Kastschenko '87, '10), and in man (Streeter '11, Hammar '11). In all instances they bear a relation as epibranchial thickenings to clefts I to IV (or more), while the ventral (caudal) portions of the respective ganglia (VII, IX, X) are intimately fused with them. In the lower forms

[3] Drüner clearly describes the epibranchial placodes in the axolotl and recognized that they contributed to the ganglia of the VIIth, IXth and Xth, although he described them as 'ectthymus' (p. 552).

[4] From unpublished observations made in the laboratory by Miss Lucy W. Smith, in the turtle, Chyrsemys marginata, and Miss M. E. Goudge, in the chick.

ganglia seem to be derived from them (Landacre, v. Kupffer, Froriep). In higher forms this is less clearly shown. The intimate association of neural crest ganglion and placodal ectoderm, however, is clearly of marked significance.

Epibranchial placode I—which, as in the territory of the propharynx, lies outside the scope of the present study—has in the 7.5 mm. embryo apparently severed its connection with the ganglion VII (geniculatum) with which it was doubtless confluent at an earlier stage. In a 6.2 mm. cat embryo (Harvard Embryol. Coll. No. 380) placode and ganglion (N. VII) are intimately associated.

In contrast with the placode I, placodes II, III and IV maintain their close association with their corresponding nerves and ganglia, and clearly play a part in the development of the ductus branchialis II and cervical vesicle, as will subsequently appear. In the 7.5 mm. embryo, placode II is a thickening of the entoderm lining, a depression behind the upper end of the second branchial cleft, in intimate contact with the glossopharyngeal nerve at the lower end of the ganglion (petrosum; fig. 25). Corresponding thickenings and depressions behind the upper ends of the third and fourth clefts, as placodes III and IV, (figs. 27, 28) are in close relation to the ganglion of the vagus nerve. With the increase in thickness of the arches and the consequent removal of the ganglion petrosum of the IX and ganglion nodosum of the Xth nerve from a more superficial position, the placodal ectoderm remains closely associated with the corresponding ganglia, while there are produced the ectodermal insinkings of the ductus branchialis II and cervical vesicle respectively.

In the 9.4 mm. embryo (fig. 19) ductus branchialis II has become quite prolonged, while the cervical sinus has deepened and narrowed, but both open to the exterior into the outer portion of the cervical sinus by separate apertures (fig. 10). By further growth, in the 10 mm. embryo the cervical vesicle and ductus branchialis II now communicate with the exterior through a common tubular opening (figs. 15–17). Figure 17 in particular shows the marked and rapidly acquired effect growth has produced in the wide separation of structures closely adjacent in

the 7 mm. embryo. The ectoderm associated with the ganglion petrosum is now prolonged into a long tube extending to the lower pole of the ganglion. The crest of the sinus cervicalis (representing the vagal placodal ectoderm) is imbedded in the lower end of the ganglion nodosum, which has been removed to reveal it. The connection of the Complex IV with the cervical vesicle, close at 9.4 mm. (fig. 19) has now by growth and descent become drawn out (fig. 17) to a slender tubular cord (D. branchialis IV), soon to be ruptured, as in the 13 mm. embryo, where on the left it is ruptured and no longer recognizable, on the right, just ruptured (fig. 20). The connection of the indrawn ectoderm, as the D. branchialis II and cervical sinus (through the ductus cervicalis) with the superficial ectoderm, becomes speedily severed and for some time (figs. 20, 22) the upper portion of the ductus persists as a dwindling vesicle close to the lower pole of the ganglion petrosum; while the cervical vesicle remains in intimate contact with the ganglion nodosum, where it gradually disappears, apparently without trace.

By growth of the surrounding structures the sinus cervicalis may thus be described as becoming divided into external[5] and internal portions, the latter again including the ductus branchialis II and the cervical sinus. These two structures obviously owe their existence and form relations in part to the intimate connection of the ganglia petrosum and nodosum respectively, with the epibranchial ectoderm and the persistence of such connection, under the expansive growth of the region. It is likewise clear that the name, ductus branchialis II, is not appropriate, since it clearly owes its existence to the persistence of a connection of nerve and epibranchial ectoderm. In its development, it is true, the second cleft becomes in part incorporated, so that the second pouch adjoins it in a characteristic manner (fig. 15), but the 'duct' projects beyond it toward the ganglion of the glossopharyngeal nerve (figs. 15, 17, 19). This conclusion is further borne out by the fact that in a 9.2 mm. embryo (Harvard Embryol. Coll. No. 734) this ductus branchialis opens

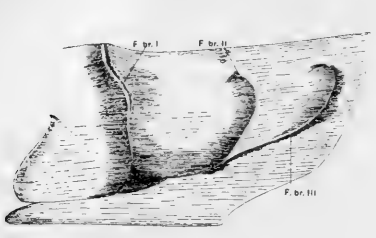
[5] Sulcus cervicalis, or sulcus precervicalis, of Hammar.

independently of the second cleft upon the ectoderm behind the cleft, i.e., at the point characteristic for the epibrachial placode II. Similar epithelial prolongations in continuity with the ectoderm in the territory of the second cleft have been described in other mammals, e.g., in the rabbit by Piersol, ('88) in the pig by Zottermann ('11) and Badertscher ('15), in the guinea-pig by H. Rabl ('13), under the name of ductus branchialis. It is not quite clear, however, what the morphological value in these cases may be, and inasmuch as it is an expression of persistent ectodermic attachment under growth expansion, its value may well vary in different forms.

Whether or not that portion of the ductus branchialis II that adjoins the second pouch persists so that ectodermal cells become incorporated with the entodermal cells, was not determined but exists as a possibility. In the same manner, the fate of the cells of the vesicles that maintain their association with the ganglion petrosum and ganglion nodosum is problematic. The connection of the vesicle derived from the ductus branchialis II with its ganglion (vesicula branchialis II, figs. 21–22) seems not so intimate as does the connection of the cervical vesicle with the ganglion nodosum, where the epithelium merges with the ganglion without sharp boundary and all appearance of a close fusion. Whether the characteristic elements of the gangilon, neuronal or other, receive from the placode or subsequently from the vesicle, augmentations, could not be definitely determined, although the histological appearances and relations suggest strongly that such may be the case—a conclusion similar to that reached by Hammar, and independently formed. Whether a definite group of neurones, the gustatory, have this placodal origin, from the epibranchial placodes—as Landacre concludes from his interesting study of the developmental relations in Lepidosteus and Amiurus—can of course be still less determined from the material and methods of the kind used in this investigation; it is a study quite apart from that of the pharynx and its derivatives.

The cervical vesicle is not only partly imbedded in the lower end of the ganglion nodosum, but in the mechanics of growth

remains closely joined to the third pouch so that as long as it persists, it is a component of the head of the Complex III. My observations, however, confirm those of previous investigators that in man it contributes nothing to the pharyngeal derivatives, whereas in some mammals it unquestionably under-



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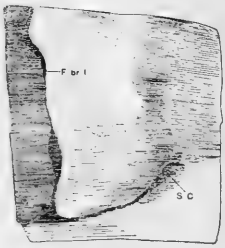

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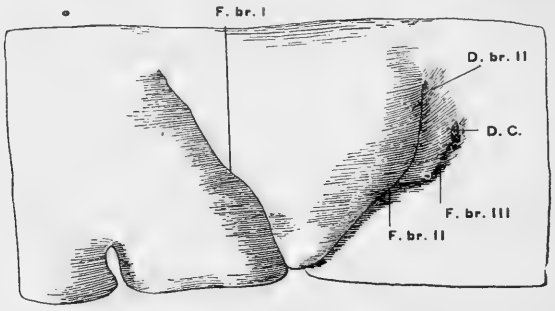

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Fig. 9 Lateral (surface) view of the ectodermal pharyngeal surface, in a 7.5 mm. human embryo (No. 256, Harvard Embryological Collection), as modelled with the entoderm (shown in figures 13 and 14).

Fig. 10 Surface (lateral) view of the branchial arches and clefts in a 9.4 mm. human embryo, as shown in a model, the posterior aspect of which is given in figure 19.

Fig. 11 Surface view, from the left side, of the branchial region in a 10 mm. human embryo (No. 1000, Harvard Embryological Collection), from a model of the ectoderm. Other aspects of the model are shown in figures 15, 16 and 17 *S.C.*, indicates the external portion of the cervical sinus (sulcus cervicalis).

goes thymic transformation, as will be commented on subsequently. Its position and relations in the Complex III are shown in figures 20, 21 and 22.

The external portion of the cervical sinus owes its existence more to the growth of the postbranchial territory, together with

the precocious and marked growth of the second arch, which as an operculum encroaches upon the boundaries of the sinus and finally causes its obliteration. The rather deep insinkings of ectoderm along the lines of the branchial clefts in the precervical region appear to be withdrawn with the growth of the mesoderm, although the inclusion and degeneration of portions cannot be excluded (figs. 9–11).

Larynx

Although the pharyngeal transformations that determine the development of the larynx and its related structures are but a part of the growth of the entire region and are affected by the shiftings in the lower pharynx, they are omitted from the present paper, since the points of interest still undetermined relate more to the mesodermic arches than to the epithelium and its derivatives, whose explanation is the question considered in the present paper.

PHARYNGEAL DERIVATIVES

In the foregoing paragraphs I have attempted to describe in a concise way the morphological transformations of the branchial epithelium, entoderm and related ectoderm, as but the expression of one side of the growth of the region with its varied stresses and strains and shiftings, which in their totality produce the anatomical relations of the adult. But a moment's reflection is necessary to establish the conclusion that it could not be otherwise, and the criticism may perhaps be offered that any morphological analysis of the regional growth is superfluous, as it can only establish conclusions that are self-obvious and which might be arrived at from *a priori* reasoning; that is, it is simply a description of changes that inevitably follow from the relations of parts in the region which can establish nothing in the way of explanation or build a foundation on which the explanations may be subsequently worked out. Such a criticism, however, in the specific instance in hand, has weight only on the assumption that there are certain pharyngeal organs predetermined in development, 'pharyngeal organs' whose growth

and evolution follow inevitably in the growth of the body from intrinsic causes not analyzable in the present stage of our knowledge. As soon as it is recognized that the existence of these organs is itself a subject of scrutiny and analysis, the determination of the developmental conditions and growth transformations becomes a matter of considerable importance, and—not to minimize in the least the ultimate importance of other sides of the problem—a correct interpretation of the morphogenesis and histogenesis remains a fundamental prerequisite for the adequate interpretation of the significance of these structures as bodily organs.

Thymus

In the case of the thymus, the prevailing tendency at the present time is to view it as an 'epithelial organ' the factors for whose appearance are intrinsic in certain cell groups in the third branchial pouch, where, in the ventral diverticulum the thymus anlage as early as 5 or 6 mm. embryos is indicated by the thicker character of the epithelium that by its growth produces the thymus body or gland. So universally have recent investigators supported this localization that it is necessary to cite specific references or quote passages; Verdun ('98), Maurer ('06), and Grosser ('11 b) have crystallized the common interpretation in their comprehensive articles on the pharyngeal derivatives. Localization of a thymus anlage in the ventral diverticulum of the third pouch, under the sway of the 'branchiomeric organs' conception leads naturally to the homologization of the comparable pockets in the second and fourth pouches (fig. 12–13) as thymus bodies II and IV. Thus Groschuff ('96) and Tandler ('09) term the ventral pocket of the fourth complex a 'thymus IV,' and Grünwald ('10) derives the palatine tonsil from the ventral pocket of the second pouch and regards it therefore as a thymus metamer. The fact that thymus bodies are unquestionably developed from the Complex IV, in man occasionally, and in certain mammals (e.g., cat) more constantly, is regarded as supporting the homology, especially since thymus bodies are developed in connection with a number of branchial

pouches in certain lower vertebrates. Kölliker ('79), Piersol ('88) and Verdun ('98) described a thymus from the second pouch in the rabbit. Hanson ('11) has more recently shown that no thymus body is derived from the second pouch in this mammal.

Despite the prevailing opinion and the evidence presented therefor, when critically examined, it is found to offer no support for the localization of thymus anlages in the branchial epithelium. As to the thicker character of the epithelium of the ventral pocket of the third pouch, it may be stated that growing epithelium tends to be thicker and that simple mechanical stresses and strains have clearly a marked effect in causing thinning or thickening of an epithelium, numerous illustrations of which might be given. The thickness of the epithelium cannot in itself, therefore, be taken as a criterion of homology; nevertheless, the tendency to do so is strong; witness the suggestion of Hammar ('11) that the thicker character of the epithelium on one side of the tubular Complex III in man may mark the localization of the thymus anlage in the epithelium and thus bridge the gap between the condition in mammals and that in lower forms. This fact just alluded to—that in mammals the thymus comes out of the ventral portion of the pouch (so interpreted) while in forms below mammals it develops from the dorsal portion of the pouch, on the assumption of a definite localization of a thymus anlage in the branchial epithelium—is confessedly an objection to the homology of the thymus bodies throughout the vertebrate series, as has been recognized by Maurer ('06), Grosser ('11 b), Hammar ('11), and others. It is only, however, on the assumption of such epithelial localization that this different mode of origin necessarily opposes such homology, whereas it does give evidence against a primary thymus-forming group of cells in the branchial epithelium.

The evidence against a distinct thymus anlage becomes markedly stronger when the development of the thymus in different mammals is considered. In man—as previously stated and as is also well known; confirmed in the work of Hammar already cited—the Complex III in the process of growth and the accom-

panying growth shiftings, becomes markedly prolonged and regionally differentiated into (1) a head containing the parathyreoid III and the branchial remnant (Hammar), closely joined to the cervical vesicle; (2) a neck or intermediary portion or cord (thymic cord, Hammar); and (3) a body, the ventral free end or apical portion of the pouch which coming to be located within the thorax undergoes thymic transformation, extending up a varying distance into and beyond the thoracic aperture. The remaining portions of the complex including the cervical vesicle degenerate and disappear, with the exception of the parathyreoid. In the pig, the entire complex—head, neck (intermediary cord, mid cervical segment, cervicothoracic cord) and body (thoracic segment), always excepting the parathyreoid—undergoes thymic transformation, as Zottermann and Badertscher particularly have shown, although their results are in full accord in this respect with the less extensive studies of earlier investigators. In the guinea-pig, it is equally clear that the entire third complex, excepting the parathyreoid-forming portion, becomes transformed into thymus (H. Rabl '13, Ruben '11). The ventral pocket or prolongation "remains very weakly developed" (Ruben) or is apparently lacking, according to the description of H. Rabl, and the complex remains purely cervical. In this form, somewhat different conditions of unequal growth, with different grouping of mechanical shiftings, may be in part responsible for the cervical position. Furthermore, the cervical vesicle becomes thymus, as Badertscher and Zottermann have shown in the case of the pig, and Ruben in the guinea-pig.

It is unnecessary further to multiply illustrations of the variability in extent of development or in position, by considering the results of investigators in the development of the thymus in other mammals (sheep, mole, cat, mouse, etc.) since the well established facts above stated—while they do indicate, as do the numerous observations on lower vertebrates, a marked and extensive tendency to form thymus bodies inherent in the branchial region—do not afford any support to the acceptance of a specific and intrinsic anlage for the thymus in the branchial epithelium, which would have to include therefore—to limit the

consideration to the mammalian thymus and the third pouch—the entire branchial entoderm and that portion of the adjacent ectoderm which becomes cervical vesicle.

It is necessary, therefore, to find some basis for understanding the development of the thymus other than the assumption that it is a representative of *branchiomeric organs* whose anlages are definitely located in one or more of the branchial pouches and potentially in all. Such a basis for its interpretation is found in the recognition that it is a structure whose appearance is determined by extrinsic factors of relation and position and not intrinsic factors located in any particular group of cells. In support of such an interpretation and giving us, I believe, a better comprehension of its morphologic significance, we have the fundamental plan of its histogenesis.

The histogenesis of the thymus, it is true, has been a mooted question, there being six different interpretations in two rather natural groups. The results of recent workers, including the extensive work of Maximow ('09–) and Hammar ('07–) and his pupils, supplented by that of Pappenheimer ('13), Badertscher ('15 b), Hartmann ('14), and others (e.g., Jolly, '11, Hart '12) show quite conclusively, I think, that the thymus is formed by extensive infiltration of the epithelium by cells from outside with the characteristics and potentialities of lymphocytes, which there proliferate. These cells are derived, according to Maximow, Badertscher and Hartmann, from the connective tissue (mesenchyme) while Hammar ('11) still recognizes the possibility or probability that they come from the blood. I believe that the correct interpretation is that of Maximow, Hartmann and Badertscher—the work of the last named having been closely followed by me. Briefly stated, and in somewhat general terms and with the attempt to emphasize the fundamental in the process: The epithelium of the third pouch complex due to its peculiar location, by growth and the attending growth shiftings, as has been described above, becomes transformed into an elongated epithelial tube. The caudal portion which becomes the thymus lobe (in man) grows extensively (fig. 24) but in a manner not in the least typical of an epithe-

lium. The lumen is lost and with it all surface relation for the epithelium, which from this time grows loosely, forming the cyto-reticulum or syncytium so characteristic of it, growth occurring particularly and irregularly in the basal layers (next the mesenchyme). The growth is further attended by an accompanying diffuse degeneration. In its relation to the underlying (surrounding) mesenchyme also the relations are atypical. The growth co-ordination of these two tissue forms would typically determine the appearance of a (connective tissue) membrana basalis. In the case in point, mesenchynal elements (so-called large lymphocytes, primary wandering cells, leucoblasts) become free and invade the cyto-reticulum, there proliferating rapidly, forming the characteristic small cells with scanty cytoplasm (the lymphocytes, small lymphocytes), the proliferation occurring mainly in the peripheral growing zone—next the mesenchyme—leading to the well known differentiation into cortex and medulla. This peculiar relation of growth and proliferation continues, in general coextensive with the presexual growth of the body, then ceases and a more or less rapid involution of the structure succeeds. Hassal's corpuscles are clearly phenomena of degeneration—cytoconglomerates—more marked and larger during the period of involution.

The thymus, from this point of view, may be regarded as an expression of a persistent and atypical growth of a non-adaptative epithelium, this being accompanied by an altered epithelio-mesenchymal relation whose characteristic feature is the infiltration of the epithelial (epithelioid) mass. The epithelial growth is attended by an accompanying degeneration which becomes more profound in the later period (involution). The thymus transformation may thus be thought of as a reaction of degeneration, prolonged and given its characteristic features by the attendant growth. Such an interpretation at once raises a number of important questions of marked biological and morphological interest, both theoretical and practical, and for the consideration of most of them the basis in fact is still insufficient. The far-reaching significance of the interpretation and

the point of view it represents will be apparent from even the scant consideration that follows.

If the thymus represents a form of 'reaction of degeneration' as outlined above, other instances of similar nature should occur, as indeed is the case. Jolly particularly has created a group of "lympho-epithelial organs" in which he has grouped Peyer's patches, tonsils, esophageal lymphoid organs of birds, the follicles of the bursa Fabricii of birds of prey, the lymphoid papillae of anal glands, placoid thymus of teleosts, bursa Fabricii (of other birds), and the thymus of most vertebrates. To these might be added a number of others, such as the amphibian tonsils (Kingsbury '12), Kiemenreste of Anura, the tubal tonsils of birds, and others will doubtless be added as the field of investigation is extended. In all these cases there is illustrated a more or less intimate reaction of epithelium and lymphoid cells (lymphocytes). In view of what seems to the writer strong and convincing evidence of the mesenchymal origin of such lymphatic cells (lymphocytes) and with the acceptance of this origin, these structures illustrate a reaction of epithelium and mesenchyme (connective tissue) characterized by free proliferation of the cells of the latter with an invasion and infiltration of the former. An essentially regressive character of the epithelial structure in many of the instances is not clearly apparent, and awaits more light on the biological significance of the part, as in the case of the bursa Fabricii of birds. In other cases the degenerative element is clear, as in the case of glands (e.g., tubal tonsils of birds) where the lymphatic cells infiltrate and replace the gland, entirely or in part.

Stöhr in 1891, in dealing with the development of the palatine tonsils, concluded with a general discussion containing the following pertinent paragraph (translation):

> It is probable that most of those leucocytes that occur outside the lymphatic nodes (lymph glands) and outside of lymph and blood vessels are the agents of resorption processes whether the same occur in the service of digestive processes or are for the purpose of removing bodily structures which have entered upon a partial or complete regression. As proof of the latter, one may point to the frequent occur-

rence of collections of leucocytes in degenerating organs, for example, in the pronephros of lower vertebrates, the gills of anura, thymus, processus vermiformis; also such a possibility may not be at once rejected in the case of the tonsils (residuum of a visceral cleft) and the so-called trachomal glands (degeneration, of the nictitating membrane?). Certain of the groups of leucocytes found in the mucous membranes are in direct relation to the degeneration of glands (p. 547).

In most places in the above quotation lymphocyte might be substituted for leuocyte for greater precision. As is of course well known, Stöhr ('06) subsequently altered his interpretation of the thymus, coming to regard the "small round cells" as of epithelial origin and a cell type *sui generis*, not to be confounded with small lymphocytes. The recent work has not, however, confirmed his second interpretation but rather the former one; with some difference of opinion, however, as to the local or general (blood) origin or source of the invading cells. The comparison to which Stöhr here refers—that of the thymus and tonsillar structures (whether palatine, lingual, pharyngeal, esophageal, coecal, conjunctival, or elsewhere occurring), as well as the comparable collections of lymphatic tissue in mucous membranes such as Peyer's patches, solitary nodules, etc. is involved in the interpretation of the thymus insisted on in this paper, and is an important one. Inasmuch as the discussion would require going into the matter in greater detail than is desirable here, and as it is the purpose of the writer to supplement later the preliminary brief paper already published by him (Kingsbury '12), he will only add that, as in the case of the thymus, so in tonsillar structures, the primary local or general origin of the 'lymphocytes' requires further consideration, as well as the relation they bear to regressive epithelial structures (glands). In this connection I quote the comment of Stöhr in a footnote to the same article quoted from above, apropos the invasion and infiltration of epithelia so characteristic of tonsils, that:

In the case of the thymus the immigration has the purpose of removing the organ that is of no further use. The leucocytes wander into the epithelium of the tonsils; this is not, however, removed since the crypts of the tonsil are hollow so that the leucocytes so commissioned with its removal suddenly come out on a free surface and reach the mouth cavity without having fulfilled their duty.

In this connection the writer, on his part, desires to offer the comment that the intrusion of a teleology, while in many instances in biological work apparently justifiable as an avoidance of complex paraphrasing and explanation, is in such instances as the above quite unnecessary. Before leaving the question of a 'lymphocytopoietic' reaction of mesenchyme (connective tissue) in the presence of regressive structures I wish to mention some other aspects. First, every histologist is familiar with the circumscribed small mononuclear cell (lymphocytic) accumulations, more or less compact, occasionally encountered in glands of the most diverse character—kidney, (thyreoid) lachrymal gland, salivary glands, as well as in minor glands of the respiratory and digestive systems—attended by the degeneration and infiltration of a certain number of the epithelial acini.

In the transplantation of tissues, where in some instances they 'take' and grow, and in other cases (as in most homoiotransplants, Loeb) they fail to do so and undergo regressive change and disappear, peculiar opportunity is given for the examination of the relation of regressive structure to its environment. The statement of Leo Loeb ('15) in summing up the results of many years of investigation in this field are so pertinent that I venture to quote in some detail:

> A certain metabolic activity on the part of parenchyma of various organs determines the attitude of lymphocytes and of fibroblasts toward the parenchyma. If the activity is normal, lymphocytes do not or only to a slight extent enter the parenchyma. The connective tissue is held in a definite state of activity (p. 728). . . . . While the vitality of the tisses has not been essentially impaired after homoiotransplantation, and they may even grow, and the metabolic changes they underwent in the different chemical environment did not therefore markedly interfere with their power to live or even propagate, these metabolic changes lead to a new condition in the host tissue, which secondarily brings about a destruction of the transplanted piece, namely, these metabolic changes cause (a) an increased activity on the part of small monocuclear cells (probably lymphocytes) and (b) a destructive activity on the part of the connective tissue of the host. The mononuclear cells collect around the transplanted tissue, penetrate into it and destroy its cells. . . . We may conclude therefore that the metabolic changes taking place in tissues after homoiotransplantation stimulate the activity of the lymphocytes and cause the altered

function of the fibroblasts which now produce fibrous tissue in large quantity. . . . The activity of lymphocytes and of connective tissue is to some extent independent of each other; while in many cases the two act together, in some cases the one prevails, in other cases the other (p. 727). . . . Connective tissue and lymphocytes may therefore be regarded as organs of attack, lying quiescent under ordinary conditions, but exerting their efforts as soon as within given limits certain pathologic changes take place (p. 728).

Might it not well be that lymphocyte infiltration and connective tissue invasion represent two sides of a fundamental mesenchymal reaction?

Finally, I desire to call attention to Metchnikoff's conception of senescence ("In senile atrophy there is always present the atrophy of the higher and specific cells of a tissue and their replacement by hypertrophied connective tissue") and to his accompanying well-known view of the frequent destruction in old age of epithelia (as in the kidney) by phagocytes—leucocytes from the blood. Without stopping to comment on his recognition of two 'distinct' reactions, the connections that the interpretation here discussed has with pathology is sufficiently obvious.

It is also recognized that the interpretation of the thymus involves the biological and morphological interpretation of the lymphatic nodes (including hemolymphatic nodes) as well as the simpler or more complex accumulations of lymphatic tissue in mucous membranes and glands. From this aspect the lymphatic nodes have scarcely been considered as yet.

Furthermore, the thymus problem is a part of the larger problem of the blood cells and hematopoiesis in general. The unitary character of the group of the blood cells, while not established beyond controversy, has been made highly probable through the work of Maximow and of Weidenreich particularly. It is customary to express it in terms of cell lineage, and to designate it as the 'monophyletic' mode of origin of the blood cells. It is a conception compatible with the acceptance of either the derivation of the primary blood cells from the entoderm, in accordance with the angioblast theory of His, or a derivation from and development out of the mesoderm, although the evidence, as the writer sees it, mainly supports the latter inter-

pretation. It is possible, however, to look at the unity in origin of the blood cells as an expression of a fundamental unity of conditions. While this view would not necessarily oppose the monophyletic interpretation under either an angioblastic or mesenchymal origin, it would not, on the other hand, necessarily presuppose it. One of the characteristic features of hematopoiesis is the ever-shifting setting of the scene, the succession of regions in which blood-cell formation occurs:—blood islands (yolk sac), liver, par- and preaxial mesenchyme (?), mesonephros (some forms), spleen, lymphatic tissue, thymus, bone marrow—as well doubtless as other places. There may well be a common metabolic feature that determines the appearance of blood-cell formation successively or simultaneously in these different places. That 'absorption'—frequently—of products of regressive change may be an element is suggested, as illustrated, for example, by the tendency of blood-cell formation to follow bone (or cartilage) absorption even when it occurs in very unusual localities (Maximow '07 b). On the other hand, it is possible that the blood-forming regions are characterized by a continued growth tendency in the mesenchyme, associated with a lack or loss of the adaptive connective tissue differentiation, such as occurs in other places such as, for example, the formation of skeletal connective tissue membranes in correlation with epithelia, etc., and that this covers factors that determine hematopoiesis. Our knowledge as yet is clearly neither detailed enough nor extensive enough to permit any comprehensive conclusions. In the case of the thymus, however, not only lymphocyte formation but also the formation of granular leucocytes (including eosinophile cells) and red blood corpuscles incontestably occur, although I question whether many of the last named find their way into the general circulation (cf. Badertscher '15, b). It should also be noted that formation of granular cells and red blood corpuscles occurs in the cervical vesicle (ectodermic) portion of the thymus in the pig (thymus superficialis), as well as in the entodermic thymus.

To return to the morphological interpretation of the thymus: It is obvious that an explanation from the point of view set forth

above removes many of the difficulties that beset the interpretation of the thymus as a branchiomeric organ definitely located in the branchial epithelium. Recognizing that the thymus-forming factors are not intrinsic but extrinsic, i.e., partly a function of position and relation, it is no longer necessary directly or completely to homologize thymus bodies in different forms, since it is obvious that different growth conditions may determine the thymus development from quite different portions of the branchial epithelium, and portions that in one form may persist and undergo thymus transformation, in others may degenerate and disappear without the characteristic reaction appearing. It is only for convenience, and in view of the final result, that the purely epithelial stage may be termed 'thymus' or 'thymus anlage.'

Turning now to the question of a 'thymus IV anlage:' In the human embryo it is clear that there is no reason for serially homologizing the ventral diverticulum IV with the thymus III. The thymus IV is variable (as indeed is thymus III) and inconstant. It has never been shown that it is distinctively or exclusively derived from this portion of the branchial epithelium. The ventral pocket is clearly but an expression of the early and temporary morphological growth relations and speedily disappears as such (Kingsbury '14 b). It is altogether probable that the thymus transformation may befall any portion of the epithelium of the Complex IV (caudal pharyngeal complex) which may persist in the mesenchyme.

It may be said in closing this consideration of the thymus that at most it may be stated that there is a wide-spread tendency to thymus-formation in the branchial region, characterized by a persistence and growth of epithelium with a characteristic (though not peculiar) reaction with the adjacent tissues, under conditions that are not yet fully analyzable. What these conditions are and what determines the development of a thymus or thymus bodies is unknown, and any attempt to determine them awaits further analysis of the growth conditions of the region, particularly in lower forms. I desire to call attention

to an entirely similar point of view regarding the homologization of tonsils in lower and higher forms (Kingsbury '12).

Parathyreoids

The characteristic number of four parathyreoids III and IV on the two sides appears in man to be quite constantly present. In the series of 60 embryos examined, many of them attaining beyond the period of differentiation of these structures from the other portions of the pharyngeal epithelium (ca 20 mm.), in but two instances were accessory parathyreoids noted. In the 40 mm. embryo, there were four parathyreoids on the right side, the two extra bodies, from their position, being undoubtedly derived from both III and IV. In the 18 mm. embryo there was a small accessory parathyroid III on the left. But little has been added to the known mode of development of these structures in man, as set forth by Grosser ('11 b) and more recently by Hammar ('11). It has been possible, however, to follow them a little farther back in development, to emphasize their fundamental morphological relations and to offer a suggestion as to their morphological significance.

They develop, as already described (Hammar '11), as a thickening of the branchial epithelium in the dorsal portion of the third and fourth branchial pouches respectively, upon their lateral, and to a slight extent (morphologically), cephalic aspects. The thickening is attended by and at first in large part at least due to a peculiar loosening or reticulation of the epithelium. Parathyreoid III was first clearly recognizable (fig. 18; also fig. 17) in the 7.5 mm. embryo, while the parathyreoids IV were not yet distinctly recognizable. In the subsequent growth their characteristic appearance is maintained. The thickening of the epithelium continues and with the (typical) degeneration and disappearance of the contiguous branchial epithelium they remain as subspherical-oval bodies in the adjacent mesenchyme. How far their increase in size in the early period of growth is due to extension of the parathyreoid-forming process to the adjacent epithelium, and to what extent it is due to only enlarge-

ment of the original anlage, it is difficult to determine. A careful study of their histogenesis in suitable material is much needed. It seems to be quite unnecessary to introduce figures in illustration of their position and general development in addition to those already published (cf. Grosser '11 and Hammar '11). It may be permitted, however, to call attention to the evident mistake in lettering in the otherwise typical figure published by Grosser ('10) and subsequently reproduced in the Keibel and Mall Handbook (vol. 2, fig. 330.) On the left, epithelial body III should obviously be epithelial body IV, and vice versa; the position is in itself characteristic. At this place I also desire to mention an error in the description of figures 1 and 2 in my earlier article ('14 b): Th. = thyreoid, thy. = thymus.

In facing the problem of the morphological significance of the parathyreoid bodies, the investigator—as in the case of the thymus—encounters the two alternative interpretations of intrinsic branchiomeric organs whose anlage is located in definite regions of the branchial pouch, and the interpretation that their development is a factor of their relative position and relations, and hence extrinsic, to a marked degree. The former is the generally accepted view, and their development from an epithelial area of definite location, seems to confirm its soundness. I am inclined to believe, however, that further investigation will support the alternative. In the early development of both parathyreoids III and IV, in embryos of (7.5 mm.) 8.3 mm. to 10 mm. the epithelium undergoing the 'parathyreoid trasformation' is that immediately adjoining the corresponding aortic arches III and IV, respectively. These arches here pass close to and in intimate contact with the branchial epithelium upon the anterior and dorso-lateral aspects of the respective pouches. It is here that the parathyreoids are being differentiated, and the mesenchyme between the two structures, vascular endothelium and branchial epithelium (parathyreoid anlage) is scanty or lacking (figs. 26, 29, 30). If this correlation possess morphological significance, it will be found in other mammals, and whether or not this is the case has not been ascertained. The existence of such an association is man in presented, there-

fore, merely as a suggestion that has several sides: (1) that possibly herein lies the significance of the fact that the third and fourth pouches are the only ones that develop parathyreoids, the third and fourth arches alone persisting; (2) the parathyreoids may thus more closely represent the primary branchial epithelium and more truly deserve the name of branchial remnants than other branchial derivatives; (3) Maurer's contention that the carotid body (in amphibia, and other forms?) represents an epithelial body II (parathyreoid II) may herein find support and confirmation. In any event, a reexamination of the origin of the epithelial bodies and their vascular relations in amphibia, as well as those forms in which the development of an epithelial body (parathyreoid) II has been described, is called for.

However interpreted as to its primary significance, the association of parathyreoid and corresponding arch persists for some time (fig. 20), and the parathyreoid follows the growth shiftings of the corresponding blood vessel, the parathyroid IV rotating toward the dorsal side, the parathyreoid III toward the ventral side of the corresponding pouch complex, as above described. Ultimately, of course, the vascular arch moves away from the parathyreoid, which remains stranded in the midst of the mesenchyme by the degeneration of the associated branchial epithelium.

Thyreoid

This derivative has received but little attention in this study, aside from its form changes already considered. The separation from the pharynx is early and complete, so that a thyreoglossal duct—in no sense a duct, any more than are the other so-called pharyngeal ducts—does not at any time normally exist, or has only a brief existence. The point of separation from the pharynx, while always recognizable from the first as a shallow depression, is only deepened subsequently (13 to 14.5 mm.) apparently mainly by growth of the adjacent mesoderm. The beginnings of a pyramidal lobe, clearly a growth of cells in the line of descent, was first found at 14.5 mm. The cavity of the thyroid is speedily lost (5 mm.) and the period of expansion begun by the peri-

pheral growth of thyreoid cell cords. The vascularization seems to take place rather gradually, being marked in embryos of 32 to 40 mm. Lumina (follicular cavities) within the cell cords appear at about this period (32 mm.) and at first contain no demonstrable colloid. In the 40 mm. embryo some of the follicles contain colloid, although not much is present in the oldest embryo (48 mm.). At 10 cm. the colloid-containing follicles are numerous.

Ultimobranchial body, postbranchial body, or lateral thyreoid

The writer desires to add nothing to the discussion of this structure already published (Kingsbury 14 b). The view then taken was that this derivative gives no good evidence of being a 'vestigial gland,' which in lower forms possesses a duct opening into branchial pouch or pharynx. Its origin, form and fate appear to be due to a persistence of a growth tendency in the branchial epithelium, as molded by the attending growth conditions and movements, but without any characteristics that mark it out as an 'organ'—of past or present usefulness—or as an externally or internally secreting gland. Its fate appears to be a degeneration within the thyreoid, although it must be confessed that this has not yet been definitely proved.

ENDOCRINE ORGANS

In view of the very large amount of work, experimental and observational, being done at the present time on the endocrine organs, by both the physiologists and clinicians, it may seem somewhat presumptuous for a morphologist to venture upon a discussion of their interpretation and significance, particularly as discordant evidence indicates—as has been insisted on by others—that the time is not yet ripe and a much greater body of facts must be accumulated before broad interpretations may be drawn. The embryologist cannot escape, however, a consideration of certain aspects of the problem; what an organ becomes is a function of its origin and development, and its activity in the adult is determined by its origin and ancestral history.

Thus to the embryologist falls the duty of contributing a respectable portion of the facts, while the character of his problems gives him a peculiar interest in certain sides of the general interpretations.

In the group of the endocrine or 'internally secreting glands'—so-called—two things stand out strongly: (a) The close correlation between them on the physiological side, showing particularly in their pathological derangement, so that hypertrophy or abnormal growth in the one is in some way correlated with abnormal changes in other endocrine organs. The organs thus particularly linked are[6] thyreoid, hypophysis, suprarenal; thyreoid, hypophysis, thymus, gonad; parathyreoid, thryoid; parathyreoid, gonad (?); thymus, thyreoid; thymus, gonad, lymphatic organs; thymus, suprarenal (?); suprarenal (medulla), pancreas (islands of Langerhans ?); suprarenal (cortex), gonad; suprarenal cortex and medulla; hypophysis, gonad; hypophysis, thyreoid; pineal body, gonad, hypophysis. The evidence that these structures are bonded by either a 'functional interdependence' or vicarious or reciprocal functioning is, it would seem, very slight or lacking; the physiological characteristics that they possess in common clearly lie deeper. (b) The second peculiarity of the group of endocrine organs contrasts, it would seem, with their common metabolic features and is at variance with the rule that organs which may be grouped in a natural physiological system also show a unity in development, so that parallel embryologic systems exist. In the case of the structures under consideration, the greatest diversity of origin prevails; their characteristic parenchyma is from all the germ layers and parts of the embryonic body regionally remote and unrelated, while material of quite distinct sources may become associated—as in the case of the hypophysis and suprarenal body. Several of them come out of the embryonic pharynx or its immediate neighborhood—hypophysis, thyreoid, para-

[6] The writer makes no claim to first-hand information in this complicated physiological field. The grouping here given is based mainly on Biedl: "Die Innere Sekretion." My colleague, Dr. S. Simpson, kindly examined the above list.

thyreoids, thymus—and this it is that causes the inclusion of the problem of the endocrine organ group as a subject of discussion in this paper. The common origin of these organs from the pharynx led Kranichfeld to assume that the pharyngeal epithelium is from the beginning and primitively 'internally secreting' (which it may well be) and that its epithelial derivatives but continue an activity that was common to them while still a part of the embryonic pharynx. He brings in support of this view no evidence, direct or indirect, and several objections might be raised in criticism. I believe it is possible, however, to view the pharyngeal derivatives, in common with the other endocrine organs, quite differently.

It is, of course, a matter of history that many of these organs have been frequently regarded as vestigial structures remnants of organs of past usefulness, but now obsolete and without 'function.' With the increase in knowledge of internal secretion, the pendulum swung the other way and there is frequently met a tendency to deny altogether the existence of anything 'vestigial,' or without function, in the body. It becomes, however, largely a matter of definition of 'vestigial' and 'function.' As far as the endocrine organs are concerned, it is quite possible that both views are correct; that they are, in a sense and from one point of view, vestigial structures and that they are peculiarly 'internally secreting;' further, even that they owe their significance as endocrine organs to their quasi vestigial nature. In the case of many of them it is clear that they arose either in the individual or in the race, out of structures morphologically quite different and physiologically of a distinct adaptative value, while the designation as organ or gland frequently becomes difficult.

The corpus luteum arises out of the wall of the Graafian follicle after its collapse, by what might be called a reaction of degeneration accompanied by growth; has, it would seem, an unquestioned influence, particularly upon the uterus through its metabolism; it finally degenerates. It might be termed a 'gland,' periodically developed and disappearing within the individual lifetime. The pineal body or gland develops out

of the epiphysis, grows for a time, approximately during adolescence, and then undergoes degenerative changes. The epiphysis, however, is an encephalic evagination, primitively developing an optic evagination, forming a parietal eye in certain lower forms. The thymus I have just presented reasons for considering as formed out of the branchial epithelium by a kind of reaction of degeneration attended by growth. The parathyreoids are likewise formed out of the embryonic branchial epithelium in its growth transformation. They are not encountered in forms breathing through gills. The thyreoid of the lamprey—which is the only form in which we obtain a glimpse of its origin—arises out of the endostyle organ, when it degenerates at transformation (Marine '13). It is customary, it is true, for zoologists to designate the endostyle organ as thyreoid. It may be pointed out, however, that the endostyle organ corresponds in no respect to the thyreoid that arises from it, in its morphology, and there is, as far as I am aware, no evidence that the endostyle organ—or, as a matter of fact, the thyreoid derived from it—possesses the influence on growth characteristic of the thyreoid of mammals. There is thus no more—nor as much—justification of speaking of the endostyle organ as thyreoid than there would be of speaking of the Graafian follicle as corpus luteum. It develops very early in the mammal and is clearly an old organ. The colloid vesicles, however, so characteristic of it, are more comprehensible, according to Dohrn's view of the colloid as stagnated and condensed secretion, primarily mucous, rather than as exhibiting any adaptative feature correlated with its influence on the bodily growth metabolism.

The hypophysis is of more obscure genetic significance. We have the attempts to interpret it as some form of ancestral mouth (Owen, Beard, Dohrn, Kupffer); as primitively a sense organ, possibly for the detection of changes in the sea-water (Sajous); as preoral branchial clefts (Dohrn); as a gland (neural gland); while the possibility is large that it may owe its development at the end of the primitive growth axis and its curious correlation with growth, to the more general growth conditions of the region, rather than to any more specific phylogenetic

tendency in its ontogeny. In connection with the last view, its development as an expression of the growth of the entire prechordal region requires investigation.

The morphological significance of the suprarenal body is equally obscure. However, while there are, as far as I have been able to ascertain, no facts available that give any suggestion as to why the cortex (or interrenal system) arises from the coelomic epithelium and in a place medial to the gonadal anlage, the derivation of the medulla cells from the embryonic sympathetic system affords a basis for further analysis and investigation of the suggestion that they are, essentially and primarily, undifferentiated sympathetic neurones. The type of cell is widely distributed in the sympathetic system, and is characterized by its strong reducing power, which is responsible, for its ready impregnation with oxydizing salts such as chromates (and dichromates), potassium permanganate, osmium tetroxid etc., and is responsible for the incorrect (Kingsbury '11) and misleading names of chromaffin cell, system and reaction, so commonly employed. It is quite in accord with this mode of origin that the suprarenal medulla cells contain a chemical substance possessing the same general effect as that of the neuronal system from which they were derived.

It is also premature to attempt an explanation of the genetic significance of the islands of Langerhans of the pancreas. We know only that they arise out of the same anlage as the rest of the pancreas parenchyma and still retain a connection with the epithelium of the duct system by cell cords (Bensley '11); but whether they represent undeveloped acini, regressive acini, or 'exhausted' acini, and what the conditions that determined—or determine—their appearance, cannot be surmised. The ancestral history of the pancreas is itself obscure.

As to the ovary (aside from the corpora lutea) and testis, there is, I believe, no evidence of the existence in either of them of endocrine glands, in the morphological sense, nor sufficient evidence of a specific gland, in the physiological sense. To the so-called interstitial cells in both instances is usually ascribed the importance of a gland. In the case of the ovary I have my-

self (Kingsbury '14 a) made, from the morphological side, a critical study of these cells and the conditions that determine their appearance, with results opposing the use of the term 'interstitial gland.' We are compelled, I think, to look to the general metabolic processes of the organ for the basis of the effect on general bodily growth and metabolism, and it is quite suggestive that regressive change is constantly an accompaniment of the growth processes therein proceeding.

Disregarding the ovary and testis, in which no distinct morphological gland is recognizable and in which regressive change is constantly present, in all the other glands grouped together as endocrine glands—even to a degree in those whose genetic significance is still obscure—there is found one feature in common. In the midst of the heterogeneity and atypicality of their origins, as apparently the one embryologic bond paralleling the physiological community that they evince, is the fact that they arise or have evolved out of structures quite different. This is most clearly shown in organs whose history is better known (corpus luteum, pineal body, thyreoid, thymus, parathyreoids). They appear by a process of metamorphosis and their persistence is attended by a 'metaphysiosis'—if one might coin the word—to which is due their influence on bodily metabolism and growth. Their activity and its peculiar effects would thus be a function of their origin, in its peculiarity. Nor could the analysis stop here; their physiological community, it would seem to me, must have its basis in the general bodily metabolism and the relation they bear to it in virtue of their unique genetic qualities. The acceptance of such a point of view would mean, however, a marked reversal of what is, I believe, the accepted interpretation, and a transference of emphasis from the organ to general bodily metabolism; from the body as a collection of organs to the body as a metabolic unit. More specifically, in the case, for example, of exophthalmic goiter, cretinismus, status thymico-lymphaticus, giantismus and acromegaly, dystrophia adiposogenitalis, or Addison's disease—I must confess it is difficult for me to conceive of the primary cause lying in thyreoid, thymus, hypophysis, gonad or suprarenal, but in

the general metabolism, particularly 'growth metabolism' as centering in the development of the reproductive phase.

In this origin out of structures of a different adaptative value, in all cases where we may follow it and where the genetic significance is not still too obscure, there appears to be an element of regressive change present; differentiations of one character, either in the individual or in the race, have disappeared and are replaced by the endocrine organs, and herein lies possibly the hint of a reason for the peculiar correlation of the structures so derived (i.e., the endocrine organs) with the general bodily growth and metabolism. As I have said elsewhere in discussing the question of the significance of the interstitial cells (Kingsbury '14, p. 81): "The assumption that the end-products of metabolism may stimulate growth (and metabolism) is not I believe opposed to the facts of general physiology, but rather the reverse. As applied to the endocrine organs in general, the suggestion has three sides; (a) that the end-products of their metabolism have a marked stimulatory effect on bodily growth (and metabolism); this would seem more or less a restatement that they *are* endocrine organs; (b) that the end-products of growth metabolism should peculiarly affect their growth; and (c) as a correlary of (b) that abnormal bodily metabolism should be associated with an abnormality of growth in these structures.

That the suggestion conveyed is, in its bearings, broader than the more specific problem of the endocrine organs is obvious on the face of it. In an earlier portion of this paper there is briefly discussed the question of a possible correlation with regressive change of the connective tissue and of the blood-forming cells. On the pathological side, the problems of the nature of the inflammatory reaction, the healing of wounds, abnormal growths, etc., are inevitably included.

To return to the primary question of the genetic interpretation of the endocrine organs: It was to be expected that the 'ductless glands' had been looked at by others from the point of view here developed. Dohrn in 1875 formulated the following "Principle of change of function:"

> Through a succession of functions whose bearer remains one and the same organ, is accomplished the Transformation of Organs. Each function is a resultant of several components of which one constitutes the chief or primary function while the others represent accessory or secondary functions. The subsidence of the chief function and the rise of an accessory function alters the total function. The total function becomes a different one and a result of the transformation is the Transformation of the Organ (p. 60).

Dohrn at this time did not apply his principle to the pharyngeal derivatives. He subsequently, however, in his "Studies of the development of the lower vertebrates," stated his conclusions (a) that the thyreoid represents a pair of branchial pockets located between the first and second in the present series (Studies VII, VIII); (b) that the hypophysis is developed from a preoral pair of branchial clefts (Study II); and (c) that the thymus (in sharks) is developed out of dorsal portions of the gill pouches which form gill lamellae, but, being overlaid by dorsal branchial musculature, become cut off and grow to form the thymus (Study IV). The first two interpretations are, I think of purely historical interest at the present day.

Willey in his interesting book on "Amphioxus and the ancestry of the vertebrates" clearly faces the question of the interpretation of the glands, which his central theme brings in for comparison, namely, the thyreoid (p. 169), the thymus (p. 29) and the hypophysis (p. 283); and as clearly recognized the principle of the "change of function." In the case of the thyreoid, the regressive element is recognized in that the "ductless gland" (the thyreoid) is a "vestige of the very actively functional endostyle or hypobranchial groove of the Ascidians, Amphioxus and Ammocoetes." He does not propound any 'principle,' however, nor does he discuss the matter in detail or more broadly.

Wiedersheim ('03), on the other hand, in a paper on "The aging of organs in the race history of man and its influence in disease processes," clearly recognizes a genetic principle involved and its broad significance for pathology. The endocrine organs are considered, but only as having their place in a larger conception. I desire to translate two passages:

I turn now to those organs of man which as their comparative anatomy and embryology teach, in the course of phylogeny have given up their original physiological duty and undergone a change of function. I have primarily in mind the thyreoid, thymus gland, pineal gland, pituitary body (hypophysis cerebri), the so-called glandula carotica, the suprarenal, and a certain territory within the nasal cavities. Many of these organs follow the gland type in their development and are primarily connected with the place of origin by means of a duct. This disappears later however so that from this time on the secretion that is formed is passed directly into the surrounding lymph or blood vessels (inner secretion). (p. 12). A typical change of function can hardly be thought of; and when we ponder it and consider what profound processes of change have taken place both anatomically and physiologically, in the thyreoid gland, for example; or, in other words, how the organ just mentioned has given up a prior clearly highly specialized function in order to enter into very important physiological relations to the *entire organism*, the question becomes quite pertinent, I think, as to whether the unusual and frequent variations in form and size, as also the very marked tendency to pathological alterations of different character, are not due first of all in large measure to the storm and stress period in its phylogeny. (p. 14).

Finally, general biologists have recognized a principle of "retrogression with change of function" (cf. Needham '10, p. 253), finding illustration in the plant and animal world. In the above quotations the word function has been repeatedly employed. It is not, however, I believe through a search for specific 'function' that a clearer comprehension of the 'internally secreting glands' is to be gained. While the adaptative aspects of the growth pattern cannot be ignored, of course, it will be through the analysis of the growth metabolism of the entire organism and the determination of the fundamental characteristics of growth that there will be established a correct basis for the understanding of this group of structures in its morphological, physiological and pathological aspects.

In conclusion, I desire again to emphasize that it is clearly due to no accident of development or chance circumstance that the branchial pharynx in its regressive metamorphosis gives rise to structures of such profound metabolic significance.

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PLATES

EXPLANATION OF FIGURES

Figures 9–11, 13–19, 21 and 22 were drawn by Miss Herford in the Department of Comparative Anatomy of the Harvard Medical School, from models by the author. The remaining figures were drawn by Miss Whitman, Cornell University.

