

MITOSIS IN THE NEURAL TUBE

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FOUR PLATES (TEN FIGURES)

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The writer's interest in the mitoses of the neural tube arose as a result of finding, in the neural plate and early neural tube stages of the chick, appearances that seemed not in harmony with the usual accounts. The neural plate of the chick is obviously a single layered columnar epithelium, whose cells are attached to each other at the free surface by terminal bars. At the basal end the cells appear not to be attached to anything, and their basal ends are not evenly lined up with each other. When a cell of the neural plate divides, it rounds up, and the act of rounding up carries the cytoplasmic mass and the nucleus toward the place where the cell is attached by the terminal bars, that is, toward the free surface of the epithelium. After division the two new cells are found to be attached to each other and to the surrounding cells by terminal bars, and stages can be found in which the cells are elongating until they again become columnar elements in the epithelium. As the neural plate of the chick thickens and passes into the neural tube, the epithelial cells become longer and thinner, and their nuclei arranged in more and more layers, yet the appearances are still such as to permit the interpretation that nuclei are drawn to the lumen before dividing, and pass away from the lumen after a division.

¹ This work was done in the laboratory of the department of anatomy, University of Kansas.

THE INTERNAL BOUNDARY OF THE NEURAL TUBE

As the attachment of the neural tube cells to each other at the surface bordering the lumen appears to play an important part in the mechanics of the development of the neural tube, the internal boundary of the tube will be described in detail.

The internal boundary of the neural tube was described by His (1889) as formed by an internal limiting membrane consisting of a feltwork of fibrils into which pass the cytoplasmic fibrils of the spongioblasts. This membrane has been accepted by later writers, and the cells of the neural tube are generally described as ending internally in such a membrane. According to the writer's observations of the chick and pig, no such membrane is present. The cells end internally in the structure that is typical of a columnar epithelium, i.e., each cell is bordered at the internal end by terminal bars which unite the adjoining cells to each other.

The terminal bar net in the ventricular surface of the neural tube may be clearly seen in sagittal sections stained heavily with Heidenhain's iron hematoxylin. Cutting in the sagittal plane permits one to find places in which an area of the ends of the cells has been sliced off, and in these one may see the terminal bars unobscured by the stain of the underlying nuclei. The terminal bars mark off the internal wall of the neural tube into polygonal areas (fig. 4). These apparently were seen by His, and were represented in a figure (1890). An earlier illustration by His (1889) probably also represents these structures. His, however, interpreted the bars as a network of cytoplasmic fibrils spreading out from the ends of the spongioblasts to pass into the internal limiting membrane. Leboucq ('09) described and correctly interpreted the terminal bar net as seen in the developing retina, which is, of course, a derivative of the neural tube. Leboucq also described and pictured the early retinal cells and their relations in a manner which agrees essentially with the present writer's concept of the structure of the neural tube.

In most of the terminal bar areas, as seen in sagittal sections of the chick or pig neural tube, there appear two small stained bodies, generally of slightly unequal size, which lie close together and present the typical appearance of diplosomes. The diplosomes usually lie at a slightly different focal level than the terminal bar net, as they are in that part of the cytoplasm which projects beyond the frame of the terminal bars. Occasionally only one centriole is present, and when this is the case it is markedly larger and darker staining than the diplosomes. In a few areas no central body is present and, when the section includes the nucleus of the cell, these areas are seen to be the ends of cells approaching or in mitosis.

In transverse sections of pig (fig. 3) and chick embryos the ends of the cytoplasmic columns of the neural tube cells are found to be evenly lined up with each other, each cell having usually a simple convex end, though the end may have a bow-like curve, or be flat, or even concave. Between the ends of the cells the terminal bars appear as stained dots, usually oval or elongated, but sometimes cross-shaped or triangular. Immediately under the ends of the cells are the diplosomes, usually in the small body of cytoplasm projecting beyond the terminal bars. In the internal end of the cell is a structure which has the appearance of a stained membrane stretched across the end of the cell between the terminal bars, and separating the part of the cytoplasm which usually contains the diplosome from the rest of the cell. The actual structure of this apparent membrane probably cannot be determined from these cells because of its thinness, which brings it close to the limit of microscopic visibility. The possibility has been considered that it may be an illusory appearance. Since the ends of the neural tube cells are only about 2μ across in the neural tube of a 10-mm. pig embryo, it was at first supposed that when the internal part of the cell is in focus the terminal bars which surround the end of the cell might be sufficiently visible to give the impression of a structure within the cell. However, the membrane is

entirely missing from an occasional cell, although the terminal bars are present. Moreover, the structure may be uniformly visible while one focuses up or down through the end of a cell, while several small particles of the same order of diameter as the terminal bars come in and out of focus in succession.