ABBREVIATIONS

A.A.III, third aortic arch
A.A.IV, fourth aortic arch
A.C., carotid artery
C.III, complex III
C.IV, complex IV
C.P., pericardial cavity
D.br.II, ductus branchialis II
D.br.IV, ductus branchialis IV
D.ph-br. III, ductus pharyngeo-branchialis III
D.ph-br. IV, ductus pharyngeo-branchialis IV
D.C., ductus cervicalis
D.thyr., ductus thyreoglossus
D.v.II, diverticulum ventrale II
D.v.III, diverticulum ventrale III
D.v.IV, diverticulum ventrale IV
E, esophagus
Epi.II, epibranchial placode II
Epi.III, epibranchial placode III
Epi.IV, epibranchial placode IV
F.br.I, fissura branchialis I (cleft I)
F.br.II, fissura branchialis II (cleft II)
F.br.III, fissura branchialis III (cleft III)
F.br.IV, fissura branchialis IV
G.IX, ganglion petrosum (IX)
G.X, ganglion nodosum (X)
N.IX, nervus glossopharyngeus (IX)
N.X, nervus vagus (X)
N.XII, nervus hypoglossus (XII)
N.symp., nervus sympatheticus
P.III, parathyreoid III
P.IV, parathyreoid IV
Ph., pharynx
S.br.I, sacculus branchialis I (pouch I)
S.br.II, sacculus branchialis II (pouch II)
S.br.III, sacculus branchialis III (pouch III)
S.br.IV, sacculus branchialis IV (pouch IV)
S.C., sinus cervicalis
T.A., truncus aorticus
Th., thymus
Thyr., thyreoid
Tr., trachea
U, corpus ultimobranchiale
V.br.II, vesicula branchialis II
V.C., vesicula cervicalis
V.J., vena jugularis

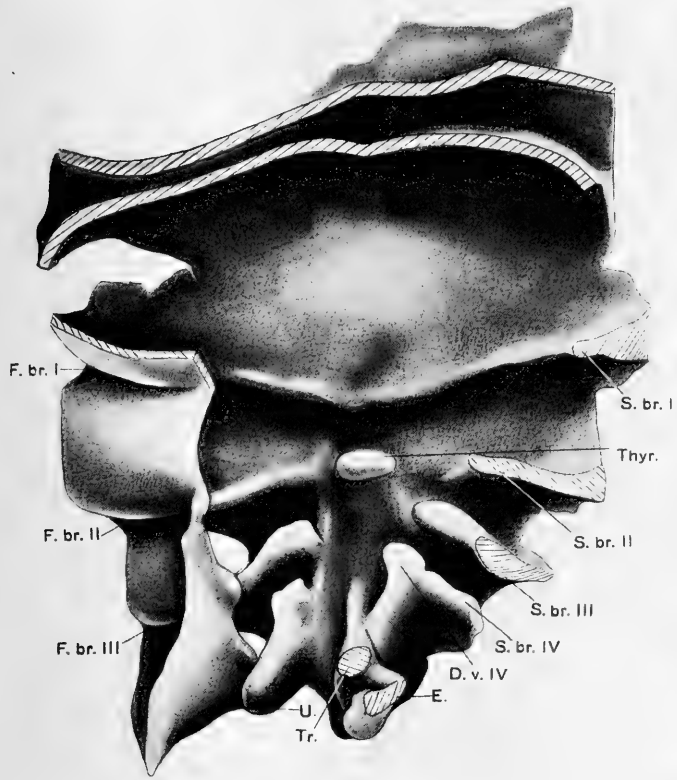
PLATE 1

EXPLANATION OF FIGURES

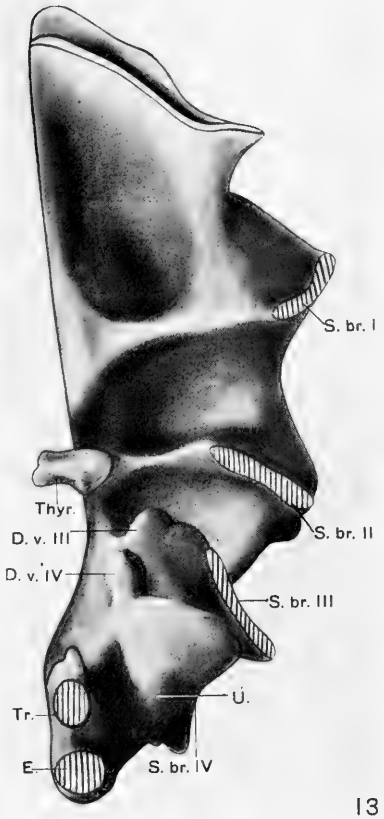
12 Ventral view of a model of the epithelium of the pharynx in a 5 mm. human embryo (Buxton embryo, No. 5, Gage Collection). × 50. Upon the right, the ectoderm is shown in position. cut surfaces are indicated by cross lining.

13 Ventral view of a model of the pharyngeal epithelium in a 7.5 mm. human embryo (No. 256, Harvard Embryological Collection). × 60. Only the left half was modelled in the anterior portion of the pharynx.

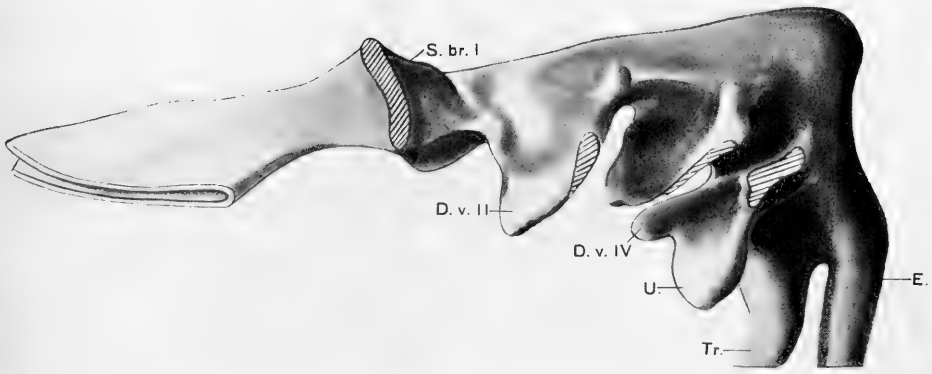
14 Lateral view of the same model. The ectoderm is removed, and the surfaces of contact are indicated by line shading, as in figure 12. × 60.


F. br. I
S. br. I
Thyr.
F. br. II
S. br. II
S. br. III
F. br. III
S. br. IV
D. v. IV
E.
U.
Tr.

12


S. br. I
Thyr.
D. v. III
S. br. II
D. v. IV
S. br. III
U.
Tr.
E.
S. br. IV

13


S. br. I
D. v. II
D. v. IV
U.
E.
Tr.

14

PLATE 2

EXPLANATION OF FIGURES

15 Lateral view of a model of the pharyngeal entoderm from a 10 mm. human embryo (No. 1000, Harvard Embryological Collection). × 50. The left half of the anterior portion of the pharynx only is shown in the model. Cut surfaces are indicated by lines, in this and figure 16.

16 Ventral view of the same. × 50.

17 View of the same model from the aspect that is toward the right in figure 15; that is, from the dorsal aspect as referred to the embryo's body. × 50.

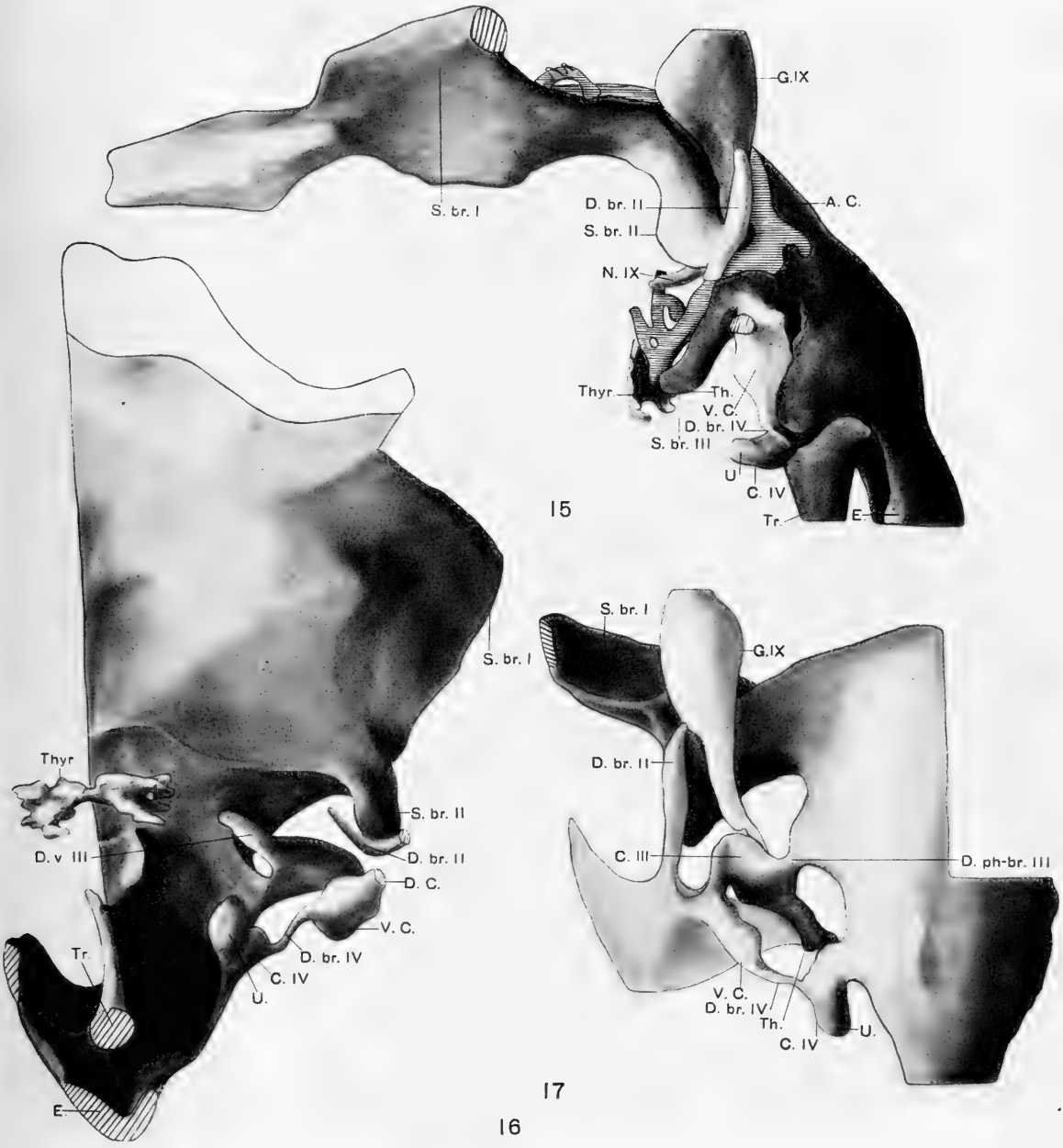

G.IX
S. br. I
D. br. II
A. C.
S. br. II
N. IX
Thyr
Th.
V. C.
D. br. IV
S. br. III
U
C. IV
Tr.
E
15
S. br. I
S. br. I
G.IX
Thyr
D. br. II
S. br. II
D. v III
D. br. II
D. C.
C. III
D. ph-br. III
V. C.
Tr.
D. br. IV
C. IV
U.
V. C.
D. br. IV
Th.
C. IV
U.
E
17
16

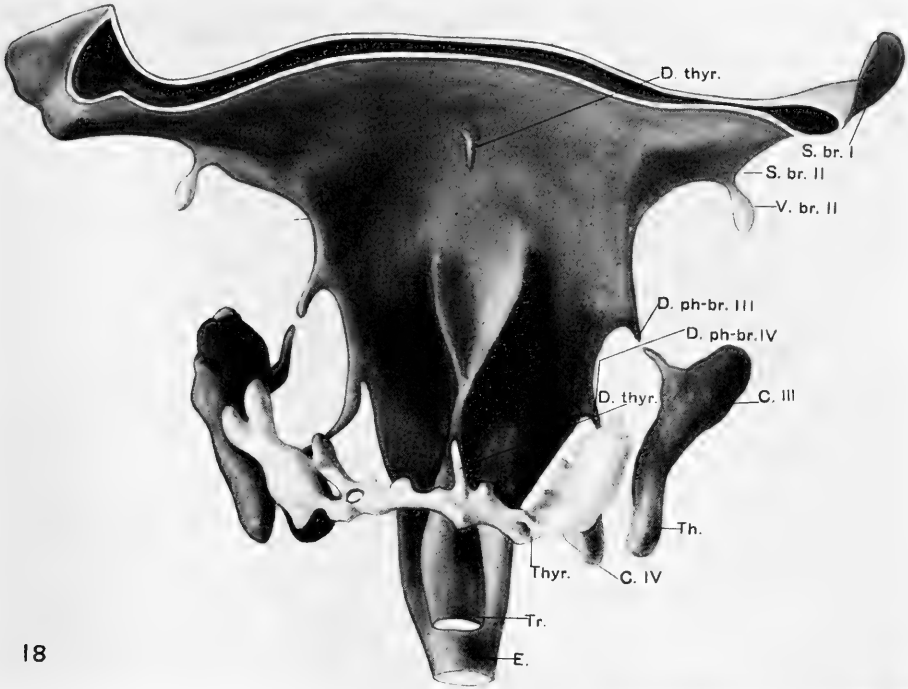
PLATE 3

EXPLANATION OF FIGURES

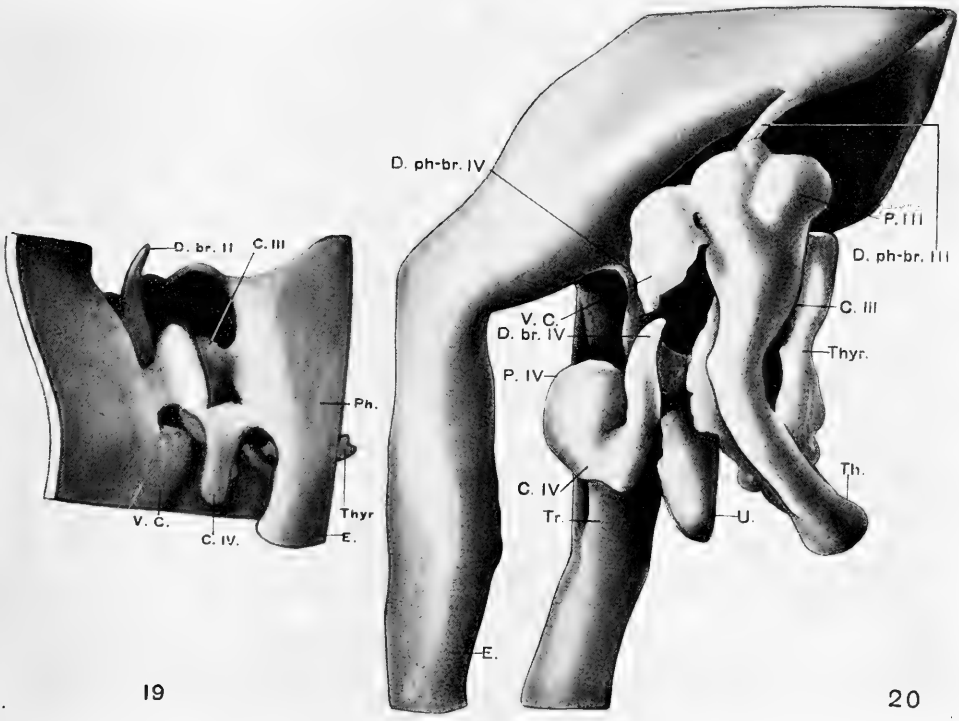
18 Ventral view of the model of the pharyngeal epithelium in a 14.5 mm. human embryo (No. 1003, Harvard Embryological Collection). × 40.

19 Dorsal (posterior) view—as referred to the embryo's body—of a model of the pharyngeal epithelium in a 9.4 mm. human embryo (No. 1005, Harvard Embryological Collection). One-half of the pharynx only is shown. The ectoderm upon the left side is included in the model, the inner surface being here revealed. × 50; reduced 1/3.

20 Lateral view of the caudal portion (metapharnyx) of a model of the pharyngeal epithelium in a 13 mm. human embryo (No. 26, Cornell University Collection). × 75.


D. thyr.
S. br. I
S. br. II
V. br. II
D. ph-br. III
D. ph-br. IV
D. thyr.
C. III
Th.
Thyr.
C. IV
Tr.
E.

18


D. ph-br. IV
P. III
D. ph-br. III
C. III
V. C.
D. br. IV
Thyr.
P. IV
Th.
C. IV
Tr.
U.
E.
D. br. II
C. III
Ph.
Thyr
V. C.
C. IV.
E.

19

20

PLATE 4

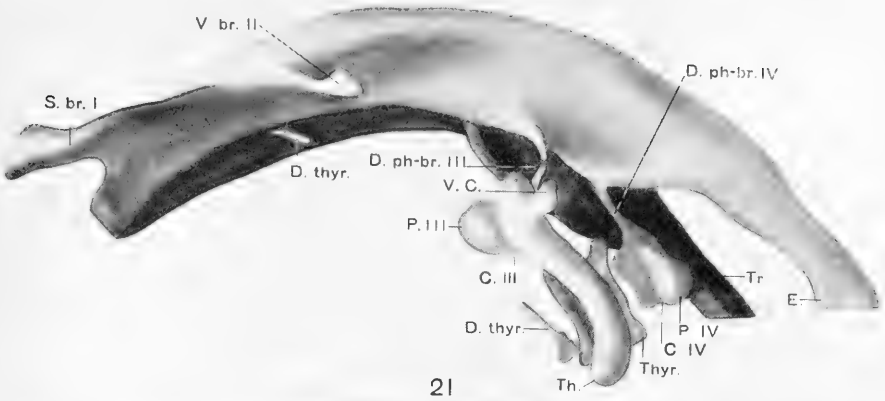
EXPLANATION OF FIGURES

21 Lateral view of a model of the pharyngeal epithelium in a 14.5 mm. human embryo (No. 1003, Harvard Embryological Collection). × 40. Other aspects are shown in figures 18 and 22.

22 Dorsal (posterior) view of the same model. × 40.

23 Photograph of section No. 317 from a 12 mm. human embryo (No. 816, Harvard Embryological Collection), showing the Complex III cut longitudinally on both sides. Upon the left side, parathyreoid III and the lumen of the complex, as well as the ductus pharyngeo-branchialis III, are particularly well shown. Note the relation of the ventral diverticulum (epithelial thymus) to the pericardium. × 50; reduced 1/4.

24 Photograph of section No. 417 from a 19 mm. human embryo (No. 828, Harvard Embryological Collection), showing the Complex III cut nearly longitudinally on both sides. Upon the left, parathyreoid III is particularly well shown. × 30.


V br. II
S. br. I
D. ph-br. IV
D. thyr.
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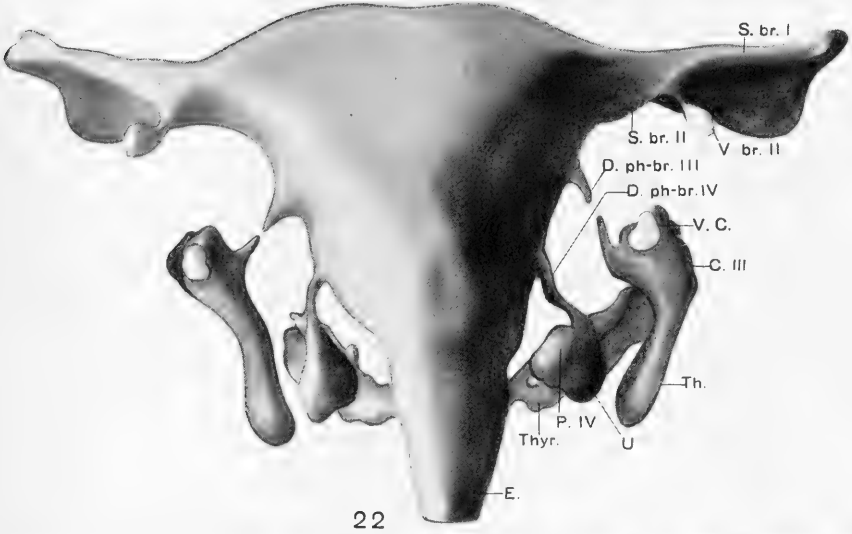
21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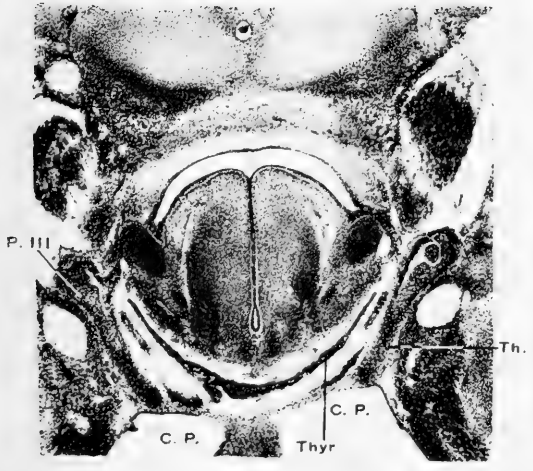
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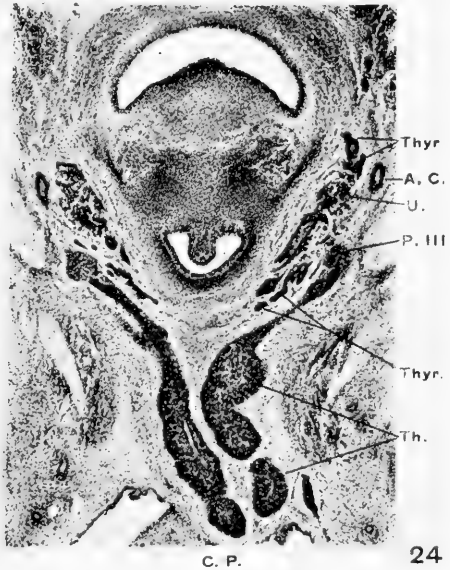
Thyr
A. C.
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Th.
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24

PLATE 5

EXPLANATION OF FIGURES

25 From section No. 127, 7.5 mm. human embryo (No. 256, Harvard Embryological Collection), showing the epibranchial placode II, and the fusion with it of the lower end of the ninth cranial ganglion (petrosum). × 100.

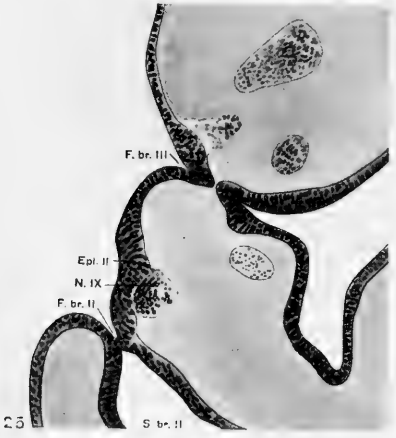
26 From section No. 125, of the same embryo, showing the parathyreoid III in its first appearance and its relation to the third aortic arch. × 100.

27 From section No. 134, of the same embryo, showing the epibranchial placode III and its relation to the vagus nerve. × 100.

28 From section No. 143, of the same embryo, showing epibranchial placode IV and its relation to the vagus nerve and ganglion. × 100.

29 Photograph of a section of a 8.3 mm. human embryo (No. 59 Cornell University Collection), showing the development of the parathyreoid III and its position in relation to the third aortic arch. × 120.

30 Photograph of a section in the same embryo on the same side, 11 sections (110 μ) intervening, to show the developing parathyreoid IV and its position in relation to the fourth aortic arch. × 120.



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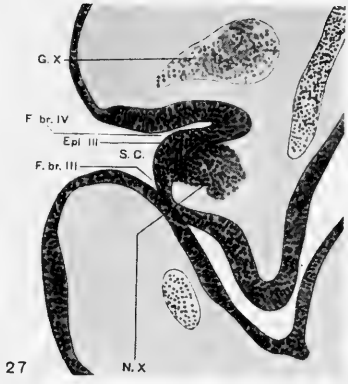

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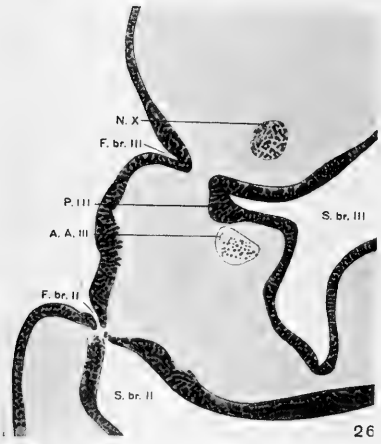

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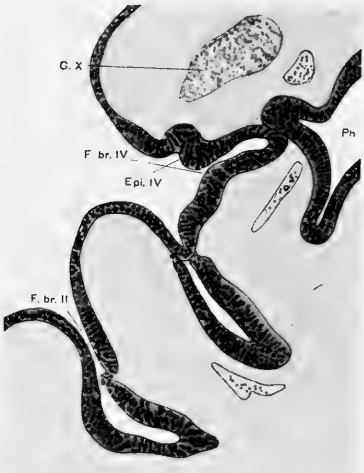

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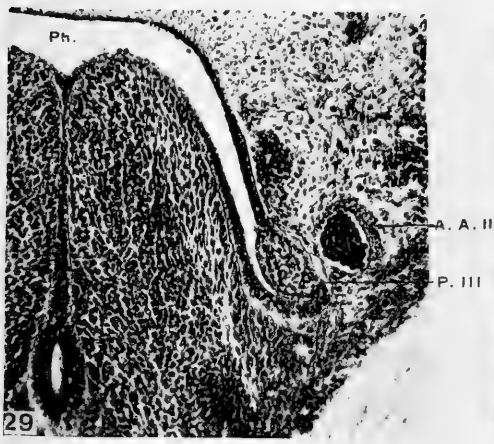

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OBSERVATIONS OF THE LYMPH-FLOW AND THE ASSOCIATED MORPHOLOGICAL CHANGES IN THE EARLY SUPERFICIAL LYMPHATICS OF CHICK EMBRYOS[1]

ELEANOR LINTON CLARK

From the Anatomical Laboratory of the University of Missouri

NINE FIGURES

INTRODUCTION

Within recent years our knowledge of the nature of the lymphatic system has been greatly extended. Researches by Ranvier, MacCallum, and Sabin have revolutionized the former conception of lymphatics as vessels which communicated, by direct openings, with the various cavities and tissue spaces, by establishing the fact that the lymphatic system is everywhere composed of closed tubes lined with endothelium.

Since the publication of Miss Sabin's first article[2] which initiated the modern investigation of the early development of the lymphatic system, much research has been concerned with the problem of the origin or primary differentiation of the lymphatic endothelium. This question is still under discussion although recent investigations have pushed back the study of the earliest lymph vessels to stages in which it had previously been supposed that no lymphatics were present.

That the growth of new capillaries, after the primary differentiation of the lymphatic endothelium, is accomplished by a process of sprouting from the endothelium already formed (a theory

[1] Many of the observations recorded in the present paper were presented before the American Association of Anatomists, at St. Louis, Mo., December, 1914. An abstract was printed in the Proceedings of the meetings, Anat. Rec., vol. 9, no. 1, 1915.

[2] F. R. Sabin, On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. Amer. Jour. Anat., vol. 1, 1901–02.

first proposed by Ranvier, from injections of older embryos, and strongly supported by the investigations of Sabin and others) has been definitely proven by the observations of Clark[3] on living lymphatic capillaries in the tail of the tadpole. He watched the same region of the same larva for several weeks and observed the sending out of delicate lymphatic sprouts which later acquired a lumen and increased by the mitotic division of the endothelial nuclei. Throughout the period of observation, the lymphatic endothelium remained entirely independent of the surrounding mesenchyme cells.

In addition, knowledge of the morphology of the lymphatic system—of the manner in which it invades various parts of the body—has been increased by many investigations. Thus, Miss Sabin and her pupils, by an extensive study of the developing lymphatics of pigs, have given a comprehensive account of the morphology of the system in a mammalian embryo. Hoyer and a number of other workers at the University of Cracow have greatly advanced our knowledge of the comparative anatomy and embryology of the lymphatic system by their investigations of the pattern of the lymphatic capillaries and the position of the main ducts, sacs and hearts in various animals and at different stages of development. F. T. Lewis, Knower, Stromsten, Huntington and McClure, and many other investigators, have contributed to this side of the subject.

On the other hand, questions of the factors which regulate the development of lymphatics, involving a consideration of the reactive powers of lymphatic endothelium and a correlation of the function and structure of early lymphatics have not been so thoroughly studied as in the case of the blood-vascular system. The observations of Clark[4] on living lymphatics have shown that the lymphatic capillary reacts continually to external stimuli by the sending out of fine protoplasmic processes, many of which are withdrawn, while others persist, acquire a lumen, and extend

[3] E. R. Clark, Further observations on living growing lymphatics; their relation to the mesenchyme cells. Am. Jour. Anat., vol. 13, no. 3, July, 1912.

[4] E. R. Clark, Observations on living growing lymphatics in the tail of the frog larva. Anat. Rec., vol. 3, no. 4, 1909.

further. On several occasions, he saw a lymphatic sprout grow out to a group of red blood cells, which had been extruded into the surrounding mesenchyme, and take them in, one by one. From these and other observations he advanced the hypothesis that the accumulation of substances (probably products of cell metabolism) and the passage of such substances through the wall of the lymphatic, stimulates the new formation and further growth of lymphatic capillaries, that the greater the accumulation of these substances, the longer and more persistent the growth processes, and that it is the varying formation of such substances which regulates the peripheral growth of the lymphatic capillaries.

Evans[5] made the observation that new lymphatic capillaries grow into tumors of connective tissue origin but that they do not invade those of epithelial origin, and concluded that the growing lymphatics respond to specific chemiotactive influences in the surrounding tissue. That the substances which act as stimuli of lymphatic growth differ from those influencing the growth of blood capillaries is shown by the fact that the latter type of tumor, described by Evans (the one in which no lymphatics were present) contained an abundant supply of newly formed blood vessels.

Knower,[6] from a study of injections of the lymphatics in living amphibian embryos, has concluded that the differentiation of the earliest lymphatics is initiated by the accumulation of various metabolic substances in the tissue spaces and has suggested a correlation between the rapid growth of the first lymphatics and the early function of the pronephros.

In the present study, an attempt has been made to continue this line of investigation of the factors which regulate the growth of early lymphatics by studying simultaneously a few of the

[5] H. M. Evans, On the occurrence of newly-formed lymphatic vessels in malignant growths. Johns Hopkins Hosp. Bull., vol. 19, no. 209, 1908. Ueber das Verhalten der Lymphgefässe bei experimentell erzeugter Peritonealcarcinose der Maus. Tübingen, 1912.

[6] H. McE. Knower, A comparative study of the embryonic blood vessels and lymphatics in Amphibia. Proc. Amer. Assoc. Anat., Anat. Rec., vol. 8, no. 2, 1914.

physiological and morphological changes which take place in the developing lymphatic system, after its primary differentiation. The problems of the origin of the lymphatic endothelium, the formation of new capillaries, and of the manner in which the lymphatics reach the various organs of the body are not considered here.

For the past three years at the Johns Hopkins University and during the present year at the University of Missouri, Dr. Clark and I have been engaged in a study of the development of lymphatics in chick embryos. In 1912, we published preliminary reports[7] of a part of this work, in which we stated that the early lymphatics are filled with stagnant blood and can therefore be seen in living embryos and distinguished from the blood capillaries. At the suggestion of Dr. Clark, I undertook to study the process by which this blood clears out of the superficial lymphatics, by injecting a few granules of India ink into the blood-filled lymphatic plexus, in living chicks, and observing their fate. It was soon found that this method afforded an opportunity for testing the early flow of lymph, since granules deposited in a vessel of the superficial plexus soon became dislodged from the point of injection and moved along definite paths, previously invisible.

METHOD

The method employed for keeping chicks alive for these experiments was the same as that previously reported for our earlier observations. A window was made in the egg-shell and the shell membrane stripped off, after which the allantois was folded back and the amnion opened and drawn aside, exposing the whole side of the embryo to view. The egg was then placed on the stage of a binocular microscope, which was enclosed in a warm chamber, heated to incubator temperature. At frequent intervals during the course of the observations, a small amount of

[7] E. L. Clark, General observations on early superficial lymphatics in living chick embryos. Anat. Rec., vol. 6, no. 6, 1912. E. R. Clark and E. L. Clark, Observations on the development of the earliest lymphatics in the region of the posterior lymph heart in living chick embryos. Anat. Rec., vol. 6, no. 6, 1912.

warm Ringer's solution was dropped on the embryo and yolk sac to prevent drying. The experiments were carried on under the high power of the binocular microscope and, for the injection tests, glass cannulae measuring from 15 to 20μ at the tip were used. The bright illumination necessary for the observations was obtained from direct sunlight or from a desk arc-light. The temperature of the warm chamber should remain between 37 and 40°C. for the success of the experiments, and all injury to the embryo, and especially to the blood circulation, must be avoided.

In chicks of 5 to $6\frac{1}{2}$ days more or less blood is always present in the superficial lymphatics and it is therefore not difficult to inject a few granules of ink directly into a lymphatic capillary. In older embryos, the lymphatics cannot be seen without injection and the direct puncture of a vessel is a matter of greater uncertainty. However, with practice and increased familiarity with the position of the lymphatics at various stages it became possible to pierce a lymphatic at practically every attempt, although small extravasations in the surrounding tissue produced no effect on the circulation of granules. It is important to insert the needle directly into the lumen of a lymphatic vessel, and not to plunge it through the opposite wall, to deposit only a very few granules, in order not to plug the vessel, and, finally, to withdraw the needle carefully so as to avoid leakage.

After testing the direction and character of the lymph-flow by observing the course of the injected granules, the superficial lymphatics were injected. The embryo was then fixed in Carnoy's fluid, dehydrated in absolute alcohol, and cleared by the Spalteholz method in benzol and oil of wintergreen.[8] In the cleared specimens, the pattern and position of the superficial lymphatics could be studied in comparison with the records of the lymph-flow in the same embryos. Injections of the deep lymphatics were also obtained at all of the stages, in order to determine their relation to the superficial vessels. For showing the relationship of the lymphatics to the venous system, cleared specimens were obtained in which, in addition to the

[8] I am indebted to Miss Sabin for this method of fixing and clearing injected specimens.

lymphatic injections with India ink, the principal blood vessels had been injected with aqueous Berlin blue.

After the 10th day of incubation, the increased thickness of the skin and the development of feathers prevented further observation of the circulation of granules in the superficial lymph vessels. For this reason, the present study is confined to the changes in the structure and function of the superficial lymphatics during their development in chicks of $5\frac{1}{2}$ to 9 days.

THE PRIMITIVE CONDITION OF THE SUPERFICIAL LYMPHATICS

The primitive form of the early superficial lymphatic system, in the chick, is that of a richly anastomosing capillary network which spreads out over a large area. This plexus communicates with the venous system at certain points but remains independent of the surrounding plexus of blood capillaries from which it can be distinguished in living embryos by its more irregular form and by the more darkly colored stagnant blood normally present in its vessels. Each set of capillaries (blood vessels and lymphatics) can be injected separately without disturbing the other. The vessels composing the primary lymphatic plexus vary in size and shape, large knob-like portions alternate with delicate connections, finer than blood capillaries, and small pointed projections. That these early lymphatics are lined throughout with endothelium is evident from the silver markings which show distinctly after the plexus has been injected with silver nitrate. The continuous endothelial lining can also be seen in microscopic sections studied with the oil immersion, where E. R. Clark[9] has shown that the endothelial nuclei have definite morphological characteristics which distinguish them from the nuclei of the surrounding mesenchyme cells.

Not all the points of origin for the lymphatics of chicks have been determined. One of them, the region in the tail lateral to the myotomes, a portion of which is occupied at a later stage by the posterior lymph heart, has been studied in detail by Dr. Clark and myself and will be reported later.

[9] E. R. Clark, On certain morphological and staining characteristics of the nuclei of lymphatic and blood-vascular endothelium and of mesenchyme cells in chick embryos. Proc. Amer. Assoc. Anat., Anat. Rec., vol. 8, no. 2, 1914.

In chicks of 5 days, the superficial lymphatic system consists of a plexus of very fine vessels and is confined to two localities—the region lateral to the myotomes on each side of the tail, where certain portions of it connect with the first five coccygeal veins, and also to the neighborhood of the thoraco-epigastric vein. At this stage, the anterior plexus extends along the side from the axilla as far posteriorly as the beginning of the leg. When it is injected and the embryo cleared, a continuation from it can be seen which runs through the axillary region and connects with a deep plexus of vessels located just dorsal to the anterior and posterior cardinal veins, near their junction to form the duct of Cuvier. The deep plexus in turn communicates with these veins at a number of places.

The primitive lymphatic plexus is characterized by a wild luxuriant growth and it extends rapidly, so that in a chick of about 5 days and 20 hours, a continuous superficial plexus of lymphatic capillaries can be injected over the whole side of the embryo, from the axilla to the tail. The size of the lymphatic capillaries of this early plexus varies according to the character of the surrounding tissue. In general, most of the vessels are larger and more distended in the lymph heart region and in the region just anterior to the leg, where the tissue is loose, while they are finer over the posterior part of the pelvis and in the axilla where the tissue is denser.

During all this primitive stage, the lymphatics normally contain stagnant blood. In one of the preliminary reports referred to, I stated that these blood-filled lymphatics were nonfunctioning. This statement now appears to have been somewhat inaccurate for it is quite probable that the very abundant formation of new capillaries which characterizes these early lymphatics is associated with the passage of fluid through their walls. Rather, it should be said that the stage in which stagnant blood is present in the early lymphatics is a stage in which there is no circulation of lymph.

It is evident from a number of observations and experiments made by Dr. Clark and myself, which can only be referred to briefly at this time, that the blood backs up into the early

lymphatic plexus from the veins with which it is connected. These communications between lymphatics and veins can be demonstrated easily by injection in chicks of five days and over. The most favorable place for testing this is in the tail region, where the whole injection can be watched and the ink can be clearly seen as it passes from the lymphatic plexus into the intersegmental coccygeal veins and then runs rapidly anteriorly in the main caudal vein.

The amount of blood present in the superficial lymphatics can be increased in a living chick by changing the position of the embryo so as to allow the force of gravity to affect various parts of the chick. For example, the posterior lymphatics, which spread out on the surface of the tail and over the posterior border of the pelvis, lie for the most part anterior to the coccygeal veins with which they are connected. By pulling on shreds of the amnion, the tail can be raised so that this part of the chick is uppermost, and the embryo held in this position for sometime. In this case, after the expiration of only a few minutes, more blood enters the lymphatics and the peripheral (lower) portions of the plexus become much redder and more distended.

Again, the amount of blood in the early lymphatic plexuses can be increased noticeably and quickly by interfering with the blood circulation. When the heart becomes embarrassed from any cause (such as the addition of strong chloretone or too high a temperature of the warm chamber) there occurs a back pulsation in the veins, which can be observed to best advantage in the large vessels of the allantois and yolk sac. Within a few seconds after the beginning of such a circulatory disturbance, the superficial lymphatics become markedly redder and more congested owing to the increased amount of blood which enters them as a result of this increased venous pressure.

From these and other observations on living embryos, we came to the conclusion that the normal presence of stagnant blood in the superficial lymphatics, during the primary stage of their development, is dependent upon:

a. The fact that the early lymphatics form a continuous plexus which is connected at a number of points with veins.

b. The absence of valves in this early stage, and

c. The fact that there is not enough fluid entering the lymphatic capillaries from without to force out the blood, or, in other words, that the pressure in the veins is higher than that in the lymphatics.

As the presence of stagnant blood indicates, there is no circulation in these earliest lymphatics. Granules introduced into various parts of this primitive plexus do not move along within the vessels, although the violent periodic movements of the embryo force them back and forth within the lymphatics.

Figure 1 represents the injected superficial lymphatics in a chick of 5 days and 6 hours, and shows the character of the two plexuses before they have united.

OBSERVATIONS OF THE EARLY LYMPH-FLOW AND ASSOCIATED CHANGES IN THE FORM OF THE LYMPHATICS IN CHICKS OF DIFFERENT STAGES

The various changes in the pattern of the superficial lymphatics are illustrated in figures 2 to 7, and the direction of the lymph flow, as shown by the movement of granules in the living, is indicated by arrows in the drawings.

The lymph-flow, in the superficial lymphatics, commences in chicks of approximately 5 days, 18 hours (measuring from 15 to 17 mm. before fixation). When a living chick of this age was observed under the binocular microscope with brilliant illumination, lymphatics containing stagnant blood could be seen in the form of a continuous plexus of irregular vessels which was present in the tail region and over the pelvis. Anterior to the leg the amount of blood was more scanty and the lymphatics consequently more difficult to see, while along the anterior part of the side and in the axillary region, only a few scattered knobs of blood indicated the position of the blood-filled plexus visible in chicks a few hours younger.

When one of the lymph vessels situated on the pelvis, in such an embryo, was punctured and a few granules of ink deposited in its lumen, they remained at the point of injection. At the time of the periodic muscular movements, the ink granules were

forced to and fro but they showed no tendency to move ahead in any definite direction. Similarly, in the region just anterior to the leg, such an injection produced no effect. However, when a small number of granules were injected into one of the scattered knobs situated more anteriorly, they soon became dislodged from the point of injection and moved anteriorly, one after the other, always following a definite though somewhat tortuous path, previously invisible, situated near the

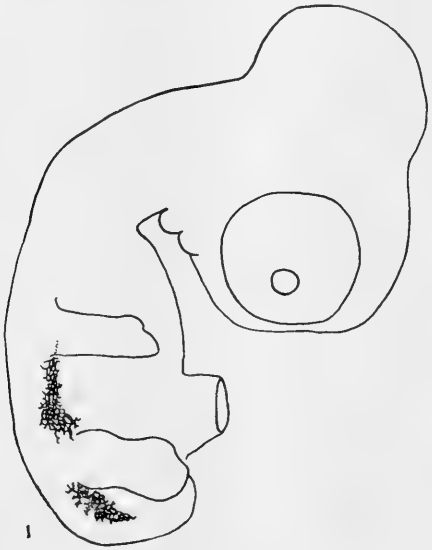

Fig. 1 Injected chick of 5 days and 12 hours (14.5 mm. before fixation), showing character of primary superficial lymphatic plexuses. No circulation. × 5.

thoraco-epigastric vein. From here the granules moved through the axillary region and disappeared in the depth, beneath the shoulder. This experiment was repeated several times in each embryo of this stage, and in every case the granules behaved in the same manner, moving steadily anteriorly and disappearing beneath the shoulder.

After the circulation had been tested in this way, the same embryo was injected and cleared. In such a specimen it was found that the whole superficial plexus over the pelvis and part of the tail (that part which had contained stagnant blood

when the egg was first opened) still showed the irregular primitive form of the lymphatics of younger embryos, but along the side, near the thoraco-epigastric vein and in the exact path which the granules had followed in the living embryo, was a distinct channel which ran through the axilla, dipping in beneath the shoulder and joining the deep plexus connected with the veins. This earliest channel to differentiate was narrow and winding but always distinctly marked out from the surrounding lymph-capillary plexus (fig. 2).

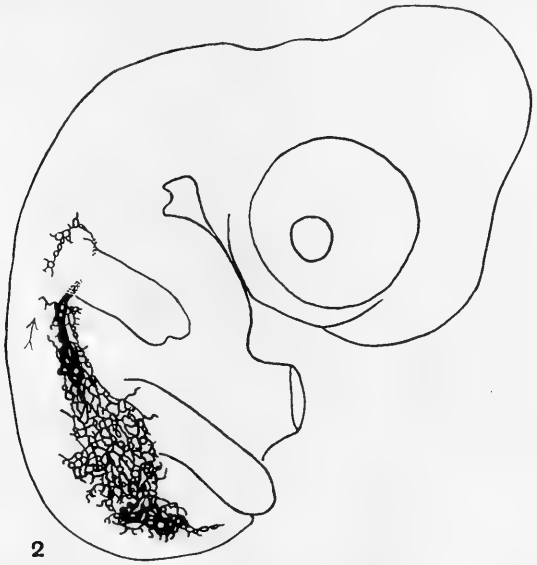

Fig. 2 Injected chick of 5 days, 22 hours (16.5 mm.). Stage of beginning lymph flow in the superficial lymphatics, showing first lateral lymphatic which differentiates in the side plexus, and the primitive character of the remainder of the superficial lymphatics. Arrows in this and subsequent figures indicate the path and direction of the circulation in the living embryo. × 5.

The earliest specimen in which any definite lymph-flow could be found was one in which blood was present throughout the superficial plexus and injected granules failed to circulate at any point except in the region just posterior to the shoulder, where they moved sluggishly but steadily into the depth, through the axilla. When this embryo had been injected and cleared, a definite channel, larger and straighter than the other lym-

phatics, was found to be present in the axillary region, which connected the side plexus with the deep jugular plexus. All the other superficial lymphatics still retained the irregular indifferent form of the primary plexus.

In testing many embryos of this stage, I found a closely graded series of chicks which showed a progressive extension of the area of lymph-flow. First, the granules circulated only in the region just posterior to the shoulder, then they moved anteriorly for about half the distance between shoulder and leg, and later for the entire area between the two limbs, and, in the oldest embryos of this stage, granules injected into the lymph vessels on the anterior part of the pelvis moved anteriorly from the point of injection until they disappeared beneath the shoulder. In each of these embryos, after injection, a definite channel was present in a position exactly corresponding to the path followed by the circulating granules. In the case of the older chicks in which the lymph-flow had been present throughout the side region and the movement of granules was more direct and rapid than in the younger embryos, injection showed that the channel was wider and straighter, as well as longer. During the latter part of this stage, a new plexus of lymphatics grows to the surface from the deep jugular plexus. It emerges in front of the shoulder and grows around dorsally to the shoulder.

It appears, therefore, that the earliest lymph-flow, in the superficial lymphatics, starts in the region between the shoulder and leg and that its direction is anterior, into the anterior and posterior cardinal veins. Associated with this first lymph flow, a channel, the first of the superficial lymph trunks, differentiates out of the irregular primitive plexus in this region. During this stage the blood is washed out of the anterior lymphatics but still remains in the posterior plexus of the pelvis and tail which retains its indifferent character.

It is probable that the pressure in the tail veins, with which the posterior lymphatic plexus connects, is higher and hence more difficult to overcome, than that of the anterior and posterior cardinal veins into which the anterior lymphatics drain, and that to this is due the anterior direction of the earliest lymph-flow.

Support is lent to this view by the fact that the main caudal vein, into which the intersegmental coccygeal veins empty, soon enters the mesonephros and breaks up into capillaries and the resistance to be overcome would thus be greater than in the neck veins which enter the duct of Cuvier near the point where the lymphatics connect with them. Further evidence for this hypothesis will be considered later in connection with various observations on the early circulation in the posterior lymphatics.

In chicks of approximately 6 to 6½ days (average length 18 mm.) the posterior lymph heart begins to pulsate. The early contractions of the lymph heart have been described in another publication.[10] In this paper Dr. Clark and I stated that the early pulsation of the lymph heart is closely associated with the periodic movements of the embryo, several beats occurring with every spasm of movements, and that during the first day and a half of pulsation, this connection is inseparable, the lymph heart beats ceasing altogether when the body movements are paralyzed with chloretone. The first pulsations commence at a time when the lymph heart is still in the form of a plexus.

In an embryo of the stage in which the lymph heart is just beginning to contract, scattered knobs of blood are still present in the lymphatics of the pelvis. The lymph heart shows as a translucent area at the angle between the tail and the pelvis. Contractions of this region occur at the time of the body movements and are plainly visible although they are feeble in comparison with the pulsations seen in older chicks. In the interval of rest between spasms (about 40 seconds in duration) the lymph heart fills up with blood and the various vessels, of which it is composed, then become visible. When India ink granules were injected into the lymphatics of the pelvis, at this stage, they moved posteriorly, taking a slow and winding course, into the lymph heart plexus, and from there into the intersegmental veins of the tail. If a lymph heart pulsation occurred while the granules were moving down over the pelvis, they were forced

[10] E. L. Clark and E. R. Clark, On the early pulsations of the posterior lymph hearts in chick embryos; their relation to the body movements. Jour. Exp. Zool., vol. 17, no. 3, 1914.

back temporarily. Immediately after each beat, however, they moved on faster than ever toward the lymph heart. Between the spasms of muscular movements, when the blood backed up into the lymph heart, the flow in the lymphatics of the pelvis became much slower and occasionally ceased altogether until the return of the next lymph heart contraction.

In embryos of this stage, the movement of granules in the lymphatics of the side region was anterior, through the axilla, and into the depth, under the shoulder, as in the stage just preceding. In addition, circulation had been established in the plexus dorsal to the shoulder, where injected granules moved anteriorly and dipped into the deep jugular plexus at the anterior border of the shoulder.

When an embryo of this stage had been injected, it showed the same channel over the side, a newly differentiated channel in the supra-scapular plexus, and also one over the pelvis (fig. 3). The position of this first posterior channel varies greatly in different embryos; it may occupy a ventral, dorsal, or median position with reference to the rest of the plexus.

In slightly older chicks, the lymph heart beats have become stronger, the circulation of lymph in the region just anterior to the hip has practically ceased, while the posterior circulation over the pelvis has become more rapid and continuous. The character of the superficial lymphatics in these embryos, as shown by injection, is practically the same as that just described except that no channels are present in the region just anterior to the hip. Instead, the vessels of this region are irregular and much enlarged.

During this stage, extensions from the supra-scapular lymphatic plexus have encircled the region dorsal to the shoulder and anastomosed with branches of the side lymphatics. No circulation is yet present in this newly formed posterior portion of the supra-scapular plexus.

In this stage of beginning lymph heart pulsations and of posterior circulation for the pelvic lymphatics, channels also differentiate in the lymph heart plexus. Their position corresponds to the paths which the injected granules follow on their way to

the veins. In this stage also the remainder of the blood is washed out of the superficial lymphatic system. The character of the lymphatics and the direction of lymph-flow for this stage are illustrated in figure 3.

In chicks a little younger than 7 days (measuring 19 to 20 mm.) ink granules injected into the posterior portion of the

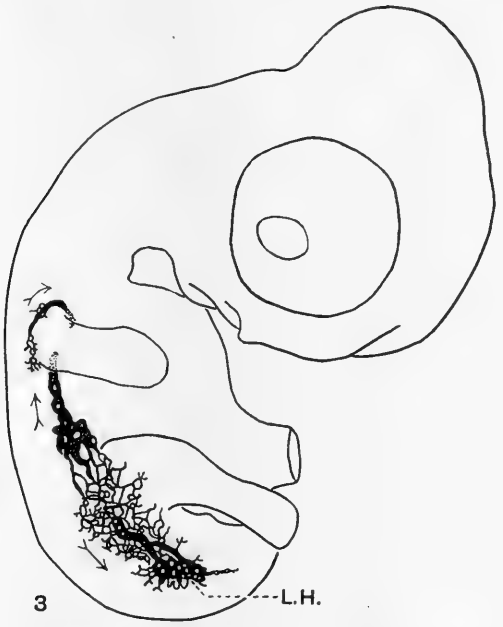

Fig. 3 Chick of 6½ days (18 mm.). Stage of beginning lymph heart pulsations and earliest posterior circulation over the pelvis, and of beginning anterior flow of lymph in the supra-scapular plexus. Injection shows first posterior channels and the first supra-scapular channel differentiating from the primary plexuses. *L.H.*, lymph heart which begins to pulsate while still in the form of a plexus. × 5.

supra-scapular lymphatic plexus make their way slowly posteriorly, pursuing a winding course to the side lymphatics. From here they move on posteriorly, more rapidly and directly, over the side and pelvis and into the lymph heart, whose pulsations have become stronger. The only parts of the superficial lymphatics in which the circulation is still anterior, at this stage, are the region just posterior to the shoulder, where the granules

still follow a definite path through the axilla into the deep plexus, and a small anterior portion of the supra-scapular lymphatics.

Injections of such embryos show definite large and comparatively straight channels over the side and pelvis. A channel

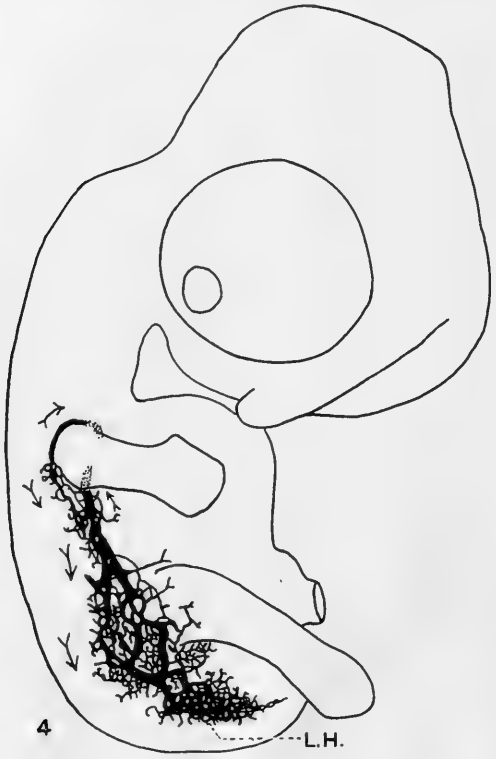

Fig. 4 Chick of 6 days, 22 hours (19.5 mm.). Stage of stronger lymph heart pulsations and posterior circulation in most of the superficial lymphatics. Flow is still anterior in axillary region and in anterior portion of the supra-scapular region. Injection shows newly formed channel connecting supra-scapular lymphatics with those of the side, and large channels over the side and pelvis. *L.H.*, lymph heart. × 5.

posterior to the shoulder, draining a small ventral portion of the side and running through the axilla is still present, while a narrow duct, connecting the lymphatics dorsal to the shoulder with the main side channel, has developed; this stage is illustrated in figure 4.

A few hours later (in embryos measuring 21 mm.) ink granules injected at any point of the superficial lymphatic system move posteriorly into the lymph heart, which beats vigorously. The flow is direct and, compared with earlier stages, rapid. The

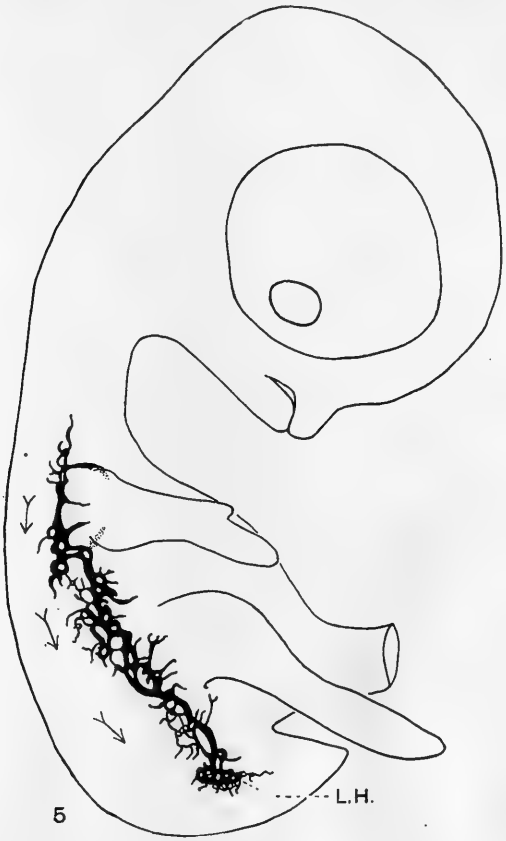

Fig. 5 Chick of 7 days (21 mm.). Stage of rapid posterior circulation into the lymph heart from all of the superficial lymphatics. Injections show large comparatively straight channels over the surface of the body and enlarged channels through the lymph heart, *L.H.* × 5.

course of successive granules can be followed readily as they move posteriorly, from a point of injection dorsal to the shoulder, over the side and pelvis into the lymph heart and thence into the coccygeal veins. In the superficial lymphatics of chicks between

7 and 7½ days the lymph-flow is all posterior, draining into the tail veins through the lymph heart.

Figure 5 represents the injected superficial lymphatics of this stage. The increased size of the channels and their moderately straight course is noticeable. The position of the main channels varies in chicks of the same stage: the main channel for the pelvis may be situated ventrally or dorsally with reference to the rest of the surrounding plexus, or two or even three main channels may develop and persist for two or three days. Frequently the two sides of the same embryo show variations of pattern, one having a ventral duct as its main channel and the other a dorsal or median one. Similarly the channel selected through the plexus connecting the supra-scapular lymphatics with those of the side may cling closely to the shoulder, or it may follow a more gradual, dorsal course, or, occasionally, channels in both positions may persist. Whatever the location of the ducts and whatever the variation in the path taken by the injected granules, the general direction of the lymph-flow, in normal healthy chicks of the same stage: is always the same.

At this stage, the pulsations of the lymph heart are still inseparable from the body movements and occur only at the time of the periodic spasms, but they are much more vigorous than in the younger chicks and the course of the granules through the lymph heart is more direct. Associated with this and also with the increase in the area drained by it, injected specimens show a change in the form of the lymph heart. Although it is still in the form of a plexus, the various channels have enlarged and many of the small capillaries have disappeared so that the heart has become more compact and regular in outline.

Injections also show similar changes in the deep plexus under the shoulder. Although no muscle develops around this plexus, various channels enlarge and many vessels disappear so that the plexus occupies a less extensive area than in the embryos of 5 and 6 days. Connections are still present between this deep plexus and the superficial lymphatics, although the lymph from the surface all flows toward the posterior venous connections. In the axillary region, a large vessel is still present, and from

its size it appears probable that a circulation of lymph from the deep axilla and the under part of the wing, into the deep anterior plexus is still present. The tissue over these vessels is too thick to test this point by watching the movement of injected granules. Posterior circulation of granules into the deep jugular plexus can be demonstrated by injection into the deep cervical lymphatic which accompanies the jugular vein.

In chicks of $7\frac{1}{2}$ days a new plexus of capillaries grows posteriorly from the supra-scapular lymphatics. These vessels spread out over the dorsal part of the side region and run parallel to the main side channel. Toward the end of the 8th day of incubation, granules injected into these dorsal side lymphatics circulate anteriorly into the supra-scapular lymphatics, and from them they enter the deep jugular plexus through the connection anterior to the shoulder. Thus the circulation, in these lymphatics dorsal to the shoulder, is again reversed. For several hours the old main side channel persists and granules injected into the ventral side lymphatics circulate posteriorly over the pelvis and into the lymph heart. This is a transition stage between those illustrated in figures 5 and 6.

In embryos of 8 days and over, the new dorsal path of drainage for the side becomes more and more prominent and gradually supplants the original side channel as the main path of circulation. Granules injected anterior to the hip, all move anteriorly following a fairly direct path into the supra-scapular lymphatics. Here their progress becomes slow and their passage into the deep lymphatics is greatly delayed. For the region over the pelvis, the lymph flow is still posterior into the lymph heart. Injections of this stage show that lymph trunks are present over the pelvis and also over the dorsal part of the side and that these vessels correspond in position and size to the course and rate of the lymph-flow as tested by the granules injected in the living. The injections also show in the supra-scapular region where the movement of granules in the living embryo, was slow, distended sac-like vessels (fig. 6). That the pressure in these vessels is very high is evident from the ease with which the injection mass extravasates. Occasionally, in living chicks, after the injection

of a slight amount of ink into these already distended vessels, the violent movement of the shoulder, at the time of the periodic muscular spasms, caused the delicate walls of this sac to assume the fuzzy appearance characteristic of extravasations.

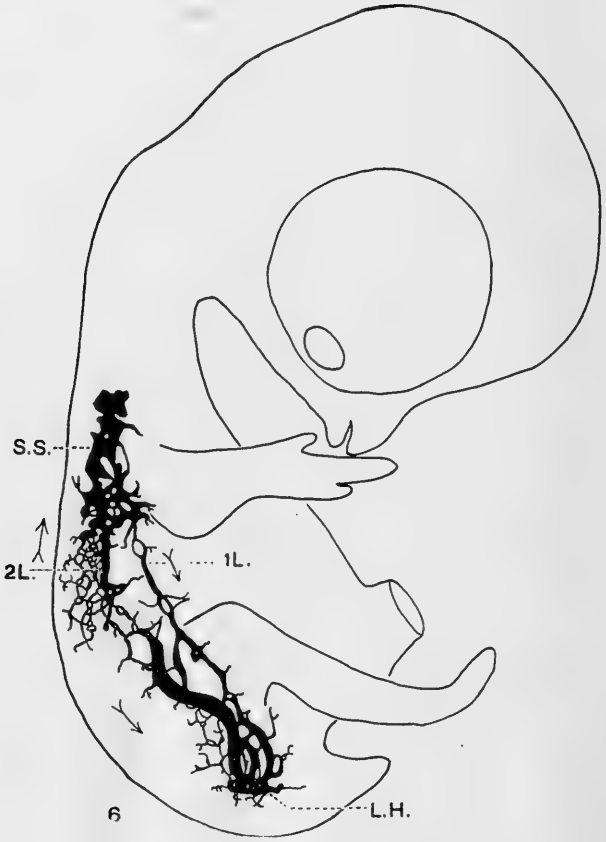

Fig. 6 Chick of 8 days (23 mm.). Stage of beginning anterior circulation in the dorsal part of the side lymphatics, retarded flow in the supra-scapular region. The circulation for the rest of the superficial lymphatics is posterior, as in figure 5. *L.H.*, lymph heart, now in the form of a sac; *S.S.*, supra-scapular sac; *1L*, original lateral lymph duct; *2L.*, newly formed lateral lymph duct. × 5.

Cleared specimens show that the newly developed cartilage of the shoulder has crowded back the deep jugular lymphatics and that the connections between these and the superficial vessels of the supra-scapular region has shifted, so that instead

of curving around ventrally and entering the deep plexus anterior to the shoulder, at a gradual inclination, it now comes off at right angles to the superficial lymphatics and pursues a steep circuitous course. It seems evident from an examination of chicks of this stage that the shifting of the deep lymphatics and the growth of the shoulder cartilages have interfered with the free outlet of the lymph from the superficial lymphatics and in consequence the fluid has become dammed back. At this stage, the subcutaneous tissue is very loose and apparently the endothelium encounters very little resistance from outside and expands in response to the increased pressure from within, resulting in the formation of a large lake or sac.

At this stage also the posterior lymph heart first assumes the form of a sac. The muscular walls appear to offer an obstacle to its expansion for its size is very little larger than in chicks of 7 days. The separate channels which formed the cavity of the heart in earlier stages have enlarged still further until the walls between them are represented merely by thin trabeculae. The venous connections of the lymph heart have now been reduced from five to three—those communications nearest the channels which differentiated from the primitive plexus are the ones which are retained.

Cleared specimens of injected embryos show that the lymph heart, at this stage, receives tributaries from an extensive plexus of lymphatics around the aorta and also from an extremely rich plexus of allantoic lymphatics. The early development of these allantoic vessels has been studied but it cannot be considered at this time. At 8 days, these lymphatics have extended for a considerable distance along the main allantoic blood vessels, and ink injected into them in living chicks can be seen to move rapidly toward the embryo, and a few seconds later the lymph heart becomes dark with the ink which has reached it in this way. The size of the channels which connect these allantoic lymphatics with the lymph heart show that there is a very active passage of fluid through the lymph heart, from sources other than the superficial lymphatics, and probably accounts for the expansion and coalescence of the channels composing the lymph heart.

In chicks of $8\frac{1}{2}$ to 9 days, granules injected into the side lymphatics moved anteriorly, following the new dorsal path into the supra-scapular lymphatics (where their movement became retarded) and finally disappeared in the depth. Over the pelvis, injected granules moved slowly posteriorly to a point near the lymph heart where they stopped and appeared to oscillate for

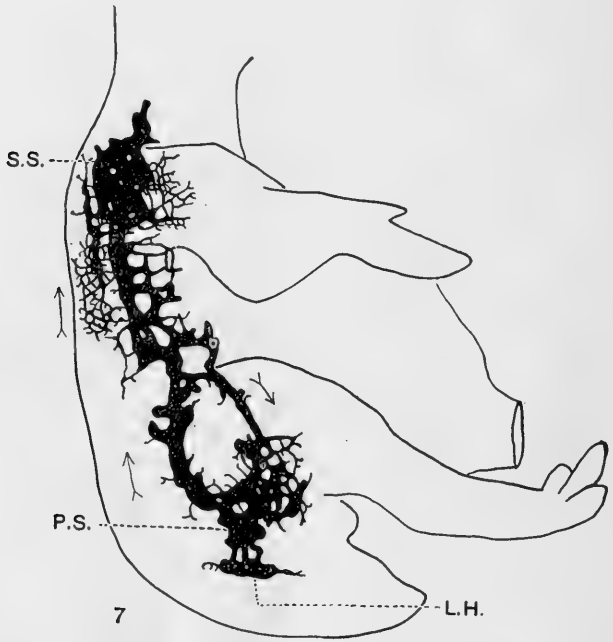

Fig. 7 Chick of 8 days, 20 hours (25 mm.). Stage of anterior circulation for side region and part of the pelvis; posterior circulation for the rest of the pelvis; retarded flow in the supra-scapular region and over the posterior surface of the pelvis; injection shows new lateral lymph channel has replaced the original ventral one. *L.H.*, lymph heart; *S.S.*, supra-scapular sac; *P.S.*, pelvic sac. × 5.

a few seconds. If the lymph heart contracted while they were in this region the granules moved rapidly posteriorly into the lymph heart, during the interval immediately after each beat. If no pulsation of the lymph heart occurred, the granules, after a few seconds of hesitation, flowed around a wide bend into another pelvic channel and from there circulated anteriorly into the side channels. In the embryo represented in figure 7, the

lymph-flow was posterior for the ventral part of the pelvis, and anterior for the dorsal portion, while in some chicks this condition was reversed. But, invariably, at this stage, there is a point over the pelvis in the vicinity of the lymph heart, where the flow is greatly retarded, and where it is modified by the beating of the lymph heart.

The injected specimens show a large dorsal side channel and two channels over the pelvis, the larger of these occupying the path in which the lymph-flow had been anterior. Dorsal to the shoulder, the supra-scapular sac has become still larger and small windows remain as relics of its former plexiform character. At the point of 'hesitation' over the posterior part of the pelvis, another sac-like enlargement is present. Both of these superficial sacs are much larger than the lymph heart or than the deep jugular vessel under the shoulder (the 'jugular sac') connected with the veins. Injections show a still more extensive circulation from the allantois at this stage which evidently has monopolized the greater part of the function of the lymph heart. At this stage the circulation in the superficial lymphatics is greatly interfered with: the fluid is prevented from reaching the posterior outlet because of the immensely increased flow from the allantois, while at its anterior outlet its flow is hindered by the mechanical obstacle presented by the shoulder joint. Also injections of the deep lymphatics of this stage show that the deep jugular sac receives an abundant lymphatic drainage from the deep vessels of the neck, lungs and heart. As a result of all this interference with the flow in the superficial lymphatics, the pressure in these vessels is greatly increased and, meeting very little resistance from the surrounding tissue, they expand into huge sacs or lakes at every point where an obstacle is encountered. Similar enlargements are also found in certain of the deep lymphatics.

Unfortunately, in chicks of 9 days and older, the lymph-flow cannot be thoroughly tested on account of the thickness of the skin and the appearance of feathers. At this stage, ink injected into the lymph heart is not forced back into the superfical lymphatics after each pulsation as is the case in younger

a
L.H.
b
L.H.
c
L.H.
d

Fig. 8 The superficial lymphatics of the posterior part of the pelvis in a series of injected chicks, showing formation of larger vessels from a primitive plexus, and the subsequent enlargement of these channels and their coalescence to form sacs (the pelvic sac). *a*, chick of 5 days, 20 hours—primitive indifferent plexus; *b*, chick of 6 days, 6 hours, first channels differentiating; *c*, chick of 7 days, showing large main ducts over the pelvis and in the lymph heart; *d*, chick of 7 days, 20 hours, neighboring channels enlarging and approaching each other; *e*, chick of 8½ days showing sacs; *L.H.*, lymph heart. × 12.5.

chicks, thus demonstrating the presence of valves between the lymph heart and its superficial connections. Injections show a still more abundant set of allantoic lymphatics which form a richly anastomosing net-work around the main allantoic blood vessels and their branches, while large lymph ducts accompany these blood vessels back toward the body. Within the embryo the dense plexus of lymphatics around the aorta is now continuous with the deep anterior lymphatics and connects with the jugular vein by means of the thoracic duct. A similar lymphatic net-work surrounds the external jugular vein. The large suprascapular sac is still present.[11]

The study of the lymph-flow and of the character of the lymphatics in chicks of later stages and after hatching would be most interesting, especially in comparison with the lymphatic system of ducks, since Jolly[12] has shown that the latter birds retain their pulsating lymph hearts and develop lymph glands in the cervical and lumbar regions, while chicks after hatching have neither lymph glands nor lymph hearts.

RELATION OF PRESSURE CONDITIONS AT VARIOUS STAGES TO THE FLOW OF LYMPH

In bringing together the results of these experiments on the early lymph-flow and of the observations of injected lymphatics the following stages appear to summarize the varied changes which occur in the lymph circulation and in the form of the lymphatics during this period of development:

1) Primary stage (in chicks of 5 to 6 days) in which the superficial lymphatic system is an irregular capillary plexus. During this period there is no circulation in the superficial lymphatics.

[11] This is the stage at which Budge first injected the lymph heart in the chick and observed its pulsations, and his conclusion that its function was chiefly concerned with the lymphatic circulation of the allantois was therefore probably correct. Not having observed the earlier pulsation of the lymph heart he was unaware of the rôle which it plays in initiating and maintaining the early lymph-flow in many of the superficial lymphatics. (A. Budge, Ueber Lymphherzen bei Hühner Embryonen. Arch. f. Anat. u. Phys. Anat. Abt., 1882.)

[12] J. Jolly, Recherches sur les ganglions lymphatiqes dea oiseaux. Arch. d' Anat. Microscopique, T. 11, 1910.

The side pressure in the veins with which they connect is higher than the pressure inside the lymphatics and consequently blood is continually forced out into the extending lymphatics.

2) The next period (in chicks of 6 to 7 days) in the developing superficial lymphatics is characterized by the establishment of lymph circulation accompanied by the differentiation of definite ducts or channels in the irregular primary plexus. The pressure of the fluid inside the lymphatics first overcomes the pressure in the veins of the neck: the lymph-flow starts in the side plexus and follows a definite course in an anterior direction. Somewhat later the lymph circulation over the pelvis begins and in this region the flow is instigated by the first pulsations of the lymph heart, still in the form of a plexus. The first circulation in the superficial lymphatics is slow and the earliest channels formed are small and tortuous. With the first flow of lymph through the lymph heart plexus, channels also differentiate here. In this stage the blood is gradually washed out of the superficial lymphatic system, first from the side region and later from the lymphatics of the pelvis.

3) The development of the superficial lymphatics in chicks of 7 to 8 days is characterized by increased pressure in the lymphatics, stronger pulsations of the lymph heart and a more rapid and steady flow of lymph. Several changes in direction of the lymph-flow occur during this period and the position of the main channels changes with the shifting circulation. The original lymphatic channels enlarge during this stage, and others differentiate from the superficial plexus. The channels through the lymph heart become larger and fewer, and the connections with those intersegmental veins nearest the selected channels are retained and the others are lost.

4) In chicks of 8 to 9 days, the pressure in the lymphatics is very high. The great increase in the flow of lymph from the allantois and from the deep lymphatics of the body and certain other mechanical factors interfere with the outlet of fluid from the superficial lymph vessels. At this stage the flow is rapid in certain regions and very slow in those places where an obstacle is encountered. Injections show that ducts or channels are pres-

ent in the former regions and large sacs or lakes in the latter. The sacs always occur at a point where there are two conflicting pressures. Because of the looseness of the subcutaneous tissue, the lymphatic endothelium encounters little resistance from without and expands in response to the increased pressure of fluid within the vessels. At this stage the lymph heart first

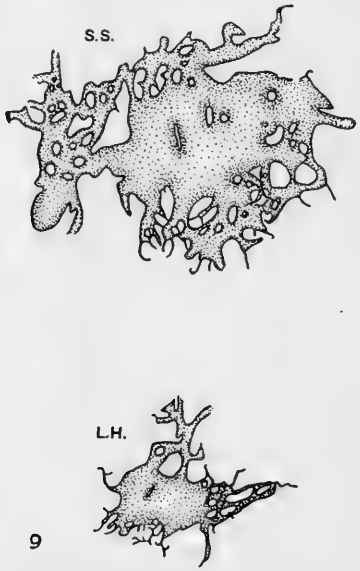

Fig. 9 Injections of the supra-scapular sac and of the posterior lymph sac (lymph heart) in the same embryo (chick of 8½ days) showing comparative size. *S.S.*, supra-scapular sac; *L.H.*, lymph heart. × 12.5.

assumes a sac-like form. Since its muscular walls offer an obstacle to its distension, it remains much smaller than certain of the other sacs (fig. 9).

OTHER FACTORS WHICH INFLUENCE THE LYMPH FLOW

In addition to the differences in pressure at the various stages, several other factors were noted, during the observations, which influenced the flow of lymph in the superficial lymphatics. Many of these have been mentioned casually in the description of the different stages of the early lymph circulation.

The lymph flow is influenced by the following factors:

(1) The muscular movements of the embryo
(2) The pulsations of the lymph heart
(3) The condition of the blood circulation
(4) The development of valves
(5) The development of new lymphatics in various regions

1) The muscular movements of the embryo. As previously stated, these embryonic movements occur periodically, about once a minute. While one of these violent spasms is taking place, injected ink granules which had previously been moving along a channel in a definite direction are brought to a standstill and then forced back and forth within the vessel and they do not resume their interrupted journey until after the cessation of the movements. This temporary alteration of the lymph-flow, produced by the embryonic movements, was noticed in all the stages studied. The movements of the embryo also exert an important indirect influence on the lymph-flow at certain stages of the early circulation owing to their intimate connection with the first pulsations of the lymph heart. In chicks of $6\frac{1}{2}$ to 8 days, the lymph heart beats occur only during the periods of body movement. Hence paralysis of the movements in such embryos alters the lymphatic circulation greatly. This will be referred to in the discussion of the next factor.

2) The pulsations of the lymph heart. The experiments demonstrated clearly that the commencement of lymph heart pulsations is the factor which instigates the lymphatic circulation in the posterior part of the body. In the earliest stage of pulsation, the lymph-flow almost ceases in the interval of rest between the group of lymph heart beats which accompany each muscular spasm, and the plexus of vessels composing the lymph heart fills up with blood. If chloretone (1:4000 in warm Ringer's solution) is dropped on the embryo, the lymph heart beats are paralyzed along with the muscular movements and in this case the lymph heart plexus and the adjacent superficial lymphatics become filled with blood, while the circulation in the anterior lymphatics continues undisturbed.

As the pulsations of the lymph heart become stronger, the area of superficial lymphatics draining into it becomes increasingly

greater until in an embryo of 7 to 7½ days the direction of the entire lymph-flow is posterior, through the lymph heart, into the veins of the tail. At this stage the function of the lymph heart is still involved with the muscular contractions and can be paralyzed by chloretone. In such a case (provided the blood circulation is undisturbed) after a few moments of uncertainty, the lymph-flow is completely reversed and granules which had previously all been travelling posteriorly from the scapular plexus, over the side and pelvis to the lymph heart, now follow the same paths anteriorly and flow into the deep jugular plexus through the connection anterior to the shoulder. This experiment has been repeated many times. In such cases the lymph heart can be stimulated to resume its function by direct puncture of its muscular wall. When this is done or when the lymph heart begins spontaneously to pulsate, after recovery from the anaesthesia, circulation is again reversed and the lymph-flow for the entire surface follows its original posterior course into the lymph heart.

These observations show that the pulsation of the lymph heart is an important factor in overcoming the high pressure of the tail veins, that it instigates and maintains the flow in the lymphatics of the pelvis, and that, until valves have formed at the venous connections, it prevents blood from backing up from the veins. The lymph heart exerts an important influence on the whole superficial lymph flow for a period of about 24 hours.

Although the fact that the superficial circulation can be reversed during this stage, shows that the contraction of the lymph heart is at no time indispensable to the flow of lymph in the superficial vessels, its absence would probably affect the lymph-flow unfavorably. This was shown by one embryo which appeared to be normal except for a stunted tail. A diminutive lymph heart about one-third the normal size, which beat frequently but feebly, was associated with this deformity. The embryo was oedematous and the lymphatics of the pelvis which drained into the lymph heart were large and distended. Although the embryo in other respects resembled a 7-day chick (the stage in which the superficial lymph-flow is rapid and

direct and the channels straight) the circulation in these pelvic lymphatics was very slow and their form greatly resembled the sacs normally present in this region in chicks of $8\frac{1}{2}$ days—the stage at which the superficial circulation is interfered with.

Moreover, at all stages up to 9 days (before valves have formed between the lymph heart and its superficial connections) the beating of the lymph heart always exerts a momentary and localized influence on the lymph circulation in nearby channels: when granules are moving steadily posteriorly over the pelvis, a beat of the lymph heart temporarily forces them back. That this effect is produced by the forcing out of fluid in both directions (into the superficial connections as well as into the veins) is shown in cases in which the lymph heart contains ink, for at each contraction a few of the granules are forced back temporarily into one of the superficial channels, while the greater part of the ink is seen to enter the veins. After each lymph heart pulsation, those granules circulating posteriorly in the nearby superficial channels, move more rapidly than ever into the lymph heart. In the older embryos in which the fluid over the posterior part of the pelvis is either moving slowly anteriorly or oscillating back and forth in the large superficial sac, a beat of the lymph heart is followed by a posterior circulation into the lymph heart which continues for a few seconds after each beat.

3) Condition of the blood circulation. The normal, regular circulation of the blood is a necessary factor for the continued lymph-flow at this early stage. If the blood heart stops beating or becomes irregular during an observation of an embryo between 6 and $7\frac{1}{2}$ days, the lymph-flow ceases immediately and does not start up again until several minutes after the blood circulation has become normal again. In such embryos no valves are present between lymphatics and veins and, since the irregularity of the blood heart is associated with a back pulsation and increased pressure in the veins, the lymphatics fill up rapidly with blood and in this particular, return to their primitive condition. This sudden filling up with blood is most noticeable in the posterior lymphatics where the change in the blood vascular circulation is also associated with a cessation of the lymph heart pulsations.

In embryos of 8 days, after the valves have formed, blood does not enter the lymphatics in case of a disturbance in the blood circulation, and the movement of lymph may continue for several seconds before it stops altogether.

In certain embryos of 6 to 7 days, which had been incubated for two or three days at an unusually low temperature, it was found, on opening, that the blood circulation was irregular and the blood vessels greatly congested. In such instances the lymph-flow was almost at a standstill and the superficial lymphatics were irregular in form, they contained blood, and in certain distended places, resembled the superficial sacs of later stages.

4) The development of valves. This point has already been discussed in connection with the effect of lymph heart pulsations and of a disturbance in the blood circulation and may be summarized very briefly here:

Up to $7\frac{1}{2}$ days, no valve is present between veins and lymphatics. Before the pressure inside the lymphatic plexus becomes sufficiently strong to overcome that of the communicating veins, blood enters the lymphatics. After the establishment of the lymph-flow an abnormal increase in the venous pressure will cause blood to enter the lymphatics. In the case of the tail region the pressure in the veins is normally greater than that of the lymphatics for several days and here a cessation in the lymph heart pulsations permits the entrance of blood into this part of the lymphatic system. With the formation of valves (in chicks of $7\frac{1}{2}$ to 8 days) no more blood enters the lymphatics from the veins.

In embryos younger than 9 days, there is no valve present between the lymph heart and its superficial lymphatic connections. In consequence each contraction of the lymph heart forces fluid back into the superficial lymphatics as well as into the veins. After the ninth day, a valve is formed and the beating of the lymph heart no longer affects the superficial lymph flow in this way. I have not studied the effect of the development of valves in other parts of the chick lymphatics.

5) The development of new lymphatics in various regions of the body. As an example of this factor, the rapid development of

new lymphatics in the allantois and the deep pelvic region, which monopolize the function of the lymph heart in chicks of 8 days and over, seems to be responsible for the second reversal of circulation in the superficial lymphatics.

ESTIMATES OF THE RATE OF LYMPH FLOW AT DIFFERENT STAGES

In keeping records of the character of the lymph flow at various stages, the rate of circulation was described somewhat vaguely as slow, moderately fast, or fast. I therefore attempted a few experiments in order to arrive at a more definite estimate of the lymph-flow. For this purpose, a few granules were injected into a superficial channel and the point of injection marked by a small extravasation. The time in seconds consumed in the passage of a single granule from this point of injection to a certain easily recognizable point such as the axilla, or the entrance to the lymph heart was then recorded. Subsequently the lymphatics were injected and the lymph channel under consideration drawn with the camera lucida. The length of this channel between the two points was measured in the drawing and the degree of magnification calculated. In making such estimates it was necessary to procure a record in the intervals of rest when the circulation was not disturbed or arrested by the muscular movements of the chicks.

The following estimates were made by this method:

1) Earliest anterior circulation in a 16 mm. chick. A granule travelled 1.6 mm in 1 minute, 30 seconds. The rate of circulation in this embryo was 1.06 mm per minute. This is the type of circulation which was designated as 'slow' in the records.

2) As an example of 'moderately fast' circulation, the flow for this same region was estimated in a chick of 19 mm. The granule under observation travelled 3.8 mm in 30 seconds, a rate of 7.6 mm per minute.

3) For an estimate of the so-called 'fast' circulation a record was made of the lymph-flow in a channel of the pelvic region during the period following the pulsation of the lymph heart, in an embryo of 20.5 mm. In this case, a granule travelled 2.44 mm in 15 seconds, a rate of 9.76 mm per minute.

SUMMARY OF RESULTS

The principal results of the present study may be summarized as follows:

1. The primary superficial lymphatics of chick embryos form a rapidly growing, frequently anastomosing capillary net-work. This primitive plexus maintains numerous open connections with the venous system in certain places. For over 24 hours the pressure in these earliest lymphatics remains less than the side pressure in the connecting veins and, consequently, there is no lymph-flow in the early plexus. Instead, it contains blood which backs up into it from the communicating veins.

2. The pressure of the fluid in the lymphatics gradually increases and finally overcomes that of the veins. The first lymph-flow which is then established is feeble and easily disturbed. The lymph-flow gradually becomes more rapid and steady, but its course is easily altered by various mechanical factors. A day later the pressure in the superficial lymphatics has increased still more, while, for various reasons, the out-flow into the veins is interfered with. At certain points two conflicting pressures are present and here the lymph-flow becomes sluggish.

3. The endothelium of these early lymphatics responds to the passage of fluid over its interior by the differentiation of definite ducts or channels out of the indifferent primitive net-work. With the increased flow of lymph these channels enlarge and more channels form. That the formation of such channels is due to the lymph-flow and to mechanical factors rather than to arbitrary predetermination is evident from the frequent variations which occur in the position of the main ducts in chicks of the same stage. The endothelial wall of the early lymphatics also responds to the increased pressure caused by interference with the lymph-flow and the damming back of fluid, by expanding to form sac-like enlargements. The size which these sacs may attain is influenced to some extent by the looseness of the surrounding tissue.

DISCUSSION

In connection with these observations of the great susceptibility of the developing lymphatics to various mechanical factors, an anomaly of the adult human lymphatic system, recently described by Dr. Clark,[13] is of interest. In a specimen from the dissecting room he found a diminutive thoracic duct which was only present as far as the ninth interspace, while all the lymphatics of the rest of the body below the ninth vertebra, drained into a large main duct which emerged below the ligamentum inguinale and ran superficially in the body wall, into the axilla and emptied at the angle formed by the jugular and subclavian veins. A significant anomaly of the hemiazygos vein was present: all the veins draining the left thoracic wall joined with a fairly large branch from the left renal vein and crossed the midline ventrally instead of dorsally to the aorta, at the exact place where the miniature thoracic duct ended and the lymphatics draining posteriorly commenced.

Dr. Clark has shown a section through this region in a human embryo of 21 mm which demonstrates the relatively enormous size of the embryonic veins and shows clearly that in a case such as the one described, where the connection during embryonic life between the two azygos veins must have been ventral instead of dorsal to the aorta, an obstacle sufficient to block the flow of lymph had been present. Since, as Miss Sabin has shown, valves in the lymphatics do not develop until a relatively late stage (7 cm. in pig embryos), such an obstacle might have prevented the flow of lymph in the thoracic duct and caused it to follow an entirely different course. Dr. Clark has pointed out that this case shows strikingly the response of the lymphatic vessels to mechanical forces "since the passage of a large amount of lymph through an unusual route had led to the formation of a duct many times larger than the vessels normally present, while the diminution of lymph-flow through the thoracic duct has been accompanied by a corresponding diminution in size."

[13] E. R. Clark, An anomaly of the thoracic duct with a bearing on the embryology of the lymphatic system. Contributions to Embryology, no. 3, Carnegie Inst. Washington, 1915.

The view presented here that the primitive form of the lymphatic system is an indifferent capillary plexus and that the main lymph trunks are formed by the enlargement of certain vessels of this net-work in response to mechanical stimuli of pressure, is of course similar to the results obtained by many investigators with regard to the development of the blood vascular system. Thoma[14] in the chick, found that the formation of the chief arteries and veins was preceded by a stage in which a plexus of simple capillaries was present. He observed that those capillaries more favorably located with regard to the circulatory stream enlarged and became arteries and veins. In discussing this process, Thoma developed a series of 'histomechanical' principles to explain the manner in which this transformation is brought about. That the formation of many of the veins and arteries within the embryo takes place in a similar manner has been shown by Evans[15], and Clark[16] has watched the development of arterioles and venules from a simple capillary plexus in a living tad-pole with a correlated study of the character of the circulation.

From the present study it seems evident that a similar process to that described for the blood-vessels takes place in the developing lymphatics. The difference in the character of the flow in the two systems (the definite pulsation in the blood-vascular system and the rapidity of the circulation as compared with the flow of lymph, etc.) undoubtedly accounts for many of the final differences in form, for the primitive network of the lymph capillaries closely resembles the non-circulating blood capillary plexus of the yolk sac.

[14] R. Thoma, Histogenese und Histomechanik des Gefasssystems. Stuttgart, 1893.

[15] H. M. Evans, On the earliest blood vessels in the anterior limb buds of birds and their relation to the primary subclavian artery. Am. Jour. Anat., vol. 9, no. 2, 1909. On the development of the aortae, cardinal and umbilical veins, and the other blood vessels of vertebrate embryos. Anat. Rec., vol. 3, no. 9, 1909.

[16] E. R. Clark, Studies of the growth of blood vessels by observations of living tadpoles and by experiments on chick embryos. Proc. Amer. Assoc. Anat., Anat. Rec., vol. 9, no. 1, 1915.

Mierzejewski,[17] in his studies of injected chick embryos, has described several of the large lymphatic trunks mentioned in the present article, and he states that they make their appearance a day or two later than the original net-like superficial plexus. He appears to think that these larger vessels are laid down in response to heredity and in this connection mentions particularly the earliest lateral trunk which occupies the position of the lateral lymphatic vessel of Amphibians. However, Mierzejewski's figures which show first an irregular superficial mesh-work of capillaries and, in later embryos, larger vessels running through the midst of this plexus, seems rather to corroborate the view presented here.

In studies of mammalian embryos, Polinski[18] found that definite lymphatic trunks develop in various regions of the embryo and in some instances his pictures show a capillary plexus preceding the formation of these trunks. In recent investigations of the developing lymphatics in pig embryos, Miss Sabin[19] has shown that the posterior part, at least, of the thoracic duct arises from a plexus of lymphatic capillaries. Stromsten's[20] injections of the anterior portion of the thoracic duct in turtle embryos show a plexus surrounding the aorta. It seems probable, therefore, that many of the factors concerned in the development of the lymphatics of the chick are present in other types of vertebrate embryos. Such a theory of origin for the main lymph ducts would serve to explain the many variations in the position of the thoracic duct which have been found in adult human subjects.

Within recent years there have been many investigations of the development of the lymph sacs and lymph hearts in various

[17] L. Mierzejewski, Beitrag zur Entwicklung des Lymphgefässsystems der Vogel. Extrait du Bull. de l'Acad. des Sciences de Cracovie, 1909.

[18] W. Polinski, Untersuchungen über die Entwicklung der Subkutanen Lymphgefässe der Säuger, in Sonderheit des Rindes. Extrait du Bull. de l'Acad. des Sciences de Cracovie, 1910.

[19] F. R. Sabin, On the origin of the abdominal lymphatics in mammals from the vena cava and the renal veins. Anat. Rec., vol. 6, no. 8, 1912.

[20] F. A. Stromsten, On the development of the prevertebral (thoracic) duct in turtles as indicated by a study of injected and uninjected embryos. Anat. Rec., vol. 6, no. 9, 1912.

types of embryos. In Miss Sabin's[21] first study of the lymphatics in pig embryos, she found, at an early stage, two pairs of sacs connected with veins. At that time she thought that these two paired sacs, the jugular and the iliac, and the thoracic duct connecting them, represented the primary lymphatic system. In 1906 F. T. Lewis[22] found, from the study of serial sections, that the jugular lymph-sacs of rabbit embryos were preceded by a plexus of capillaries containing blood. He believed them to be blood-vessels which had formed a part of the circulating blood-vascular system at one time and had subsequently separated from it. Later, Miss Sabin[23] found this same primary plexus in sections of early pig and human embryos and Huntington and McClure[24] found similar conditions in cat embryos. In studying, in living chicks, the region in the tail later occupied by the pulsating lymph heart in the form of a sac, Dr. Clark[25] and I observed that the plexus which occupies this region, in stages before the formation of the lymph heart, is continuous and composed of blood-filled lymphatic capillaries instead of isolated portions of formerly circulating blood-vessels. Lately, West[26] has confirmed this observation. In her more recent publications, Miss Sabin[27] has described similar blood-filled plexuses which remain after the blood-vessels have been washed out with Ringer's solution, and which precede the formation of the mammalian lymph sacs. According to Baranski[28] and Fedorowicz,[29] the

[21] F. R. Sabin, Am. Jour. Anat., vol. 1, 1901–02.

[22] F. T. Lewis, The development of the lymphatic system in rabbits. Am. Jour. Anat., vol. 5, 1906.

[23] F. R. Sabin, The lymphatic system in human embryos, with a consideration of the morphology of the system as a whole. Am. Jour. Anat., vol. 9, 1909.

[24] G. S. Huntington and C. F. W. McClure, The anatomy and development of the jugular lymph sacs in the domestic cat. Am. Jour. Anat., vol. 10, no. 2, 1910.

[25] E. R. Clark and E. L. Clark, Anat. Rec., vol. 6, no. 6, 1912.

[26] Randolph West, The origin and early development of the posterior lymph heart in the chick. Am. Jour. Anat., vol. 17, no. 4, 1915.

[27] F. R. Sabin, The origin and development of the lymphatic system. Johns Hopkins Hosp. Reports, Monographs, New Series, no. 5, 1913.

[28] J. Baranski, Die Entwicklung der hinteren Lymph herzen bei der Unke (Bombinator). Extrait du Bull, de l'Acad. des Sciences de Cracovie, 1911.

[29] S. Fedorowicz, Untersuchungen über die Entwicklung der Lymphgefässe bei Anurenlarven. Extrait du Bull. de l'Acad. des Sciences de Cracovie, 1913.

lymph hearts in Amphibians are formed from two or three lymphatics instead of from a luxuriant plexus, as in birds and mammals. However, Knower[30] and Kampmeier[31] state that in frog and toad embryos the anterior lymph heart is formed from numerous lymphatic capillaries. The majority of recent investigations, therefore, seem to show that the lymph sacs of mammals and the pulsating lymph hearts of Amphibia and birds are secondary structures whose appearance is preceded by a plexus of lymphatic capillaries.

The manner in which the lymph sacs are formed from a capillary plexus has been named 'confluence' by Ranvier[32] and consists in the gradual enlargement of various vessels of a plexus until the walls between them become thin trabeculae and are finally absorbed. The method of formation of those lymph sacs and hearts connected with the veins is similar to that of the subcutaneous lymph sacs of Amphibia, which has been described by Goldfinger.[33]

In the present study it has been found that the method of formation of a lymph sac is essentially the same, no matter what its location or relation to the veins. The only difference is found in the case of the posterior lymph sac in the tail, which develops muscular walls and is the only real lymph heart present in the chick. The formation of all the lymph sacs studied in the chick, including the lymph heart, is preceded by a primitive plexus of lymphatic capillaries and by a stage of increasingly rapid circulation during which certain of the capillaries enlarge to form channels. The sacs are then formed by the enlargement of a single channel or, more frequently, by the expansion and confluence of neighboring channels. In the deep jugular plexus that vessel next the vein, which has served for approximately 48 hours as the main duct, gradually enlarges to a sac-like form.

[30] H. McE. Knower, Anat. Rec., vol. 8, no. 2, 1914.

[31] O. F. Kampmeir, On the origin of lymphatics in Bufo. Am. Jour. Anat., vol. 17, no. 2, 1915.

[32] L. Ranvier, Morphologie et développement des vaisseaux lymphatiques chez les Mammifères. Arch. d'Anat. Microsc., T. 1, 1897.

[33] Gizella Goldfinger, Ueber die Entwicklung der Lymphsäcke in den hinteren Extremitäten des Frosches. Extrait du Bull. de l'Acad. des Sciences de Cracovie, 1907.

The posterior lymph sac (lymph heart) and the supra-scapular sac are both formed by the flowing together of neighboring main channels whose walls remain for a time as thin trabeculae. And over the posterior pelvic region a sac is formed sometimes by the expansion of a portion of a single main channel, or, more frequently, by the enlargement of two or more nearby vessels.

The observations of the character of the lymph-flow and the pressure conditions in various regions and at different stages have helped to explain the development of these embryonic lymph sacs, since they have shown that a lymph sac forms at any point in the early lymphatic system where conflicting pressures occur. For example, a sac develops in a place such as the supra-scapular region where there is an obstacle to the outlet of fluid, in regions like the posterior lymph heart and the deep jugular plexus where the pressure in the lymphatics encounters the side pressure in the connecting veins, or at a point of hesitation such as the superficial pelvic region, where the lymph-flow is now in one direction and now in the opposite.

In describing the development of the lymphatics of the chick, there appears to be no adequate reason for a division into primary lymph sacs and peripheral lymphatics, since the mode of development is essentially the same throughout the early lymphatic system after its differentiation. The peripheral lymphatics have spread over the surface of the body before any lymph sac develops and there appears to be no real distinction, either in the time of their appearance or in the manner of formation, of the sacs which may develop near the venous connections of the early lymphatics (in those regions where the first lymphatic capillaries make their appearance in much younger embryos) and the sacs located in other portions of the lymphatic system. Sacs may form at any point in the early system where the mechanical conditions favor such an enlargement and all of the sacs are secondary structures. The primary form of the developing lymphatic system, in chick embryos, is a rapidly growing, richly anastomosing, irregular blood-filled plexus out of which channels or ducts and lakes or sacs develop secondarily, in accordance with the circulation of lymph which is determined by pressure

conditions in the lymphatics and in the veins with which they connect.

It seems reasonable to assume that somewhat similar conditions are present during the early development of the lymphatic system in other vertebrates and that the variations in the number and position of the lymph sacs as well as the difference in pattern of the lymph vessels in various forms are associated with differences in the condition and direction of the lymph flow.

It appears probable, from the descriptions of many investigators, that the jugular lymph sac of mammals develops and attains a large size at an earlier period than the lymph sacs of the chick. In this connection I should like to mention again the enlargements of the superficial lymphatics just anterior to the leg, which occur normally in embryos of 6 days. These are sac-like in form and compared with the other lymphatics of that stage, they are very large. These 'sacs' occur in a loose tissue at a stage of hesitation when the lymph-flow, whose direction in this region had been anterior for several hours, is about to shift and move posteriorly. Moreover, in the case of the chick with the stunted tail, described above, whose lymph heart was small and inefficient, large sacs were formed in the posterior pelvic region fully twenty-four hours earlier than the time at which they normally appear. These two examples show that if the right mechanical conditions are present a sac may form at an early stage in the chick. In the case of the jugular lymph sac of mammals such conditions probably make their appearance earlier than in the chick. It should also be remembered that before it was known that the primary lymphatic system contained blood and could be studied in the living and injected directly, the early lymphatics were constantly mistaken for blood-vessels or overlooked altogether. It is therefore possible that more extensive lymphatic plexuses than have hitherto been described may be present in mammalian embryos before the jugular plexus has become transformed into a sac.

The view that the lymph sacs of embryos represent temporary reservoirs of lymph and that they are isolated portions of a developing lymphatic system which has not yet become continuous and is

unconnected with the veins, is inapplicable to the superficial lymphatics of the chick. Here a continuous plexus of lymphatic capillaries can be demonstrated by a study of the living, by injection with fine glass cannulae, and by the microscopic study of sections with the oil immersion, hours and often days before any sacs develop. Moreover, the earlier the stage of development of this lymphatic plexus, the narrower and more delicate are the capillaries composing it. The formation of the sacs of the chick is preceded by an active circulation of lymph associated with the formation of channels, and although the circulation of lymph is very sluggish in these sacs, it never ceases entirely. The connection of the lymph sacs with the rest of the lymphatic system, and through it with the veins, was never lost during the stages studied. Miller[34] has represented such a supposedly isolated sac in the jugular region of the chick at a stage (7 days) in which an active circulation through it can be demonstrated, in a living embryo, by injection of a few ink granules into the deep cervical lymphatic channel, which accompanies the jugular vein.

It has been shown by the researches of Clark and of Evans that the growth of lymphatic endothelium after its differentiation is dependent on its reaction to external stimuli—that it responds to substances outside and to the passage of fluid through its wall by the sending out of new capillary sprouts. The present study seems to show that lymphatic endothelium reacts also to the pressure and flow of the fluid inside the walls of the vessels, and that the formation of lymph trunks and of lymph sacs from a primitive plexus represents a response to such stimuli.

[34] A. M. Miller, The development of the jugular lymph sac in birds. Am. Jour. Anat., vol. 12, no. 4, 1912.

ORIGIN OF THE DEFINITIVE SEX-CELLS IN THE FEMALE CHICK AND THEIR RELATION TO THE PRIMORDIAL GERM-CELLS

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EIGHT FIGURES

CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

The question as to the origin of the definitive ova has for a long time been of interest to the biologist. Apparently definitely settled, it has again sprung into prominence owing to the discoveries made in regard to the primordial germ-cells—the so-called gonocytes or primordial gonocytes of the French authors, or ureier or urgeschlechtszellen of the German writers.

Briefly stated, the definitive sex-cells are either lineal descendants of the primordial germ-cells or they are developed from peritoneal cells in the differentiated region called the germinal epithelium.

Waldeyer ('70) may be called the first investigator who really attacked the problem. In his work "Eierstock und Ei" he advanced the opinion that the definitive ova arose from the ureier. These ureier were the larger clear cells, which he ob-

served in the germinal epithelium of the younger animals studied. This conclusion of his was weakened to a certain extent by a faulty premise, namely, that the ureier were developed from the cells of the general epithelium by a process of differentiation. As will be shown later in this article, in the same forms studied by Waldeyer, the ureier, or primordial germ-cells, arise long before the appearance of the germinal epithelium.

Balfour ('78) in a study of the development of the vertebrate ovary agreed in the main with the findings of the previous investigator in attributing to the germinal epithelium the origin both of the definitive ova and follicular epithelium. Balfour's principal investigations were made on Scyllium, an Elasmobranch, a form in which the primitive ova appear in the germinal epithelium at an early stage. Although he believed that the primitive ova—primordial germ-cells—were derived from the germinal epithelium, he, however, thought that the primitive ova in the female were not the true ova but the parent sexual cells which gave rise to the definitive ova.

Schmiegelow ('82), to quote Bouin, saw in the germinal epithelium of a young chick embryo the large primordial eggs described twelve years before by Waldeyer. He observed that the germinal epithelium was sharply limited on its deep surface by a basal membrane and that in older embryos anastomosing cords of cells grew down into the subjacent ovarian tissue. These cords contained some primordial eggs and there were others in the stroma between the cords. The author believed that the latter did not emigrate down from the germinal epithelium in the cellular cords, but that they differentiated in situ from the mesenchyme. He believed that both kinds of primordial eggs could give rise to definitive eggs.

Von Mihalcowics ('85) was the first investigator to observe and appreciate the two proliferations which the germinal epithelium undergoes in the female. In his work on the embryos of Amniotes—reptiles, birds and mammals—he also observed the germinal epithelium at the time of its formation from the coelomic epithelium. Among the cells contained in it he found primordial ovules or 'grandes cellules sexuelles.' Then this

epithelium, producing a first series of buds, sent into the underlying stroma sexual cords which contained some of the large primordial germ-cells. After a short period of rest, the germinal epithelium became active again and sent down into the sexual cords cells which he characterized as primordial eggs. He called the cells surrounding these primordial eggs follicular cells. This author differed from Schmiegelow in believing that the primordial eggs of the sexual cords as well as those seen in the germinal epithelium were of peritoneal origin.

Prenant ('89) in a very complete series of chick embryos studied carefully the evolution of the gonad and particularly the origin of the primordial eggs—the so-called primordial germ-cells. In the three-day chick, he found on the medial surface of the Wolffian body lateral to the root of the mesentery an elongated ridge covered with peritoneal epithelium. This enlargement, which he called the genital ridge, was covered at three and one-half days with the elongated cells of the germinal epithelium. In this epithelium and the mesenchyme beneath, which forms the main mass of the genital ridge, he discovered the large primordial eggs. Since he did not see any mitotic figures and since these large cells increased in number, he was drawn to the conclusion that they were produced by a process of differentiation from the cells of the germinal epithelium. The author did not hazard any opinion as to the relation of the primordial germ-cells to the definitive ova.

Just as the work of Waldeyer is the starting point of one epoch in connection with research done on sex-cell formation, so the work of C. K. Hoffmann ('93) should initiate another.

Up to this time the true value of primordial germ-cells or primordial eggs had not been appreciated. True, a great many investigators, some of whom are mentioned in the preceding pages, had seen and described them, but not one had found them in the embryo previous to the formation of the germinal epithelium. Even the theory as to their origin in the gonad, faulty as it was, remained in controversy. Waldeyer, Balfour, von Mihalcowics and Semon believed that their sole seat of origin was the germinal epithelium; Schmiegelow and Prenant, although not

denying that some could originate there, believed that others might arise by a process of differentiation either from the cells of the sexual cords, or from the mesenchyme cells which make up the columns of cells connecting the Wolffian body with the germinal epithelium in the young embryo.

If the origin of these primordial germ-cells was in doubt their fate was certainly a greater mystery. According to some—Waldeyer and Semon, for example—they developed directly into the definitive ova, while Prenant would not hazard a guess as to their issue.

It remained for Hoffmann to prove that the primordial germ-cells were a thing apart from the elements making up the germinal epithelium and ovarian stroma; that they existed a long time before the appearance of the germinal epithelium and gonad, and that the definitive ova were in a direct line of descent from the true primordial germ-cells.

Hoffmann employed in his work twelve species of birds, included in the orders Natatores and Grallatores. In three species, Haematopus ostralegus, Sterna paradisea, and Gallinula chloropus, there was sufficient evidence brought out to prove that the primordial germ-cells did not originate 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.

In another form, Numenius arcuatus, Hoffmann worked out the later history of the primordial ova. He observed that the primordial germ-cells at first remained quiescent in the thickening germinal epithelium. When the germinal epithelium sent down into the underlying stroma of the gonad the first series of

buds—the first sexual cords as described by von Mihalcowics ('85)—a number of primordial ova were involved. Others, however, remained in the germinal epithelium to which he now gave the name layer of primitive ova. At this time the germinal epithelium, or layer of primitive ova, according to Hoffmann, is composed of two kinds of cells, the ordinary prismatic peritoneal epithelial cells and the large spherical primordial ova. Following the appearance of the first sexual cords the primordial germ-cells in the germinal epithelium were observed to be dividing actively, which accounted in part for the increasing thickness of the layer of primordial ova.

At this point lobulations began to appear on the deeper surface of the germinal epithelium. These were the beginnings of the second series of sexual cords, and in them the primordial ova began to transform themselves into ovarian ova. In addition, and this is most important, the young ovarian ova now began to invest themselves with a layer of cells, which Hoffmann called 'membrane granuleuse.' These cells resembled in every way the second type of cell found in the germinal epithelium, which were of undoubted coelomic epithelium descent.

The following quotation from Hoffmann will be of interest:

> Les observations de Waldeyer auront toujours une grande valeur, puisque c'est lui, qui le premier a montré que de l'oeuf primitif se developpe l'oeuf ovarien. D'apres lui la membrane granuleuse et l'ovule primordial dérivent d'une même couche germinative. Je l'ai cru aussi autrefois, mais à present je ne partage plus cette manière de voir parce que je suis arrivé à la conclusion, que les ovules primordiaux ne se forment pas de l'epithelium péritonéal, mais qu'ils viennent s'ébaucher déjà dans les périodes de développement, quand des cellules péritonéales n'existent encore, comme cela a été expliqué plus tôt.

Bouin ('00), Allen ('04), von Winiwarter and Sainmont ('08), using Rana temporaria, the pig and rabbit, and the cat, respectively, drew the conclusion from their researches that the definitive ova are derived from the germinal epithelium.

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 tissues were differentiated from them, the cells of the tissues acquired the rod-shaped mitochondria. In this way he was able to distinguish 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 are descended from the primordial germ-cells.

Firket in 1914 published a very complete account of the organogenesis of the indifferent gonad and ovary in the chick, which was the last article dealing with the primordial germ-cells and their relation to the definitive ova. According to Firket ('14) a great majority of the definitive ova were derived from the cells of the germinal epithelium, but he admitted that there was no reason why certain of the primordial germ-cells might not produce some of the definitive ova. Firket distinguished two lines of germ-cells—the so-called primary gonocytes or primordial germ-cells and the secondary gonocytes which are derivatives of the germinal epithelium. As the chick embryo advanced in development a majority of the primary gonocytes degenerated and those which remained, having lost their vitellus, and other distinguishing characters, could not be separated from the secondary gonocytes.

Firket thought it necessary to consider the primary gonocytes of Vertebrates "comme etant un rappel phylogénique des gonocytes définitifs des classes inférieurs notamment des Cyclostomes et des Acraniens."

Any further account of the earlier literature on the subject of the origin of the definitive ova would be useless. The reader is referred to the excellent bibliographies of Bouin ('00) and Firket ('14) for the articles on this subject.