A probable explanation of the apparent membrane is available from the observation of epithelial cells larger than those of the neural tube. In the epithelial cells of the blastula of the toadfish (*Opsanus tau*) there appears, stretching across the ends of the cells between the terminal bars, a network of cytoplasmic fibrils. When the blastula has been stretched between the points of two needles before fixation, these fibrils appear in sections to have a very straight path from one terminal bar to another, as if they had been pulled taut by the stretching, while in a blastula fixed without stretching the fibrils have a somewhat wavy course. These fibrils have been seen in toadfish blastulas sectioned in a plane tangent to the blastula surface, as well as in transverse sections, and they have been observed in living cells in the blastula of *Fundulus*. Similar fibrils passing across the ends of cells between the terminal bars have been observed in the epithelial cells of the intestine of a 10-mm. toadfish embryo, and in the medullary plate cells of chick embryos. This latter is especially significant, since the cells in question give rise to the cells of the neural tube. The neural tube cells of the chick have the apparent membrane, just as do those of the pig, and its position and relations are similar to those of the fibrils across the ends of the medullary plate cells. The reduction in diameter of the columnar cells between the neural plate and neural tube stages is such that the fibrils could hardly be separately visible in the ends of the neural tube cells, but would blend into an apparent membrane, such as is actually observed there.

While evidence from analogy with other epithelial cells is, of course, inconclusive, I am inclined to regard the structure in question as a network of fibrils, of such dimensions in the

neural tube cells that the fibrils are not separately visible. It is evidently not an impervious structure, as the diplosomes frequently lie deep to it, rather than in the end of the cell.

As the structure just described has not, so far as the writer is aware, been previously described or given any name, I shall call it the terminal web.²

MITOTIC STAGES IN THE NEURAL TUBE OF THE PIG

In the phases of mitosis to be described the appearance of the internal ends of the cells undergoes changes. The diplosomes are absent from the ends of cells approaching or in mitosis. The single large centrioles noted above are found in the ends of cells whose nucleus is being reconstructed after mitosis. The terminal web disappears during mitosis.

The typical resting cell of the neural tube of the pig embryo is shown in figure 1a. It is, of course, only rarely that the entire length of the cytoplasmic strands of such a cell lies in one section. The cell shown is one of a small group of cells which happened to lie sufficiently parallel to the plane of the section that they could be traced throughout their entire length. The end of the cell bordering on the lumen (the upper end in the figure) has terminal bars and diplosomes as described above. The diplosome in this cell was so turned that it does not appear as a double body. From the internal end the cytoplasm narrows, as it passes outward, into a strand of varying thickness. From sagittal sections it is evident that this strand, as well as that which passes from the nucleus to the external limiting membrane, is of irregular cross section, frequently ribbon-like rather than cylindrical. The nucleus of the cell is oval, with a clearly marked nuclear membrane, and an internal reticulum with several large clumps of chromatin and numerous smaller chromatin clumps at the intersections of the threads. Around the nucleus is a layer of cytoplasm, sometimes not clearly visible, but frequently thicker than that pictured, tapering out into the internal and external cytoplasmic strands. At

² The writer is indebted to Dr. H. C. Tracy for the suggestion of this name.

the periphery the external strand widens slightly as it reaches the external limiting membrane.

Among the cells of the neural tube there may be seen nuclei (fig. 1 b) which are sharply pointed, the point being directed toward the lumen. Figure 1 b shows only the nucleus and surrounding cytoplasm of such a cell. The cytoplasmic strands passing to the lumen and to the external limiting membrane are similar to those of the cell shown in figure 1 a. The structure of the nucleus is still that of an interkinetic nucleus, but, besides the pointed form, it differs from the majority of the interkinetic nuclei in that it is larger and has a more basophilic ground substance. A fibril is usually visible in the internal cytoplasmic strand, attaching to the tip of the nucleus. Between cells of this type and those shown in figure 1 c there is a continuous gradation.

Cells of the type shown in figure 1 c are found only with the nucleus relatively close to the lumen. The figure shows the entire length of the short internal cytoplasmic strand which contains, as is usual, several fibrils. The external cytoplasmic strand is not shown in this and the following stages, as it can seldom be traced for more than a short distance before it passes out of the plane of the section. The nucleus is still somewhat pointed at this stage. The chromatin of the nucleus has begun to clump into a number of rounded bodies suspended in a network of threads. The basophilia of the nuclear ground substance is less pronounced than in stage *b*.