MATERIAL AND METHODS

The chick was selected as the animal best adapted to this investigation for several reasons: 1) Because of the ease with which material may be procured. 2) As will be seen from the preceding paragraphs the question as to the origin of the definitive ova in this species is still an open one. Especially

is this so as regards the rôle played by the primordial germ-cells. 3) It seemed especially desirable to continue the study of the germ-cells since the writer had just finished an investigation dealing with the origin and early history of these cells in the chick.

In selecting a fixative for the very young chick embryos, those having fewer than 20 pairs of somites, it was found that fluids containing osmic acid had a decided drawback. This was due to the fat staining properties of the acid. In these very young embryos the germ-cells are loaded with vitellus and when this is blackened by the osmic acid it is almost impossible to see any cytological detail. This property, however, becomes an asset in the germ-cells in embryos of three days incubation and over, since it facilitates identification. In these embryos the germ-cells possess comparatively little vitellus, so that when stained no detail is occluded.

Fixatives such as Meves' modification of Flemming's fluid, the acetic-osmic-bichromate mixture, and Hermann's fluid, all of which contain osmic acid, were found to be best adapted for this investigation, in which the embryos employed were all older than three days. They preserve such cytoplasmic structures as mitochondria and the attraction-sphere excellently, and because of their osmic acid content, bring out the yolk spheres plainly.

In embryos between 3 and 8 days' incubation it was found that the ordinary acetic-osmic-bichromate mixture was most efficacious, but in older embryos, Meves or a modified acetic-osmic-bichromate fluid worked best. The latter fixative was modified by using two drops of acetic acid instead of the usual one.

There is no need of entering into detail in regard to all the staining methods employed since they are described by Bensley ('11), and Cowdry ('12). However, one method has not been published and I shall describe it in detail. It is called the Bensley anilin-acid fuchsin-Wright's blood stain method. It is used after the acetic-osmic-bichromate mixture preferably. The pieces of tissue are imbedded and cut in the usual way. This method is as follows:

1. Pass down through toluol, absolute, 95, 70 and 50 per cent alcohols into water.

2. One per cent aqueous solution potassium permanganate about 5 seconds; this time, however, is dependent upon the age of the embryo and the thickness of the section.

3. Five per cent aqueous solution oxalic acid about 5 seconds or less.

4. Stain 3 to 6 minutes at 60°C. with Altmann's anilin-acid fuchsin.

5. Wash thoroughly in water.

6. Differentiate with Wright's blood stain. This stain is allowed to remain on the section 10 to 20 seconds.

7. Remove the excess of the differentiator with 95 per cent alcohol.

8. Absolute alcohol, toluol and into balsam.

This method gives a preparation which looks like a Benda stain and has the advantage that it is much more rapid. All sections were cut 4μ in thickness. Table I will show at a glance the number of embryos employed, their age, together with the methods used in fixation and staining.

TABLE 1

METHOD	$3\frac{1}{2}$ DAYS	$3\frac{3}{4}$	4	$4\frac{1}{2}$	5	$5\frac{1}{2}$	6	$6\frac{1}{2}$	7	8	9	10	11	14
Meves' fixation and iron-hematoxylin stain										2	2	2		
Acetic-osmic-bichromate fixation: Bensley's anilin acid fuchsin and methyl green stain											1			
Bensley's anilin acid fuchsin and Wright's blood stain	1	2	6	2	2	1	2	1	2	2	3	2	1	2
Hermann's fluid and iron-hematoxylin stain			1						2					
Trichlor acetic acid and corrosive sublimate mixture; iron-hematoxylin stain										1	1	1		

ORIGIN AND MIGRATION OF THE PRIMORDIAL GERM-CELLS

Before commencing a description of the evolution of the gonad and its tissues, it seems best to enter into a short account of the early history of the primordial germ-cells, which are present in the germinal region before the appearance of the gonad, and which enter into it at its inception.

The primordial germ-cells of the chick, according to Swift ('14), arise from germ-wall entoderm anterior and antero-lateral to the forming embryo. This genesis occurs during the primitive streak stage and continues until the embryo possesses about 3 pairs of somites.

This region of the young embryo is not invaded by mesoderm until later so that the germ-cells are first seen free in the space between ectoderm and entoderm. They later, on account of their amoeboid properties, enter the mesoderm, and a great majority of them get into the forming blood channels. They are carried in the blood vessels to all parts of the embryo and vascular area. The primordial germ-cells remain generally distributed in this way until the embryo has about 20 pairs of somites.

Dantschakoff ('08) described certain cells in the chick embryo, which resemble in every way the primordial germ-cells during their intra-vascular life. She called them entodermal wander-cells, and stated that they disappeared from the blood vessels when the embryo had about 22 pairs of somites.

The primordial germ-cells are still found in the blood vessels of embryos having from 20 to 22 pairs of somites, but are becoming more numerous in the vessels of the splanchnic mesoderm, while in embryos possessing 23 to 25 pairs of somites a majority of them are found in the mesodermal plate of the splanchnopleure near the coelomic angle.

Many investigators, among whom may be named Nussbaum ('80, '01), Kiebel and Abraham ('00), Hoffmann ('93), Rubaschkin ('07), and von Berenberg-Gossler ('12), have described the germ-cells in the splanchnopleure of bird embryos having 22 to 23 pairs of segments. At this age, as will have been seen from the foregoing, they have just passed out of the vessels.

In embryos having 33 pairs of segments (about 3 days of incubation) the primordial germ-cells are distributed in the radix mesenterii, the coelomic epithelium and the mesenchyme beneath, on both sides of the coelomic angle.

Recently von Berenberg-Gossler ('14), working with duck embryos, has confirmed the findings of Swift ('14) in all the essential points. The primordial germ-cells in this form, however, do not leave the blood vessels of the splanchnopleure until the embryo has from 24 to 32 pairs of somites.

LATER HISTORY OF THE PRIMORDIAL GERM-CELLS

1. Primordial germ-cells and the indifferent gonad

As an introduction to this section dealing with the indifferent sexual primordium, I must say that my findings, relative to this period of development coincide in all essential details with those of Firket ('14). For this reason, and because the reader may refer to his excellent monograph, I shall pass over this period of organogenesis rapidly.

On opening the body of a $3\frac{3}{4}$ day (90 hours) chick, and on removing the abdominal viscera so as to expose the posterior wall, the Wolffian bodies are seen. They have a pink color and have a length of about 3 mm.; their long axes correspond to the longitudinal axis of the animal.

On the medial surface of the rounded ventral face of each Wolffian body—the surface towards the mesentery—there is another elongated, narrow, whitish body, which has the appearance of a ridge. This is the young developing gonad. The whitish color of the area is due in part to the thickened bloodless coelomic epithelium, the so-called germinal epithelium. The gonads are about 1.5 mm. in length and their anterior extremities begin some distance behind the cephalad extremities of the Wolffian bodies. Even at this early stage the hand lens, or even the unaided eye, will show that the left gonad is more massive than the right.

EXPLANATION OF FIGURES

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 figures except no. 3, for which Zeiss 8.0 mm. objective and ocular 8 were used. The camera lucida was employed for all the figures 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 except figure 3, which has a magnification of 205 diameters. 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 4μ in thickness.

alb., albuginea
at.sp., attraction sphere
c.c., cortical cord or cord of second proliferation
coelom, coelomic cavity
f.c., deep indifferent cell or follicular cell
germ.ep., germinal epithelium
l.gon., left indifferent gonad
m.c., medullary cord or cord of first proliferation
med., medullary region of ovary
mes., mesentery
oog., oogonia
pr.o., primordial germ-cell
pr'.o'., primordial germ-cell in state of division
r.gon., right indifferent gonad

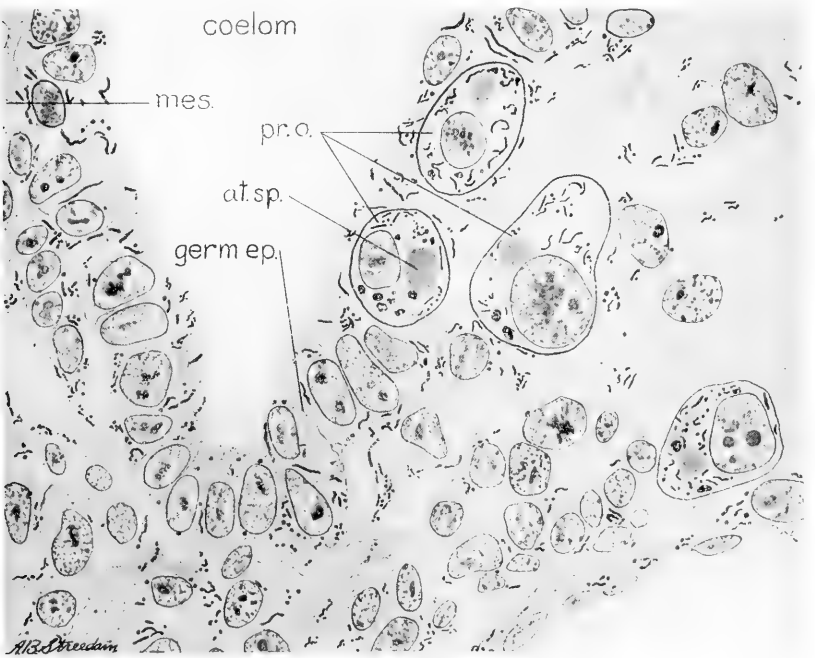


Fig. 1 Portion of a transverse section through anterior third of Wolffian bodies of a $3\frac{3}{4}$-day chick embryo. This section shows the appearance of the germinal epithelium just after its formation, the position and characteristics of the primordial germ-cells, and the tissue beneath the epithelium.

On examining with a microscope a section through the anterior third of the sexual region of the Wolffian body the following facts concerning the genital anlagen may be observed.

Each gonad appears as a rounded hillock on the medial surface of the mesonephros, and is separated from its fellow by the mesentery. Even at the first glance it will be noticed that the gonad is composed of three tissues (fig. 1). The epithelium covering the gonad is a single layer of elongated prismatic cells. This epithelium is the germinal epithelium of Bornhaupt and Waldeyer ('70) and of succeeding authors. This epithelium is not confined sharply to the gonad, but, beyond its medial and lateral boundaries, merges with the flat coelomic epithelium (fig. 1). At this time and in the two later stages the germinal epithelium covers the mesentery for some distance before it disappears. The germinal epithelium commences to differentiate from the coelomic epithelium when the embryo is $3\frac{1}{2}$ days old so at this time it is well developed.

Under this germinal epithelium is a compact mass of tissue, the cells of which, although more closely packed, resemble those of the general embryonic mesenchyme. This compact tissue makes up the mass of the gonad, occupies the concavity of the germinal epithelium, and hence gives rise to the rounded form of the germinal hillock.

If the section happens to be rightly situated, the mesenchyme material under the germinal epithelium will be observed to extend into a cord or strand, which runs obliquely towards a Malpighian corpuscle. According to Firket ('14) there are sixteen of these cords and they together with the tissue under the germinal epithelium make up the urogenital connections or "organ of Mihalkowics" of Sainmont ('05). There has been much discussion as to the origin of these cords of urogenital union, as to whether they are derived from peritoneal epithelium, the capsules of the Malpighian corpuscles or the mesenchyme. My preparations from embryos of this age, that is 90 hours, and from one of 84 hours, would seem to show that the cords of urogenital union are derived from the mesenchyme. This is at variance with the opinion of Sainmont ('05) who described them

in the cat as coming from the epithelium of Bowman's capsule; neither is this in accord with Allen's ('05, '06) findings in reptiles (Chrysemis) who described them as derived through invaginations of the germinal epithelium. The opinion of Firket ('14) that they are merely condensations of mesenchyme is, in my opinion, correct. The cells of which they are composed differ in no way from those of the general mesenchyme. They merge without any change into the other portion of the "organ of Mihalkowics" under the epithelium and this in turn merges with the general embryonic mesenchyme. The cells of these cords are not epithelial in character and do not resemble the cells of the germinal epithelium nor those of Bowman's capsule. The primordial germ-cells in them can be explained by migration.

The third tissue in the gonad is made up of primordial germ-cells. They are present between the cells of the germinal epithelium, on both sides of the coelomic angle, in the mesenchyme tissue under the epithelium and in the cords of urogenital union (fig. 1). They are not all confined to the gonad for some are present in the germinal epithelium lining the base of the radix mesenterii, while others are present in the mesenchyme of the same structure. Most of them are found in the medial portion of the gonad near the coelomic angle, although an occasional one will be found in the germinal epithelium of the gonad far removed from this spot. Their appearance at this age is the same as that of those described by Swift ('14) in younger embryos. The factors which distinguish them are size of cell, clearness of cytoplasm, size of nucleus, persistent droplets of vitellus and the large attraction-sphere (figs. 1 and 4). They are not numerous—from one to ten in a single section—nor are they dividing to any extent. One interesting point which will be taken up at length in the next stage to be described is the fact that there are more of them in the left gonad than in the right.

In connection with the next stage, that of approximately 4 days, seven embryos were studied. The gonad has about the same length but projects more into the coelom. This increase in size is due in part to the increased thickness of the germinal epithelium, which is now composed of two or three layers of cells,

but in greater measure to an increase in the amount of the underlying tissue (fig. 2).

As regards the primordial germ-cells, there has been practically no change in their appearance; however, their distribution is quite different from that described in the last stage. Although a majority are in the medial portion of the gonad, yet they are not grouped as in the preceding embryo, but they are more evenly

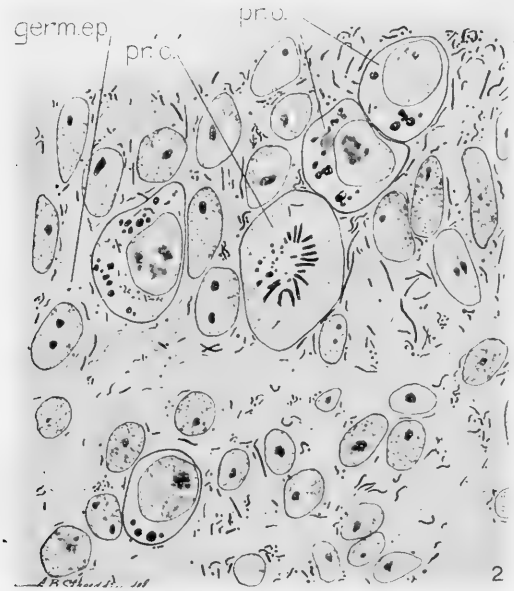


Fig. 2 Portion of a transverse section through indifferent gonad of a 4-day chick embryo, to show the thick germinal epithelium, and primordial germ-cells, one of which is dividing—a rare occurrence at this stage.

distributed in that portion of the epithelium and mesenchyme near the mesentery. This change in position can be accounted for by growth of the gonad and to a certain extent by their power of amoeboid movement. The germ-cells are not entirely quiescent for in the seven embryos studied about a dozen were found dividing (fig. 2). That some division is taking place is also evidenced by the fact that an occasional group of 8 to 15 of them are found in which the cells are solely germ-cells without any tissue whatever between, indicating that a number of successive mitoses had probably occurred.

In one embryo the number of primordial germ-cells was counted and was found to be 743; in another embryo the number was found to be 860. These numbers are only approximate but are probably 90 per cent correct. In the first embryo there were about twice as many on the left side as on the right, while in the second embryo there were about five times more numerous on the left side (fig. 3). The number of germ-cells in the other embryos

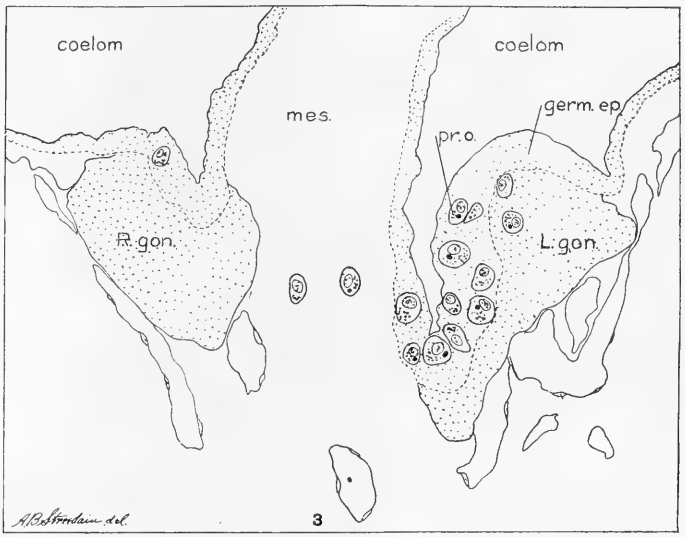


Fig. 3 Portion of a transverse section through indifferent gonads of a 4-day chick embryo. The drawing is semi-diagrammatic, although the outlines, position and number of the germ-cells are exact reproductions of conditions existing in a single section of this embryo. Notice the greater number of primordial germ-cells in the left gonad.

was not counted, but it could be easily seen that the number in the left gonad was far in excess of that in the right gonad (fig. 3). This very suggestive fact was published by Firket ('14) for the first time. It is of course proof positive that the germ-cells migrate actively and are not distributed by tissue displacements as was advocated by von Berenberg-Gossler ('12). This unequal distribution of the germ-cells in favor of the left side may account for the fact that the gonad of that side is, from its first appearance, the more massive.

As regards the embryos of ages $4\frac{1}{2}$ and 5 days, respectively, the changes are not sufficient to warrant an extended description. The gonads, in these stages, have increased only slightly in length, but their volume, as shown by the cross-section, is considerably greater. This increase of the area of the transverse section is due solely to hyperplasia of the elements of the stroma, which are

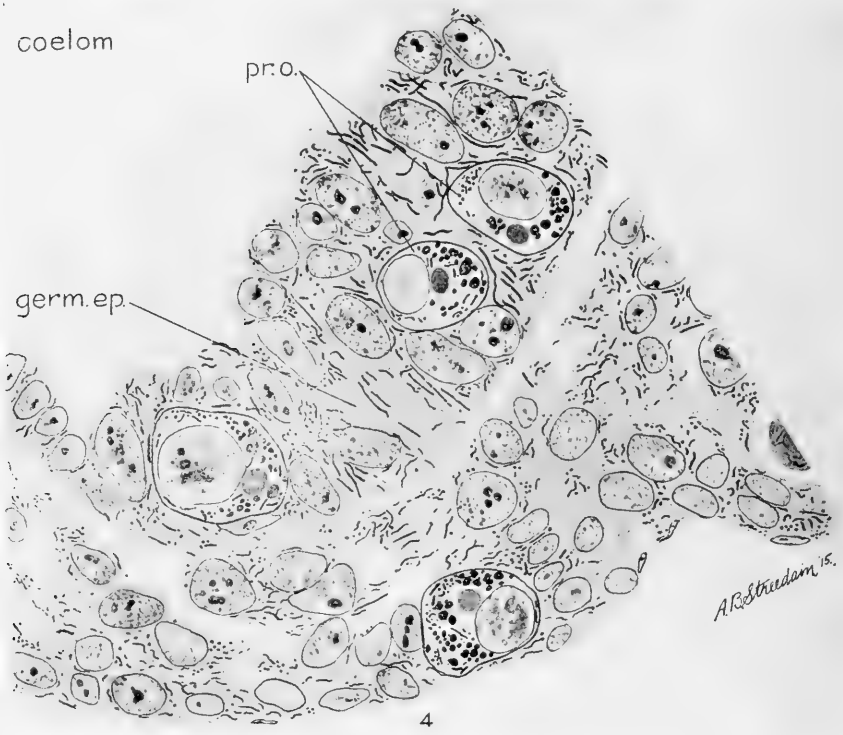


Fig. 4 Portion of a transverse section through indifferent gonad of a 5-day embryo. In this embryo the germ-cells retained a quantity of vitellus.

of mesenchymal origin. Proof of this is found in the numerous mitotic figures. The germinal epithelium still is composed of two or three layers of cells and the limiting basement membrane is as distinct as of old (fig. 4).

The primordial germ-cells have about the same distribution, and are still quiescent, since only one or two in each embryo are found in a state of division (fig. 4).

2. Primordial germ-cells, germinal epithelium and medullary cords of the embryonic ovary

The next three embryonic stages studied, namely, $5\frac{1}{2}$, 6 and $6\frac{1}{2}$ days, are of great interest, for in them the first true sexual cords, or cords of first proliferation, make their appearance. In the male they become the semeniferous tubules and in the female form the medullary cords which later have much to do with the constitution of the ovarian medulla.

The gonad of the $5\frac{1}{2}$-day chick has a stroma, which is composed of only one kind of element, the mesenchyme tissue. The cells of this tissue are more closely packed than in the general embryonic body, but their round or branching character can readily be seen. There are many karyokinetic figures in this tissue, in which are to be seen also a few primordial germ-cells.

The general thickness of the germinal epithelium remains about the same but in several places there are projections or buds on its deep surface. An occasional finger-like projection of the epithelium into the stroma is present. The bud is the beginning, and the projection is the completion of the cord of first proliferation. These buds and the completed cords are due to local increase in activity of the germinal epithelium. It is true that they may contain one to several primordial germ-cells, but I have never seen any evidence that they played any rôle other than a passive one in the local hyperplasia, which gives birth to the sexual cord (fig. 5).

The cords of first proliferation are seen at their best in the next two stages—embryos of 6 and $6\frac{1}{2}$ days respectively.

In these stages the evolution of the cords is to be seen—the bud, the cord still attached to the germinal epithelium, and the cord which has become separated from the latter by an ingrowth of stroma (fig. 5). In this connection it may be said that the growing cord does not remain attached to the epithelium for any length of time. At first the basement membrane of the epithelium is continuous around the lengthening cord but very soon this continuity is lost (fig. 5). After separation from their mother tissue they become autogenous and increase rapidly

in size. They also become broken up and soon make up most of the mass beneath the epithelium. Although widely scattered and fragmented these cords in general retain the same orientation, which they had at their inception, before becoming detached

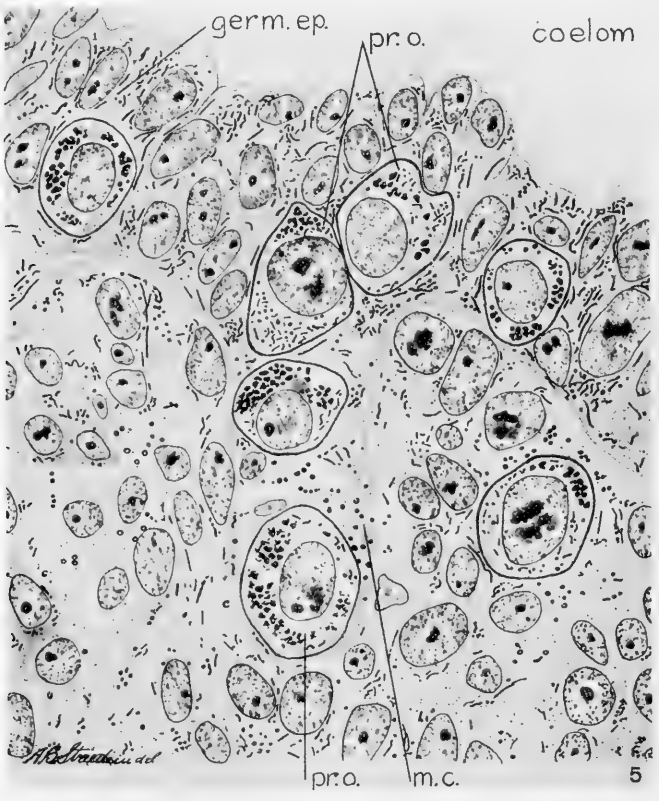


Fig. 5 Portion of a transverse section through ovary of a 6½-day chick embryo. This drawing, made from two sections, shows conditions during differentiation of sex. Notice the cord of first proliferation, and the thick germinal epithelium, containing many primordial germ-cells—a fact characteristic of the female just after the appearance of the medullary cords.

from the germinal epithelium, that is, their long axes are perpendicular to the axes of the epithelium. Before dismissing the subject of the formation of these cords of first proliferation, I must again repeat that they are purely of germinal epithelium

origin, and that the primordial germ-cells found in the cords are there because they happened to be present in the localized region of proliferation which gave rise to them (fig. 5).

At this time, that is to say, during the 6th and 7th days of development and in fact for some days to come, there are three tissues in the mass beneath the germinal epithelium. The old mesenchymal tissue between the cords, the cords derived from the germinal epithelium, whose cells are like those of the parent tissue, and the primordial germ-cells (fig. 5). The latter have entered the stroma of the gonad in various ways, that is, some of them have been in it from the beginning, others entered it from the germinal epithelium by individual migration, while still others were brought in by the cords of first proliferation.

For the evolution and degeneration of the cords of first proliferation in the female chick the reader is referred to an excellent account written by Firket ('14).

When the chick embryo has reached the 156th hour of development ($6\frac{1}{2}$ days), the formation of cords of first proliferation ceases rather abruptly and it is about this time that the sex of the individual can be definitely determined.

In the determination of sex there are three criteria on which reliance can be placed. These are—the relative size of the two gonads, the germinal epithelium, and the number of primordial germ-cells in the germinal epithelium.

In all the embryonic stages which I have studied the right gonad is smaller than the left, but towards the end of the sexual cord formation this disparity in size becomes marked in the female. The great increase in size of the left gonad in the female is due to several factors. In the first place the cords of first proliferation in this gonad grow rapidly and there is also an increase in the thickness of its germinal epithelium.

The germinal epithelium of the left gonad of the female possesses several more layers of cells than does that of the male. In the male gonads and in the right female gonads the epithelium remains relatively thin. This increase in thickness of the germinal epithelium takes place synchronously with the formation of the cords of first proliferation.

An interesting fact, and one to which attention has not been previously called, is the presence of more primordial germ-cells in the germinal epithelium of the left female gonad than in the epithelium of the male gonad (fig. 5). Since, as has been previously stated, the germ-cells play a passive rôle only, this can be accounted for only by the fact that the germinal epithelium of the left female gonad is increasing in thickness during the genesis of the cords of the first proliferation and so in this way

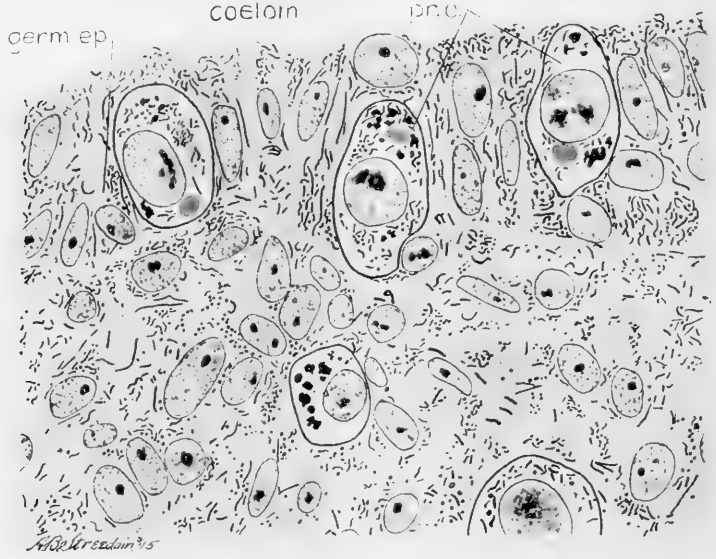


Fig. 6 Portion of a transverse section through embryonic ovary of a 7-day chick. This drawing shows the thick germinal epithelium, containing many germ-cells, which is characteristic of the female gonad before appearance of the cords of second proliferation.

many of the primordial germ-cells are taken away from the sites of local hyperplasia which give rise to the sexual cords.

All these factors are of relative value only, and it is only by invoking them all, that any certainty as to the sex of the individual may be reached.

In the 7-day female gonad or embryonic ovary the germinal epithelium is composed of several layers of cells (fig. 6). The layers are not very definite and cannot be traced for any dis-

tance in the epithelium, nor are the cell outlines distinct. The layers can be distinguished more by the nuclei of the cells and their relative position than by cell walls (fig. 6). However, where a single cell can be seen in its entirety, it will be noted that the form is columnar and the nucleus constituted as in the past (compare figs. 1, 2 and 6).

The region of the gonad under the germinal epithelium, which may now be called the medullary region, is composed in greater part of the medullary cords. They can be easily seen as cords of epithelial cells, distinctly delimited, containing here and there a primordial germ-cell, and at the same time showing evidences of continued growth in the many mitotic figures.

The gonad at this stage is increasing in all diameters. This enlargement, as far as breadth and thickness is concerned, is due in great measure to the increase in the medullary cords, which fact is plainly evident on careful study of the transverse section.

The primordial germ-cells in the germinal epithelium are more evenly distributed throughout this tissue than in any previous stage (fig. 6). The greater number are still in that portion of the epithelium near the mesentery but in an average transverse section 6 to 12 are distributed in the rest of the germinal epithelium over the main portion of the gonad. There are as yet no evidences of division in the germ-cells but on the other hand the vitellus is much reduced in amount. There also has been a slight change in position of the rod-shaped mitochondria, which are numerous in these cells. In former stages these bodies had been rather evenly distributed in the cytoplasm, but now there seems to be a tendency, rather slight it is true, for them to collect in the region of the centrosphere. This is not true of all the germ-cells, as is the case in older embryos; however, this fact can be observed in a majority.

Up to the stage now about to be described, that of 8 days, there has been but little evidence of division of the primordial germ-cells and most of that evidence has been of an indirect nature—an increase in the actual number of germ-cells present in each section from stage to stage, and their arrangement, at times, in groups, as if indicative of past mitoses.

This stage of 8 days is the beginning of a period of rapid multiplication of the germ-cells, which reaches its height, as will be described later, during the 9th and 10th days, but which continues with decreasing activity for some days longer.

CORTICAL CORDS AND ORIGIN OF THE DEFINITIVE SEX-CELLS

The 8th, 9th, 10th and 11th day stages may be described together, since they are characterized in common by one fact, that is, germ-cell division.

As has been mentioned from time to time, as the various stages were being described, there has been little change in the germ-cells. Their size has remained constant, as has that of their nuclei and spheres. The vitellus, which they acquired from the germ-wall entoderm, although constantly diminishing in quantity, has been easily seen up to this stage. Their relatively constant size and ever present vitellus have been due to a lack of division. These facts have been remarked by all describers of them but Rubaschkin especially, in the guinea pig, has accentuated their primitiveness.

At 8 days the primordial germ-cells in the germinal epithelium are more numerous than in previous stages. In one section 18 germ-cells were present in the epithelium. The majority still have a little vitellus but in some cells this is absent. They are present in the deeper portions of the epithelium next the slight quantity of connective tissue, which separates the medullary region from the germinal epithelium. This connective tissue is the primordial albuginea. Most important, however, as far as the germ-cells are concerned, some are in a state of division. In one 8-day embryo 7 dividing primordial germ-cells were counted.

At 9 days the primordial germ-cells are actively dividing. Little groups of 3 and 4 with karokinetic figures are found in the deeper parts of the germinal epithelium (fig. 7). The lower part of the epithelium, next the albuginea and medulla begins to have a lobulated appearance as has been described by d'Hollander ('04) and Firket ('14). These lobulations are due to the groups of germ-cells, just described, and elements resembling those of the more superficial layer, and are the first evidence of the cortical

cords or cords of second proliferation. Sainmont ('05), von Winiwarter and Sainmont ('08), d'Hollander ('04), and Firket ('14).

The majority of the cells in these lobulations are primordial germ-cells, or rather oogonia, since active division has begun and since they are in the direct ovuliferous line. They are not related to the cells of the germinal epithelium in any way, be-

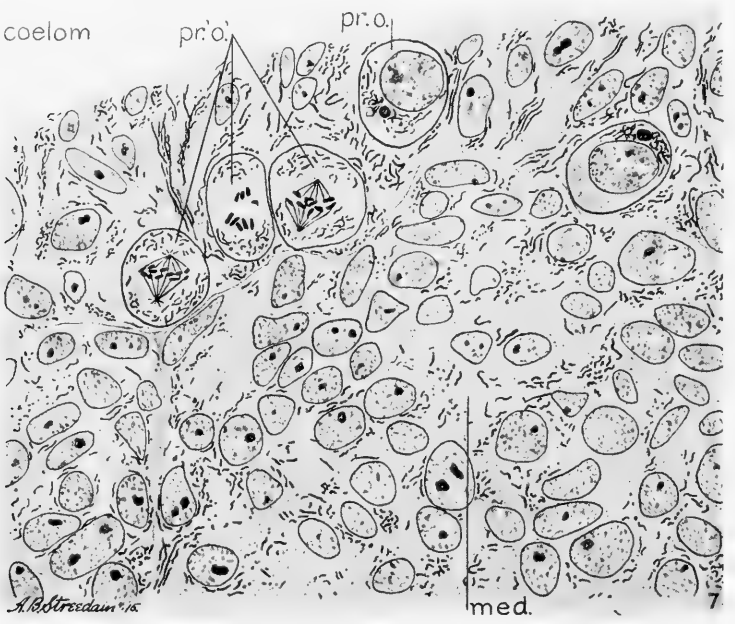


Fig. 7 Portion of a transverse section through embryonic ovary of a 9-day chick. This drawing shows the germinal epithelium with dividing germ-cells; these little nests of dividing primordial germ-cells give rise to the cortical cords or cords of second proliferation.

cause, in the first place there is no resemblance, secondly there are no stages which are intermediate, and thirdly there are present in the groups of oogonia, or primordial germ-cell descendants, typical prismatic cells, which resemble in every way cells of the germinal epithelium.

Now to take up the first point more at length: The epithelial cell is prismatic, long for its size, has a small nucleus, a sphere that can hardly be demonstrated and mitochondria which are

usually scattered in all parts of the cytoplasm, but which may be at the margin of the cell (fig. 7).

The oogonia or descendants of primordial germ-cells on the other hand are round or oval in all cases, have a large nucleus, with rather coarse chromatin granules. The attraction sphere is large and just as characteristic as at 4 days (fig. 7). The mitochondria are grouped around the sphere (fig. 7). This is not a new character, since there was a suggestion of it at 7 days, which had become constant at 8 days.

The only difference in the oogonia in the 9-day chick as compared with the primordial germ-cell of the 7-day stage is the disappearance of the vitellus, the decrease in size of the cell, and the grouping of the mitochondria (compare figs. 6 and 7). The last began to be evident at an earlier stage and the first two can be explained by the fact of active cell division.

To repeat, there is very little difference between the oogonium at 9 days and the germ-cell in the 7-day stage. There is a vast deal of difference between the oogonium and the germinal epithelium cell at any stage (figs. 7 and 8).

In the 9-day chick it may be of interest to compare the primordial germ-cells found in the medullary cords and the oogonia in the germinal epithelium and primordial cortical cords. In the first situation the germ-cells have retained all their primitive characters, but the following are to be especially noted: The cell is never seen dividing, the vitellus is still present, and the mitochondria are scattered in the cell. Cells of this character are never seen in the germinal epithelium, except in its medial portion, where a few still retain all these primitive characters.

The oogonia, although resembling the primitive type of germ-cell in many ways, are yet distinctly different in others.

At 9 days the ovary is becoming massive, due in great measure to a change in the medulla. The medullary cords in that portion of the gonad next the urinary organ have lengthened, become thicker and some are developing a cavity. These cavities are lined by cells, which are arranged like epithelial cells around a lumen, and radiate towards the germinal epithelium. The albuginea has also thickened.

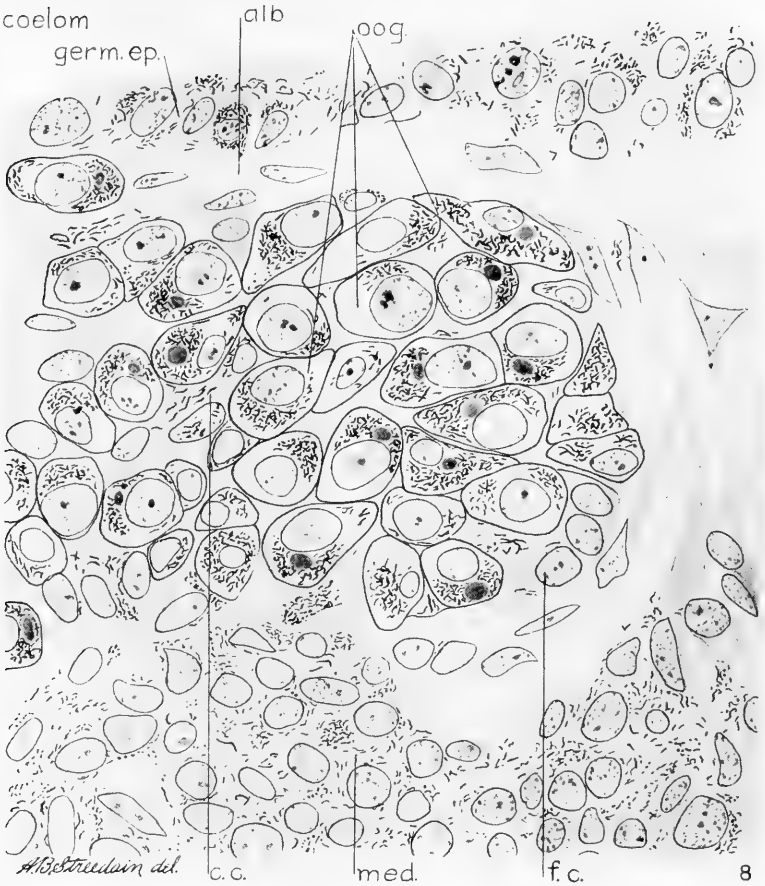


Fig. 8 Portion of a transverse section through ovary of a 14-day chick embryo. The groups of oogonia, resulting from division of the primodial germ-cells, now extend down into the medulla as cortical cords. The oogonia produce the definitive ova and the follicular cells the follicular epithelium.

The ovary of the 10-day chick closely resembles that of the 9-day chick, which has just been described.

The oogonia are still dividing, regular nests of mitoses are seen occasionally, in which 3 to 6 cells may be seen dividing at the same time. The lobulations on the deep surface of the germinal epithelium have increased in size, due of course to the increased number of oogonia. These lobulations are now quite distinct from the germinal epithelium, to which they are at-

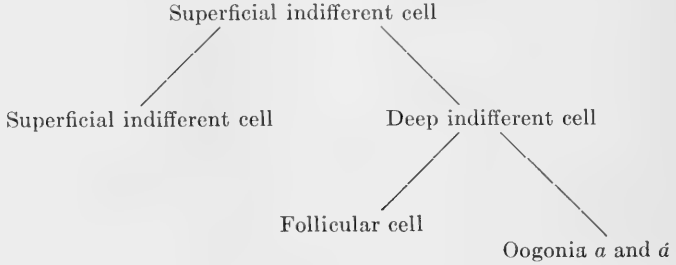
tached by a constricted portion or neck. Between the lobulations there is much connective tissue, which is also present between the medulla and the superficial region which may be called the cortex.

The medullary region at this time has a rather striking appearance due to the large cavities in the deeper portions of the medullary cords. These large spaces have unstainable contents since they never show any evidence of stain.

This 10-day stage is of special interest because it was at this stage that d'Hollander ('04) began his study of oogenesis in the chick. At this stage of development according to d'Hollander, the cortical region consists of two zones, the layer of superficial indifferent cells or epithelial layer, and the deeper layer of germinative buds, which later become the cortical cords.

The epithelial layer is made up of cylindrical cells, with elongated nuclei and long axes directed perpendicularly to the surface of the ovary.

The germinative buds are made up of several types of cells. One kind, which he calls indifferent deep cells, is scattered irregularly throughout the buds. According to d'Hollander, these cells resemble the superficial indifferent cells from which they are derived. The deep indifferent cells give rise to oogonia of two kinds *a* and *á*. The oogonia of type *a* are true oogonia, those of type *á* are intermediate in character, resembling, on the one hand, the deep indifferent cells, and on the other, the true oogonia. The deep indifferent cells may also by division produce follicular cells, which at this stage cannot be distinguished from the parent cell. D'Hollander's scheme is as follows:



Except as regards the origin of the oogonia, I agree with d'Hollander in his findings.

Sainmont ('05) in his investigations on oogenesis in the cat described the cords of second proliferation as composed of oogonia, which were derived from primordial germ-cells. However, he retracted this opinion in 1909 in an article on spermatogenesis and oogenesis in the cat, in which he collaborated with von Winiwarter.

Since 1904, the date of d'Hollander's article, all investigators with the exception of Rubaschkin and his pupils have adopted the opinions of d'Hollander as regards the origin of the oogonia in the chick.

The ovary of the 11-day-old embryo resembles closely that of that 10-day embryo.

The cell buds have now attained a size which warrants their being called cords of the second proliferation. In the cortical cords the oogonia still show signs of activity, although it is not so marked as in the two preceding stages. The oogonium itself resembles closely the same element as described in the 9-day ovary. In between the oogonia are a few much smaller cells, which show occasional mitotic figures, and which are derived from the cells of the germinal epithelium. These are the deep indifferent cells of d'Hollander, the elements from which the follicular epithelium is derived in later stages.

The next ovary studied was from a chick embryo of 14 days' development.

The cortical cords are much more massive—in some as many as 30 oogonia may be counted in a single section of one (fig. 8). The cords now extend down into the medulla for some distance, although they are still separated from it, as well as from each other, by a thick layer of connective tissue (fig. 8). In places the cortical cords are separated from the epithelium by a layer of this connective tissue, which in this position, under the germinal epithelium, may now be called the true albuginea (fig. 8). The germinal epithelium now consists of a single layer of cubical to columnar cells.

In the cortical masses or cords there are, as formerly described, two kinds of cells—the oogonia and the follicular cells (fig. 8).

The further history of the oogonia in the chick has been admirably presented by d'Hollander ('04) and Sonnenbrodt ('08), who have described in detail the changes, nuclear and otherwise, which the oogonia and oocytes undergo in the process of oogenesis. The reader is referred to their accounts for the details of the processes which transform the oogonium into the ovum and some of the deep indifferent cells into the follicular epithelium.

SUMMARY AND CONCLUSIONS

1. During the two days subsequent to the development of the germinal epithelium, which occurs between the 80th and 90th hours of incubation, a majority of the primordial germ-cells are found between the cells of that tissue. Some of the remaining primordial germ-cells are present in the mesenchyme beneath the germinal epithelium, others are found in the cords of urogenital union, while still others remain in the root of the mesentery. The latter may be seen in that situation in some embryos until the 9th day of development, when they degenerate.

During the 4th and 5th days of development it is possible to count, with a considerable degree of accuracy, the germ-cells in the gonads, and if that is done, it will be found that the number on the left side exceeds that of the right in the proportion of 2 to 5 to 1.

2. During the 6th and 7th days of development the germinal epithelium sends down into the subjacent tissue a first series of sexual cords or cords of first proliferation. These cords are produced as a result of localized activity of the germinal epithelium. The cords of first proliferation are epithelial in character, and the germ-cells, which are present in them, apparently have nothing to do with their formation, however, it is not beyond a possibility that certain of the germ-cells play a metabolic rôle in stimulating the epithelial cells around them to activity.

The cords of first proliferation in the male become the seminiferous tubules and in the female the medullary cords.

3. Immediately following the formation of the first sexual cords it is possible to determine the sex of the individual being studied (fig. 5).

In the male the number of primordial germ-cells remaining in the germinal epithelium after the formation of the cords of first proliferation is small, while in the case of the female the number does not seem to be greatly diminished.

4. Beginning with the 8th, but especially during the 9th, 10th and 11th days of development, there is a rapid increase in the number of primordial germ-cells in the germinal epithelium of the female. Three or four dividing germ-cells may be seen in a small area (fig. 7). Collected into groups, which are the result of successive mitoses, the primordial germ-cells, or oogonia as they may now be called, give rise to lobulations which appear on the deep surface of the epithelium. These lobulations, or buds, composed principally of oogonia, but including also cells of peritoneal parentage, increase in size and become the cords of second proliferation or cortical cords (fig. 8).

5. The oogonia become the definitive ova, while the peritoneal cells of the germinal epithelium, present in the cortical cords, develop the follicular epithelium.

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A MODEL TO ILLUSTRATE THE PROBABLE ACTION OF THE TECTORIAL MEMBRANE

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SEVEN FIGURES

In some studies of the cochleae of the pig, the writer ('08 and '15) became convinced that the tectorial membrane and not the basilar membrane is at least the chief vibratory structure in the mechanism for hearing. With this conviction, the construction of a model was undertaken with the hope that it might illustrate something of the possible behavior of the mammalian tectorial membrane when energy represented by sound waves is imparted to the fluid in which it lies.

Of the many experiments with apparatuses and the several models described by others, none have attempted to reproduce the form and arrangements of the parts of the actual auditory apparatus and but little of the conditions under which these parts are supposed to act in the animal. It seemed to the author that more instructive indications of the process of hearing might be obtained with a model in which the outer, middle and inner divisions of the peripheral part of the auditory apparatus are represented and the forms and relative arrangements of the structures comprising each division imitated, and a model to which might be applied the disturbances giving rise to sensations of sound in the actual ear. The construction of such a model must necessarily be preceded by detailed studies of the structures of the ear and especially of those structures considered acted upon by sound waves in a way to arouse the various sound impulses in the auditory neurones. Also it seemed advisable that the studies of the structures be carried to the labyrinths of adult animals, rather than confined to those of foetuses, and the writer's paper

of 1915 describes some observations upon adult cochleae, chiefly those of the adult hog.

The purpose of this paper is to describe a model and to mention some of the results obtained with it suggesting the action of the tectorial membrane when actual sound waves are applied to the tympanic membrane. It is thought advisable to precede this description by a brief résumé of the anatomical observations made in previous papers which have led to the conviction that the tectorial membrane is the structure best adapted for the vibratory activities required. The model lays no claim to completeness of plan. Both its form and excellence of construction leave much to be desired. Consideration of only the few simpler and most evident of the results obtained with it will be undertaken. Many of the phenomena it presented appeared so mixed and complex in the physics involved that, considering the great coarseness and imperfection of the model as compared with the cochlea itself, attempts to interpret them seemed needless as well as almost hopeless. Some figures are given for review of certain of the anatomical features mentioned as well as figures to illustrate the construction of the model. Citations to most of the literature consulted are given in the previous papers and but few of these will be repeated here.

RÉSUMÉ OF THE THEORIES OF HEARING AND OF OBSERVATIONS UPON THE SUPPOSEDLY VIBRATORY STRUCTURES RELATED TO HEARING

It is considered established (1) that the cochlea is the essential organ of the peripheral part of the auditory apparatus, that it contains the neuro-epithelium in which auditory impulses are aroused and is generally thought to be so constructed as to be especially capable of serving, in conjunction with the central nervous system, in the analysis of sound; (2) that the auditory impulses imparted to the fibers of the cochlear nerve are aroused in the hair cells of the neuro-epithelium (organ of Corti) by impingement of the hairs of the hair cells against the basal surface of the tectorial membrane, and that the impingement results from vibratory motion induced by that form of atmospheric disturb-

ances known as sound waves; (3) that sensations of sounds of the highest pitches of which the apparatus is capable are mediated by the basal end of the coil of the cochlea. For example, Munk destroyed the basal end of the spiral organ in dogs, operating through the fenestra cochleae (rotunda), and found deafness to high notes to result, and Baginski destroyed the cochlea of one side entirely and then operated to destroy parts of the cochlea of the other side, obtaining results confirming those of Munk.

The most commonly accepted theories of hearing may be divided into two: that elaborated by Helmholtz, comprising ideas involving phenomena of resonance purely, and the Telephone Theory, comprising ideas involving little or no resonance.

One of the earliest advanced ideas was that the hairs of the hair cells are themselves agitated selectively, or in resonance, by the different sound vibrations imparted to the cochlea. This was quickly abandoned as untenable on the ground that the hairs are neither suitably constructed, long enough, nor vary sufficiently in length to be effectively acted upon by the vibrations as transferred to the endolymph in the cochlear duct. The Helmholtz theory is a resonance theory wholly and was applied solely to the basilar membrane, that narrow, thinnest span of the membranous spiral lamina. Practically all who have tried to apply it to given phenomena have had to modify it. Based upon erroneous descriptions by others (Nuel, 1872, for example) of the basilar membrane as composed of independent, radially disposed fibers and upon the fact that the membrane increases in width (in length of the fibers) in passing from the basal to the apical end of the cochlea, the theory requires the sympathetic or selective vibration of these fibers in resonance with the various vibration frequencies imparted to the endolymph. The hairs of those hair cells resting over fibers of the basilar membrane with a length, or natural vibration frequency, corresponding to the vibration frequency of a given note, were thought to be thrown against the basal surface of the tectorial membrane by the selective vibration of such fibers. Thus the theory assumed that the basilar membrane is composed of fibers of lengths vary-

ing according to the vibration frequencies of all the sounds the organ is capable of appreciating, that mixtures of sounds are analysed in the cochlea by the resonant vibration of the fibers in different parts of it corresponding to the different tones contained in the mixture.

Aside from the many physiological difficulties met in applying it, the Helmholtz theory is not supported by the later found anatomy of the cochlea: (1) The basilar membrane is not composed of independent fibers. The structure given the name is composed of three layers of white fibrous tissue, one of which courses at right angles to the others. That part of it which is radially arranged is nothing more than a flat tendon, the fasciculi of which are abundantly connected with each other by collateral branches. (2) Were the basilar membrane composed of radial fibers capable of vibrating independently, it is blanketed on both its sides by continuous and thick layers prohibitive of any such action. On its apical or vestibular side is spread the spiral organ (of Corti) and the membrana propria of its epithelium; on its basal or tympanic side extends the continuous layer of epithelioidal syncytium with its blood vessels and the continuous endothelium lining the scala tympani. (3) Were the membrane as the theory assumes, the cells of the spiral organ are so closely associated and cemented together that individual hair cells or groups of hair cells overlying the fibers concerned with a given note could not be made to impinge separately against the tectorial membrane by the resonant vibration of the fibers. (4) It is questionable whether the fibers, were they independent and free, are long enough for the resonant vibration assigned them by the theory, especially for the sound waves of the lower notes appreciated. Liberal measurements in the human cochlea give the supposedly vibrating part of the membranous spiral lamina a width of only $\frac{3}{10}$ mm. (304μ) at its apical end where it is broadest and $\frac{3}{18}$ mm. (168μ) at its basal end. Measurements of the same for the adult hog gave 258μ as the average width at the apical end and 185μ as the average width at the basal end. Helmholtz himself appreciated the doubt whether fibers so short as the width of the basilar membrane can be

thrown into vibration by sound waves. (5) Sometimes in the cochleae of the pig, and perhaps other mammals, a part of the organ of Corti in the basal end may rest upon the bony spiral lamina instead of the basilar membrane.

The idea involved in the telephone theory was first suggested by Rinne in 1865, or about thirty years before the elaboration of the Helmholtz theory. It was more fully worked out by Rutherford in 1886 and since modified by Waller in 1891, Meyer in 1898, Ewald in 1899, Gray in 1900, and others. Originally it assumed that the vibrations imparted effect the cochlea as a whole. Rutherford at first suggested that all the hairs of the hair cells are thrown into vibration by each note and the impulses thus aroused in the cochlear nerve are merely similar in frequency, intensity and quality to the vibration frequency, amplitude and quality of the notes acting upon the apparatus. Therefore, the analysis of sound would be wholly cerebral. This idea that the impulses are aroused by the hairs being acted upon directly was early abandoned by Rutherford and the telephone theory became applied to the basilar membrane and all the later modifications of it have applied it to this membrane. Waller and Meyer assumed that the vibrations as transferred from the tympanic membrane to the endolymph of the cochlea affect the basilar membrane as a whole. Meyer supposed that the wave motion produced by each note, as it passes in the scala vestibuli, affects an extent of the basilar membrane just in the proportion that the amplitude of the vibration is not decreased by the resistance to be overcome in its passing toward the apex of the cochlea. Thus certain wave motions will affect greater extents of the basilar membrane than others and therefore will cause the hairs of a greater number of hair cells to impinge against the tectorial membrane. Waves of lesser amplitudes (intensity), in overcoming the resistance met in passing from the basis of the stapes toward the apex, earlier become too faint to sufficiently agitate the basilar membrane, each note involving an extent of the membrane according to its amplitude or intensity. In this idea pitch depends upon the vibration frequency (the number of stimulations of the hair cells per unit of time) and intensity is

expressed in the total number of hair cells (extent of membrane) irritated. Obviously some analysis of sound may thus be made in the cochlea.

The telephone theory differs from the Helmholtz theory in that the latter supposes the basilar membrane composed of fibers of varying length, those of given lengths vibrating in resonance with waves of given vibration frequencies, making entire analysis of sound by the cochlea possible, while the telephone theory as now modified assumes that the basilar membrane vibrates as a whole to every note in such extent as the amplitude of the wave motion and the resistance to its propagation will allow, the auditory neurones transmitting to the brain impulses of frequencies and intensities corresponding with those of the vibrations concerned.

Leave is here asked to submit below a modification of the telephone theory applied to the tectorial instead of the basilar membrane. Siebenmann ('98) seems to have first recognized the importance of the tectorial membrane in the auditory apparatus, noting that it begins in the animal series with the beginning of 'musical hearing' and that its absence or deformity results in deafness. He was the first to suggest that it may be thrown into vibration by sound waves. Von Ebner ('02) suggested that the tectorial membrane, especially its free zone, may serve as a mechanism for sympathetic vibrations. Following von Ebner, Kishi ('07) and Shambaugh ('07) attributed powers of resonance to the tectorial membrane but considered it attached to the spiral organ and composed of independently vibratory elements ('lamellae').

The anatomical studies undertaken by myself have had to do with the tectorial membrane of the foetal and adult pig and the adult ox, rat and human. Most of them have been made upon cochleae of the pig. My conviction that the tectorial membrane is by form, nature and position the best adapted of the structures in the cochlea for the vibratory activities required in the process of hearing is based upon the following observations:

(1) The tectorial membrane is strictly coextensive with the spiral organ (organ of Corti). It is developed in company with

and from the same variety of cells as the spiral organ (both being of ectodermal origin). The so-called basilar membrane is not always coextensive with the spiral organ.

(2) In position, the tectorial membrane lies over the apical or vestibular surface of the spiral organ and thus may be first and more directly acted upon by the usual sound vibrations, since these are imparted by the basis of the stapes directly to the fluid in the scala vestibuli. The basilar membrane lies under the basal side of the spiral organ and next the scala tympani, the thick spiral organ, the tectorial membrane and the vestibular membrane intervening between it and the scala vestibuli.

(3) In the other sense organs, and all sensitive surfaces, the impulses are aroused at the peripheral or outer surface of the epithelium. The tectorial membrane lies over the peripheral surface of the spiral organ while the basilar membrane lies under the organ. The membranes of the maculae and cristae, whose origin is directly homologous with that of the tectorial membrane, arouse impulses at the peripheral surface of their respective neuro-epithelia.

(4) When the two are teased out for examination in the fresh, the tectorial membrane is found to be far more flexible than the basilar membrane.

(5) The tectorial membrane is considered more adapted for application of the telephone theory, capable of being more freely agitated, in that one edge of its supposedly vibratory portion, its outspanning zone, is free, while the basilar membrane is merely a flat tendon, one edge continuous from the tympanic lip of the spiral lamina and the other continuous into the spiral ligament. Furthermore, not only are both edges of the basilar membrane attached or continuous with the wall of the labyrinth, but it is loaded on its two sides by thick layers of other tissues likewise continuous beyond its edges and thus it must be less readily agitated than is the tectorial membrane, certainly by the vibrations of lesser amplitude.

(6) Figure 1 is given to show the shape of the tectorial membrane of the adult hog viewed on the flat from the basal surface, its coil slightly opened to obviate the overlapping in its natural

form. It is seen that its vibratory portion or outspanning zone (*OZ*, this figure and figure 2) increases gradually and regularly in width from its basal end (*BE*) to its apical end. Measurements of its width in its different turns recorded in my paper of 1915, show that this zone is about 7 times as wide in the apical as in the basal end. The ends terminate bluntly rounded. Liberal measurements of the width of the basilar membrane (the portion of the membranous spiral lamina supposed to vibrate) show that its apical end is only about 1.8 times wider in the human and averages 1.4 times wider in the hog than its basal end. Measurements of the attached axial zone of the tectorial

EXPLANATION OF FIGURES

REFERENCE LETTERS

AM, 'Accessory membrane'
AS, Adjusting screw
AZ, Attached axial zone
B, Battery
BE, Basal end
BL, Bony spiral lamina
BW, Bony wall of cochlea
BM, Basilar membrane
BS, Basis of stapes
C, Cork
CD, Cochlear duct
CN, Cochlear nerve
CP, Copper plate
CW, Copper wire
ED, Endolymphatic duct
EM, External auditory meatus
FC, Fenestra cochleae (rotunda)
FV, Fenestra vestibuli (ovalis)
GP, Glass plate
GT, Glass tube
H, Horn
HT, Huschke's teeth (edge of vestibular lip of spiral limbus)
L, Boundary line between outspanning and attached axial zone. (Line of imprint of Huschke's teeth)
M, Manubrium of Malleus
MB, Metal Japan button
O, Auditory ossicles
OZ, Outspanning zone of tectorial membrane
PW, Platinum wire
R, India rubber to make water-tight
RC, Cover for organ reed
RT, Rubber tube
S, Switch
SS, Spiral sulcus
SC, Set screws
SG, Spiral ganglion
SL, Spiral lamina (basilar membrane)
S Lg, Spiral ligament
SM, Signal marker
ST, Scala tympani
SV, Scala vestibuli
TL, Tympanic lip of spiral limbus
TM, Tectorial membrane
TpM, Tympanic membrane
WB, Heavy wooden block
WS, Wooden strip
VM, Vestibular (Reissner's membrane)

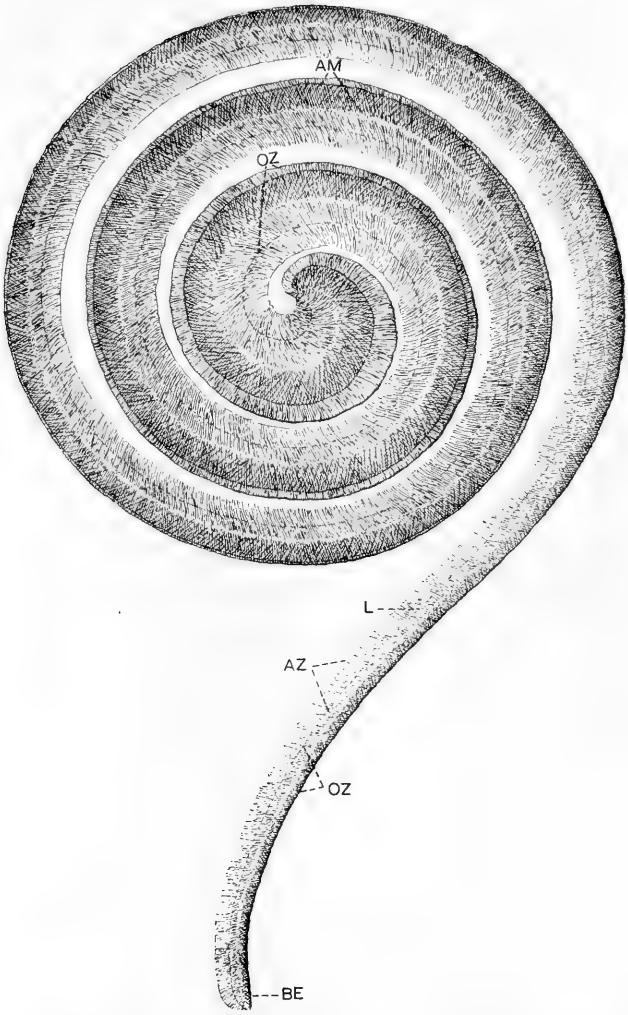

Fig. 1 The basal surface of the tectorial membrane from the left cochlea of the adult hog drawn with the coil opened slightly to obviate the overlapping of its edges existing in its natural coil in the cochlea. Drawing made from several teased out membranes, some whole but slightly damaged in places, others broken in removal and the pieces mounted in their order on the slide.

membrane of the hog (*AZ*, figs. 1 and 2) show that it varies very little in width throughout its entire length. It is slightly narrower in the basal turn. The free edge of the outspanning zone extends well beyond the hair cells in the apical coil but barely over the outermost of the outer hair cells in the basal coil. Section *A* of figure 2 passes some distance from the actual basal end of the cochlea.

Measurements of the thickness of the tectorial membrane, necessarily made from sections of dehydrated and stained specimens in which the membrane was no doubt somewhat shrunken, show that its outspanning zone likewise increases gradually and regularly in thickness in passing from the basal to the apical end and that at the apical end it is 3 times as thick as at the basal end. There is practically no variation in the thickness of the adult basilar membrane; any little variation shown is never regular. The usual variations in the thickness of the membranes are indicated in figure 2, which represents transverse sections of the 1st, 3rd, 5th and 7th half turns in a section of one of the cochleae of the adult hog used for the measurements recorded in the previous paper.

Considering that the usual vibrations are imparted at the basal end by the basis of the stapes, then these vibrations in passing toward the apical end must, according to their vibration frequencies and amplitudes, be damped out in overcoming the inertia of the membrane itself as well as the resistance offered by the walls of the labyrinth and the fluid contained. Thus, it may be argued that the tectorial membrane, varying far more in width and thickness than does the basilar membrane in passing from the basal to the apical end, is adapted for being affected by a far greater variety of vibratory activity, has a much greater possible scale of activity, than the basilar membrane.

Variations in width and thickness are but indications of variations in volume and variations in the volume of the membrane, or the load it carries, are the most important factors to be considered determining the extent to which it may be thrown into vibrations by given vibratory disturbances. Not only must a more voluminous segment absorb a greater amount of energy,

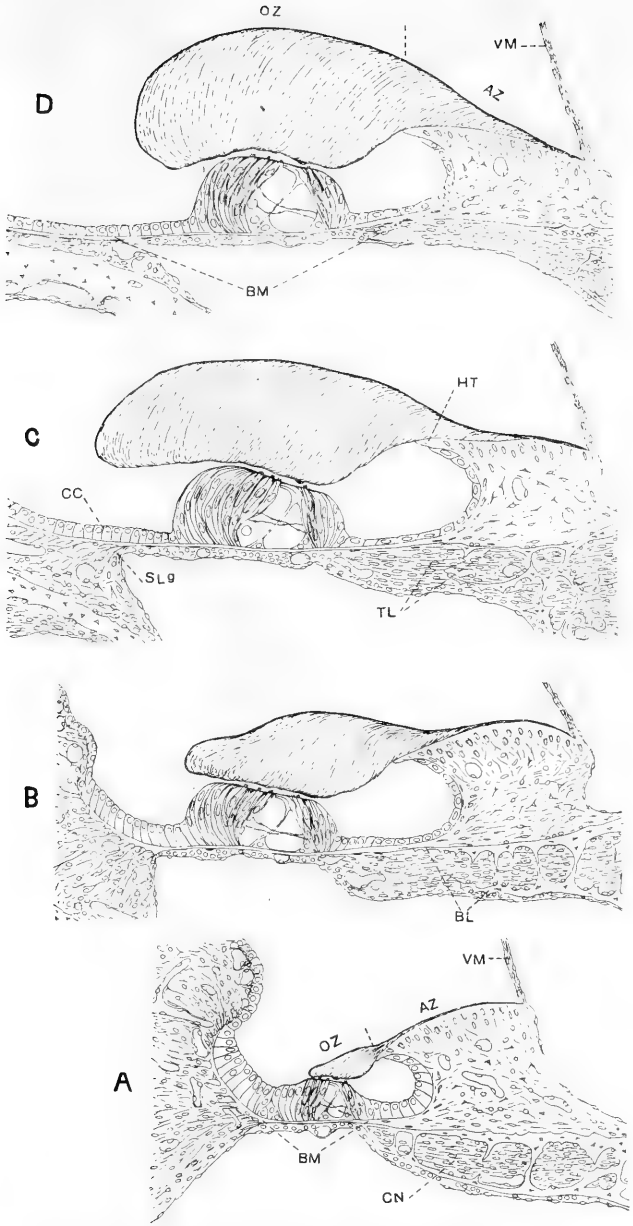

Fig. 2 Transverse sections of the spiral organ and its tectorial membrane taken from one side of a vertical section of the cochlea of an adult hog. *A*, through the 7th half-turn of the coil, represents a section near the basal end of the organ; *B*, *C* and *D* represent respectively sections through the 5th, 3rd and 1st half turns of the coil.

offer greater resistance to being thrown into vibration sufficiently for the necessary impingement upon the hairs of the hair cells, than the less voluminous segment, but it must possess a different natural vibration frequency. The natural vibration frequencies of strips of material, or the vibration frequencies with which they may act in resonance, vary according to their volume or load as well as according to their length. Computations, given in the previous paper, based upon the areas obtained of its transverse sections, suggest that the volume of a given very short length of the basal end of the outspanning zone of the tectorial membrane may increase as much as 40 times in grading to the volume of the same length of the apical end. Calculated in the same way, the volume of the assumed vibratory portion of the basilar membrane at its basal end increases only about 1.4 times in passing to its apical end. The average thickness of the basilar membrane of the adult hog, measured under the spiral tunnel, was found to be 2.8μ. It varies irregularly from 1.8μ to 3.7μ. Usually it is found to be thicker in its basal instead of its apical end.

The length of the tectorial membrane of the adult hog is about 26 mm. It comprises nearly 4 turns.

(7) The shape and attachment of the tectorial membrane may suggest that it is of other use than that of a mere foreign body spanning over the spiral organ for impingement of the hairs against it upon vibration of the basilar membrane. Its outer edge is free in the developed mammalian cochlea; its most voluminous and thus most varying portion is over the organ; it suddenly thins toward its attached zone as though the thinner part of this side of the outspanning zone may serve somewhat as a hinge; and the contour of the basal surface over the organ is always parallel with the apical (peripheral) surface of the organ while other parts of the basal surface are not.

(8) The structure and consistency of the tectorial membrane suggest that it may be especially sensitive to vibrations in the fluid in which it lies and that it may express such disturbances almost wholly in motions vertical to the surface of the spiral organ. It is composed of fibrils imbedded in a gelatin-like matrix

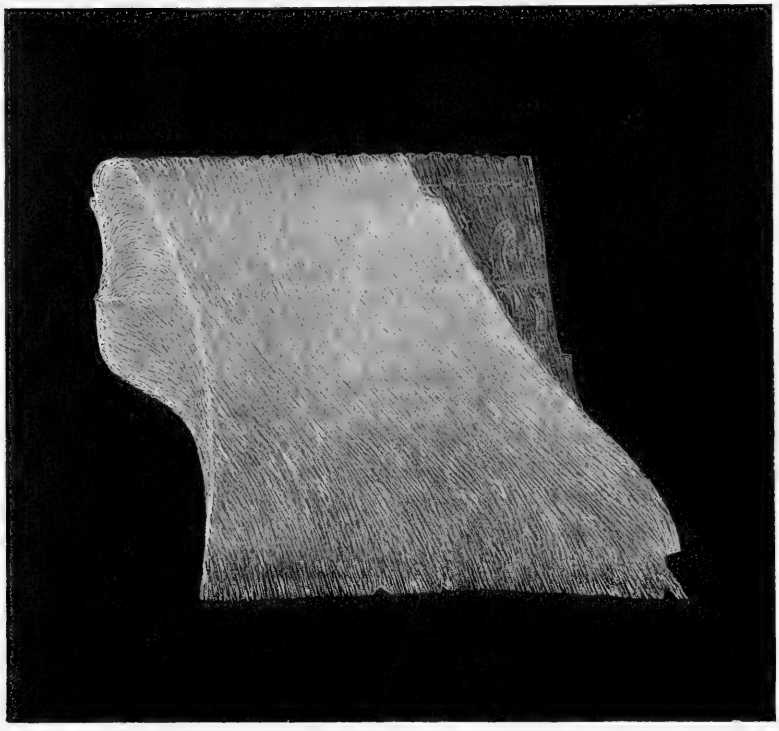

Fig. 3 Piece from the 5th half turn of a teased out tectorial membrane of the pig viewed from its apical surface. Given to show more nearly the actual appearance of the membrane as seen over a black surface and to show the course of its fibrils embedded in the matrix. The right hand side of the figure represents the appearance presented where the membrane had been torn across in teasing. The cut surface shown in the left side was not drawn from this specimen but added onto the figure as suggested in the vertical sections of this turn of the coil. The bottom part of the figure represents the attached axial zone of the membrane. The line showing through the outspanning zone is 'Hensen's stripe.'

and its specific gravity appears practically the same as that of the endolymph in which it lies. Its fibrils are not arranged in it transversely but course in it obliquely, their direction inclined toward the apical end. Those in its apical side are more inclined toward its apical end than those in its basal side, thus intercrossing in the structure of the membrane rendering it less fragile for, in teasing it out even after fixation, it never breaks transversely but always tears apart (fig. 3). In its natural

state, it is most inconceivably flexible. In the cochlea while being teased out and in the water under which it is teased, it becomes tangled or adheres to the needle at the slightest touch with most exasperating readiness. In the fresh state especially, it is extremely sensitive to agitations of the fluid surrounding it. This flexibility is much greater in the transverse directions. While wafted about in the water of the dish it manifests sufficient elasticity for its apical turns to resume their normal coil before it settles upon the bottom of the dish. Its thinner basal turn will not do so. It manifests considerably more elasticity in the direction of its width, or against stress applied parallel to its length. Except where it may become twisted, even its very thin axial zone is never found folded upon itself in mounts of the whole membrane nor in pieces of it. With its thin axial zone adherent upon the vestibular lip of the spiral limbus, this latter elasticity is considered sufficient to hold the outspanning zone in its position in proximity to the hairs of the hair cells whatever the position of the head of the animal, especially since the membrane manifests a specific gravity but very little greater than the fluid surrounding it. It is suggested that this greater elasticity in its width may allow a delicate spring-like action of the thinner axial part of the outspanning zone, and the adjoining part of the attached zone which overlies the projecting edge of Huschke's teeth (*HT*, fig. 2, *C*). Such action may aid in controlling such undulatory motion as the outspanning zone may assume. The form of movement of this zone is suggested to be possibly coarsely represented by that of a flexible ribbon attached along one edge and immersed in water, plus the elasticity and varying proportions manifest in the tectorial membrane.

In constructing the model, the following considerations were in mind.

The spaces within the cochlea are completely filled with lymph in the normal condition. The vibratory motion imparted to this lymph by the basis of the stapes must be of the form shown by experiment to be propagated in a column of water upon percussion at one end of the column, namely in the form of compression

waves, or alternate phases of condensation and rarefaction, moving longitudinally. The pressure under which the endolymph and perilymph exists is probably the same as the general blood pressure of the animal. Should it be greater or less at any time, equilibrium may be regained chiefly by way of the endolymphatic duct and cranio-spinal fluid and by way of the blood vessels of the labyrinth. Considering the spiral lamina (osseous and membranous) the most rigid partition in the cavity of the cochlea, the two scalae, continuous with each other at the helicotrema, may be considered as a column of fluid along which at least the fainter of the vibrations imparted by the percussions of the basis of the stapes may pass apexward in the scala vestibuli and basalward in the scala tympani. Considering the lymph in the labyrinth as incompressible by such force as may be imparted to it by the basis of the stapes, pressure resulting from strong pulsations of the stapes may be compensated or relieved by the membrane over the fenestra cochleae and also, at need, through the endolymphatic duct by way of the ductus reuniens and sacculus. The membranes bounding the latter and the vestibular (Reissner's) membrane are considered so delicate as to allow scarcely appreciable differences in the vibratory movement in the fluid on their two sides, namely, in the perilymph and endolymph.

Sound waves affecting the tympanic membrane are transformed by the ossicles, the amount of work transferred to the stapes being somewhat controlled by the muscles of the middle ear. The basis of the stapes fits accurately into the fenestra vestibuli (ovalis) and is joined to its bony walls in a piston-like joint. Taking into consideration the difference between the area of the tympanic membrane and the area of the basis of the stapes, together with the leverage afforded by the form and arrangement of the ossicles, it has been computed that the actual force of the motions of the tympanic membrane produced by the atmospheric disturbances may be increased thirty times in its transformation and transference to the perilymph, and that on the other hand the amplitude of the atmospheric vibrations may be reduced as much as seventy times. The amount of the increase in force

and the decrease in amplitude depends upon the tensity of the tympanic muscles and also upon the pressure of the air in the tympanic cavity. Thus it may be borne in mind that the vibrations imparted to the lymph of the cochlea by the stapes must *correspond* to the atmospheric waves but *resemble* them only in frequency of vibrations and quality of vibration, for the quality of the vibrations transferred to the endolymph must depend upon the quality or form of the atmospheric vibrations acting upon the tympanic membrane.

The lumen of the scala vestibuli decreases in passing from the base to the apex of the cochlea and that of the scala tympani increases in passing from the apex to the base. The cochlear duct increases in diameter in passing to the apex where it ends blindly. In the adult hog (fig. 4), the space on the apical side of the spiral lamina, namely the scala vestibuli and cochlear duct combined, increases in size in passing from base to apex. The lumen of the scala tympani increases in passing basalward much more than either or both the spaces on the apical side of the spiral lamina, enlarging very greatly in its basal or longest turn. These variations in the lumen of the lymph spaces must result in variations in the resistance offered by their walls to the vibratory motions passing from the base toward the apex of the cochlea. Waves passing apexward in the scala vestibuli are thought to be imparted simultaneously to the cochlear duct, the vestibular membrane between being considered only to control slightly or damp their force as affecting the tectorial membrane. In the cochlea of the adult hog, there appears to occur in the third turn of the coil a slight constriction of the scala vestibuli (fig. 4). This seems present but less apparent in the adult beef but not noticeable in the adult human and rat. No attempt was made to reproduce it in the model, though when present it must make an additional variation in resistance offered to transmission of wave motion toward the apex. The coiled character of the walls of the cochlea must give complicated results of resistance, some of them being no doubt phenomena due to reflections of the vibratory motions. In the model no attempt was made to reproduce the coil of the cochlea because of the

difficulties of construction involved. It was decided that such evidences of vibration as could be obtained with the cochlea represented as a straight canal would be suggestive of actual activities though perhaps simpler.

The two scalae are relatively larger in the human cochlea than in those of the hog, beef and rat.

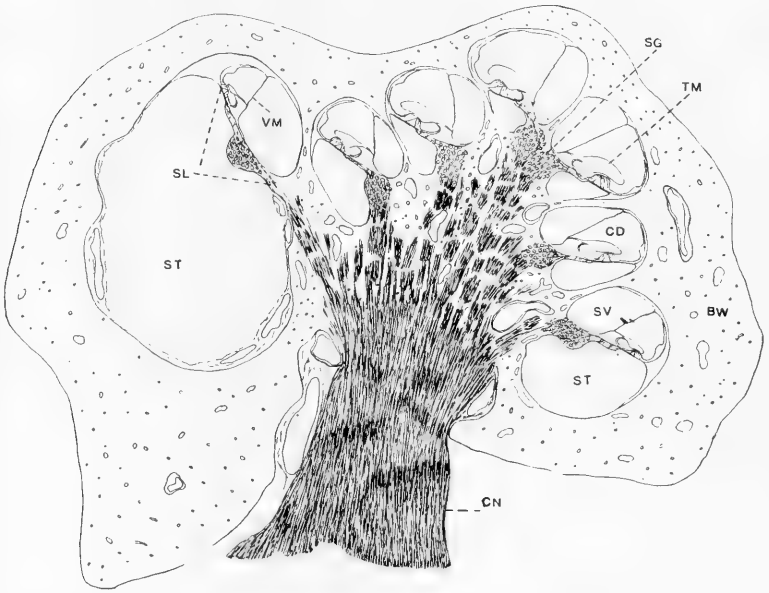

Fig. 4 Outline drawing of a vertical section of the cochlea of the adult hog showing variations in the scalae vestibuli and tampani and in the proportions of the tectorial membrane. For meaning of reference letters, see page 478.

Figure 5, *A*, is a line drawing of the model entire with the accessory parts attached as used in most of the work with it. Figure 7, *A*, shows that end of the model which represents the basal end of the cochlea and figure 6 represents in detail a transverse section of the model.

For the labyrinth of the cochlea, three pieces of wooden board $1\frac{1}{4}$ inches thick and 44 inches long were so cut and joined together as to make a water-tight trough with inside dimensions of $2\frac{1}{4}$ inches square and 42 inches long when the ends, cut from the

same board, had been put on. The joints, cut as shown in figure 6, were coated with a fresh xylol solution of asphaltum immediately before screwing them together. The spiral lamina, with its membranous portion and its vestibular and tympanic lips was represented by a piece of wood $2\frac{1}{4}$ inches wide and trimmed out as indicated by *SL*, figure 5, *C*, and figure 6, the spiral sulcus, *SS*, being attained by cutting a lateral groove in the wood. The lamina was fitted as a water-tight partition along the trough, so slanting that the space above it, representing the scala vestibuli, *SV*, figure 6, increased in passing from the basal toward the apical end and thus the space below it, the scala tympani (*ST*) was smallest at the apex and increased in passing toward the basal end. At the basal end (*BE*, fig. 5) the lamina was fitted water-tight against the inner side of the end of the trough, spanning between the openings in this end representing the fenestra vestibuli above it and the fenestra cochleae below it. The basal end of the model showing these openings, *FV* and *FT*, closed by their respective membranes, is illustrated in figure 7, *A*. At the apical end the lamina was cut about $1\frac{1}{2}$ inches shorter than the length of the trough, thus leaving a helicotrema or continuation here of the scala vestibuli with the scala tympani. The top side of the trough could be closed water-tight by means of a plate of glass (*GP*, fig. 6) cut to fit and placed upon strips of India rubber upon which it was pressed down firmly by means of wooden strips placed along its edges and held so pressed by means of metal Japan buttons (*MB*, fig. 5, *A*, and fig. 6) set at intervals along the wall of the trough.

Even an approximate imitation of the tectorial membrane was found very difficult to attain. A material was desired with low specific gravity and a texture sufficiently tough to hold when trimmed to the shape and proportions of the actual tectorial membrane. Gelatin cast in the desired shape and fixed with formaldehyde was tried but the thin, attached axial zone was not sufficiently resistant for the purposes of the experiments nor would the gelatin hold the wires which were decided to be passed through it at intervals for making electrical contacts. Chamois-skin has a specific gravity but little greater than water

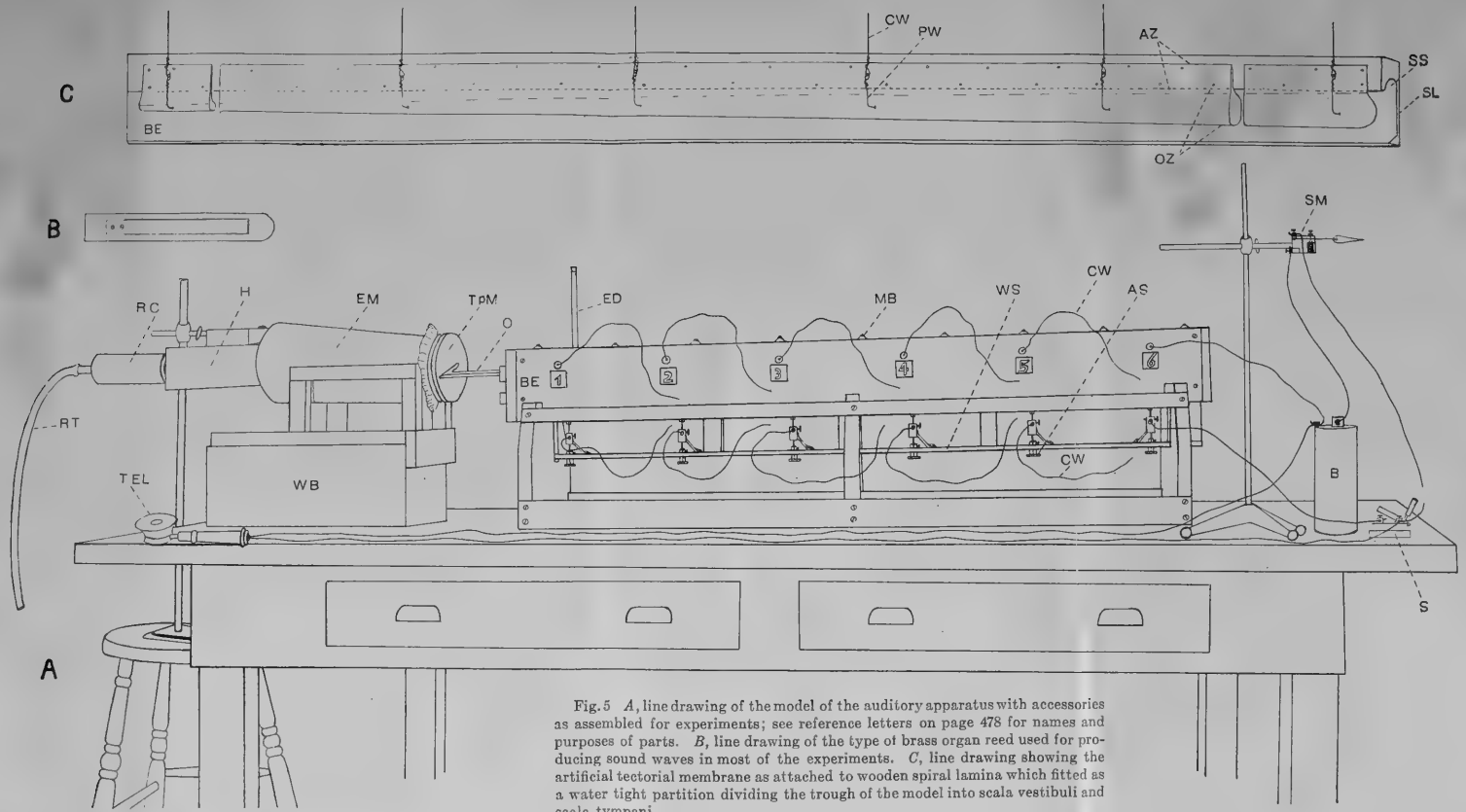

Fig. 5 *A*, line drawing of the model of the auditory apparatus with accessories as assembled for experiments; see reference letters on page 478 for names and purposes of parts. *B*, line drawing of the type of brass organ reed used for producing sound waves in most of the experiments. *C*, line drawing showing the artificial tectorial membrane as attached to wooden spiral lamina which fitted as a water tight partition dividing the trough of the model into scala vestibuli and scala tympani.

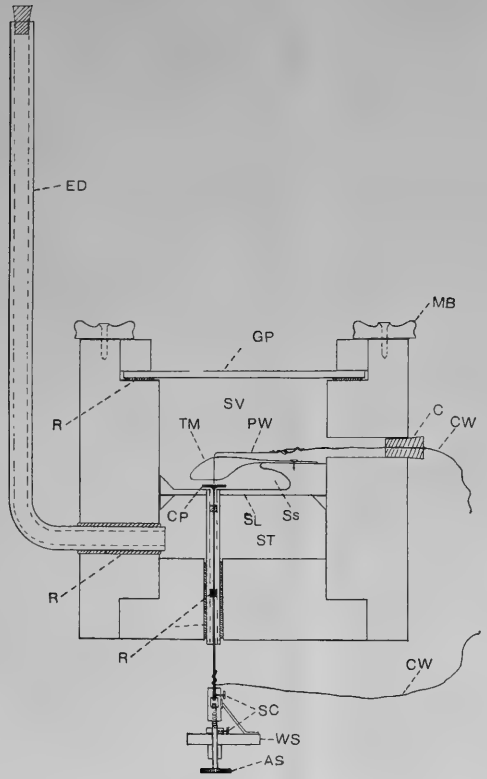

Fig. 6 Line drawing of cross-section of model to show position and relation of parts within; see reference letters on page 478 for names of parts.

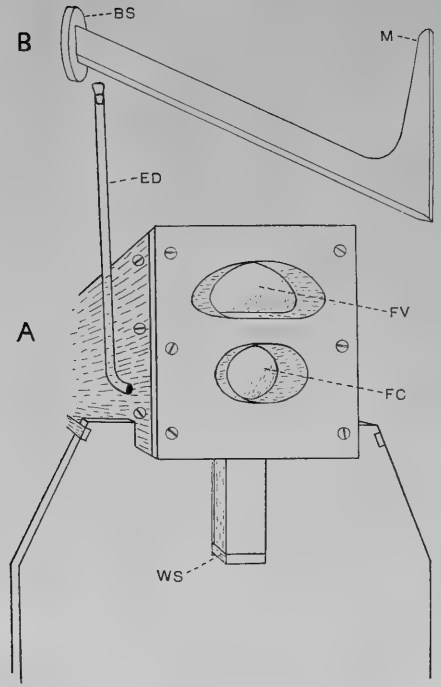

Fig. 7 *A*, line drawing of 'basal end' of model to show the two fenestrae as represented and the position of the 'endolymphatic duct' and the support of the model. *B*, the form finally used to represent the auditory ossicles. *BS*, basis of stapes; *M*, manubrium of Malleus. For other reference letters, see page 478.

when wet and immersed. It could not be obtained thick enough to at all represent the thickness of the tectorial membrane. Some results were obtained in using a strip of chamois skin, one edge of which was turned over to represent the varying width of the outspanning zone of the membrane and stitched down along a line corresponding to the line of the edge of Huschke's teeth, while the remaining width of the strip represented the thin, attached axial zone. The long, tapering sac formed by the turned edge was then filled with a melted 8 per cent solution of gelatin, allowed to set, then fixed in 10 per cent formalin. When immersed, in water this appeared some heavier than was desired but when tacked down by its thin edge along upon that part which represented the vestibular lip and arranged under water in the adjusted model, it gave considerable evidence of vibratory activity. However, after the prolonged soaking necessary to remove the formaldehyde, this contrivance began to show inequalities from beginning disintegration of the gelatin after some time in the water in the model. It of course at no time could be allowed to dry out. Such final results as were obtained came with the use of an imitation but little more satisfactory than the preceding, except that it was more permanent. At a harness factory I found a piece of very thick leather which was said to be elk's hide. It was so tanned as to somewhat resemble the result obtained in chamois skin, being soft and easily wet, and more than usually flexible when wet. Any thick leather tanned by the same process would do as well probably, though there is apparent reason for the impression that the hides of the deer family are more porous than the beef hides. The strip of elk's hide was cut the length and width required and trimmed from the 'flesh side' to the approximate relative proportions of the tectorial membrane. It was kept in frequently changed water for several days to remove any substances soluble in water. During this washing it was often kneaded to increase its flexibility. Figure 5, *C*, gives an idea of it as tacked down by its thin edge (attached axial zone) along upon the vestibular lip of the spiral limbus. In this figure, drawn in larger scale than the model below, it is shown, to sug-

gest its shape in transverse section, as though two small segments had been cut out. When not surrounded by water in the model, its weight caused it to rest limply upon the spiral lamina below, but with the trough filled with water, its thin attached edge was sufficient to support it in a horizontal position. It may be noted that the outspanning zone is trimmed so as to gradually taper and is made much narrower in its basal end (*BE*).

With the spiral lamina fixed so as to divide the trough into the two scalae, pieces of platinum wire (*PW*) were forced through the outspanning zone of the leather tectorial membrane at six intervals. These wires were bent at right angles and joined by interlocking loops along the inner side of the trough with copper wires (*CW*) which passed through corks driven into holes bored in wall of the trough at the corresponding intervals (fig. 6). The platinum and copper wires were joined by looping them together to avoid as much as possible the additional rigidity that would be given the membrane by continuous wires, the loops being thought to act somewhat as hinges during movement of the membrane. That end of each of the platinum wires which passed through the outspanning zone of the membrane was arranged so that it could come in vertical contact with the surface of a small copper plate (*CP*) soldered onto the end of a heavier copper wire which passed through a small glass tube (*GT*, fig. 6) inserted in holes bored vertically through the spiral lamina and the bottom of the trough below. The passage of the glass tube through the bottom was rendered water-tight by forcing it in surrounded by a piece of rubber tubing, and, for the same purpose, the heavier copper wire passed through plugs of rubber which were forced into the glass tube (*R*, fig. 6). Each of the six heavier copper wires (with the copper plates affixed upon their upper ends) was inserted below into a small brass block and held by set screw, and the lower end of each brass block had threaded into it an adjustment screw (*AS*, figs. 5 and 6) by which the copper plate above could be raised and lowered to adjust the contact between it and the end of the platinum wire. The adjustment screws were held fixed by attachment in a wooden strip (*WS*) extending along beneath the model. To the lower

end of each of the heavier wires was attached a piece of copper wire of the same size as that interlooped with the platinum wire above. Thus, a battery interposed between these two copper wires would make possible an electric circuit which could be made and broken at the contacts between the platinum wire and the copper plate by vibrations of the artificial tectorial membrane. Figure 5 shows a battery (*B*) interposed in the circuit of the sixth interval, the circuit of the part of the model representing the apical end of the cochlea.

The object of the similar arrangements of the wires at the 6 intervals was for the experimental determination of the behavior of the different regions of the tectorial membrane toward a given form of vibratory activity imparted to the fluid in the model at its basal end (*BE*). In addition to the necessary use of an ordinary switch (*S*, fig. 5) in the electric circuit, a simple electric signal-marker (*SM*, fig. 5) such as is used in physiological laboratories, and a telephone (*TEL*) were interpolated for use in observing results. A small piece of white paper was glued vertically on the end of the vibrator of the signal-marker that vibrations in it could be more easily seen. The noise of the horn (*H*) usually obscured its clicking to the ear of the observer. The telephone was found to be far more efficient than the signal-marker used. It gave evidence of vibrations of all frequencies whatever induced in the model with sufficient force and amplitude to make and break the circuit, while the signal-marker was found incapable of recording the higher vibration frequencies of which the model was capable. In other words, with the application of sounds of higher vibration frequencies, there could be heard a continuous buzz in the telephone when the vibrator of the signal-marker was practically motionless. An ordinary telegraphic 'sounder' was tried in the earlier stages of the experiments but had to be discarded because found capable of service only with vibrations of lower frequencies.

Before the use of the electric current was suggested, small mirrors were tried with the hope that vibratory activities could be read in the behavior of the small spots of light reflected by them. Thin circular cover-glasses were silvered on one side

and cemented with thin asphalt to very minute cork blocks stuck at intervals along the upper surface of the leather tectorial membrane. The apparatus was placed in direct sunlight with the idea that evidences of vibration could be read in the movements of the spots of light reflected by the mirrors upon the ceiling of the laboratory, or on specially devised screens tried later, the long light-levers magnifying the movements. This plan, however, was found disappointing because of the very indefiniteness of its results. The light reflected from the surfaces of the mirrors had to pass through the water in the apparatus and the glass plate of its top side and was so refracted that the boundaries of the spots upon the screen or ceiling were not sharply defined. Continuous vibration could not be distinguished from intermittent, vibrations of higher frequencies were doubtfully discerned and all results seemed exasperatingly confused. The mirrors gave evidence, however, of the first result obtained, namely, that vibrations may be induced by sound waves in a structure imitating the proportions and position of the actual tectorial membrane.

Distilled water was used to represent the lymph in the cochlea. The actual lymph of course differs in viscosity from distilled water and may be better adapted for the conduction of vibratory motion. But in using the electric current for indicating vibratory activity in the model, the fluid used had to be as nearly as possible a non-conductor of electricity. An oil with a low boiling point would of course have been most efficient as a non-conductor, but so far oil has not been used because of the difficulty with which its leakage could be prevented with a construction of the apparatus as here employed and because it was considered that a difference in viscosity between an oil and the lymph would be greater than that of water. It is realized that the ordinarily used distilled water is not absolutely a non-conductor of electricity. Its conductivity is less than tap water and much less than the animal lymph. That the distilled water in use in the model might be as pure as possible, the trough was soaked in distilled water, frequently changed, for about two weeks before the experiments using the electric current began. Further,

the water was replaced with fresh at the beginning of each series of trials with the model. Thus since the work extended intermittently through a number of months, it is thought that the water used toward the last at least was fairly free of extracts from the wood and other material used in construction.

Instead of the copper plates employed for contact with the ends of the platinum wires, very small copper cups containing a drop of mercury were at first tried on the ends of the pieces of heavier copper wire. It was found, however, that the movements and changes in shape of the drops of mercury due to changes in surface tension when charged and uncharged seemed to render the contacts less definite and delicate and adjustment of the distance through which contact was made much more difficult than with the use of the copper plates. The plates were kept burnished.

The trough was always filled completely with water. The bent glass tube, *ED*, figures 5, 6 and 7, was put in the model to represent the endolymphatic duct of the labyrinth. It was considered a precaution for compensation in any changes in pressure produced by vibratory pulsations and by changes in temperature. By means of this tube the filling with water could be completed after the glass plate had been put on and pressed down watertight. Remaining air bubbles were gotten rid of through a small hole drilled in the glass plate at its apical end, the hole being closed with a plug of rubber.

The end of the model representing the basal end of the cochlea is shown in figure 7, *A*. The fenestrae were represented by cutting large holes through the wooden end piece and closing these holes with thin skin. To accomplish this cuffs of sheet copper were made to fit the holes and over one end of each of these was stretched and tied a piece of thin raw-hide of the sheep known as 'drum-head skin.' The end of the cuff carrying the skin was driven into the hole till the skin, or membrane, became flush with the inner surface of the trough. Driving in thus made the membranes tense. Then melted wax was poured around the cuffs making their insertion water-tight. The fenestra vestibuli (*FV*, fig. 7) was made oval in shape and larger than the

fenestra cochleae (*FC*) below it. These differences were probably unnecessary.

The external auditory meatus (*EM*, fig. 5, *A*) was represented by a wide funnel made from block-tin. A wooden collar was fitted upon the smaller end of this funnel that the tympanic membrane could be stretched over it and secured more safely and easily. For the tympanic membrane (*TpM*), a sheet of that thin preparation of white fibrous connective tissue known as 'Gold-beater's skin' was wet and stretched over the wooden collar of the meatus and bound firmly about it with both twine and rubber bands. The membrane became tense upon drying. The collar was so trimmed that only its periphery was in contact with the membrane, thus making the area of the vibratory part of the membrane greater than the area of the circle formed by the small end of the tin funnel. The area of this membrane was about 30 times the area of the skin closing the opening representing the fenestra vestibuli in the basal end of the model. A tympanic membrane represented by sheet-rubber was found useless in that it absorbed rather than transmitted its vibrations to the "ossicle" pressed against it.

The ossicles of the middle ear were finally represented by a piece of wood trimmed as shown in figure 7, *B*, and figure 5, *A* (*O*), with an oval shaped piece attached to one end to serve as the basis of the stapes (*BS*, fig. 7). At first pieces of hard wood were cut and joined together to imitate the three ossicles with their joints and lever arrangement and with rubber bands for the tensor tympani and stapedius muscles. This arrangement, however, was found difficult to keep in place during the experiments and it absorbed the vibrations of the tympanic membrane considerably more than the single piece shaped as shown. During the experiments, a straight single piece was tried placed perpendicular to the center of the tympanic membrane and to the membrane closing the fenestra vestibuli. This piece was of the same thickness as that illustrated in the figures and its "basis of the stapes" was similar, but its end representing the manubrium of the malleus was fan-shaped and was used extending in both directions across the center of the tympanic membrane

instead of having a single arm extending from the periphery of the membrane to its center as shown in figure 5. The planes of the two ends were cut at right angles to its long axis instead of in the form shown in figure 7. It was of interest to find that the vibrations of the tympanic membrane were transferred to the fluid in the model better and more definitely by the arrangement of a single arm extending from the periphery to the middle of the tympanic membrane, similar to the arrangement of the manubrium of the malleus in the mammalian ear, than by the straight piece pressing across the center of the membrane and extending at right angles to both its surface and that of the fenestra vestibuli. The form found most efficient was somewhat similar to the columella of birds.

Sound producers of various types were tried, including pipes giving different tones, trumpets (a cornet and a trombone), horns of different kinds, and finally an assortment of ordinary brass organ reeds. Organ reeds were used chiefly in the work for two reasons: first, instruments producing sound waves by means of vibrating tongues seemed to act upon the apparatus more definitely than any of those tried which gave in other ways the alternate phases of condensation and rarefaction, and second, the vibration frequency of each of the organ reeds was known, the name of the note it produced being stamped upon it. With the other instruments, the vibration frequency of a given sound had to be previously determined by means of a standard tuning fork and the kymograph, used with a writing arm registering the vibrations of the tympanic membrane. The form of the brass organ reed used is shown in figure 5, *B*. In order that its vibrations could be conducted directly into the external auditory meatus, a wooden box or horn (*H*, fig. 5, *A*) was constructed into the end of which fitted a removable cover for the reed (*RC*). This cover was so constructed that reeds could be interchanged at will. Air was blown through the reed by way of the rubber tube (*RT*). The whole sound producing apparatus was held in place by a heavy, ordinary filter stand and no part of it was allowed to touch the model nor the table upon which the model sat. The end of the horn was inserted into the large end of the

external meatus but could not be allowed to touch it during the experiments. Trumpets such as the cornet, held in the hand, were merely directed into the meatus.

The external meatus with the tympanic membrane had to be mounted upon a heavy wooden block (*WB*, fig. 5, *A*) and even then it was found necessary to brace it against a cleat fastened upon the table, for certain vibrations of low frequency would cause it to move gradually away from the ossicle pressed upon the membrane.

The adjustment of the distance between the copper plates and the ends of the platinum wires during the experiments was made to the point at which the least movement of either plate or wire would make or break the electric current. Usually a position just at the brink of the make of the current was employed. This could be determined by observing the vibrator of the signal-marker while manipulating the adjustment screws, the battery being interposed.

RESULTS

It was realized that an apparatus so infinitely larger, heavier and coarser in construction than the mammalian auditory apparatus could not be expected to give many results trustworthily representing the behavior of the actual apparatus. Many of the apparent phenomena with the model were so confused and seemed so complex that no attempt will be made to describe them on the ground that they were more the result of imperfections in the model than representing behaviors of the normal apparatus. Interpretations of only the few simpler results will be undertaken and these interpretations will be limited.

I have described the construction of the model in considerable detail with the hope that its repetition with the possible improvements, may be suggested to other investigators and that the experiments may be repeated and extended.

Of the results given by the model indicating the action of the tectorial membrane, the following are considered most directly suggestive.

(1) A structure imitating the tectorial membrane in shape, proportions and environment can be thrown into vibrations by sound waves so applied that their energy is transferred to the fluid in which the structure lies and in a way similar to that employed in the actual ear.

(2) With sounds of low vibration frequency, at least, the tectorial membrane is thrown into vibrations of the same frequency as those of the sound giving rise to them. This fact was determined by allowing the vibrator of the signal-marker to write upon the drum of a kymograph, on which drum had been already traced the vibrations of a standard tuning fork. With the sound waves of higher vibration frequencies by which the model was capable of being acted upon, the signal marker either did not vibrate at all or acted irregularly.

(3) With the distance between the copper plates and ends of the platinum wires adjusted, a heavy step on the floor of the room, a slight blow upon the table or a hand laid upon the apparatus would produce vibrations of the artificial tectorial membrane sufficient to cause a series of makes and breaks of the current. This may be advanced as illustrating the possibility by which sensations of sound are aroused by way of other parts of the body than the tympanic membrane, stimuli explained as conveyed to the bony labyrinth, and thus to the structures within it, directly by way of the skull bones, teeth, etc. Sensations received in this way are among those of lower pitch.

(4) The model showed slightly less sensitiveness to vibrations imparted to the membrane covering the fenestra cochleae (rodunda) than to the same vibrations applied at the fenestra vestibuli. This slight difference is explained as due to damping out effects or absorbtion of the vibrations by the fluid in the scala tympani before they pass directly into the scala vestibuli by way of the helicotrema at the apex of the cochlea.

(5) Jarring of the model and notes of low vibration frequency applied to the tympanic membrane by way of the horn, were found to throw the entire tectorial membrane into vibration. This was determined by interposing battery, signal-marker and telephone at each of the regions of the membrane from 1 to 6,

(positions indicated in figure 5). It was found that all notes with vibration frequencies below that of the note *a* (concert pitch, 220 vibrations per second) resulted in constant vibration of all regions of the tectorial membrane sufficiently strong to make and break the current. This suggests that within the lower range of the natural auditory scale, all sound waves affect the entire spiral organ.

(6) The extent of the tectorial membrane that could be thrown into vibration by certain notes depended upon the intensity or amplitude of vibration with which the note was sounded. For example, the note *g* (196 vibrations per second), the next whole note below *a*, when sounded faintly into the external meatus, failed to result in vibrations of the apical end (region 6) of the membrane sufficient to make and break the current, while if sounded forcibly, this note caused vibrations in all regions. All sounds and notes with vibration frequencies below that of *g* resulted in vibration of the entire membrane, regardless of the amplitude that could be employed. Ewald ('99) applied a modification of the telephone theory to the basilar membrane. He constructed an arrangement in which narrow strips of thin sheet-rubber were adjusted under water and observed with the microscope the behavior of these strips when sound vibrations were imparted to the water. His narrowest strips was 0.5 mm. broad. From the behavior of these strips he suggested that the basilar membrane vibrates throughout its entire length in response to every note applied. The above observation suggesting the action of the tectorial membrane, and the one following, do not fully support Ewald's conclusion for the basilar membrane as based upon the action of his rubber bands. His bands did not vary in thickness, or load carried, along their length. Meyer ('98) constructed an apparatus by which he applied the telephone theory to the basilar membrane. His apparatus was designed to indicate the results of varying amplitudes of vibration rather than varying pitch, using electrical contact and electric lamps as indicators. No attempt was made to imitate the basilar membrane or any other structures of the auditory apparatus. His results led him to conclude that the greater the amplitude of the vibration,

the greater the extent of the basilar membrane involved: that with a faint back-and-forth movement of the stapes only the basal or beginning part of the basilar membrane is set in motion; the greater the back-and-forth movement of the stapes, the further toward the apex of the cochlea will the motion of the basilar membrane extend. This suggestion for the basilar membrane agrees with that offered above as to the behavior of the tectorial membrane, namely, with a note of a given pitch or vibration frequency, a greater amplitude will throw into appreciable vibration a greater extent of the tectorial membrane than a lower amplitude, the extent beginning with the narrower basal end of the membrane and ascending, with the amplitude, along the gradually increasing membrane toward the apex of the cochlea. This action, shown by the model, is one to be expected, since the vibratory motion, imparted at the basal end of the cochlea, necessarily must be gradually absorbed in overcoming the resistance offered by the walls of the cochlea, the inertia of the fluid and the increasing inertia offered by the increasing bulk of the tectorial membrane itself in passing toward the apex of the cochlea. The variations in the diameter of the two scalae in passing from base to apex may result in additional decrease in the efficiency of the vibrations imparted. It is suggested that in the functional scale of any auditory apparatus there must be notes sounded whose amplitudes are gradually decreased by the resistance till the vibrations are completely damped out.

(7) It was further indicated by the model that frequency of vibration or pitch of a sound, considerably more than its amplitudes of vibration, determines the extent or length of the tectorial membrane that may be thrown into vibration. The note *a* (220 vibrations per second) produced with whatever amplitude, would not produce vibrations in the apical end of the tectorial membrane (region 6) sufficient to make and break the current, while it did produce vibrations in region 5. Note *b* (247 vibrations per second), the next whole note above *a*, also produced vibrations in region 5 when sounded with highest amplitude, but none in region 6. Both *a* and *b* produced vibrations in regions

1, 2, 3 and 4. The note *c* ('middle C,' 261 vibrations per second) produced vibrations in regions 1 and 2 but none in the other regions, regardless of amplitude. Judging from the preceding, this note was expected to give vibrations in region 3 at least and I was unable to determine a reason for its not doing so. Also the notes *d* and *e* (294 and 329 vibrations per second respectively) gave vibrations in region 1 but not in the other regions, and the note *f* (349 vibrations per second) at greatest amplitude gave in region 1 only a slight hum in the telephone. Notes with vibration frequencies above that of *f* produced no evidences of vibration in the model. The note *f* and the three or four notes above *f* that were tried produced appreciable vibrations in the artificial tympanic membrane.

Beginning with the note *b*, the signal-marker ceased to be of service with the higher vibration frequencies. At times it would start vibrating at the first puff of the air through the reed and then become silent though a continuous hum might be heard in the telephone. The fact that notes *c* and *d*, *e* and *f* could not be made to give more graded results must have been due to imperfections in the construction of the model. Perhaps the intervals of the membrane arranged for were not short enough. However, such results as were obtained seem to indicate a relation between the vibration frequency of sounds and the extents of the membrane thrown into vibration by them. In explanation of this may be mentioned the familiar fact that of sounds given the same amplitude, those of lower vibration frequency are damped out less quickly in their transmission through a medium, or will travel farther, than those of higher frequency. For example, fog-horns of low vibration frequency or pitch are known to be more efficient for warnings at a distance. Also in atmosphere, sounds of high vibration frequency are not only sooner damped out in overcoming resistance than those of low frequency, but their speed of transmission decreases more rapidly as they become fainter. This damping out must occur much more quickly in transmission through a medium like the endolymph than in atmosphere and in overcoming the resistance

offered by the walls of the very small canals of the cochlea and the structures contained within them.

As noted above, the size and volume of the tectorial membrane so increases in passing from the basal to the apical end that the volume or load carried by a given short length of the apical end is about 40 times that carried by the same length of the basal end. This increase seems to occur uniformly throughout, or perhaps a little less rapidly through the basal turn of the coil. It is noted that these variations in the proportions of the tectorial membrane are far greater than can be claimed for any other structures of the cochlea, especially the basilar membrane. Increase of volume or the load carried by a structure means increase of its inertia. The much thinner and narrower basal end of the tectorial membrane extends in the region of the fenestra vestibuli (ovalis), or the region at which the vibrations are imparted to the endolymph by the basis of the stapes. Obviously, a thin, narrow strip offers less resistance to agitation than a thicker, broader strip of the same material. Thus, the thin, narrow basal end of the outspanning or vibratory zone of the tectorial membrane may be thrown into vibration by notes of such high vibration frequencies as would be wholly damped out in overcoming the inertia alone of a more voluminous portion of the membrane. The volume of the outspanning zone of the membrane increases with its distance away from the basis of the stapes and, therefore, among the appreciable sounds of higher vibration frequencies, those of different frequencies must be capable of throwing into vibration different lengths of the membrane, beginning at its basal end. The resistance offered by the endolymph and walls of the labyrinth, as well as the inertia of the membrane itself, contribute to their being damped out at their respective distances toward the apex of the cochlea. Since vibrations of higher frequency are damped out more quickly in transmission through a medium, there may be sounds whose vibration frequencies as imparted to the endolymph are such that they agitate the basal end of the membrane alone sufficiently for the required impingement against the hairs of the hair cells. And, of course, there must be vibration frequencies of similar

character but so high as to be damped out by the other structures of the auditory apparatus to an extent rendering them unable to produce effective stimulation of any of the hair cells at all. It is stated that the auditory apparatuses of certain individuals are capable of appreciating higher pitches (vibration frequencies) than others and that certain species of animals have an auditory range including high pitches not heard at all by other species. It is suggested by the model that the lower vibration frequencies may affect all auditory apparatuses. The range of the model extended no higher than the note *f* above 'middle *C*. Considering its relatively enormous size and coarseness of constuction, it is interesting that it had a range so high, even though the end of the horn was inserted directly into the external meatus.

The quality of a note is supposed to depend upon the form of the wave-motion producing it. The peculiar form of a vibration must be represented in its transmission to the endolymph as well as its amplitude and frequency, and the consequent form of the undulations of the tectorial membrane while striking the hairs of the hair cells must give rise to interpretations of quality by the central nervous system. Sensations of pitch must be determined by the number of stimuli applied in a unit of time upon a unit area of sensory surface, that is, by frequency of vibration. Also, among the higher frequencies, it may depend somewhat upon the extent of the sensory surface involved by a given sound: the greater the extent, the lower the note. Intensity of sensation must depend upon the force of the impingement of the hairs of the hair cells (amplitude) and also upon the extent of the sensory surface involved. Of two sounds of the same vibration frequency, that of the greater intensity involves the greater extent of the tectorial membrane. It is suggested that sensations of intensity and quality of sound are produced in ways entirely comparable with those recognized for the other organs of special sense, namely, in all the interpretation of the sensations is correlated with the number, quality and intensity of the stimuli and the area of sensory surface involved.

(8) As to the question of resonance, very little could be determined with the model. The suggestion that, within the

higher part of the range of the organ, notes of varying vibration frequencies involve correspondingly varying extents of the tectorial membrane and that sensations of pitch are determined by the number of stmuli applied in a unit of time to a unit area of hair cells might be so elaborated as to satisfy some of the simpler requirements of the idea that analysis of sound is accomplished in the cochlea or peripheral part of the auditory apparatus.

It is known that the natural vibration periods of strips of material vary according to their proportions: that a thin, narrow strip has a higher natural vibration frequency than a thick, broad strip of the same length, and that a shorter strip has a higher natural frequency than a longer one of the same thickness and width. A load added to a strip lowers its natural vibration frequency. Of the number of musical instruments constructed on these principles, the xylophone may be preferably mentioned here. However, there is no available information as to what would be the behavior of a series of such varying strips joined continuous with each other end to end in their natural sequence, and especially if of a material soft and flexible as that of the tectorial membrane. The tectorial membrane is not only soft and very flexible but its vibrating part or outspanning zone is attached all along one side, and thus its vibratory behavior must necessarily be different from that of a metal or hard-wood strip shaped in the same proportions and lying free as to both its sides. Very possibly, being flexible and attached, the natural vibration frequency of each segment comprising the whole would be modified by that of the segments adjacent to it, that is, the natural frequencies of adjacent regions would overlap into each other as it were, and yet a given region of the whole membrane would have a natural frequency differing from that of another region. If such is possible, then a region would, in its center at least, have a tendency to vibrate in resonance with imparted vibration frequencies corresponding to its own natural frequency. Thus it would be possible that, when the apparatus is subjected to a given sound, a given region of the membrane may vibrate with sufficient excursion to impinge upon the hairs of the hair

cells, while regions both above and below it may not so vibrate. In other words, the regions immediately adjacent to that vibrating in resonance, having nearly the same natural periods, might be agitated but to a degree less effective and decreasing as the distance from the region most affected increases. Or, again, since loading a vibrating body lowers its natural period or pitch, if the load be uniformly distributed, the vibration frequency of all its components will be lowered, but if the load be placed at one end of the vibrating body then one of the complications resulting would be a lowering of the natural vibration frequency of that end more than the other. If the tectorial membrane may be considered as a vibrating body with a load gradually increasing till the load carried by its apical end becomes 40 times that of its basal end, then it may at least be assumed that the natural period of its different regions must be lowered as the apical end is approached and sound waves imparted to the endolymph may act upon it accordingly. Several waves, of course, may be transmitted in the same direction simultaneously through a medium.

Results with the model, however, show that sounds of lower vibration frequency throw the entire tectorial membrane into effective vibration.

It may be mentioned that in line with the above a possible suggestion of resonance with the higher sounds was offered by the model. In three cases a note seemed to produce stronger and more evenly continuous vibration of one region of the artificial membrane than in any other of the six regions. For example, the note *a* (220 vibrations per second), while causing vibrations in all regions except region 6, seemed to cause a more definite and more evenly continuous buzz of the signal-marker and telephone at region 4 than in other regions. In region 2, the indication in both signal-marker and telephone was more or less intermittent in contrast, regardless of the amplitude given the note. The note *d*, above *a*, gave the same in region 1, and, less to be expected, the note *g* below *a* gave the same in region 4, the same region as that in which the note *a* gave it. The fact that *a* and *g* gave the evidence in the same region leads to the

conclusion that all these suggestions might have been due to lack of uniformity in construction of the model or faults in arrangement of parts. Certainly the model showed no evidence of resonance in the definite way advanced by the theory of Helmholtz as applied to an assumed structure of the basilar membrane. Altogether, with the suggestions so far obtained, the question may be revived as to whether analysis of sound is not accomplished in the interpretations of the stimuli by the central nervous organ. It may be a question whether any actual analysis is accomplished by the cochlea at all other than to such extent as can be possible in the indications that, dependent upon the vibration frequencies and amplitudes of the sound waves applied, varying lengths of the tectorial membrane are thrown into efficient vibration, the lengths beginning at the basal end. Instead of its different regions manifesting selective resonance, the membrane may lie limply passive, agitated by given wave motions only in so far as the resistance offered by itself and the perilymph and endolymph will permit, and, just as in the sense of touch for example, differences in qualities, intensities and frequencies of the stimuli are interpreted by the central nervous system as qualities, intensities and pitch of sounds. A number of different stimuli applied to the unit area of the skin give rise, within the peculiar possibilities of skin innervation, to an interpretation different from one stimulus applied to that area, and stimuli involving varying extents of skin, above certain limits, are interpreted differently. The same may be urged in general for the other sense organs, within the variations of their special differentiation. Gray ('00) in applying a modification of the resonance theory to the basilar membrane, notes, among others, that a mixture of tones of closely approximate vibration frequencies cannot be analysed by the auditory apparatus. If of varying amplitude or intensity, that of the maximum intensity in the mixture alone is perceived. Such a mixture of notes must all involve so nearly the same extent of the vibratory mechanism and hair cells and with so nearly the same frequency of impingement upon the unit area that the different components are not separately interpreted, just as when two or more points

applied to the skin close enough together and simultaneously are interpreted as a single stimulus (the sum of the several stimuli), but if one is given greater intensity, that one is appreciated by the brain, while the other images produced by the mixture may be neglected. Impulses mediated by given sense organs are distributed to given areas of the cerebral cortex and differences in the development of certain of these cortical areas are claimed in cases of absence or extraordinary development of the function of the corresponding sense organs. Auerbach ('11) found in his study of the brain of Bernhard Crossman, a famous musician whose specialty was the violincello, that, compared with the average type, the surface of the superior temporal gyrus of both sides was considerably greater than normal, with the middle temporal gryus taking part in the enlargement to a slight extent. With proficient musicians, the chief of the differentiations in the function of the auditory apparatus are the power of analysis and tone memory. The supra-marginal gyrus of Crossmann's brain was described as being also extraordinarily developed. Helmholtz himself concluded that the power of analysis depends largely upon 'attention.'

Whether a note produced vibrations in the artificial tympanic membrane could be easily determined by holding the finger in very delicate contact with the membrane while the note was being sounded. With one of the lower notes used this membrane seemed to manifest a sort of resonance, vibrating with greater excursion than with other notes sounded with approximately the same amplitude. The membrane vibrated apparently as a whole. When the 'ossicle' was removed, its vibrations were even audible. The vibrating diaphragms of the telephone must exercise some form of resonance for it is common experience that certain voices and certain tones are transmitted much more loudly and definitely than others.

(9) With the notes by which the model was capable of being affected, two further phenomena may be mentioned. (1) Occasionally a note would produce a constant buzz in both signal-marker and telephone in one region of the artificial tectorial membrane while in another region the buzzing would manifest

arising and falling in intensity and, more rarely, the result was actually intermittent while the sounding of the note by the horn was continuous. (2) With some of the low notes employed, while a low intensity would result in a continuous buzzing at all regions, if the intensity was greatly increased, certain regions of the membrane seemed to 'buckle' or be lifted away from contact of the platinum wire with the copper plate. Or, in some other cases the current remained made in a region, indicating that the membrane in the region had been forced in closer contact than the adjustment made for a make and break of the current. Ewald and Jäderholm ('06), experimenting with an apparatus producing sounds of known vibration frequency, formulated the conclusion that "all intermittent noises give intermittent tones." This conclusion I think is necessarily correct, but also, could the construction of the model be trusted, the above would suggest that there may be evenly continuous forms of vibration (noises) which can give sensations of intermittent tones. Obviously also the manifestations of rise and fall of intensity or throbs in the buzzing of the telephone suggest the phenomena described as beats and perhaps overtones as well. As is known, the vibrations of the tongue of the organ reed do not give pure tones.

Tinnitus aurium could be produced by a region of the tectorial membrane being so forced down upon the hairs of the hair cells as to remain adherent to them temporarily, or for a long period as sometimes happens, after very violent auditory stimulation. In origin, the tectorial membrane is directly analogous to the otolithic membranes. These latter are normally loaded with calcarious deposits. Under pathological conditions and with advancing age the tectorial membrane may become so loaded, as are other tissues of the body. A light load or a load in a part of the membrane, increasing its inerita, might give rise to 'tone islands' among the higher pitches; a heavier load equally distributed could contribute to the 'failing ears' of old age.

It is not intended here to claim that the tectorial membrane is the only possibly vibratory structure in the cochlea, but, that it is the most adapted in every way and must be the chief vibra-

tory structure contained. In my paper of 1915 attention was called to the fact that the spiral organ (of Corti) or the load carried by the basilar membrane increases appreciably in both width and thickness in passing from the base to the apex of the cochlea. This increase in proportions is, however, by no means so great as is the case with the tectorial membrane. The possibility that wave energy imparted to the fluid in the cochlea may also result in some vibration of the membranous spiral lamina, or basilar membrane, as a whole is not denied. While it is a flat tendon stretched with both edges attached instead of having a free edge and lying limply passive, the fact that it is a little wider at its apical end (averaging 1.4 times) and that its chief load, the spiral organ, increases nearly two times in size in passing to the apical end seem suggestive of an adaptation for vibratory activity. But it must offer greater resistance to undulatory movement than the tectorial membrane, being far less flexible and of different proportions as well as of different structure and position. It is possible that vibrations of low frequency and high amplitude may throw both the tectorial and the basilar membrane into vibration, while the lesser amplitudes may affect the tectorial membrane only. If the two were equally affected by given vibrations, then their undulations would be parallel and, the two remaining the same distance apart, the required stimulation of the hair cells would not occur But, if the position and greater flexibility of the tectorial membrane allow in it vibrations of greater excursion than in the basilar membrane, then the hair cells may be stimulated and with the frequency of the wave motion imparted. It might be advanced that in case of very violent stimulation, the undulation of both membranes is a necessary and economical arrangement, for in such cases injuriously forcible impingement of the tectorial membrane, which alone stimulates the hair cells, may be avoided while yet the tectorial membrane is kept within working distance of the hairs. The increasing load (spiral organ) upon the basilar membrane must act as suggested for that of the tectorial membrane, making it possible, by its increasing resistance with the increasing distance from the basis of the stapes, that varying extents of it may be affected according to the intensity and vibration

frequency of the wave energy transferred to the surrounding fluid. In the waves of less amplitude and higher frequencies, vibrations must be reached which are unable to agitate the lamina at all but may agitate the tectorial membrane alone, and then those which agitate decreasing extents of this and with decreasing excursion or amplitudes of vibration up to the functional upper limit of the auditory apparatus.

CONCLUSIONS

Discussion of most of the usually described physiological phenomena of hearing is not undertaken in this paper. It is felt that the model, as compared with the actual auditory apparaatus, is so crudely constructed and so gross in size that attempts, based upon its behavior, to explain many of the seemingly complex phenomena would be not only difficult but unprofitable at the present stage of the study. The discussion is confined to the more evident and more probably trustworthy suggestions offered by the behavior of the model:

(1) That the tectorial membrane is a vibratory structure and from its consistency, shape, proportions and position, is the chief vibratory structure in the cochlea.

(2) That it may vibrate in such a way that a modification of the telephone theory of hearing may be applied to it.

(3) That sound waves of low vibration frequency produce vibrations in the entire tectorial membrane regardless of amplitude while those of higher frequency produce vibrations in varying extents of it, depending upon their amplitude.

(4) That among the sound waves of the higher vibration frequencies capable of being appreciated, the extent or length of the tectorial membrane in which efficient vibrations may be induced depends more upon the vibration frequency or pitch than upon the amplitude of the sound waves. The higher frequencies being damped out more readily than the lower, varying extents of the membrane are affected according to the vibration frequencies of the waves applied, the highest frequencies affecting the thin, basal end of the membrane alone.

(5) Some evidences of what may be considered resonance in the tectorial membrane were indicated but were thought question-

ably trustworthy as such. No resonance of the form demanded by the Helmholtz theory is possible. It is suggested that no resonance or analysis of sound is accomplished by the cochlea in itself other than such as may be possible in the evidences that, beginning with its basal end, different extents of the tectorial membrane may be thrown into vibration by different sound waves, depending upon the frequency and amplitude of vibration.

(6) It is admitted that waves of low frequency and high amplitude may induce vibrations also in the less flexible and otherwise less adapted basilar membrane as a whole. Attention is called to the fact that its chief load, the spiral organ, increases in size in passing from the base to the apex of the cochlea and that this suggests a vibratory behavior possible in it similar to but not so comprehensive as that claimed for the tectorial membrane.

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DEVELOPMENT OF THE BLOOD VESSELS OF THE MAMMARY GLAND IN THE RABBIT

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SIX FIGURES

The mammary glands receive their blood supply from the superficial blood vessels of the thoraco-abdominal walls. The chief arteries distributing blood to these superficial vessels in the rabbit are the thoraco-epigastric (external mammary), arising from the subclavian, and the superficial epigastric arising from the femoral. The internal mammary furnishes several small branches which pass between the ribs to the skin and a large epigastric branch. A small branch from the hypogastric artery passes to the region of the inguinal gland. There is a fairly constant branch from the lateral thoracic to the thoraco-epigastric. The intercostals furnish little of the blood supply. From the main arterial trunks branches are freely distributed. Anastomoses are frequent between the branches.

The larger veins as a rule parallel the arteries. The thoraco-epigastric vein is an exception in that it enters the axillary rather than the subclavian vein. The smaller veins and arteries are less apt than the main trunks to parallel one another. The terminal branches interdigitate in such a way as to insure an even distribution of blood in the capillaries.

The main branches lie in the subcutis superficial to the cutaneous muscle. From these vessels rami pass, on the one hand, toward the skin, on the other, out over the surface of the musculature, on which an extensive rectangular plexus is formed. From both sets of branches, as well as from the main vessels, rami pass into the mammary glands.

During embryonic development the cutaneous vessels are formed from the capillary plexus which is developed on the

outer side of the anlage of the body wall as this extends forward at the expense of the membrana reuniens to enclose the thoraco-abdominal viscera. This plexus at first drains forward through the membrana reuniens into the umbilical vein, but later the thoraco-epigastric vein becomes differentiated so as to drain the plexus into the main vein of the arm. From a similar plexus on the inner surface of the body wall, which at first drains into the cardinal veins, later also into the subclavian and femoral veins, the intercostal, internal mammary and deep epigastric vessels are differentiated. Between these and the superficial vessels numerous anastomoses exist. At a later period the territory of the thoraco-epigastric vein comes to drain into the femoral vein as well as into the axillary vein and the arteries corresponding to the superficial veins are formed.[1]

Although the mammary line arises comparatively early it appears to exert no influence on the formation of the chief ventral cutaneous vessels. The latter are formed in response to the needs for more direct vascular connections in the ventral region of the abdominal wall as the umbilical veins change their courses and the distance from the dorsal axis increases.

The first noticeable influence of the gland region on the cutaneous vessels appears at the period when the individual gland anlages begin to differentiate in the mammary line. At this time in the deep layer of the skin the branches of the thoraco-epigastric vein spread out widely and anastomose. Superficial to these larger branches there is a network of smaller capillaries. This network becomes especially well developed about the lenticular thickenings of the epidermis which mark the anlages of the glands (fig. 1). As the epithelial buds of the ducts project downwards and the connective tissue begins to condense about their bulb-like extremities a special capillary plexus develops in this connective tissue sheath.

By the time the ducts extend laterally from the primitive gland anlage veins and arteries are differentiated in the capillary

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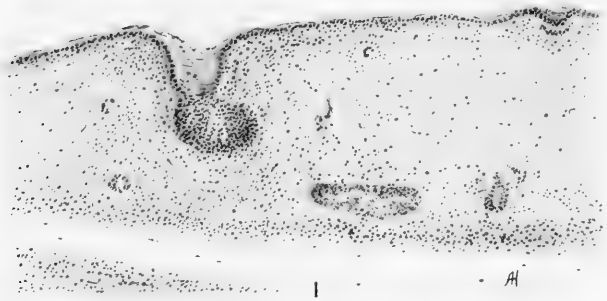

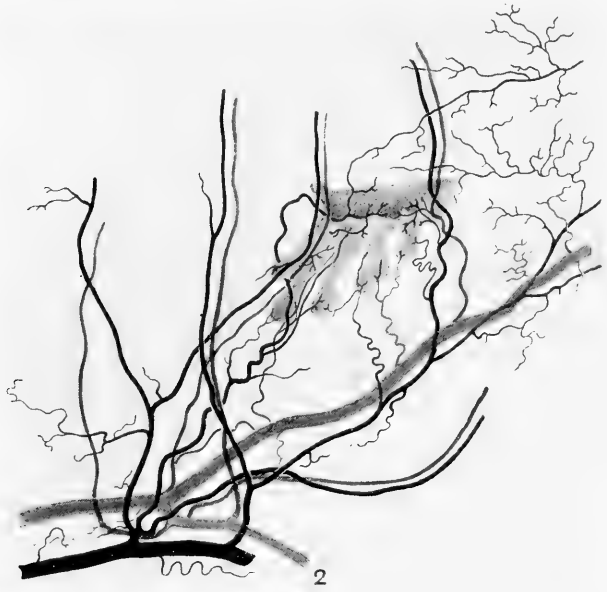

Fig. 1 Cross-section through the skin of a rabbit embryo 23 mm. long, showing the anlage of a mammary gland and sections of several blood vessels. Magn. 66 diam.

Fig. 2 Mammary gland of a newborn rabbit, seen from below; the epidermis and nipple have been removed from the piece of skin in which the gland lies. The skin has been lightly stained with carmine, cleared and mounted in balsam. The blood vessels are injected; the veins are shown lighter colored than the arteries; no attempt has been made to show the capillaries. The smaller ducts of the gland have been bent back over the nipple area in mounting the specimen and are shown darker than the rest of the gland; length of longest duct, 2 mm. Magn. 8 diam.

plexus surrounding this anlage. These vessels may be readily followed in a new-born rabbit (fig. 2). At this period the ducts spread out a short distance from the nipple anlage and have narrow necks and dilated extremities. The chief superficial blood vessels and their branches are distributed essentially as in the adult. From the vascular trunks and their main branches and from the chief branches to the superficial layer of the dermis and to the cutaneous muscle lying near the gland anlage rami may be followed to the nipple region and upwards in the connective tissue surrounding the necks of the ducts into a plexus lying immediately beneath the epidermis covering the anlage of the nipple. As a rule, at this period the arterial and venous rami take somewhat independent courses, although frequently they parallel one another for a part of their course. The longer ducts extend toward the main blood vessels and the rami coming from these run parallel to the ducts. Frequently an arteriole will run on one side of a duct, a venule on the other side. Capillary plexuses surround the ducts, but are less well developed about the necks of the ducts than about their expanded extremities.

Two months after birth the ducts have extended considerably further from the nipple region and show many bulging projections, the anlages of branches (fig. 3). The vascular supply of the gland is much elaborated. Special vessels to the nipple region supply the capillary plexus about the necks of the ducts and that part of the ducts lying near the nipple, while other vessels from the cutaneous and muscular plexuses are beginning to supply the extremities of the ducts. The nipple branches are long slender tortuous vessels which give off few rami until near the nipple. Some of them lie superficial to and others beneath the free parts of the ducts. At the base of the nipple they turn upwards toward the apex of the nipple which at this period is a small bullet shaped projection. Usually one or more main rami may be followed into the arterial rete about the mouth of the ducts. Some of these rami lie at the periphery of the group of ducts in the nipple, others in the midst of the group. Both sets send branches to the capillary plexus about the necks and mouths

Fig. 3 Mammary gland of a rabbit two months old, prepared like that shown in figure 2. The ducts are cut off near the base of the nipple; only a few of the larger blood vessels are shown; length of longest duct 5 mm. Magn. 8 diam.

Fig. 4 Two main ducts and their branches of a mammary gland of a fullgrown virgin rabbit, prepared as described for figure 2. A few of the arteries supplying the gland are shown; length of longest duct 10 mm. Magn. 8 diam.

of the ducts. The peripheral vessels also send branches into the integument surrounding the nipple. For each of the larger arterial rami there is usually a vein which takes a corresponding course but does not always run close to the artery. About the free extremities of the ducts as they extend outwards from the nipple region a rich capillary plexus is developed. Near the tips of the ducts this capillary plexus is continuous with that of the surrounding stroma, but toward the nipple it exhibits a greater independence. As this independence is established there become developed in the capillary plexus of the stroma arterioles and venules which supply the capillary plexus of the ducts. First an arteriole is differentiated, then a venule, so that the capillary plexus of the ducts is supplied alternately by arterioles and venules as one passes from the nipple region outwards. At the growing tips of the ducts one usually finds either a developing arteriole or a developing venule. The branches which supply these arterioles and venules arise directly from the main subcutaneous vessels, and from the overlying cutaneous or from the underlying muscular vascular plexuses. The chief venous and arterial rami supplying the venules and arterioles to a given part of the duct may parallel one another or the venule may come from a direction different from that of the arteriole.

In the fullgrown virgin rabbit (fig. 4) the ducts have ramified out extensively from the nipple region. The glands are from 2 to 3 cm. in diameter. The ducts branch in a plane parallel with the surface of the body but no branches project perpendicular to this plane. The blood supply of the nipple region corresponds essentially with that described for the rabbit at two months, but the plexus about the necks of the ducts is more fully developed. As a rule, the terminal venules and arterioles enter the plexus on opposite sides of a given segment of the duct. The arteries and veins distributed to the free parts of the ducts are far more extensively developed than at the preceding stage, but otherwise are similar in origin and distribution. As the ducts extend outwards branches from the superficial cutaneous vessels and the superficial muscular plexus are called upon more and more for a vascular supply, although the chief subcutaneous vessels also

furnish new branches to them. The alternation of venules and arterioles continues as at the preceding stage. In the capillary plexus about the ducts, however, venules and arterioles are beginning to appear. The venules are better developed than the arterioles and exhibit a greater tendency to form longitudinal trunks accompanying the main ducts and joining the main vessels which pass to the gland directly from the chief subcutaneous vessels.

During the early stages of pregnancy the ducts expand rapidly and their ramification increases (fig. 5). If the territory supplied by a single main duct and its various branches be called a lobe it soon becomes impossible on mere gross inspection to distinguish one lobe clearly from another owing to the interdigitation of branches from neighboring lobes. The territories of the chief branches of the main ducts similarly become difficult to distinguish clearly as sub-lobes. As pregnancy advances the ducts of the smaller compound units of structure, the lobules, begin to appear on the ends and sides of the main ducts and their branches. The first lobular ducts to appear may arise from a plane perpendicular to the skin on both the superficial and deep surfaces of the ducts. The lobular ducts may be simple or compound. The simple ducts arise as solid bulbular processes which elongate and become hollowed out. The sides of these ducts become studded with knob-like processes which later expand into alveoli. The compound lobular ducts give rise to branches of one or more orders and these, in turn, become studded by alveoli.

Meanwhile, in the nipple region the necks of the ducts become much enlarged to form the lacteal sinuses, although in sections the walls appear irregular and collapsed. The mouths of the ducts, hitherto expanded and funnel-shaped, become greatly constricted. At the base of the nipple the ducts are likewise constricted but beyond here they again dilate.

At first the vascular development proceeds essentially along the lines described in the growing animal. The capillary plexuses about the ducts, however, become much more richly developed than before and the development of arterioles and venules in

these plexuses becomes much more active, the differentiation of the veins of the ducts continuing as before to exceed that of the arteries.

The subcutaneous vessels in the region of the gland and all of the vessels supplied to the gland meanwhile increase rapidly in size. Not only do the vessels enlarge in diameter, but they also elongate with the outward growth of the ducts and send rami to the branches which arise from the ducts. The new ducts are supplied in part by branches thus derived from the vessels of the parent ducts and in part by new vessels developed in the capillary plexus of the surrounding stroma. As mentioned above, there is a greater tendency for the new veins than the arteries to be derived from the preëxisting duct vessels.

In the latter part of pregnancy the chief interest lies in the development of the vascular supply of the glandular lobules. At first the anlage of the lobule is surrounded by a network of capillaries continuous with the capillaries of the duct. As the lobular anlage enlarges the surrounding capillary plexus becomes more richly developed and one or more arterioles and venules are developed between it and the arteries and veins of the ducts. As the alveolar ducts develop at the periphery of the lobular ducts the same process is repeated, venules and arterioles for the alveoli being developed between the capillary plexus about the alveoli and the vessels of the lobule (fig. 6). Frequently, however, the arterioles, and less frequently the venules, of the capillary plexus join vessels which approach the lobule from the side opposite the duct. Both the veins and the arteries may extend into a lobule from the extremity opposite the duct. The venules and arterioles supplied to the alveoli both however, almost invariably extend from the neck of the alveolus outwards into the capillary plexus surrounding it.

During lactation the gland still further increases in size owing to the expansion of the existing alveoli and the formation of new ones. Between the expanded secreting alveoli the connective tissue becomes very slight in amount. The ducts become greatly distended with milk. There is a great elaboration and

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Fig. 5 A duct and its branches of a mammary gland of a rabbit in the early stages of pregnancy, prepared as described under figure 2. The smaller blood vessels are not shown; length of longest duct 15 mm. Magn. 8 diam.

Fig. 6 Cross-section through a duct and its branches of a mammary gland of a rabbit during lactation. Magn. 17 diam.

enrichment of the vascular supply of the gland, but the essential features are as above described (fig. 6).

Within two weeks after weaning the gland appears much thinner than during lactation owing chiefly to disappearance of milk from the ducts and to the retrograde metamorphosis which becomes well marked first in the alveoli and later in the ducts. The alveoli first shrink in size, then the alveolar cells degenerate and are absorbed, but usually a duct stem remains as a small group of epithelial cells with merely a small lumen or no lumen. The stroma appears relatively greatly increased as the alveoli disappear. Different parts of the same gland undergo quite unequal retrograde metamorphosis. The alveoli of one duct may disappear, while along another duct they may appear to be still in the secreting stage. Alveoli of this kind may persist for at least several months after lactation. The walls of the main ducts and their branches first collapse as the contained milk secretion is absorbed and then gradually shrink in size.

As the alveoli are absorbed the surrounding capillaries disappear so that in the lobule the relatively thick-walled venules and arterioles seem disproportionately large compared with the capillary field which they supply. The capillaries about the ducts likewise in part disappear. The various arteries and veins of the gland appear for a time tortuous and shrunken, but gradually they come to resemble more and more the vessels of the gland of the virgin adult. Several characteristic differences, however, remain. The ducts are much longer and more ramified than in the virgin animal and the number of veins and arteries supplying them is greater. The larger arteries are more regularly accompanied by veins and the large veins by arteries.

To sum up briefly: The blood supply of the gland during development and rest appears in the main to be secondary to the blood supply of the skin and the subcutaneous muscles, but during functional activity it becomes more independent, the blood supply of the alveoli being connected with the vessels of the ducts and to a large extent, at least, independent of that of the stroma. The irregularity in the retrograde metamorphosis of the gland and the changes in the blood supply are suggestive from the standpoint of cancer formation.

A STUDY OF WANDERING MESENCHYMAL CELLS ON THE LIVING YOLK-SAC AND THEIR DEVELOPMENTAL PRODUCTS: CHROMATOPHORES, VASCULAR ENDOTHELIUM AND BLOOD CELLS[1]

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THIRTY-FIVE TEXT-FIGURES

INTRODUCTION

The aim of the present consideration is an analysis of the histogenetic changes passed through by the mesenchymal cells in the living yolk-sac. A study of the origin and development of the blood and vascular endothelium in normal teleost embryos, and in other specimens in which the circulation of the blood had been experimentally prevented, made it evident that a detailed

[1] The present contribution is a continuation of the author's study of "The origin of blood and vascular endothelium in embryos without a circulation of the blood and in the normal embryo" which appeared in the September number of this journal. This first part is referred to in the following pages as 'the previous paper.'

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investigation of the development of the living yolk-sac would be most instructive for a comprehensive knowledge of the behavior of mesenchyme in forming the blood cells and vessels. The yolk-sac had been thoroughly investigated in sections and the appearance and location of the earliest blood islands and vascular formations were already familiar.

The egg of the Teleost is particularly adapted to the investigation of such a problem, since its yolk-sac has no definite mesenchymal layer and the freely wandering mesenchyme cells may be clearly seen between the ectoderm and yolk periblast. The remarkable extent to which the cells migrate and the great numbers of such wandering cells impress one with the importance of this cellular movement in embryonic development. The appreciation of this phenomenon also emphasizes the great danger of interpreting developmental processes from a mere study of serial sections. Sections fail to produce a correct impression of what is actually taking place in an area vasculosa. The study of living embryos is absolutely necessary and through it one quickly becomes acquainted with the remarkable rôle played by wandering cells in the formation of the heart and vessels, as well as the production of the future blood cells.

These facts have been pointed out long ago, but with little effect, as is indicated by the enormous literature containing the endless interpretations and guesses of numerous authors after studies of fixed and sectioned material. It is not intended to under rate the importance of the study of sections. However, such a study to be fully comprehended must be accompanied by observations on the living material as far as is practical. These observations are further greatly clarified by an experimental modification of the normal developmental processes where such is possible.

Almost thirty years ago Wenckebach ('86), at that time a young medical student in Holland, described his observations on the living embryos of the developing bony-fish. In this contribution he lamented the fact that the knowledge of the embryology of the bony-fish, as well as of other vertebrates, was based almost entirely on studies of sections of the embryos. The

germ layers were described actually as growing layers and from this layer formation the different organs were built or produced by foldings. Each cell was thought of as being passive and only through its division was the formation of the organs brought about. This initial investigation, and the only one so far as I know, by young Wenckebach gave him an entirely different view point regarding the processes of embryonic development.

Wenckebach readily observed the cells of the mesoblast independently wandering in amoeboid fashion, often with extraordinarily long protoplasmic processes, within the body of the embryo as well as upon the hypoblast-free yolk. The wandering cells move as with aim and purpose to form certain definite organs. In the formation of the anlage and further development of the heart, as well as the vessels and other structures, this independent wandering of the mesoblast cells performs a most important part.

Unfortunately, Wenckebach's scientific efforts ceased and his early study was unappreciated since on only one occasion was it considered by an investigator, Raffaele ('92), who studied similar material. Today the student gathers from text- and hand-books as well as descriptive embryological contributions much the same orthodox conception of the layers and foldings as all important factors in the origin and development of embryonic organs. No doubt the growth of layers and folds does contribute its part, but this part is almost negligible in a study of the development of the vascular system and blood.

Wenckebach and Raffaele, in their observations of the wandering cells on the yolk-sac failed to recognize the erythroblast. They were also unable to follow the processes in the differentiation of pigment and endothelial cells in the way at present possible with improved microscopes. The experimental embryos without a circulation of the blood are also most instructive for comparison in such a research.

The wandering mesenchymal cells on the yolk-sac differentiate into four distinct types of cells. The chromatophores of two varieties, one black and one of a reddish brown color, endothelial lining cells of the yolk vessels and islands of erythroblasts may

all be seen to form in the living specimens. The association of the four types is also clearly determined. In addition to these cells the large periblast nuclei are conspicuously seen. The periblast never gives rise to any type of tissue or cells, but finally the nuclei become swollen and distorted and degenerate in the manner Wenckebach long ago described.

MATERIAL AND METHODS OF STUDY

The material used for these observations and experiments was the embryo of the Teleost, Fundulus heteroclitus. The eggs of this fish are very transparent and may be readily observed by transmitted light with a high power microscope. A compound binocular microscope made by E. Leitz has been found to serve splendidly for such observations since with this instrument an oil immersion lens may be used to study the embryo suspended in a hanging drop from a thin cover glass over a hollow slide. A double or triple lens condenser facilitates the regulation of light and in a darkened field the almost transparent cells may be seen while granular or pigmented cells are distinctly outlined.

The mesenchyme cells are of sufficient size to be readily followed with a Zeiss DD objective while the eggs are grouped on the bottom of a watch glass. An ordinary microscope serves almost equally well for these observations but the Leitz binocular has the advantage of producing an apparently stereoscopic effect, since it permits the observer to look with both eyes at the same time. One is also enabled to see much better and look much more continuously than with one eye. An ordinary binocular microscope is unfit for the finer observations on account of the poor arrangements for condensing the light, and the magnification is insufficient for details of structure.

Observations have been made on the normal embryo at all developmental stages. Specimens in which the circulation of the blood was never established were also used, since in these the behavior and development of the cells of the yolk-sac are in no way contaminated by the introduction of additional cells brought in the circulating current. Such specimens enable one to hold the yolk-sac in its original condition so far as cellular elements

go, and further give an opportunity to test the influence of the circulation on the mode of differentiation and function of the mesenchymal cells.

The prevention of the circulation has been accomplished in the same manner as employed in the previous investigation and fully described in the September number of this journal. The eggs shortly after being fertilized are placed in solutions of alcohol in sea-water. The series of solutions most advantageously used is prepared as follows: 1.5 cc., 2 cc., 2.2 cc., 2.4 cc., 2.6.cc., 2.8 cc., and 3 cc. of 95 per cent alcohol is added to 50 cc. of sea-water. These solutions are renewed after twenty-four hours and after another twenty-four hours the eggs are placed in pure sea-water.

Such a series gives, of course, gradiations of the effect. Eggs in the weaker solutions develop normally in many cases, while other individuals develop slightly slower than the normal and have the circulation of their blood arrested to different extents. Many individuals in all of the solutions fail entirely to establish a blood circulation and although the heart pulsates feebly it does not propel the plasma for one or another reason which has been previously discussed. In spite of the failure of the blood to circulate, the development of the cells on the yolk-sac progresses in an almost normal fashion and vessels and blood corpuscles arise in this region and may be carefully observed throughout the life of the embryo.

The observations on the living yolk-sac have been supplemented by a study of fixed and cleared specimens. Embryos at different stages of development are fixed in a saturated solution of corrosive sublimate to which 5 per cent of glacial acetic has been added. Eggs are left in this mixture for 4 or 5 minutes, then rinsed in tap water and placed in 10 per cent formalin. The formalin is changed after about one-half hour when it has become slightly cloudy. This method if carefully handled brings out in a most beautiful way the cell outlines of the ectoderm of the yolk-sac (fig. 34). The yolk remains rather transparent and the mesenchymal cells may be observed beneath the clear cut net-work formed by the ectodermal cell borders. This method has been frequently employed by other workers and I have used

it myself for ten years on Fundulus eggs, but have never before succeeded in getting this heavy outline of the cells. It seems scarcely possible that so striking an appearance could have been overlooked, yet it is perfectly simple to obtain. Fundulus yolk-sacs fixed in this way are equally as beautiful as silver preparations of cell boundaries.

In addition to the above, I have used for the first time another solution which renders the specimen still more transparent. This is a mixture of strong formalin 5 parts, glacial acetic 4 parts, glycerine 6 parts, and distilled water 85 parts. Eggs are placed directly into this and left for two days, and then transferred to 10 per cent formalin for permanent preservation. The fluid mixture causes the egg to swell to some extent but it leaves the yolk as clear as in life and by fixing the cells causes them to stand out in beautiful contrast (figs. 5, 6, etc.). The mixture of glycerine and glacial acetic has been used for a long time in preparing transparent specimens of invertebrate eggs. Wilson in 1892 used it with Nereis eggs. The proportions here employed have been used by several students at Woods Hole and are not original with me, except that others leave the eggs permanently in the mixture while it seems better to put them in formalin after two days. The eggs remain equally transparent in formalin.

The cleared specimens are most valuable for use in connection with the studies of the living. But the remarkably beautiful filamentous processes of the wandering mesenchyme cells and the endothelial lining cells of early vessels are not so extensive as during life. Some shrinkage or contraction of these processes always accompanies fixation. The movement of the processes in life also gives one a much better conception of their form and structure.

THE EARLY WANDERING CELLS

About two hours after fertilization the eggs of Fundulus heteroclitus have undergone the first division and are in the two-cell stage. The cleavages then continue in a more or less regular fashion to form a discoidal mass of cells as a cap on the yolk. At eighteen to twenty hours the germinal disc is begin-

ning to flatten or thin out in order to begin its expansion to cover the yolk sphere. After the fourth or fifth cleavage some of the peripheral cells of the germ disc are somewhat fused with the yolk mass and do not present a clearly formed distal cell wall. The nuclei of such cells continue to divide and begin to wander or are pushed out into the superficial yolk material. In this way are formed the so-called periblast nuclei, or more correctly periblast syncytium, of the teleost. This periblast syncytium precedes the germ disc in its descent over the yolk, so that one observes loosely scattered nuclei of unusually large size forming an advance border around the periphery of the germinal disc. The nuclei multiply and finally lie scattered over the entire yolk surface by the time the germ ring or blastodisc has completely covered the yolk (figs. 5, 7 and 8).

These periblast nuclei are of interest to us in the present consideration only on account of the fact that they are located in a superficial syncytium covering the yolk. It is over this syncytium that the mesenchymal cells wander. The periblast of the hypoblast-free yolk-sac of the teleost, in so far as position is concerned, may be compared to the endodermal covering of the yolk-sac in other meroblastic eggs.

The outer cover of the yolk-sac in Fundulus is formed by the germinal disc as it grows over the yolk. This constitutes the ectoderm of the sac which is its only true or typical layer. Thus the yolk-sac consists of an outer-continuous ectodermal layer beneath which are freely wandering mesenchymal cells and below these the periblastic syncytium fuses into the yolk material itself.

The periblast nuclei were interpreted by Agassiz to represent the survival of the nuclei which had at one time in phylogeny controlled the segmentation of the yolk. These were the nuclei of the former yolk laden cells in the holoblastic cleavage of the ancestral teleost. Others have thought that they played some part in the formation of the ventral wall of the gut, etc. In Fundulus, however, they take no part in the formation of the body tissues or organs, but may be observed to degenerate in the late embryo. The periblast nuclei become very much vacuolated,

irregular in shape and huge in size before their final degeneration. After the embryo has hatched the remains of the yolk contain a protoplasmic mass in which the periblast nuclei are packed together and the whole is finally absorbed.

The blastodisc is separated from the yolk by a space which arises during the early hours of development. This space between the ectoderm and periblast has been interpreted by Agassiz and Whitman ('84), Ryder ('87), Wilson ('90) and others to represent the blastocoel or segmentation cavity. It is actually into this space that the wandering mesenchymal cells migrate and we shall later recall this fact as of importance in interpreting the nature of the vascular lumen in connection with other body cavities or spaces.

Twenty-four hours after fertilization the germinal disc is from one-quarter to one-third way over the yolk-sphere. Its wall has thinned out centrally and around the periphery is seen a thickened border, the so-called germinal ring. After about 48 hours the germ-ring has traveled almost completely over the yolk-sphere and now surrounds the small remaining uncovered pole of the yolk which may be considered a yolk-plug. A shield-shaped thickening, beginning about the twenty-fourth hour, extends from one region of the germ-ring towards the animal pole. This is the embryonic shield and along its median line a second thickening begins to appear which is the first indication of the embryonic body.

From the edges of the embryonic shield and from the germ-ring as it finally encloses the yolk, an early migration of mesodermal cells takes place. The cells apparently do not wander far and some of them may again be included in the embryonic body. After the germ-ring has enclosed the yolk, from 45 to 50 hours usually, a very active migration of mesenchymal cells begins from the caudal and posterior lateral portions of the embryo. Figure 1 shows outlines of a few such cells wandering out from the side of an embryo of 45 hours. This figure is a camera sketch from life and even at this early time there are some cells inclined to be more or less spindle or stellate in shape with long delicate filamentous projections, while other cells are

of more irregular shape with short amoeboid processes. Figures 3 and 4 show the cells highly magnified and in active movement; figure 3 the spindle-shaped delicate process type and figure 4 the heavier more amoeboid cell. These cells are actively wandering and changing in shape as figure 4 shows. From *A* to *E* are the outlines presented by a single cell at five minute intervals.

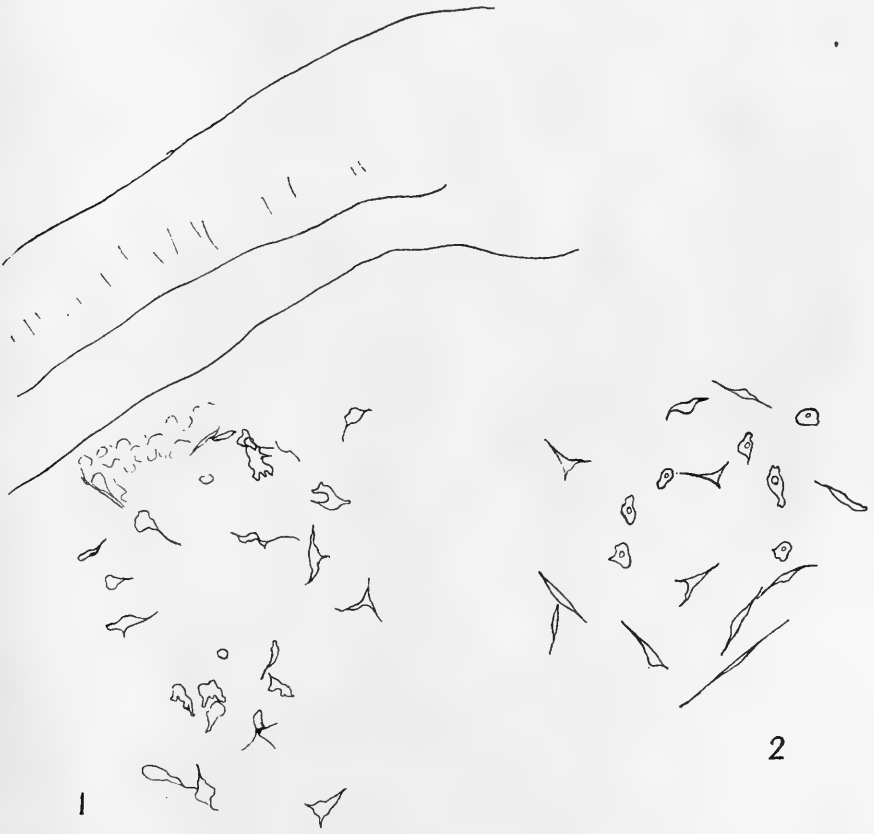
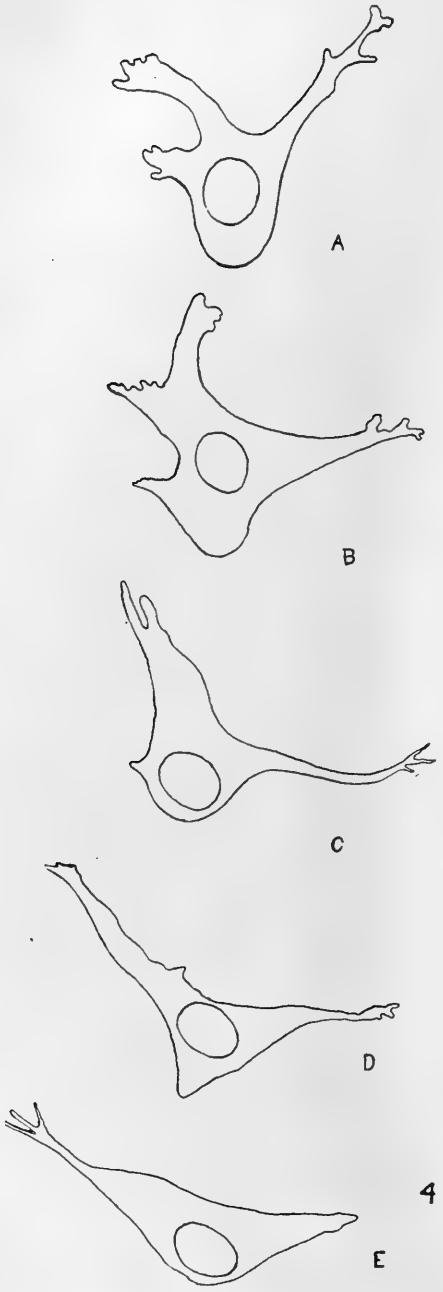


Fig. 1 A group of mesenchyme cells indicated in outline, camera lucida sketch, wandering out from the side of an early embryo, 45 hours old. Two kinds of cells are seen, one with delicate filamentous processes and another amoeba-like cell.

Fig. 2 A similar group in one microscopic field from an embryo 48 hours old, again showing the two types. The elongate spindle cells are future endothelial cells.



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It is, therefore, difficult to state that the spindle-shaped cells may not change to the heavier amoeboid pattern and vice versa. But we shall see that these two forms are the probable if not actual forerunners of two groups of cells later, at any rate, with permanent shapes and structures differing in much the same way as the early appearances now differ. Figure 2 again shows a sketch from life of wandering cells on the yolk-sac of a 48 hour embryo, and here as usual the spindle cells are contrasted with the amoeboid ones. The spindle cells are assumed to be future vascular endothelial cells and the amoeboid cells are probably the future chromatophores of the yolk-sac.

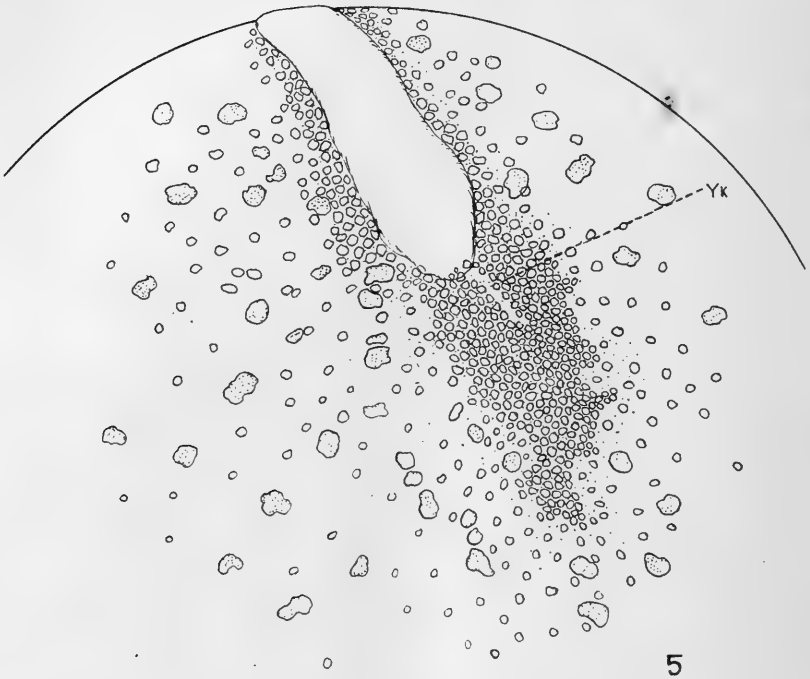
The places from which cells wander out most actively are the borders of the tail, and particularly from that mass of cells representing the obliterated germ-ring. Figure 5 shows the cellular arrangement around the tail end of a 48 hour embryo fixed and cleared. The cells have withdrawn their processes. The open space in the cell group at the tip of the tail, *yk*, is the place still remaining between the borders of the germ-ring. Later, the tail of the embryo grows over this cell group so that it is less conspicuous. Figure 8, the tail end of a 56 hour embryo, shows such a condition.

The important fact which we shall later consider is that these cells form a mass continuous with the mesenchymal cells within the tail end of the embryo. The wandering cells may be interpreted to grow out from the end-bud or blastopore lip. They are a scant rudiment of the peripheral or ventral mesenchyme usually growing away from the blastopore lip over the yolk mass in the reptile and the bird. It will be presently shown that such an interpretation is upheld by the nature of the products to which these wandering cells give rise.

Figure 6 illustrates the wandering away of cells from the lateral mesoblast of an embryo with two pairs of somites, 48 hours old. Figure 7 shows the head end of a 56 hour embryo. Scarcely any

Fig. 3 Camera outlines of wandering mesenchyme cells 48 hours old, all of the future endothelial type, highly magnified. *A* and *B* are two outlines of the same cell at a 6 minute interval.

Fig. 4 Camera outlines of one cell drawn at 5 minute intervals *A* to *E*. The cell is a migrating future chromatophore in an embryo 50 hours old (3b. DD ob.)



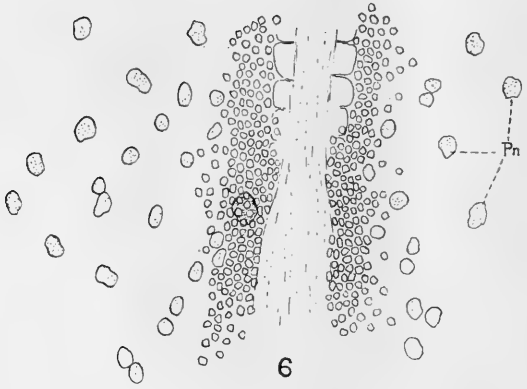


Fig. 5 Camera lucida outline of the tail end and caudal yolk region of an embryo 48 hours old, fixed and cleared in glycerine-formalin. The germ ring just closing over the yolk pole, numerous mesenchyme cells beginning to wander away from the caudal end, huge periblast nuclei indicated in outline and stipple over yolk. *Yk*, polar bit of yolk just being covered by union of germ-ring border.

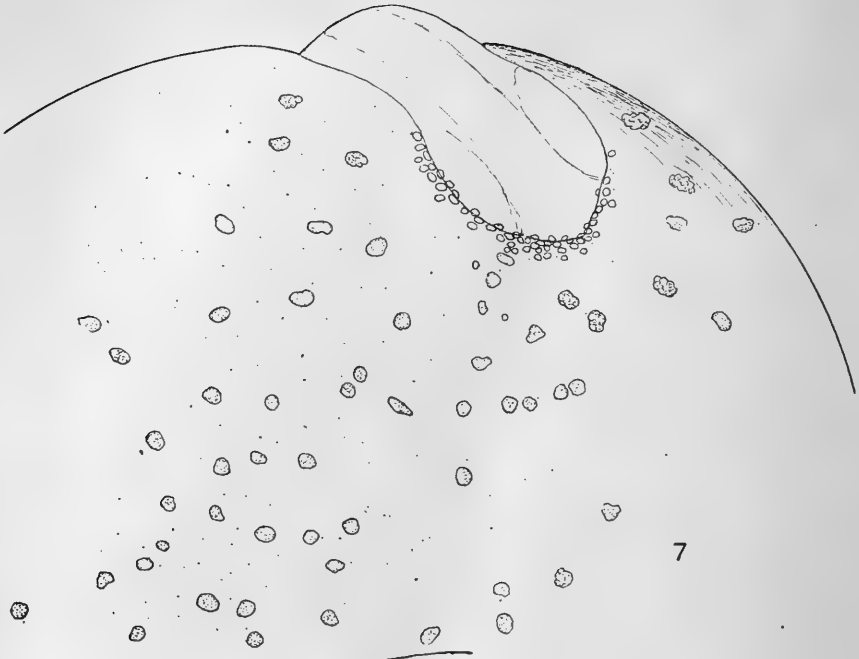
Fig. 6 Portion of the caudal half of an embryo of 48 hours showing the first two pairs of somites recently formed, cells of the lateral plate mesoderm extend out upon the yolk as shown in outline, some beginning to wander away. Periblast nuclei outlined and stippled. Glycerine-formalin specimen.

cell migration is taking place from this region. A few mesenchyme cells are found along the border of the head; these cells later take part in either the formation of the heart or pericardial wall. The tail end of the same embryo, figure 8, shows a remarkable contrast; here there is an enormous wandering out of cells from the mesoblast of the embryo. The two figures show the huge periblast nuclei to be widely distributed throughout the surface of the yolk sphere. These drawings are from cleared specimens and the cell outlines are more or less circular without the beautiful processes characteristic of the living.

The tail end of a living embryo 72 hours old, some time before the blood began to circulate, is illustrated by figure 9. The circle beneath the tail represents Kupffer's vesicle. The various shaped mesenchymal cells are represented in the act of wandering out over the nearby surface of the yolk. The embryo and yolk are beautifully transparent in life and the cells are clearly seen as they move upon the surface of the periblast.

An entire embryo, except the anterior portion of the head which extends beyond the curve of the yolk, is shown in figure 10 at a lower magnification. This specimen was 76 hours old when drawn. The heart had begun to contract slowly and feebly but no circulation of fluid had begun. Groups of mesenchymal cells are seen wandering away from the lateral and particularly the caudal regions of the embryo and are now scattered broadly over the yolk surface; there being very few, however, in the anterior region. The lateral plates of the mesoderm are seen at the sides of the head, and a circle at the caudal end indicates the Kupffer's vesicle which is always clearly shown at this stage.

In embryos of 72 hours, and somewhat earlier, there are wandering out from the tail region a number of cells slightly smaller than the two types mentioned above. These small cells tend to be more or less circular in outline but show slow amoeboid movement as they send out short blunt processes. They group themselves into small clumps and are to give rise to erythroblasts or future red blood corpuscles in the yolk-sac as shall be discussed beyond. Figure 31, page 569, shows six such cells from the living yolk-sac of an embryo 90 hours old;



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a circulation was partially established in this specimen but these cells had not yet been taken into the vessels.

The various wandering cells then represent the mesodermal layer of the yolk-sac in the teleost. They never assume a membranous layer-like arrangement, but finally differentiate into the characteristic structures of the yolk-sac. As is shown by the illustrations, these cells are very numerous and during their earlier stages are actively changing their shape and moving over the yolk surface.

We may now consider the further development of such cells the living embryos.

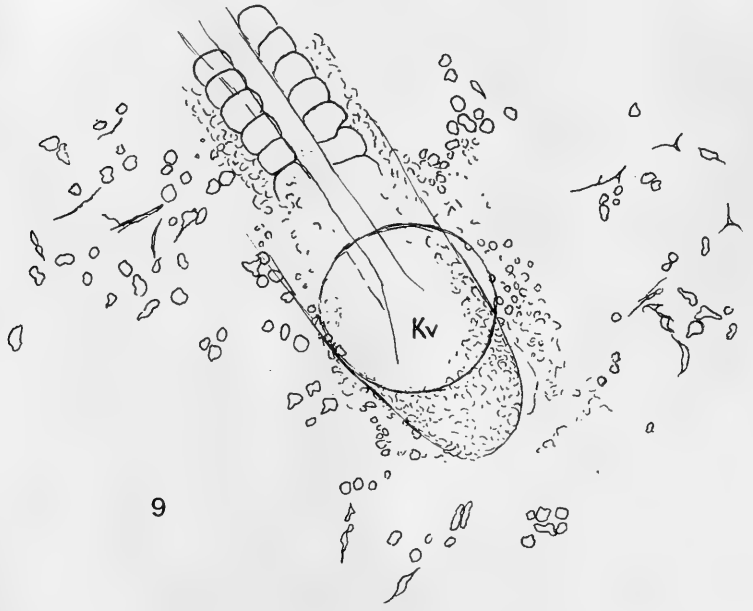

Fig. 7 Outline of the head end of a 56 hour embryo, scarcely any wandering mesenchymal cells in this region. Large periblast nuclei scattered over yolk surface.

Fig. 8 The caudal end of the same embryo; note the great contrast in the abundance of out-wandering mesenchymal cells. Glycerine-formalin specimen.

Fig. 9 The caudal end of a living normal embryo of 72 hours, with beautifully delicate mesenchymal cells wandering away from the body; *Kv.*, Kupffer's vesicle (3b. 2/3 ob.).

Fig. 10 A camera sketch of an entire embryo of 76 hours except the anterior end of the head. The mesenchymal cells wandering away from the tail region and to a less degree from the sides of the body.

DEVELOPMENT AND DIFFERENTIATION OF THE WANDERING CELLS

1. *Chromatophores*

a. The black type of chromatophore. The first to be considered of the four types of cells which develop on the yolk-sac are the black chromatophores. These are the largest and most conspicuous cells of the yolk-sac. In the early stages discussed above, one notes even in embryos of 2 days that certain cells of the yolk mesenchyme are considerably larger than others.

These large cells may be followed through their development and they will be found to differentiate into one or the other of the two types of chromatophores. The amoeboid cell shown in different stages of movement already referred to as figure 4, from a 52 hour embryo, is of this large type, and concluding from my observations on great numbers of embryos, this is an early condition of the future black chromatophore before any pigment granules are deposited.

Slightly older stages, figures 22 and 26, show the same cells containing a light amount of pigment granules. Between the second and third days the pigment granules appear and in an embryo 72 hours old, end of the third day, the chromatophores are already well differentiated freely moving huge cells.

A black chromatophore from an embryo 72 hours old is shown in figure 11 with one of its processes overlying the body of a brown chromatophore, the type to be considered in the following section. The black cell is loaded with coarse granules. The nucleus occupies a central position and is clearly shown on account of the displacement of the pigment granules by its transparent body. Several pseudopod-like processes project from the chromatophore which is actively moving. The clear cytoplasmic tip of the pseudopod extends beyond the granular mass.

Figures 12 and 13 are two other illustrations of the same cell after 15 minute intervals. Its shape is constantly changing and it is slowly moving in a direction towards the right side of the page. The brown pigment cell is also moving and their rates of progress are indicated by the increasing distance between them.

This movement of the chromatophores continues until about the end of the fourth or middle of the fifth day in the normal embryos. By this time all of the black pigment cells of the yolk-sac with few exceptions have taken up more or less permanent positions along the walls of the blood vessels or around the surface of the pericardial space. The individual chromatophores have increased enormously in size as is seen by comparing figures 11, 12 and 13 with figure 14, all drawn at the same magnification, though figure 14 is one-third more reduced in reproduction.

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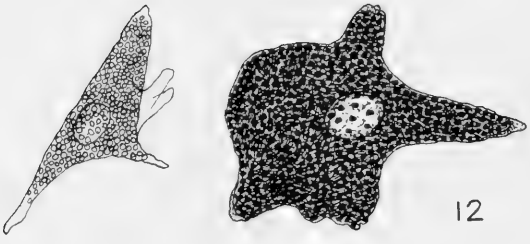

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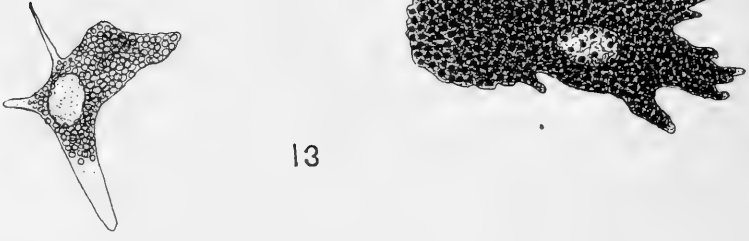

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It must be appreciated, however, that some of the difference in extent is due to the flattening of the cells in figure 14.

Figure 14 shows two huge pigment cells on the yolk-sac of a 5 day embryo in the act of arranging themselves along a vessel wall. The granules are not so densely arranged as in the younger stages, since the cell body is greatly thinned out in pressing around the vessel. A number of granules are often arranged in solid black lines and masses as indicated in the figure.

The two cells are close together and a very peculiar phenomenon is taking place. Each cell sends out short processes to meet similar processes from its neighbor. The processes fuse, and finally the two cell bodies melt into one thus forming a pigmented syncytium about the vessels of the yolk-sac. The syncytia continue to expand along the vessels as enclosing sheaths (fig. 15). The dense black of the young chromatophores becomes a steel grey as the granules are more thinly spread along the vessels.

In order to test whether the cells had actually joined or fused to form a true syncytium, I attempted to contract them, thinking that this should pull them apart unless they were actually united. The various solutions of KCl which Dr. Spaeth has found to contract the chromatophores within the embryo's body failed entirely to produce any change in the chromatophores of the yolk-sac. Solutions of adrenalin of one to 1000, one to 10,000 and one to 100,000, which Dr. Spaeth so kindly supplied me, were then tried. These solutions contract the pigment cells on the brain of the embryo until they appear as small black dots, but neither the black nor brown chromatophores on the yolk-sac respond in the slightest degree. Such specimens were preserved to show the extreme contraction of the chromatophores over the brain of the embryo in contrast to the unchanged pigment cells of the yolk-sac.

Fig. 11 A black and brown chromatophore lying in contact on a yolk-sac of 72 hours. The black cell is much the larger with broader pseudopod-like processes; both are in active movement as shown by comparing figure 12, of the same two cells 15 minutes later and figure 13, the same cells 20 minutes after figure 12. (3b. DD. ob.).

From this it would seem as though the material of the chromatophore had lost its contractile or wandering power after once becoming arranged around the yolk vessels. Those black chromatophores which retain their cellular individuality along the borders of the pericardial space also fail to contract when treated with KCl or adrenalin.

Although this physiological test failed to serve the purpose for which it was used I feel certain, after many observations, that the black chromatophores actually do form true syncytial masses as they surround the vessels.

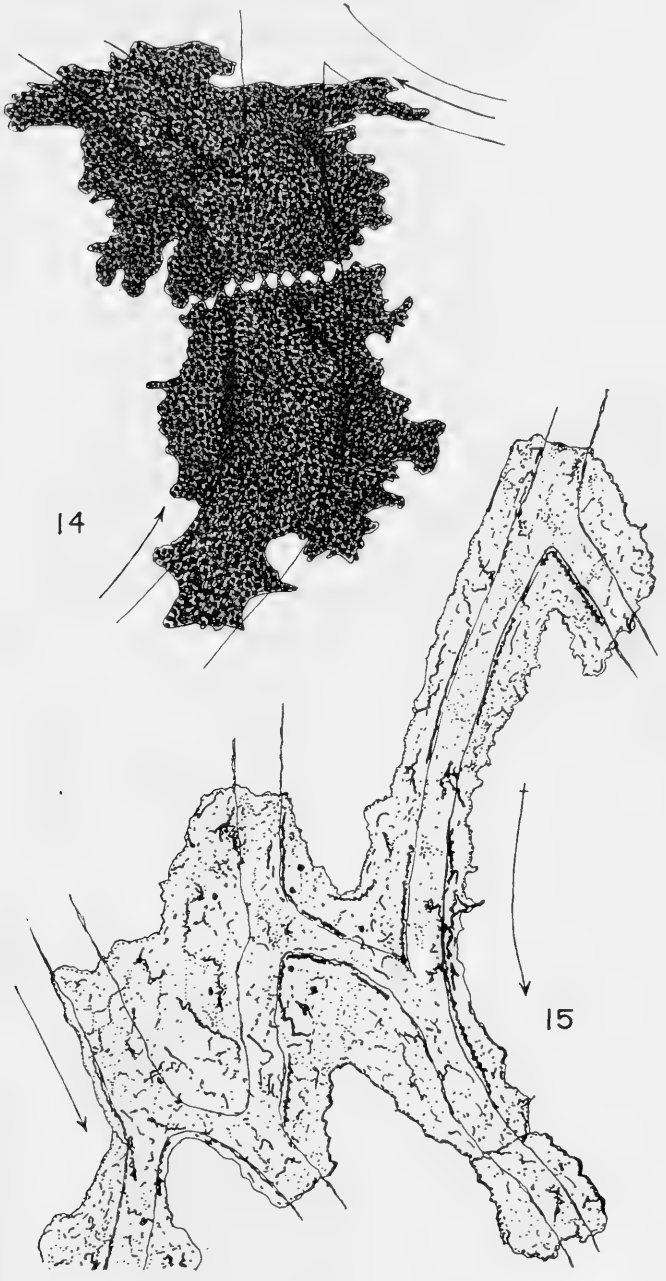
b. The brown type chromatophore. The brown chromatophores differentiate on the yolk-sac at about the same time as the black. They are always somewhat smaller and more delicately formed cells than the black, and react in a slightly different manner. Figures 22 and 26 show several brown chromatophores before the end of the third day. They are paler in appearance and more elongate in shape than the black cells.

The two types of cells are well contrasted in figures 11, 12 and 13 referred to above. The brown cell is smaller, with more delicate processes and is the more rapidly moving of the two. The three figures indicate its condition in embryos of 72 hours.

These pigment cells also wander to the vessel walls and yolk spaces and take on their permanent condition about the fifth day. Figure 16 illustrates one of the exquisite brown pigment cells in a yolk-sac of 5 days. The nucleus is still distinguishable in life while it is not in the black cells of this age. The mossy branched processes projecting from all sides give to this cell a most fascinating form.

Fig. 14 A camera lucida drawing of two huge black chromatophores lying upon a yolk vessel of a 5 day embryo. The adjacent sides of the chromatophores are beginning to fuse to form a syncytium. The direction of blood flow is indicated by the arrows.

Fig. 15 A syncytial mass of black chromatophores forming a sheath about the vitelline vessels. The chromatophores become so thin that the pigment granules are spread apart giving a less intense color. The individual cells are completely lost in the syncytium (3b. DD. ob.).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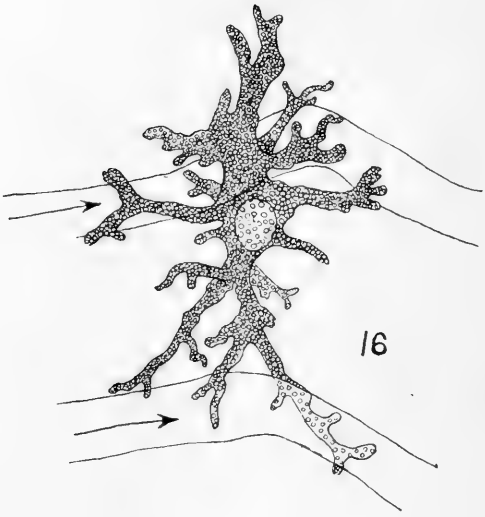


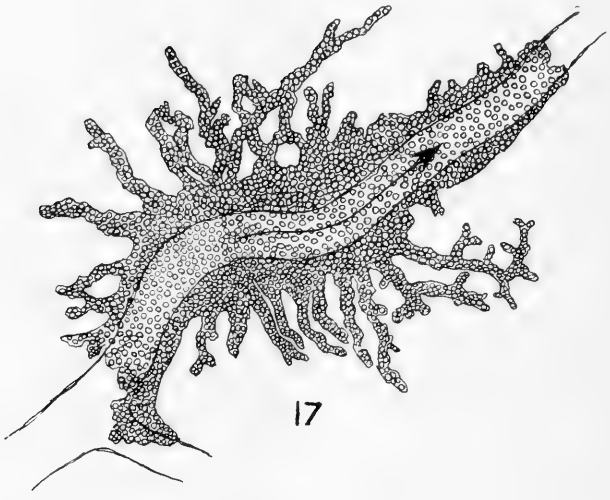


Fig. 16 A brown chromatophore on the yolk of a 5 day embryo. The cell is coming in contact with two vessels shown in outline. The moss-like processes extend from all sides of the cell.

Fig. 17 A similar cell 12 days old surrounding a yolk vessel. The complex processes from this cell are quite in contrast to the almost smooth border of the black chromatophores of figures 14 and 15. The brown cells never fuse to form syncytia.

Finally, in older embryos the cell body often surrounds a vessel, as shown in figure 17, but the processes persist and project from it in all directions, forming a striking contrast to the more or less smooth outlines finally assumed by the black cells, figures 14 and 15, as they surround the vessels.

The brown chromatophores do not group themselves together or form a syncytial mass as the black pigment cells are prone to do. They remain individually separated and many really never become associated with vessel walls, but lie scattered on the yolk surface.

In early embryos, from 72 to 90 hours, the brown pigment cells may sometimes, though rarely, get into the blood stream. I have never been able to observe one in the act of entering the current. Yet in a quiescent state they might become surrounded by endothelial cells along with the erythroblasts, and finally be swept away. They might, on the other hand, actually migrate through the porous wall of an early vessel.

The enormous brown pigment cell presents a smooth circular outline as it is carried along in the blood current. On account of its size the chromatophore often meets with difficulties in passing narrow portions of the vascular system. Several such cells were seen in the blood circulation of different embryos during the course of the observations, and when once located in the current the same cell could be seen periodically for a long time as it came around again and again through the vesssel within the field of study. There is no question of the identity of these cells, as their characteristic reddish brown color and coarse granular structure is readily recognized. It is most improbable to think of them as becoming changed into any type of blood corpuscle, and it is doubtless entirely by accident that they occasionally become entrapped within the vessel wall and washed away by the current.

c. Behavior of the chromatophores in specimens with no circulation. The behavior of both the black and brown types of pigmented cell is distinctly different in embryos without a circulation of the blood from that described in the two previous sections for normal embryos.

During the early stages, up to the beginning of the fourth day, the cells wander in amoeboid fashion much the same as in ordinary specimens. In other words, at this time the condition is the same in all embryos since the blood has not begun to circulate in any. At about 72 hours the blood circulation begins in the normal embryos and the pigment cells seem to be attracted to the vessel walls, as already pointed out. If the circulation does not begin at this age, the plasma accumulates in various spaces, chiefly the pericardial sac and Kupffer's vesicle at the caudal end of the embryo. The excessive accumulation of plasma in these spaces causes them to be in many cases hugely distended. The heart in such specimens also becomes a sacular structure filled with plasma which it is unable to pump on account of one or another deficiency in the vascular system.

Large numbers of chromatophores of both types tend to aggregate about these plasma filled spaces and partially cover their walls. The spaces are thus rendered more conspicuous. In some specimens this coating of the distended plasma sacs by pigment cells is most remarkable, but such an arrangement is not invariable and in a number of individuals the pigment cells are irregularly scattered over the yolk-sac with no recognizable pattern or system.

The heart of embryos in which there is no blood circulation is almost without exception covered with chromatophores. These cells often form a perfect sheath about such hearts whether the heart is a plasma filled sac or a mere string. The patterns of these arrangements are illustrated by numerous figures, particularly figures 15 to 20 in the previous paper.

A point of much interest in this connection is the fact that the heart of the normal embryo is entirely free of pigment cells.

The behavior of the chromatophores of the yolk-sac in normal individuals where they tend so decidedly to arrange themselves along the blood vessel walls along with their affinity for the plasma filled spaces in the non-circulating condition would seem to indicate that the chromatophore was attracted by the plasma itself, or some element which it contains. The distended plasma filled heart in the non-circulating cases is covered with pigment

as would be expected, yet the solid string-like heart present in many such specimens is also covered with pigment though it of course is entirely empty of plasma. In this last case, however, the string-like heart actually stretches as an axis through the pericardial space which is distended with fluid. The cells arrange themselves around the wall of the pericardium and on reaching the venous end of the heart migrate along it and so cover the heart string or tube in their effort to come in close proximity to the plasma. The distended condition of the pericardium may in this way account for the pigment arrangement along the heart in the cases with experimentally arrested circulation.

The normal heart is constantly pumping the plasma through itself, yet pigment cells are never present in its wall since they are all arranged along the vessels of the yolk-sac. In non-circulating cases many vessels form on the yolk-sac and some become quite well developed, while others actually surround the blood corpuscles of the yolk islands. Such vessels are at times covered with pigment but probably through accident as the pigment is irregularly scattered over the entire yolk-sac. Yet the pigment cells on such vessels never arrange themselves in the definite sheath-like fashion characteristic of the vessel pigment in the normal embryo.

Figure 18 illustrates the lack of arrangement of the chromatophores on the yolk-sac of a 16 day embryo without a circulation; compare figures 15 and 17 of pigment in the normal embryo. Figure 35, see beyond, also illustrates in a striking way the irregular grouping of black chromatophores in the neighborhood of a collection of stagnant blood islands in an embryo of 14 days that never had a circulation of its blood.

All of these reactions cause one to wonder what is the actual function of the pigment cells upon the yolk-sac. The entire egg is rather transparent and their function might be to protect the vessels from the light, yet the vessels are never completely covered and the development of the eggs in the dark is normal but not in any way supernormal.

The pigment cells form such a complete sheath about the vessels in some cases that one might be led to imagine that their

expansion and contraction would serve as vaso dilator and contractor. Yet when they are along the vessel wall I have failed to see them contract or expand even when treated with substances such as KCl and adrenalin that violently contract the chromatophores within the embryonic body. These experiments have not been carried sufficiently far since they were directed towards another point, still they indicate at least that the pigment sheath of the vessel wall does not respond as a delicate vaso-motor apparatus.

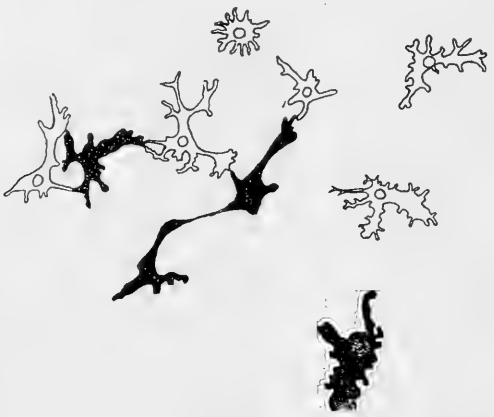


Fig. 18 A group of brown, indicated in outline only, and black chromatophores on the yolk-sac of a 16 day embryo in which the blood has never circulated. There is no arrangement of the pigment cells on vessels and no real syncytium of black chromatophores as compared with the conditions in the normal embryo.

Wenckebach ('86) found in certain pelagic eggs containing a number of oil drops which invariably floated up that pigment cells often completely surrounded the oil globules, and as he thought prevented these globules from focussing heat or light on parts of the embryonic body. The oil drops in the demersal Fundulus yolk do not particularly attract the pigment cells and they are rarely found to lie against the oil globule.

The function of the pigment in the yolk-sac of Fundulus, if it has any function other than its own existence, is most difficult to determine. The same is true of the abundant pigment in the coelomic wall and other internal structures of many animals.

d. Relationship of chromatophores to blood and endothelial cells. There has been much discussion in the literature regarding the relationship of the chromatophores to blood corpuscles and to endothelial cells. The actual relationship of these cells is clearly brought out by a careful study of the living yolk-sac in Fundulus. The cells are completely different and their structures when once established are consistent in their particular type.

The black chromatophores, the brown chromatophores, the endothelial cells and blood corpuslces are all derived from mesenchymal cells which wander away mainly from the caudal region of the embryo during early stages of development. These cells come to lie in the primary segmentation cavity of the yolk-sac beneath the ectoderm and over the periblast syncytium. The mesenchymal cells very soon begin to show certain differential characters in structure and behavior. When certain ones of the cells incline in a definite direction their development progresses continuously along this line.

Observations on the normal living embryos and comparison with those individuals without a yolk-sac circulation lead one to conclude as follows regarding the wandering mesenchymal cells. At the time these cells leave the embryo proper to wander over the yolk, differentiation has proceeded to some extent in the embryo and the mesenchyme cells are probably somewhat limited in their potentialities. Certain of them are derived from the same portion of mesenchyme that gives rise to the intermediate cell mass or future red blood cell forming mass within the embryo. This mass is located towards the caudal end of the embryo and the wandering cells derived from it finally come to lie on the posterior and ventral surfaces of the yolk-sac and form islands of red blood corpuscles. Few if any of these cells reach the anterior regions of the yolk-sac before the circulation of the blood begins. In embryos that *never* have a circulation the blood islands lie beneath the tail of the embryo and on the ventral yolk surface. The future endothelial cells wander out from the caudal end and side of the embryonic body and finally line up to form vessels in a manner to be described beyond. The pigment cells also wander out from the lateral regions and differentiate into chromatophores of either the black or brown variety.

It would seem that these cells must have some potential differences at the time they come to lie in the yolk-sac, since from that time on they all appear to be in an identical environment. Two cells lying side by side in the yolk-sac above the periblast and beneath the ectoderm would be expected to develop and grow in similar fashion unless there were some internal difference between them. I have thus concluded that the mesenchymal cells which wander in the yolk-sac of the Fundulus embryo must be potentially of four different classes when they first wander out, although all have the ordinary appearance of embryonic mesenchymal cells. Otherwise, it is difficult to conceive why they should develop into four distinct types of cells while all are surrounded by an identical environment so far as is possible to discover. Differentiation in various directions must be due either in the first place to similar cells developing in different chemical or physical surroundings, or in the second place it may result from potentially different cells developing under identical conditions.

The four types of cells are all derived from mesenchyme, just as the thyroid follicles and pulmonary epithelium are derived from endoderm but from different endodermal anlagen, and further than this there is no relationship. Pigment cells and blood corpuscles are perfectly separate and distinct types derived from different mesenchymal analgen and are not in any way transmutable.

2. *History of the endothelial cells*

The endothelial cells on the living yolk-sac of Fundulus embryos are readily recognized. Their entire behavior in the formation of the earliest yolk vessels may be traced in a manner to fully repay the patient observations necessary in order to follow through the processes.

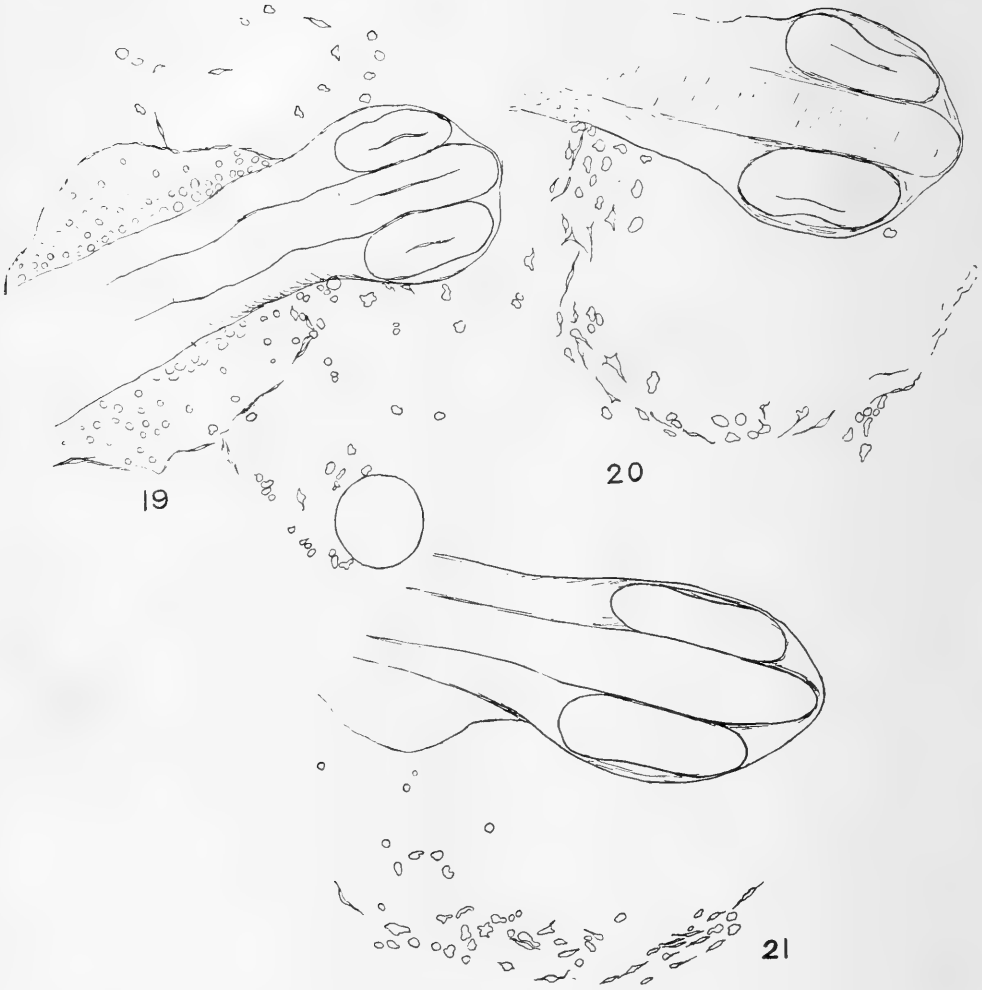
Among the early wandering cells migrating away from the lateral borders and caudal end of the embryo it is noted that certain ones assume a delicate spindle shape with filamentous processes extending from their ends and occasionally projecting

from their long sides. In a 48 hour embryo these cells already present the appearance of individual endothelial cells. They migrate indefinitely for a few hours and then tend to group themselves in more or less irregular collections.

Up to this time no one from mere observation could be absolutely certain that the cells of this rather characteristic appearance are actually to become vascular endothelial cells in all cases. The possibility, of course, exists that the elongate spindle cells may at times round up and then assume the more amoeboid shape of the probable future chromatophore. Yet since the shape of these cells is so characteristic and such shapes are so constantly present, one is inclined to believe that the same cell may actually retain this character until it really becomes a component part of a vascular endothelial arrangement.

Figures 19, 20 and 21 illustrate the region along the side of the embryo's head at 48 hours old. It is in this region that the first large yolk vessel develops. This vessel carries blood from the body of the embryo around a short circuit to reach the venous end of the heart and thus in a way relieves the flow that otherwise would force itself through the small poorly formed vessels in the embryonic body. This vessel is, therefore, of necessity one of the earliest to develop. The three figures, 19, 20 and 21, show variations in the arrangement of the wandering mesenchymal cells in the region of the future vessel.

In figure 19 there is really no definite cell aggregation except along the edges of the head mesoblast as it spreads somewhat over the yolk, yet a few of the cells show the typical spindle shape. Figure 20 indicates a tendency of the mesenchymal cells to line themselves in a group exactly along the course of the coming vessel. Many of the cells in this group give the actual appearance of an endothelial cell after it is fully developed and forming one of the units in a vessel wall. The embryo illustrated by figure 21 shows much the same condition. Very few mesenchymal cells occur between this cell aggregation and the side wall of the head. Lateral of the vessel group the cells are also not numerous and have no system of arrangement.



Figs. 19, 20 and 21 Outlines of the head regions of three living embryos from 48 to 50 hours old, showing different conditions in the grouping of mesenchymal cells on the yolk which later give rise to the large vessel that short circuits blood from the side of the embryo around over the yolk to the venous end of the heart. The future vessel wall is now separate spindle shaped mesenchyme cells.

This cellular aggregation may then be regarded as the actual anlage of the vascular endothelium of the future vessel. The anlage consists merely of a group of separate wandering mesenchyme cells, and not of a capillary net in any sense.

A slightly older embryo shows a still more definite alignment of the mesenchymal cells and still later presents the appearance of cellular strings or cords as illustrated in an embryo of 67 hours by figure 22. Here the wandering mesenchyme cells have differentiated to such an extent that they are readily distinguishable as black and red chromatophores and elongate endothelial cells.

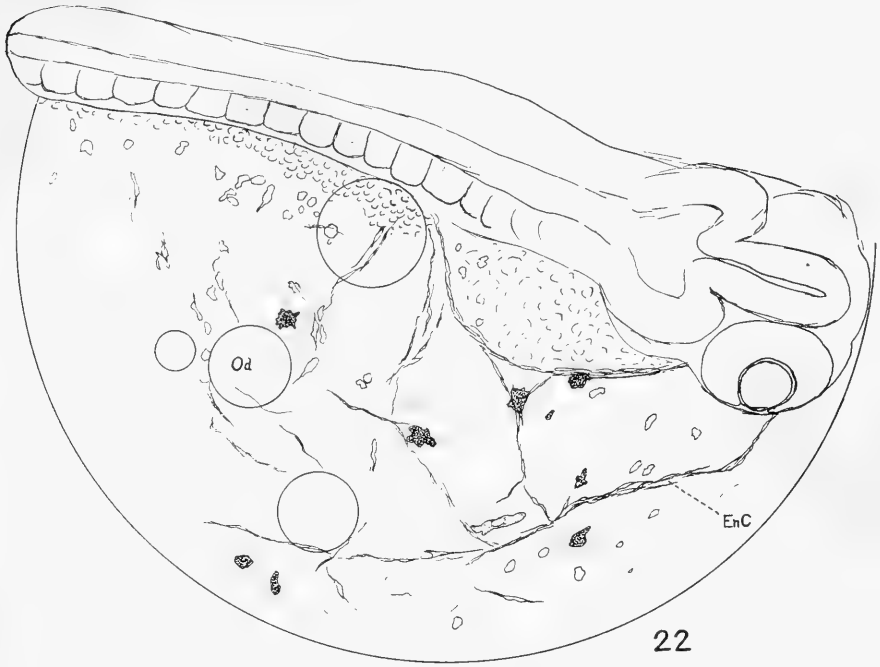


Fig. 22 A sketch of a 67 hour embryo showing the stage in the origin of yolk vessels in which the mesenchymal cells have a linear arrangement. Early black and brown chromatophores are also shown in the yolk-sac. *Od*, oil drops.

Early erythroblasts are also seen on the caudal region of the yolk-sac in such an embryo, but are not shown in the aspect here illustrated.

The endothelial cells are strung out in various directions and several linear groups are more or less isolated from the rest. The string to be the future large vitelline vessel is not clearly continuous posteriorly, but anteriorly it is well outlined extending towards the venous end of the now forming heart which has not yet

begun to pulsate. Here there is no further doubt that these elongate spindle cells are the elements which will make up the endothelial lining of the vessel wall.

There is considerable variation in the rate of development of the yolk vessels in embryos of the same number of hours. Some individuals may be in the condition just described, while others of this age may have already begun to establish a circulation of the blood. Figure 23 shows the yolk region lateral of the head in another embryo at 67 hours. In this specimen an incipient circulation has begun and the cord of cells illustrated in figure 22 has now become a small hollow tube sufficiently open to allow the passage of a single file of corpuscles from the side of the embryo around to the venous end of the heart. The individual cells composing the vessel are distinctly seen and their nature is clearly made out with a higher magnification. They retain the same general appearance presented before entering into the vascular arrangement.

Near this vessel is shown in figure 23 a partially formed vascular plexus which is broken in several places and entirely disconnected from the large vein through which the blood is flowing. There is of course no circulation of fluid in this partially formed plexus.

Figure 23 was sketched with a camera lucida at 12 M. and about three hours later at 2.45 P.M., figure 24 was sketched from the same field. The main vessel in accord with Thoma's ('93 and '96) first law of vessel growth has increased in calibre on account of the increased flow and pressure of the circulation. It now permits the passage of three or more corpuscles abreast and is a strongly developed vessel. The former disconnected vascular plexus has grown towards the large vessel and two of the projections shown in figure 23 have now met the wall of the vein and joined with it. One of the first corpuscles from the circulation to enter the plexus is shown in the figure tightly held in the small vessel. Immediately opposite this vessel a sprout is seen growing away from the wall of the large vein.

Figure 25 illustrates the state of arrangement at 6.00 P.M., three hours older than figure 24. The third process from the

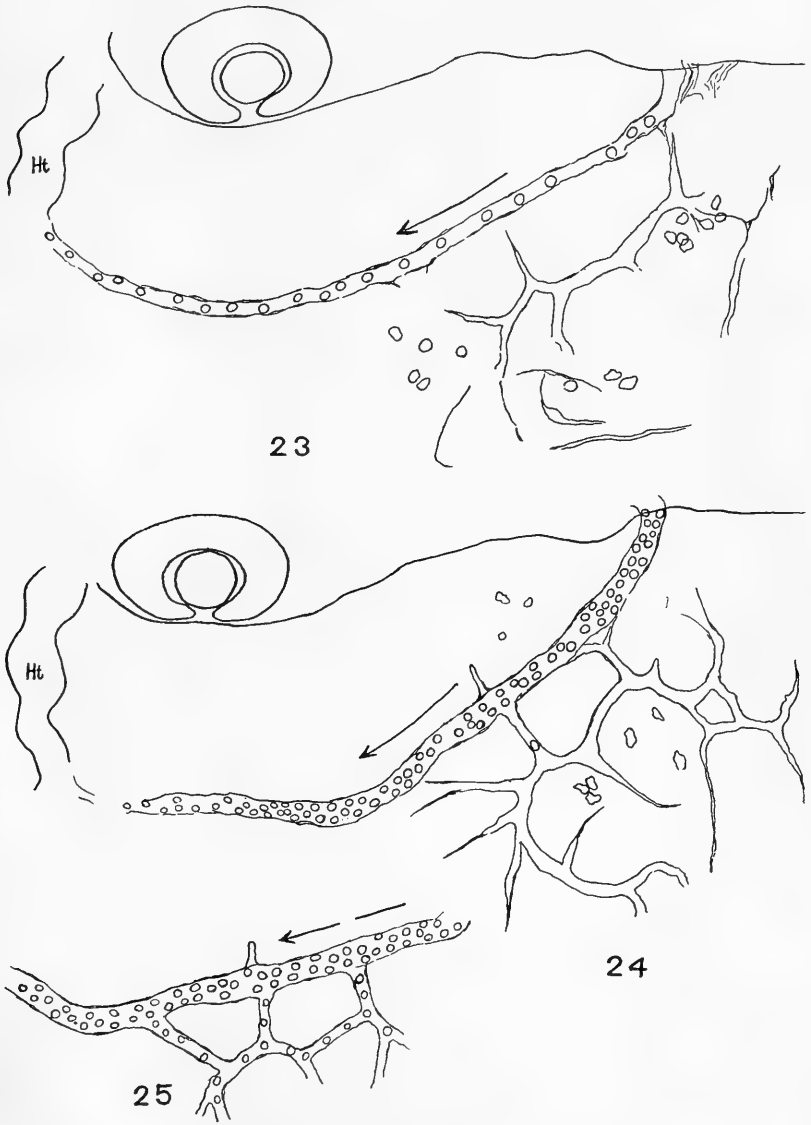


Fig. 23 The large vitelline vein in an embryo of 67 hours just beginning to permit the passage of blood through its lumen. Corpuscles moving in single file. This becomes the largest vessel of the embryo and arose from the arrangement of the freely wandering mesenchymal cells of figures 19, 20 and 21. In the

plexus has here joined the vein and corpuscles are freely passing into the vessels. The sprout from the vein wall is still seen opposite the entrance of the middle vessel. The small plexus arose in loco entirely independently and subsequently became connected with the larger vessel which also arose as we have seen from a group of mesenchymal cells.

Figure 26 shows another lateral view of the head region of an embryo of 67 hours in which the large vein is filled with circulating corpuscles and the beginning of the same plexus followed in figures 23, 24 and 25 is seen lateral of the vein. At this stage the plexus is entirely disconnected and separated from the vein.

In the formation of the large yolk-sac vein, as well as all other vessels arising upon the yolk, there is nothing to be seen of the forerunning capillary plexus so strongly emphasized by Thoma in the yolk-sac of the chick. There is no selection and dilation of certain channels in the capillary plexus of the teleost's yolk-sac to form the veins. Here the veins seem to arise in rather definite localities and soon expand into their full form after the circulation has become established.

This method of the formation of vessels was beautifully brought out by Wenckebach ('86) in the early study already referred to so often. He concludes: "Aus diesen Beobachtungen geht hervor, dass Mesoblastzellen durch selbständige amoeboïde Bewegungen die Wände der Blutgefässe des Dotters bilden." Raffaele ('92) later confirmed this observation and further was strongly of the opinion that in selachians and other vertebrates a similar process of vessel building from wandering cells also takes place.

From my present studies on the normal and abnormal Fundulus embryos, I can see no way to doubt that the endothelial wall of the primary yolk vessels in the bony-fish is formed by arrangements of wandering mesenchymal cells.

lower part of the camera sketch is shown an independent capillary plexus not yet connected with the vein. *Ht*, heart.

Fig. 24 The same vessels 2 hours and 40 minutes later. The main vessel has increased in caliber and two branches of the capillary net have joined the vessel.

Fig. 25 The same vessel three hours later, a sprout is given off opposite the union with the middle capillary and corpuscles now enter all three capillaries. The arrows indicate the direction of blood flow.

Wenckebach's description cannot be fully agreed with in all detail. He thinks, for instance, that cells forming part of the vessel wall are brought by the blood stream. These cells have small protoplasmic processes but they are not in any way to be confounded with the "definitiven Blutkörperchen." Such cells have never been observed in the Fundulus embryos and if they exist, which is very improbable, their part in vascular formation is extremely insignificant.

Wenckebach observed the three primary vessels on the yolk to bud and give off sprouts forming other vessels. The wandering cells also formed small separate tubes which later became connected to form a portion of the vascular net. By these methods the complex vascular net of the yolk-sac was finally formed. This agrees closely with what may actually be seen to occur in the embryos of Fundulus.

Considerable variation occurs in the position of vessels and a number of actual abnormalities are found in which the bilateral arrangement is completely disturbed. These abnormalities are frequently very instructive for a thorough understanding of the origin and development of the yolk-vessels. Occasionally, a group of cells will form a completely isolated endothelial space which may fail to associate itself with a vessel. Figure 27 shows such an isolated space in a yolk-sac of 90 hours old; solid endothelial tips project from the space, yet it is completely isolated so far as can be determined with the highest power, and at the same time every part of it is clearly and distinctly seen.

When the early yolk vessels are studied under high magnification, the individual cells may be clearly observed and they are strikingly the same as before they became associated to form the vessel. The cells are not closely arranged but distinct intervals and spaces exist between them and filamentous processes often project far into the lumen and may actually at times fuse with a similar process from a cell on the opposite side of the wall. These filaments thus stretch across the vessel and may even persist after the blood has begun to flow. They are well seen by focussing so as to get an optical section through the cavity. Cor-

Fig. 26 Outline of the yolk vascular condition in the head region of a 67 hour embryo. Blood is circulating freely in the large vessel but the yolk net of vessels is not yet connected with the current. Early black and brown chromatophores are also shown.

Fig. 27 An isolated endothelial cavity with solid projecting tips, no connection can be seen with any other vascular spaces. From an embryo of 90 hours.

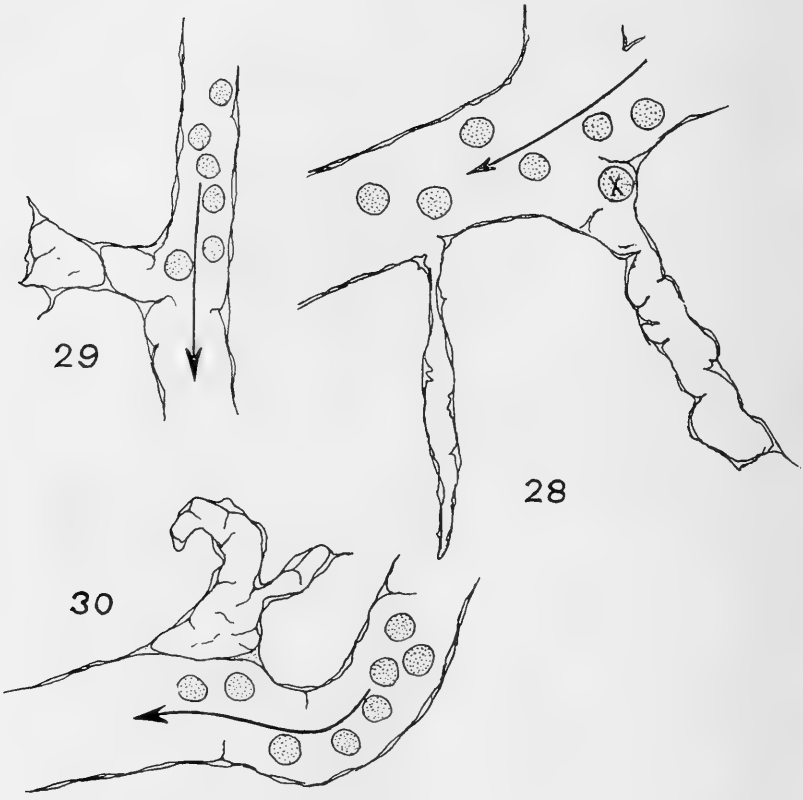
puscles often strike against the cell processes and cause them to wave back and forth as the current flows past.

The cells of the vessel wall thus maintain much of their individuality and may actually separate themselves or loosen away from the small growing tip of a vessel. The tips of the vascular sprouts probably break up or disassociate in this manner to include small groups of corpuscles which may be seen to enter the vessels from the yolk surface.

A most instructive specimen for a study of the cellular elements of the vascular endothelium is one in which the circulation has just begun. The vessels in such an embryo are still growing in length and sprouting off branches rather actively. Figure 28 illustrates such a vessel with its incipient branches. Corpuscles are passing through the vessel in the plasma current; one of these, X, is seen harbored behind an endothelial cell at the base of the outgrowth to the right. This corpuscle remained in position for more than one hour, being protected from the current by the projecting endothelial process. Such a condition is frequently seen and conveys some idea of the actual irregularity of the vascular wall.

The cells constituting the walls of the outgrowths from the vessel are changeable in shape and doubtless move their positions to some extent. The cells at the tip of the growing sprout may be seen to send out processes as if they were actually creeping along. The behavior of these vessel walls is strikingly similar to that which Clark ('09 and '12) has so clearly described for the growing lymphatics in the tail of the tadpole.

Figure 29 shows a similar vessel with a projecting bud. The cells of this bud are seen to exhibit a most indefinite arrangement; their processes project across the space and join the cells of the opposite side and none are completely elongated or flattened as are the cells of the main vessel wall. The tip cells might still be described as stellate mesenchymal cells. The appearance shown in figure 30 is much the same. The walls of these early vascular buds are extremely loose membranous arrangements with irregularities in their surfaces and openings and spaces between the cellular components.



Figs. 28, 29 and 30. Portions of vessels from three yolk-sacs of 6 day embryos. The vessels show blind endothelial sacs projecting from their walls. The constituent cells of these sacs are distinctly seen, and still retain their wandering mesenchymal characters. Filamentous processes from these cells may extend entirely across the lumen and fuse with processes from the cells on the opposite side of the wall. Corpuscles are often entangled in the filaments as well as the spaces between the endothelial cells. *X*, a resting corpuscle harbored behind an endothelial projection.

These porous or incomplete endothelial walls permit the blood cells to occasionally escape from the vessel cavity and become free within the space of the yolk-sac; or, on the other hand, a growing vascular tip may be observed at certain stages to come in contact with a group of erythroblasts, or actually a blood island unsurrounded by vascular endothelium. The tip of the

vessel seems to disorganize to some extent and its cellular elements slowly surround the group of corpuscles which are later taken into the circulation as the current becomes established in the including vessel.

Unfortunately, I have never been able to observe the consecutive steps in any one case of this kind, so that an absolutely positive statement cannot be made at present. Yet numerous observations of the contact of vascular sprouts with groups of uncovered corpuscles and the ends of such sprouts containing corpuscles, as well as other arrangements, would indicate that the behavior of the endothelium in surrounding the naked groups of erythroblasts on the yolk-sac probably takes place about in the manner just outlined.

Figure 32, page 568, illustrates a highly magnified field on the yolk-sac of a 90 hour embryo. This field shows a very interesting composite of the vascular condition at such an age. The rapidly flowing blood current is freely passing through the vessel on the right. A short circuit is forming across below the curve of an arch in the vessel. This small vessel permits only a single line of corpuscles to pass. At this time only one corpuscle has entered and it is caught in the narrow tube. This corpuscle remained fixed for a long time and so enabled a comparison of its structure and appearance with the erythroblasts forming the group just below the huge black chromatophore. The cells of this group are uncovered by endothelium. On the left of the figure a portion of a vascular net not yet connected with the circulating current presents the typical appearance of an early blood vessel formation. The individual cells are loosely associated and the tip projecting towards the right slowly changes position. This tip later approached the group of erythroblasts and finally these cells were all included within the vessel by a process which, as stated above, I was not able to follow definitely.

After closely studying these early vessels in a large number of living yolk-sacs, the observer is able to establish very clearly the actual relationship between the vascular endothelial cells and the erythroblast or early blood corpuscles. The corpuscles on the yolk are always of a distinctly different shape and size, and

lie, as described below, in small groups with originally no endothelial cells around them. The groups are later either surrounded by endothelium or taken into the early vessels as already indicated. Nothing has been observed during a long study of these cells on the living-yolk sac of normal embryos with a free circulation, or on the yolk-sacs of embryos experimentally prevented from establishing a circulation, or finally in sections of embryos of various ages, that would indicate even a tendency of endothelial cells to change into any type of blood corpuscle. The endothelial cell, whether in the free and wandering mesenchymal state or constituting a part of the vessel wall, presents a typical shape and clear appearance entirely distinct from the wandering erythroblasts.

In observing the early yolk vessels certain things may be seen which are most important in interpreting sections supposedly showing the transition of vascular endothelial cells into erythroblasts or primitive blood cells. Frequently, one or more corpuscles become entangled within the spaces and filaments existing between the cells of the vessel wall. Other corpuscles flowing in the current strike these entangled ones and beat against them sometimes for hours before they become disentangled from the vascular pits and holes, to flow again in the current. This is an extremely common sight during the early hours of the circulation of blood in any of the yolk vessels.

It may now readily be imagined that if the embryo was killed and fixed while the corpuscles were tangled in the spaces of the vascular endothelium, a study of sections might produce the impression that the cells of the vessel wall were "protruding into the lumen and assuming the typical characters of primitive blood cells," according to the description of many that imagine the occurrence of such things. I have previously offered another explanation of these appearances and many phenomena observed on the living yolk-sac bear out the point of view. It may sometimes happen when the vascular endothelium encloses a group of primitive corpuscles that one or more of these future blood corpuscles come to lie in the plane of the vessel wall, or may actually seem to form one of the cellular components of the wall.

When the circulation begins, however, this cell becomes loosened away from the wall for mechanical reasons, the lack of long processes, etc., and projects into the lumen to be finally washed away. Any one may readily observe such occurrences who will study the living yolk-sac of Fundulus with a high power microscope and a strong condenser so as to use a darkened field.

All of these observations lead one to conclude that the only connection between vascular endothelium and primitive blood cells is one of association. The endothelial cells never metamorphose into blood cells. It is important here to recall the fact previously emphasized by the author that in those specimens in which there is never a circulation of the blood or plasma the vascular endothelium develops in a perfectly normal manner in the aorta and other intra-embryonic vessels, as well as in the vessels on the yolk-sac, yet in none of these does one find any appearance indicating a tendency of the lining cells of the vessel wall to change into any type of blood cell. Numerous other details from my notes might be enumerated which would bear on this question, but sufficient care has been taken to definitely establish the above crucial points as facts. This may not of course hold for all types of animals but it does for those studied.

I have seen a number of sections on which other investigators have based their claim that vascular endothelial cells do change into primitive blood cells and although inclined towards the acceptance of such a view from a mere acquaintance with the literature, a study of such material has convinced me that the negative interpretation is equally plausible in all cases.

3. *Blood corpuscles on the yolk-sac of teleost embryos*

In all meroblastic eggs except those of the teleost a great sheet of mesoblast is found extending over the yolk as the so-called peripheral or ventral mesoderm, or subvitelline mesoderm. It is this peripheral mesoderm that gives rise to the blood islands of the yolk-sac in selachians, reptiles, birds and mammals The teleost, however, presents a unique case in that the ventral mesoderm does not spread out over the yolk but is included

within the embryonic body. The differentiation of this mesoderm within the embryo is much the same as that of the peripheral ventral mesoderm in the yolk-sac of other groups. Thus in the bony-fish the great bulk of blood formation takes place within the so-called intermediate cell mass, the probable homologue of a portion of the yolk-sac mesoderm of other vertebrates.

The intermediate cell mass first described by Oellacher ('73) and later fully studied by Ziegler ('87), Swaen and Brachet ('99, '01), and numerous others, is derived from the primary lateral plate mesoderm, separating away from the median border of this plate. The cell masses of the two sides remain apart in some species and form the future cardinal veins and red blood corpuscles, while in others the two masses unite in the median line to form the conjoined cardinals or stem vein, which is loaded with the primitive erythroblasts—the red blood anlage.

All recent workers on the development of the blood in the bony-fish have considered this intra-embryonic blood formation as being the only source of blood cells in these animals. Several authors, however, Swaen and Brachet among them, have recognized that the cells of the stem vein may become so packed and crowded within the embryo that masses of them are directly pushed out laterally upon the yolk. They have also thought it possible that a very few cells might wander upon the yolk-sac and there form blood, but this has been considered questionable in all cases. No one has recognized the actual occurrence of blood islands upon the teleostean yolk-sac. Even Wenckebach in his study of living embryos, although he made so many important observations on the formation of the periblast and yolk vessels, entirely overlooked the early or primitive yolk-sac blood cells. This is probably due to the fact that he studied only normal embryos. In embryos without a circulation of the blood one observes the yolk islands much more readily as the cells finally become filled with haemoglobin and present a bright red color. When they are once located in these experimental embryos it becomes much easier to trace them in the younger normal individuals, and finally the observer readily locates these cells and may follow completely their migrations and association to form the yolk-sac blood islands.

The arrangement of these wandering cells in the yolk-sac, figures 7, 8 and 10, suggests in a way the regions of growth of the yolk-sac mesoderm in other meroblastic eggs. About the caudal region there is an 'area opaca' formed by the great number of wandering cells, while around the head end the scarcity of mesenchymal cells might be considered an area pallucida.

All of the yolk-sac blood islands in the Fundulus embryos are formed from certain of the early wandering mesenchymal cells on the yolk. During the early wandering stages when the future endothelial cells have a spindle shape and the future chromatophores are large amoeboid cells, other mesenchymal cells on the yolk are small and more or less circular in outline. These small circular cells move slower than the other types and throw out short thick pseudopod-like processes.

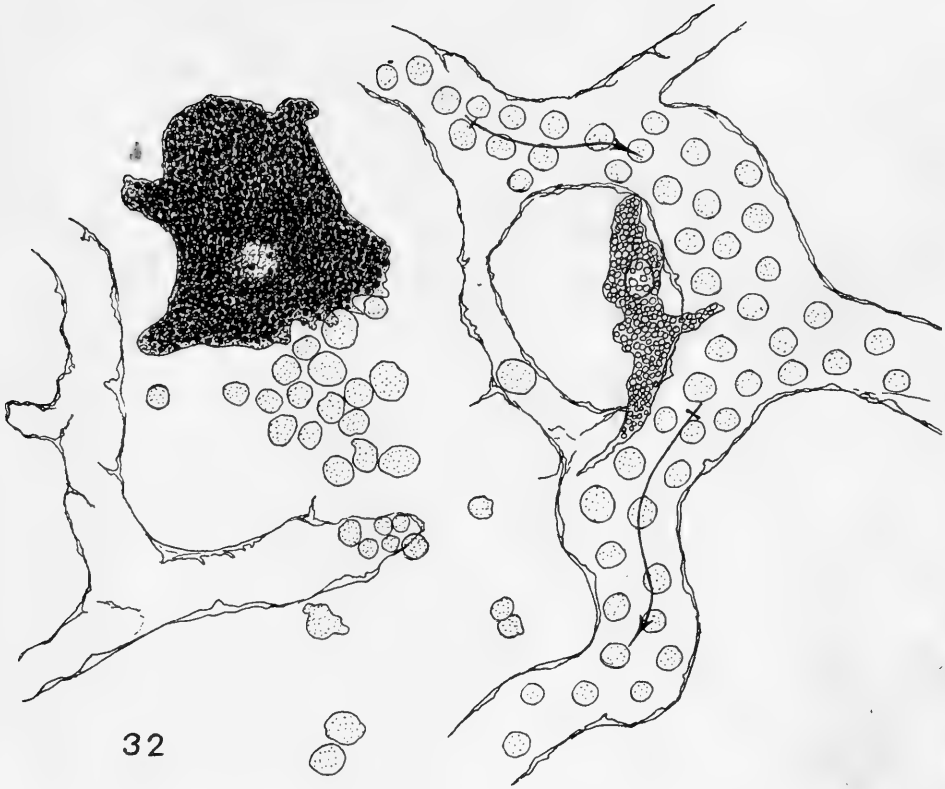
Whereas the spindle shape cells wander away from the embryo along its entire lateral border as well as the caudal end, and the large amoeboid future chromatophores have almost an equally extensive place of origin, the small round cells wander out only from a limited region. The earliest ones of these to be seen are near the caudal end of the embryo before the tail fold has completely separated the caudal end from the yolk surface. As the tail is moulded free from the yolk-sac, the point of outwandering of the circular cells follows the place of union between the ventral wall of the tail and the yolk-sac. Just at this place the mesenchyme of the embryonic body extends itself out on to the yolk as free wandering cells.

In a study of sections this mesenchyme is found to lead directly to the end-bud, Endknospe, which may be considered to represent the blastopore lip. The cord of mesoblast which has been designated as intermediate cell mass leads caudally to the end-bud which is well out in the tail. The ventral cells from this mass wander away into the yolk-sac from the extreme caudal position and other cells also wander away laterally from the intermediate cell mass. Figure 8, the tail end of an embryo 56 hours old, illustrates very well the place of outwandering of the round cells. Few, if any such cells wander out from the lateral borders of the embryo in regions more cephalad than this.

This confined local origin of the round cells and the period at which they wander out, along with their general appearance, lead one to believe that these cells are actually derived from the same general mass or group of cells which goes to form the intermediate cell mass or red blood anlage within the embryo. All of these slowly wandering circular cells finally differentiate into red blood corpuscles, as described below, just as cells of the intermediate cell mass will finally do.

In this connection a most instructive defect is frequently found among embryos developing in the stronger solutions of alcohol. Many such embryos are of the common short type with their tails split, cauda-bifida, figure 4 of the previous paper. This defect is due to the fact that the germ-ring descends over the yolk in a slow or arrested fashion and may never succeed in fully enclosing it. The caudal end of the embryo is thus divided and the two tail moieties remain spread apart laterally along the line of the germ-ring. This condition renders the caudal portion incapable of including all of its usual median tissues and such cells extend past the angle of the split and lie between the two parts of the bifid tail. The interesting thing is that the cells constituting the blood-forming intermediate cell mass lie in just this position.

Figure 33, a diagram, illustrates the embryonic body with a bifid caudal end. The great lake of blood corpuscles is situated beyond the angle of the split tail. Such an abnormality liberates the future blood forming mass from the body of the embryo and the mass spreads posteriorly over the yolk surface, yet not in a diffuse manner since it maintains its densely packed cellular structure. We might consider that here the evolutionary events are reversed. The blood anlage in the primitive fishes, the selachians, is spread over the yolk in the area opaca. In the normal teleost this primary yolk-sac blood anlage has been included within the embryonic body and localized in the intermediate cell mass. While in the abnormality here considered the intermediate cell mass is again outspread upon the yolk somewhat suggestive of the old ancestral selachian arrangement. This abnormality, in other words, may give some notion of the actual



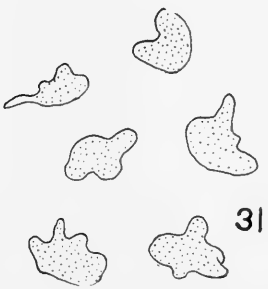


Fig. 31 A group of six early erythroblasts unsurrounded by endothelium on the yolk-sac of a 90 hour embryo. Short amoeboid processes project and the cells move very slowly.

Fig. 32 A camera lucida sketch of a microscopic field on the yolk-sac of a 90 hour embryo. All four derivatives of the wandering mesenchymal cells are shown. The circulation is established in the vessel to the right and the current follows the direction of the arrows. To the left is a small vessel not yet connected with the current; its wandering endothelial tip is approaching a group of erythroblasts still uninclosed by endothelium as they lie near the huge black chromatophore. A brown chromatophore is seen on the large vessel.

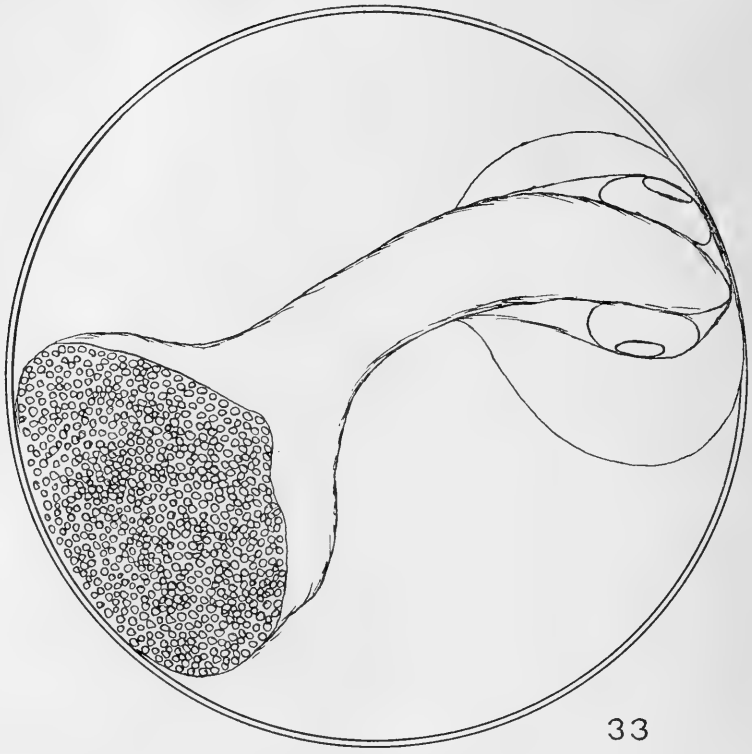


Fig. 33 An outline of a short 6 day embryo from a strong alcohol solution. The embryo presents a cauda-bifid condition and the normally intra-embryonic blood-forming mass is represented by a densely packed expanse of corpuscles outside the body of the embryo and spread upon the yolk.

incorporation of the primitive blood-forming mesoderm of the yolk-sac into the body of the teleost embryo.

The situation may be pictured as follows. In the reptiles and birds, for example, the peripheral mesoderm is outspread over the yolk and in it differentiates the blood islands of the area vasculosa. The peripheral mesoderm in Fundulus and teleosts generally does not become outspread over the yolk, but is concentrated into a median cord or mass within the caudal half of the embryo. Yet even here there is actually a tendency for the cells of this mass to be attracted to the regions of the yolk-sac, and during the early stages of development many cells sepa-

rate from the mass and wander freely on the yolk. The extent of such wandering is probably variable in different species. Yet in all eggs with an extensive vitelline circulation the wandering of these future red blood cells probably takes place to a considerable extent in spite of the fact that the cells have been so generally overlooked. A fact easily accounted for when one realizes that most of the studies in blood origin in teleosts have been confined to sections of the embryos of the salmon and trout. Sections are extremely slow in revealing the significance or even existence of wandering cells in development.

We may now consider the individual wandering cells and their subsequent differentiation. Figure 31 shows a group of six such cells. They are small when compared with the huge chromatophores but are about as large as the endothelial cells, though completely different in shape, texture and behavior. Figure 32, a camera lucida sketch, serves well to illustrate the relative sizes of the different type cells on the yolk-sac. In this figure are shown all four types, the enormous black chromatophore, the very large brown chromatophore, the delicate elongate endothelial cells of the vascular wall with their filamentous processes, and the almost circular erythroblast, small when compared with the first two types, but as large or even larger than the endothelial cell.

Figure 34 was drawn from an embryo that had been fixed in such a way as to render conspicuous the cell outlines of the ectodermal layer of the yolk-sac. Below the ectoderm groups of erythroblasts forming blood islands are shown, and the extremely small size of the erythroblasts in comparison with the enormous dimensions of the ectodermal cells is most striking.

As mentioned before, the erythroblasts tend towards a circular shape but send out short processes, while they move in a sluggish amoeboid fashion. The cytoplasm of these cells is slightly greyish and not so perfectly transparent as that of the spindle cells; this difference between the two types is not so marked during the early stages but is readily noticeable in embryos of 80 or 90 hours.

The future erythroblasts very slowly wander away from the tail region of the embryo and down the posterior surface of the

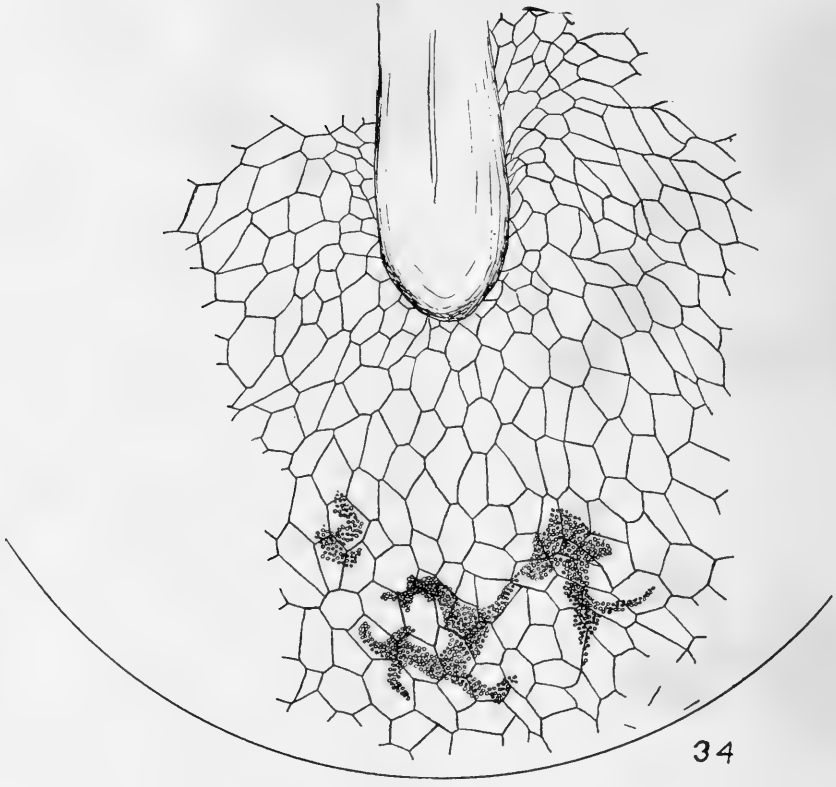


Fig. 34 A camera lucida outline of the caudal region of the yolk-sac in a 96 hour embryo with the ectodermal cell borders made visible by mercury fixation. An island of blood corpuscles is indicated by small circles which give some idea of the minute size of the erythrocytes as compared with the huge ectodermal cells.

yolk to reach its ventral surface. At various places on the posterior and ventral yolk surfaces the cells collect into groups and become less active, although their movement does not entirely cease. These groups constitute early blood islands. They are at first not surrounded by endothelial cells but later become enclosed or taken into the ends of the incipient vessels as briefly described above.

When the circulation first begins on the yolk-sac of a normal embryo a great many of these islands are present, but are more or less isolated or out of the channels through which the fluid is

flowing. The taking up of the islands by the circulation is most interesting to observe. At first those cells near the tail of the embryo, which are enclosed by endothelium, are taken. A few of the corpuscles are shaken by the current and these strike against the other members of the group until all become loosened and move slightly to and fro; finally one or two are suddenly washed away, then others follow—few at first, until the entire group loses its stand and is swept away by the current. One and then another of the islands may be seen to perish in this manner before the irresistible force of the tiny stream.

Yet even after the yolk circulation is fairly well established a number of islands of the round cells may still exist unsurrounded by the endothelial vessels. Figure 32 shows such a case. A well established current flows through the vessel to the right, while to the left is an incipient vessel not yet connected with the current. In the center of the figure is a group of corpuscles below a huge black chromatophore. These round cells constitute a blood island still unenclosed by vessel endothelium; in the course of a few hours, however, they too will become enclosed by a vessel and subsequently be included among the circulating blood cells.

The early erythroblasts which are in this manner included within the circulation assume a circular outline as they float in the current. In the previous paper, figures 31 and 32 of cross sections through the intermediate cell mass, and figures 34 and 35 of cells in a yolk island, all from a young 72 hour embryo, illustrate the definite circular outline of these cells. In life they may be carefully observed in the small vessels where a single cell passes with difficulty, and are here seen to be globular in shape and to retain their slight amoeboid movement. The form of the early erythroblast is readily changeable for the first one or two days after entering the current. After two or three days, that is, in embryos five or six days old, the cells in the blood current assume a typical erythrocyte appearance, becoming elongate and elliptical in shape when seen from one position, while they are thin in profile view. They are now ellipsoidal nucleated red blood corpuscles. At about the time they begin

to change from the globular to the elipsoidal shape, the accumulation of haemoglobin takes place and the cells begin to show a typical straw color.

It may then actually be seen in the living that the early or primitive erythroblast is really a more or less globular amoeboid cell without haemoglobin and resembling more closely a lymphocyte or early leucocyte than a fully formed erythrocyte. This is probably true of the early stages in many cases of cytomorphosis, yet the globular amoeboid cells of the yolk islands are not indifferent 'primitive blood cells' in the sense Maximow ('09) has concluded, but they are definitely future erythrocytes.

This point is established by a study of these cell groups in the normal as well as in embryos without a circulation of the blood. In the latter individuals the islands arise by the formation of local aggregations of the early wandering cells in an exactly similar manner to that described for the normal embryo. In fact, the observer cannot distinguish between the two specimens in many cases, yet one fails to establish a circulation and the islands are thus enabled to retain their positions on the yolk-sac.

The constituent cells of such a permanent island may be observed from time to time or continuously, and will be found to pass through changes exactly identical with those which take place in the island cells that become swept into the blood stream of the normal embryo. They are for a few days globular in shape, but capable of slightly changing their form and sending out short pseudopod-like projections. When the embryos are five or six days old the cells in these islands then become flattened ellipsoidal corpuscles and attain a haemoglobin content exactly as in the normal embryo. The blood islands now appear bright red in color and are quite conspicuous on the yolk-sac where they permanently remain. The globular colorless cells are thus seen to differentiate directly into the typical ictheoid erythrocyte.

From a study of the living embryos alone one could not, of course, be certain that all of the cells of these islands had differentiated into red blood corpuscles. However in the previous study of the non-circulating embryos, I have examined a large number of yolk islands completely and have never seen any

type of blood cells other than erythrocytes in such a position. The same is true of the cellular products of the intermediate cell mass; in hundreds of sections studied with the oil immersion not one case has been found of a lymphocyte or leucocyte arising from a cell of the original intermediate cell mass. It is thus concluded that this mass is the red-blood anlage of the teleost and the wandering cells of the yolk-sac which are very probably derived from the same mesodermal stem that forms the intermediate cell mass are likewise a portion of the red blood cell anlage.

The embryos that fail to develop a circulation are most important material for the study of these questions of relationship among the different types of blood cells. The observations on the living show definitely the qualities of the early blood forming mesenchymal cell in its assumption of the globular slowly wandering type, which would fully satisfy the descriptions of Maximow's 'primitive blood cell' as being closer in appearance to a lymphocyte than to a red corpuscle. When one follows these supposedly indifferent primitive blood cells which are claimed to possess the power of differentiating into either a leucocyte or an erythroblast, he invariably observes them to differentiate only into erythrocytes. Although all cells of the early islands are distinctly visible, they are not mixed in type, but are of one class. A long study of these early islands in sections also fails to reveal any other than red blood cells. The leucocytes are not very numerous in the blood, yet they should be seen if present in these islands, as they are found in other positions and are well represented in the blood of the adult Fundulus.

The further history of the cells in the stagnant masses on the yolk-sac of an embryo in which the blood has never circulated is instructive in several ways. In the first place, all of these islands seem to become surrounded by endothelium, yet the endothelial arrangement cannot be completely traced in every case and it rarely extends much beyond the island mass so far as one can observe in life.

Pigment cells are irregularly scattered in the neighborhood of the islands, as they are throughout the yolk region. The

chromatophores do not, however, assume any arrangement with reference to the islands of cells and as mentioned above they retain their original condition as separate cells instead of fusing into syncytial masses, as is the case in specimens with a free blood circulation.

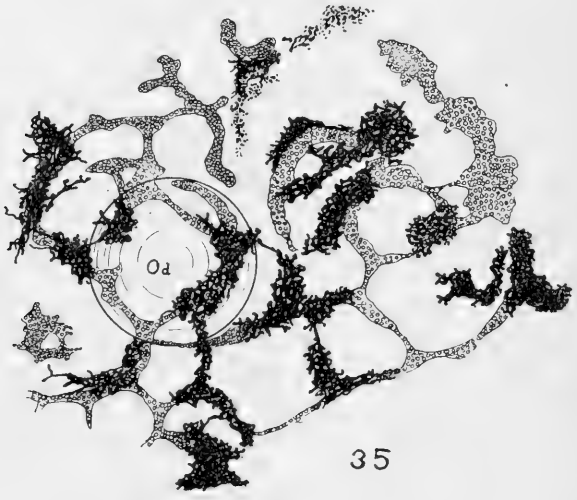


Fig. 35 The arrangement of red blood corpuscles in more or less connected endothelial sacs on the ventro-lateral surface of the yolk in an embryo 14 days old that had never had a circulation of fluid in its vessels. All of these blood cells wandered away from the caudal body regions of the embryo during early stages. The chromatophores are irregularly scattered among the old blood-islands. *Od*, oil drop.

Figure 35 illustrates a group of blood masses on the yolk-sac of an embryo 14 days old. In this specimen there had been absolutely no circulation or movement of the blood fluids within the vessels *at any time.* This is most important to know in the case of all specimens without a circulation at the period they chance to be examined. I shall return to this point below. The cell groups in the specimen without a circulation are arranged somewhat like a vascular net in the region illustrated by figure 35, yet they present a deadly still appearance as contrasted with the lively movement of the corpuscles within the yolk vessels of a normal individual. The erythrocytes forming these islands

are brilliantly red in color and their shape and size are apparently normal.

The blood corpuscles are thus found to differentiate in a typical fashion and to retain their haemoglobin reaction for a long period without having circulated in the vessels. The function of an erythrocyte would thus seem to be entirely independent of its circulation so far as its capacity to form oxyhaemoglobin goes. These cells are also able to accumulate oxygen within the intermediate cell mass in its central position in the embryonic body. The embryo from which figure 35 was taken had lived 14 days without its blood having circulated, which is about the period required for the young fish to hatch and become free swimming.

In older embryos the erythrocytes begin to degenerate and in many they lose their red color, the haemoglobin probably breaking down. The islands on the yolk then become pale in color and finally almost white, as if the cells were dead. The color of the blood cells seems to fade within the embryo earlier than on the yolk-sac as a rule, probably due to the better chances of obtaining oxygen on the thin yolk-sac than in the thicker embryonic body. The non-circulating specimens often continue to live for a long time even after the blood cells have lost their color. Some such specimens may exist for more than 40 days, which is a very long time considering that the normal embryo may hatch when from 11 to 20 days old. The specimens without a circulation are always weak and delayed in development and of course never succeed in hatching from the egg membrane.

In a study of the embryos treated with weak alcohol solutions, one very frequently finds cases in which the circulation of the blood may start almost normally and finally stop permanently, although the embryo continues to live. Other embryos may fail to establish a circulation at the proper time and yet may develop a freely flowing circulation of their blood some days later. Again, an embryo may have a fairly normal circulation and for some reason lose it for a few hours, or for one or two days, and then regain it, at first slowly and finally in a fairly strong fashion.

All three of these phenomena have also been observed in eggs developing in ordinary sea-water when they were not properly

separated so as to allow free respiration. The egg membrane is covered with long hair-like filaments which become entangled with those of neighboring eggs and in this way masses become closely packed. The central eggs of such a mass develop in a poor atmosphere and go more slowly than their neighbors on the outside of the mass. These centrally located eggs show many abnormal and arrested conditions of much the same type as may be obtained by treating the eggs with various injurious solutions.

The changeable states in the circulation offer many pitfalls for one attempting to determine the sites of origin of blood cells in the non-circulating embryos. Old embryos are seen in which there are beautiful blood islands on the yolk-sac and great clots of blood in the head or other unusual position. The heart is very frequently completely loaded with corpuscles, and yet there is not the slightest movement of the blood cells or any sign of a circulation at this time. The heart itself may be pulsating feebly or even practically stopped.

Another source of blood movement which is slight, yet to be guarded against, is that due to the muscular twitching of the embryo's body. This movement may frequently serve to push cells from the intermediate cell mass out on to the yolk-sac, but usually by way of the vessels. These dangers are to be taken seriously in experiments of this kind. Since one is able to be *absolutely certain* that the blood never circulates in a great number of embryos, only such embryos should be considered in a study of blood origin. During a study of this exact problem now extending over four spawning seasons, I have seen blood in almost every conceivable position in embryos without a circulation at the time of the observation. The accumulation of blood is more frequent in certain positions and regions than in others. The venous end of the heart is a most common place for a clot, the sides of the head, the large vessels of the yolk just lateral to the body, and various places on the anterior and lateral yolk surfaces.

When, however, the experimenter collects a number of embryos that have really never experienced the slightest flow of their blood, the case is very definite. No blood clots ever occur in regions other

than the 'intermediate cell mass,' within the embryo, and the islands on the caudal and ventral yolk surfaces which have been formed as described by the early wandering cells that migrate away from the caudal region of the embryo. All embryos whose history for lack of circulation throughout their existence is actually known show the blood pattern most consistently, there being of course a certain amount of variation in the extent and position of the yolk-islands but not enough in any case to confuse the problem.

These observations may readily be made by any observer, but can only be made in a reliable fashion with the high power microscope and strong condensers so that the light may be sufficiently regulated to observe the most transparent cells. The movements and differentiation of these cells should be carefully followed through every step in a number of cases, in order to fully appreciate the significance of their position and behavior.

The cells may be seen even with an ordinary binocular microscope to some extent, but the arrangements for light regulation and the magnification are insufficient for determining the important details. After the red blood cells have formed, they are readily located even with a low power yet such an examination could only determine their places of origin provided the embryo has been carefully watched with a high power magnification to make certain that it has had no blood flow.

The condition of the yolk-sac mesenchyme must be fully understood and must always be considered in interpreting the origin of blood-islands and clots. For example, clots seen at the venous end of the heart or on the extreme anterior surface of the yolk must be most cautiously considered, remembering the scarcity or even absence of the wandering mesenchyme in these regions. Clots in such places probably always result from a partial circulation of short duration and there is abundant evidence to support such a view.

Although the future red blood cells migrate upon the yolk in their early mesenchymal stages, after they once group themselves and differentiate into erythrocytes their powers of wandering become very much limited if they exist at all. I have never

seen anything to indicate that a fully formed erythrocyte was capable of automatic migration. Yolk-sac blood islands of all ages have been examined in great numbers, but never has an erythrocyte appeared wandering away from such an island into neighboring regions. This fact is most important in the study of the blood-islands in the non-circulating specimens.

When the slightest flow does exist for any length of time, there is a definite tendency, as mentioned above, for the blood to accumulate in certain sinuses and vessels. The positions of accumulation vary somewhat with the stages at which the circulation ceased, as well as the manner of stoppage of the flow, whether it was gradual or sudden.

When the circulation stops during early stages, there is a great accumulation or massing of the blood over almost the entire ventral surface of the yolk. In other words, there is a hemorrhage or bleeding into these spaces or vessels until no more blood is left in other regions of the embryo, the heart gradually becomes empty of corpuscles and no longer passes them along. The packing of the yolk vessels probably clogs or blocks the circulation so that it ceases. Again, the circulation may stop more suddenly and the venous end of the heart or the entire heart may be seen packed with corpuscles while the vessels immediately entering and leaving it are comparatively or entirely empty. In older embryos there is the tendency to accumulate red cells in the vessels of the head so that brilliant red clots are frequently seen in these positions.

In all cases it is interesting in these individuals in which the circulation has ceased at one or another period in development, and doubtless for different reasons in different specimens, to observe the way in which the blood sooner or later accumulates in one or another vascular space and does not remain uniformly distributed throughout the vascular system. Only when the heart is suddenly stopped and the blood quickly fixed by some strong killing fluid does one get a good pattern of the vascular system loaded with corpuscles throughout most of its extent. In rare cases, three during the present summer in some hundreds of embryos examined, will a specimen without a circulation at

the time observed show almost all of its vessels loaded with blood cells, and this is probably due to a slowing down gradually of the circulation on account of the heart itself which finally stops with the vessels in a balanced state.

The study of the yolk-sac in the living embryo enables one to observe every phase in the development of the red blood corpuscles from the early time when they wander as amoeboid mesenchyme cells to collect into groups of globular cells with short processes, the 'primitive blood cells' of descriptive histologists, to be later surrounded by vascular endothelium, and then to change from the globular wandering cells into the flattened ellipsoidal erythrocyte loaded with haemoglobin, and finally freely floating in the current of the blood stream. The fully formed corpuscles apparently become incapable of independently migrating even when not carried by the circulation.

DISCUSSION AND CONCLUSIONS

In the previous paper on the origin of blood and endothelium, a somewhat full discussion of the problems of blood formation in the teleosts and other vertebrates was entered into. A consideration of the questions of origin and development of vascular endothelium was also undertaken in the light of the results there presented and the more recent general literature bearing on this subject. The experimental results then contributed seemed in the light of the past literature to render highly probable, if not to actually prove, the polyphyletic origin of the various types of blood cells, as opposed to the now extremely improbable monophyletic theory of origin of blood cells and vascular endothelium. For a general consideration, the reader is referred back to these discussions.

A number of particularly significant points are brought out in the present study of the living normal and experimental embryos which bear directly on several of the past theories and speculations regarding the origin of vessels and blood. Only these special points will be briefly considered and analyzed at this time.

In the first place, the writer cannot resist the impulse to highly recommend that all students of haematogenesis and vascular origin spend some time at least in a study of living mesenchymal cells and their cytomorphosis. Such a study will soon convince one of the great disadvantages under which an investigator labors in attempting to solve the origin of blood from observations on dead material in serial sections. The problem becomes so simplified and devoid of laborious uninstructive technique that it seems almost superficial. One may learn as much from the living yolk-sac in an hour of careful study as in almost a week's perusal of sections. Most important is the fact that certain things may actually be seen to occur that sections could scarcely stimulate the mind to imagine. The only disadvantage is that the worker may be led to wonder whether so apparently simple a problem is actually of scientific importance. Fortunately this mental state is soon passed over on realizing the necessary care and precaution which must be taken in following the movement and changes in the living cells.

Each cell must be recognized as a living complex and the observer will realize the importance as well as the difficulties of thoroughly understanding and interpreting correctly its manifold changes and behavior. Material which to some extent allows such a study is often available. The Fundulus yolk-sac, however, is exceptionally adapted to this study on account of the beautiful simplicity of its structure, as well as the remarkable clearness with which each cell may be observed.

An investigation of the Fundulus yolk-sac readily supplies a crucial answer to the old question regarding the relation of the blood vessel lumen to other body cavities and spaces. Ryder ('84) was right in describing the blastocoel of the bony-fish as remaining an extensive cavity for some time. This is the space between the ectoderm and yolk and is identical and continuous with the cavity which arises very early beneath the blastoderm and above the yolk periblast. Agassiz and Whitman ('84), as well as Ryder ('84), Wilson ('90), and others, have identified this correctly as the cleavage cavity, the blastocoel. Later in development, the blastocoel extends over the yolk, forming the

space into which we have seen the free mesenchyme cells wander, and finally within this space groups of these cells form the yolk-sac vessels.

Wenckebach ('86) described very clearly the origin of vessels from the free mesenchyme within the segmentation cavity. My study of a somewhat similar yolk-sac confirms the main points brought out by Wenckebach and all serve as crucial facts in support of the early theory advanced by Bütschli ('82) in his "Die phylogenetischen Herleitung des Blutgefässapparates der Metazoen." Bütschli held that in the Metazoa the lumen of the blood vascular system was derived from the blastocoel. Later, Hubrecht ('86) supported the same standpoint from his studies on Nemertines. Hubrecht also found wandering cells playing an important rôle. Ziegler ('87) gives a most careful analysis of the continuity of the vascular lumen with the blastocoel in his studies on the development of the bony-fish.

The foregoing description and figures of the origin of vessels on the yolk-sac of Fundulus leaves no doubt that the vascular lumen in these animals, coenogenetically at any rate, is continuous with the blastocoel or primary body cavity and is in no way related to the coelom.

Almost twenty years ago, Felix ('97) advanced the opposing theory that the vascular lumen was really a localized or separate part of the secondary body cavity, or true coelom. The many decided objections to this theory from the standpoint of comparative anatomy, the presence of blood vessels before the acquisition of a true coelom in the animal kindgom, and the numerous embryological contradictions in its path were pointed out in the discussion of this matter in the previous paper.

Very recently Bremer ('14) has advocated the theory of the origin of vessels as parts separated from the coelomic cavity, or strands of cells from the coelomic epithelium. In the first place, the material on which his investigation was based, early human embryos, will scarcely permit such generalizations. At least more suitable material could be found for the analysis of this problem. Further than this, his consideration of the questions involved does not lead one to form a definite idea of

the exact direction he considers his evidence to lead. He credits Bütschli ('82) with having originated the coelom theory that accords, so he thinks, with his evidence. This is entirely incorrect, at Bütschli's theory is exactly on the other side. The morphology of the yolk-sac of the chick, sheep and numerous other animals, as the literature of the subject readily shows, is entirely out of accord with such speculations. The yolk-sac of the bony-fish shows this view to be really impossible and there should be no longer any doubt that vessels arise from loose and wandering mesenchymal cells in many animal species, and certainly not from ingrowths from the coelomic epithelium in any species.

The formation of vessels on the yolk-sac of the teleost further limits the generalization of the origin of larger vessels from capillary nets. Thoma ('93) in his masterly study of the vasculogenesis of the yolk-sac of the bird, held that "the first vascular spaces, the rudimentary capillaries, were formed by the secretory activity of the cells forming their wall." These capillaries formed an extensive net and the arteries and veins arose secondarily and differentiated from the capillaries on account of the flow of blood set in motion by the beat of the heart. The anlage of the vascular system was the capillary.

These principles of Thoma are not, however, applicable to the development of vessels in the embryonic bony-fish. The aorta arises as one or two vessels independent of any flow of blood or the existence of a capillary net. The first vessels on the teleostean yolk-sac are the large vitelline veins, as described by Wenckebach, and the median vitelline vein or the net of vessels in its place. These large important channels arise entirely independently and separated from the capillary net if such exists at the time. They also develop entirely independently of the blood flow, and not as a result of the pressure due to the heart beat. The capillaries and other vessels in many cases arise separately or away from these primary vessels and finally connect with them in a way similar to the connection formed between the Randvene and the venous end of the heart. Other capillaries and small vessels arise as buds or sprouts from the first formed veins on the yolk-sac.

More recently Evans ('09) with a very efficient and delicate method of injection has shown many of the larger intra-embryonic vessels of the chick embryo to develop from a foregoing capillary network. He found the same principles of development that Thoma had observed on the yolk-sac to hold for the development of certain vessels within the embryo. These principles of vascular development Evans thought applied to vertebrates generally, but such is certainly not the case, the large vessels of the teleost embryo arise directly from associated mesenchymal cells and are not preceded by a capillary net.

His evidence was derived from injected vessels and could not justify the statement, p. 512, of "The presence *always* in the embryo of a united vascular system"—"a single branched endothelial tree." Such a united vascular system is rather late in its establishment in the fish embryo and there is no "single branched endothelial tree" present when the first blood vessels are formed. These facts may readily be demonstrated on the living embryo by direct observation.

The vessels of the yolk-sac and several of the larger vessels within the body of the teleostean embryo form independently of any foregoing capillary network. *In the teleost, then, the anlage of the vascular system is not the capillary but the mesenchymal cells which directly give rise to the chief arteries and veins, as well as to numerous groups of isolated capillaries.* Other small vessels and capillaries grow as branches or sprouts from the arteries and veins.

Thoma advanced three laws for the formation and growth of vessels. The first law was considered the most important, but rather destructive evidence is thrown against it by the present study. The law may be stated as follows: "The increase in the size of the lumen of the vessel, or what is the same thing, the increase in the surface of the vessel wall, depends upon the rate of the blood current." The vessel increases in size when the rate is exceeded, becomes smaller when the rate is slowed, and *disappears when the flow is finally arrested.* Thoma ('96) states: "This law which brings the growth of the surface of the vessel into dependence upon the rate of the flow of the blood is, I con-

sider, the first and most important histo-mechanical principle which determines the state of the lumen of the vessel under physiological and pathological conditions."

Thoma again states this principle thus: "In development the vessels in which the blood stagnates degenerate, and in those in which the rapidity is too great the lumen in enlarged."

No one could fail to admire the splendid manner in which Thoma attacked the problem of vasculogenesis in the yolk-sac of the bird, or the ingenious way in which he attempted to analyze the problem and deduce his three laws of histo-mechanical processes. Yet the "First and most important histo-mechanical principle" does not apply to the development of vessels in Fundulus embryos where there is no circulation of the blood. *Many vessels grow in size or "what is the same thing, show an increase in the surface of the vessel wall" without any "rate of the blood current."* The aorta in old embryos that never had their blood to circulate and in which the heart is actually a solid string of tissue, grows and attains a well developed lumen and a wall lined with endothelium and surrounded by concentric fibers of connective tissue as is shown in figure 49 in the previous paper, drawn from such a specimen. This vessel is very slow to degenerate, in fact, it shows no sign of degeneration and actually persists as long as the embryo is able to exist without a circulation, for 30 days or more. Vessels also develop upon the yolk-sac without ever having a fluid to circulate through their lumen. *Other vessels are developed around the blood cells of the intermediate cell mass and the yolk-sac islands and in such vessels 'the blood stagnates' from the first yet the vessels degenerate very slowly, in some cases scarcely at all.*

In still other cases the blood may have circulated for a while and then stopped for some time, but the vessels do not degenerate as is proven by the fact that the circulation through them may again be resumed. Such a sequence of events may be occasionally observed in the experimental embryos. *The function of the vessel as a blood conductor, therefore, seems in these embryos of Fundulus,* both the early normal and those without a circulation of the blood, *to have little if anything to do with its early development and not much effect on its ability to survive.*

On the other hand, when Thoma has a straight normal case, the lumen may readily be seen to increase in size with the rate of flow. Yet in the entire absence of this action the vessel is still capable of increasing in size and it becomes questionable whether the rate of flow is ever an actual cause of size increase beyond mechanical stretching.

These facts are most significant in a consideration of the influence of function on growth and development, auto-differentiation. Here it is seen that the structure both grows and develops in entire absence of its function. In normal cases the function of the vessel as a blood conductor exerts more likely a physical rather than a biological effect on development.

Thus the development of blood vessels on the yolk-sac of the living Fundulus embryo proves that the capillaries are not universally the anlage of the arteries and veins, but that these larger vessels may arise directly from wandering mesenchyme cells. Such arteries and veins may grow and persist without a circulation of the blood through their lumen and even though stagnant masses of corpuscles may crowd the vessel cavity.

The development of vessels in Fundulus also directly disproves the claims made by Sobotta ('02) that the vessels in the teleost grow over the yolk entirely as branches from those near the embryo and without the wandering cells taking part. This assumption is probably due to the difficulty of estimating the part played by wandering cells from a study of serial sections. From a study of the living yolk-sac there is no question of the major part played by the wandering cells in the origin and formation of vessels on the yolk.

Sobotta also advances the opinion that the entire yolk vessels may sprout from the heart. This is much of the same nature as the ingrowth or parablast theory of His ('75), and is obviously defeated by the same array of facts which long ago relegated the parablast theory to a place of mere historic interest, in spite of the fact that it is so often revived for literary reasons.

Finally, we may consider the study of the developmental products of the early wandering mesenchymal cells on the yolk-sac of the Fundulus embryo as a problem of cell lineage carried

to its ultimate end. The primordial mesoderm cell or cells carry within their bodies all the potentialities of the mesoderm and may give rise to a series of cells which are capable of developing muscle, cartilage, bone, connective tissue proper, blood cells, vessels, etc. Yet after a few cell generations the individuals in the series derived from these early cells containing all the mesodermal potentialities no doubt become somewhat limited as to their potentialities. In a certain generation there may be definite cells more or less generally distributed which possess the capacity to give rise to muscle cells, but to no other type of mesodermal tissues. Still later in development these cells may come to be even more limited in their developmental capacities and thus have the power to produce only a certain type of muscle cell and no other type.

Collections of such cells would then be designated embryologically as the anlage of striated muscle, smooth muscle, or heart muscle as the case might be Yet it is not to be forgotten that at this stage there might be really no means of distinguishing between the several different types of mesodermal cells.

Limitization of potentialities in the individual cells has apparently reached a comparable stage just about the time when the mesenchymal cells begin to wander upon the yolk-sac of Fundulus. We have seen these cells as they wander out and have noted how very soon they may be separated into four distinctly different types, and following the development and behavior of these types it has seemed evident that they are entirely separate and do not intergrade or transmutate. The black chromatophore does not change its nature or divide off other cells which become different in type from the parent cell. Neither do the endothelial cells lining the vessel walls change into chromatophores or into erythroblasts, or vice versa.

From all the observations on these yolk-sacs we must conclude that the four types of cells described above have developed from four different anlagen, although these anlagen were not necessarily localized groups of cells, but were diffusely scattered mesenchymal cells capable of developing into a definite product, either normal or abnormal, depending upon the nature of the

developmental environment. Therefore, the four distinct mesenchymal anlagen each give rise to a perfectly typical and distinct cell type although all develop in, as far as one can judge, an identical environment, the cavity of the yolk-sac between the ectoderm and the periblastic syncytium. The differences among the four cell types produced are from the standpoint of our present knowledge in all probability due to the potential differences among the apparently similar mesenchymal cells from which they arose. The four types including endothelial cells and erythrocytes we must consider from an embryological standpoint as arising from different mesenchymal anlagen.

SUMMARY

The yolk-sac of the teleost egg is a most beautiful object for observing the movements and migrations of cells in the developing embryo. Such a yolk-sac has only one really definite continuous membranous cell layer, the ectoderm; a true endodermal layer is absent, though a superficial syncytium, the periblast, fuses with the actual yolk surface. The mesodermal layer is represented by numerous separate wandering mesenchymal cells. These freely wandering mesenchymal cells may be clearly observed through the perfectly transparent ectoderm as they move over the surface of the periblast.

The present contribution attempts to give a full account of the movements of the mesenchyme cells and their manner of development and differentiation in the yolk-sac. Observations have been made on the normal embryos from the earliest stages at which the mesenchyme wanders out upon the yolk up to the late embryo in which a complex vitelline circulation is fully established, and all of the products of the yolk mesenchyme completely differentiated. The study has been greatly facilitated by a comparison of the normal embryos with specimens in which the circulation of the blood was experimentally prevented from taking place. In such specimens the cells on the yolk-sac never became confused or contaminated with other cellular elements introduced by the circulating blood. The

wandering cells may thus be completely followed through all stages in their isolated position.

The behavior of the migrating cells impresses one with the very important rôle of such elements in the formation of tissues and organs, particularly the blood vessels and certain blood cells. The observer is also struck by the fact that such phenomena are extremely difficult if not actually impossible to interpret from a mere study of dead specimens cut in serial sections. Of course the study of sections greatly aids the observations on the living, and but for the fact of a long acquaintance with the Fundulus yolk-sac in sections, the writer would have found it much more difficult to identify many of the cells in life.

The results of this investigation of wandering mesenchymal cells may be summarized as follows:

1. The wandering cells begin to migrate away from the embryonic shield or line of the embryonic body at an early period, when the embryo is about 40 hours old, the germ ring having almost completely passed over the yolk sphere to enclose its vegetal pole. The cells migrate away chiefly from the caudal end of the embryo, only a few wandering out from the head region. The regions of the yolk-sac thus suggest an area opaca about the tail end and an area pallucida around the neighborhood of the head.

All of the cells wander into the so-called subgerminal cavity, the space Wilson ('90) and others consider a late stage of the segmentation cavity, between the yolk-sac ectoderm and the periblast syncytium.

When the cells first appear they are all closely similar in shape and about the same size. Very soon, however, they begin to exhibit certain differences. Many become elongate spindle cells with delicate filamentous processes, sometimes producing a stellate appearance. Others are more amoeboid in shape with conical pseudopod-like processes which are constantly being thrown out at one place and withdrawn at another.. Still a third class of cells appear somewhat later than the other two; these are more circular in outline with short thick pseudopods and are more slowly moving.

The movements of these extremely numerous cells and their changes of position may be readily followed with a high magnification. In embryos of about 60 hours, still some time before the heart begins to beat or the blood to flow, four clearly distinct types of cells may be recognized among these originally similar mesenchymal cells, and the further history of the four types has been completely traced.

2. The amoeboid cells with conical pseudopod-like processes shortly after 60 hours begin to show an accumulation of pigment granules within their cytoplasm. Just at this time they are seen to be of two distinct varieties, one depositing a black and the other a brownish red pigment.

The black chromatophore increases rapidly in size and by the end of the third day becomes an enormous amoeboid body wandering over the yolk. These cells are attracted to the walls of blood vessels and plasma filled spaces, such as the pericardial cavity becomes in specimens without a blood circulation. When the embryo is five days old the chromatophores are abundantly arranged along the walls of the vitelline vessels, but the pigmented cells are distinctly separate. After this time neighboring cells begin to fuse along their adjacent borders and large pigment syncytia are formed which completely surround and ensheath the vessels. A single syncytium is often of considerable extent, as shown in figure 15.

The brown chromatophores have a somewhat different history. They never become so massive as the black, and their processes are more delicate and graceful in appearance. Yet these cells also attain a large size and in embryos of 72 hours are scattered over the entire yolk-surface. After the third day when the blood begins to flow in the yolk vessels, the brown chromatophores likewise become attracted to the vessel wall. These exquisitely branched cells apply themselves to the wall of the vessel and may often completely surround it, as shown in figure 17. This type of chromatophore, however, always maintains its cellular individuality and never fuses with other cells to form a syncytium as is the case with the black type.

The function of the chromatophores on the yolk-sac is most difficult to decide, but one thing is certain, they never become

changed into any type of blood cell. The brown chromatophore in early stages may accidentally reach the blood current; it then becomes spherical and may be readily observed for a long time on account of its huge size as compared with the blood cells. It never, however, changes in type.

In specimens without a circulation of the blood both types of chromatophores arise in a normal manner and differentiate normally. Their arrangement along the vessel walls fails to occur and they remain scattered over the yolk or collected about the plasma filled spaces. The heart in such embryos is sheathed with pigment, while the normal heart never has a chromatophore on it.

3. The elongate spindle cells with their delicate filamentous processes are small in comparison with the two chromatophore types. These spindle cells retain in general their original appearance, but their behavior is most important. In embryos of about 48 hours such cells aggregate into certain rather definite groups; later, these groups become more linear in shape and finally these lines of cells arrange themselves so as to form tubular vessels. Several of the larger vessels arise independently upon the yolk, and certain ones of them later become connected with the venous end of the heart, while in all cases capillary nets which also arise independently become connected with the larger vessels. These processes may actually be followed through every step in the living yolk-sac.

The wall of the early vessels is very irregular with spaces existing between the component cells. Corpuscles are often caught in these spaces or entangled in the filamentous processes of the endothelial cells. Such conditions in sections would appear as though the corpuscles actually formed a part of the endothelial wall and might incorrectly be interpreted as endothelial cells changing into blood cells. Nothing has been seen in the living embryos to indicate that an endothelial cell has the power to produce a blood cell or to change into a blood cell of any type, but much has been seen to the contrary.

The generalization particularly made by Thoma ('93) that larger vessels arise from a net-work of capillaries is not true for the large vitelline vessels on the fish yolk-sac. It is also found

in the specimens without a circulation of the blood that the vessels arise and increase in size and persist for a long time without ever experiencing any effect of the blood current upon their walls. In many embryos the circulation after having begun may stop for a time and then later be reestablished, the vessels having persisted in a normal condition. Thoma's so-called laws of vessel formation are, therefore, rudely violated by the development of the vascular system in these embryos.

The vessels arising from independent mesenchymal cells in the space of the blastocoel in the teleost yolk-sac entirely overthrow any notion that vessels arise ontogenetically as portions of the coelomic epithelium. The vascular lumen is originally continuous with the primary body cavity, the segmentation cavity, and never with the secondary body cavity or coelomic cavity.

4. The fourth class of cells wander out from the embryonic body somewhat later than the three former types. These are small globular cells with short pseudopod-like processes. They move very slowly, but finally collect into groups on the posterior and ventral regions of the yolk-sphere.

The round cells wander away only from the caudal region of the embryo and probably are derived from the so-called intermediate cell mass which is the anlage of the red blood corpuscles in the fish embryo.

The groups of round cells are slow in their differentiation but just before the circulation of the blood begins, they are seen to be circular erythroblasts. The observer may follow the disappearance of the islands of cells one by one as they are enclosed by the vessels and swept into the circulating stream. About the fifth day these circular erythroblasts become flattened ellipsoidal erythrocytes filled with haemoglobin, the typical red blood corpuscle. The complete change from wandering more or less spherical mesenchymal cells into typical haemoglobin bearing corpuscles may be followed in the living yolk-sac.

In several instances the entire body proper of the embryo failed to develop or else degenerated very early, yet the yolk-sac formed or persisted with numerous blood islands fully differentiated.

The embryos in which there has been no circulation of the blood form the blood islands from the wandering cells on the yolk-sac, and the constituent elements of these islands differentiate perfectly and may maintain their red color for many days. Yet they never leave the locality in which they have differentiated. The fully formed red blood corpuscles have little if any power of migrating. When the observer can be positive that the blood has never circulated, and this requires very consistent watching, the islands of the yolk are always limited to certain regions and never occur so far anteriorly on the ventral surface of the yolk as to reach the venous end of the heart.

5. On the yolk-sac of Fundulus embryos one thus finds four distinctly different products differentiating from the apparently similar wandering mesenchymal cells. The environment in which the four types differentiate is identical as far as is possible to determine, and the only explanation of their various modes of differentiation is that the original mesenchymal cells that wandered out were already of four potentially different classes. These differences in potentiality within the early cells gave rise to the four different directions of cytomorphosis in one and the same environment. The four resulting types of cells are then in an embryological sense derived from different mesenchymal anlagen.

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