A closer approach of the nucleus to the lumen, together with an increased distinctness of the chromatin masses, brings the cell to the stage shown in figure 1 d. The chromatin has assumed the form of chromosomes, which appear as oval bodies. Some are suspended in the reticulum within the nucleus, but increasing numbers of them lie against the inside of the nuclear membrane.

Stage *e* shows the chromosomes fully formed as elongated bodies lying against the nuclear membrane and joined together by very fine fibrils. The basophilia of the nuclear ground

substance has decreased continually as the chromosomes form, and the inside of the nucleus now appears quite clear and empty. There is no spireme stage; the chromosomes of a slightly later stage shown in figure 1 e' have reached their maximum elongation.

Figure 1 f shows a cell in which the nuclear membrane has disappeared, leaving the chromosomes free within the cell and irregularly arranged. The cytoplasm about the nucleus is now relatively abundant, due, evidently, to the cytoplasmic strands of the cell having been drawn in as the cell prepares for division. Cells of this stage of nuclear arrangement may have a spindle-shaped cytoplasmic body, or may still retain a peripherally directed cytoplasmic strand. It is evident that as the cell approaches mitosis there is a tendency for it to round up and draw in the cytoplasmic columns. The peripheral strand may have been completely drawn in by the time the cell reaches stage *f*, or, on the other hand, a peripheral cytoplasmic projection may be present as late as the metaphase, stage *h*. In the anaphase the cell is invariably rounded and without any cytoplasmic projections.

Stage *g* shows the chromosomes arranging themselves in the equatorial plane in preparation for metaphase. The cytoplasm now has a reticular appearance after fixation—a feature that has become increasingly prominent as the cell approaches division. From analogy with other cells, it is probable that the diplosome, which no longer appears in the end of the cell, has migrated to the poles to form the centrioles. Stages of this migration have not been identified with certainty, as the cytoplasm contains a number of granules which might easily be mistaken for migrating centrioles.

The direction of the spindle in metaphase is almost always paratangential, or nearly so; that is, a line joining the two centrioles is nearly parallel to the inner surface of the neural tube wall. In a few cells in metaphase the spindle is more or less oblique to the surface, and in a very few it is at approximately right angles to the surface. The appearance of cells in telophase, however, leads me to believe that in

such cases a rotation brings the spindle to a paratangential direction before the completion of the division, since the division of the cytoplasm appears always to take place with the plane of division at right angles to the epithelial surface. If divisions take place with the division plane parallel to the epithelial surface, so as to form two daughter cells of which one borders the lumen and the other does not, such divisions must be very rare. Certainly, they are not sufficiently numerous to account for the rapid appearance of the early free neuroblasts in the margin of the neural tube.

Figure 1 i shows the completion of anaphase. The oval or rounded form which these cells invariably take, and the fact that they must push aside the adjacent closely packed cells to take such a form, suggests a considerable turgidity of their cytoplasm.

Figure 1 j shows a cell in telophase. The mate to this cell lies at a deeper focal level. Remains of the spindle fibers pass from one group of chromosomes to the other. Cells of approximately stage *j* are frequently ruptured in the preparation of the material, in specimens which otherwise show no signs of damage. This may be due to handling the embryo before fixation, or possibly to osmotic changes in fixation. In many pig embryos there appear 'puffs' of granular material projecting into the lumen of the tube, and these are found to proceed from the ends of cells whose nuclei are as represented in figure 1 j. In some embryos such ruptured cells are not present at all, but when they are present they are apt to be found in considerable numbers. The loss of cytoplasm from the cell may be so great that hardly more than the chromosomes are left, and these are then clumped into a thin cylindrical mass. Apparently the cells of stage *j* are decidedly more fragile than cells of other stages.

Stage *k* shows a pair of cells in which the chromosomes have begun to take an arrangement suggesting in size and shape the nucleus they are to form. There now appear again the fine fibrils connecting them together, as in the prophase. The remains of the spindle fibers are seen here passing from

the chromosomes to the line of junction between the two cells. This and similar pairs of cells give strongly the impression that the fused and swollen centers of the spindle fibers may develop into the terminal bars.

Stage *l* shows the reappearance of the nuclear membrane, which has become more clearly marked in stage *m*, as the nucleus begins to move away from the internal surface of the neural tube wall. The fibrils between the chromosomes are clearly visible in these stages, and the chromosomes lie just under the nuclear membrane. The cytoplasm here, as in all stages following the metaphase, has a granular rather than a reticular appearance, though fibrils are visible within it. Prominent cytoplasmic fibrils from the nucleus to the internal end of the cell are a frequent, though not a universal characteristic of the stages in which the nucleus is beginning to move away from the lumen. These fibrils sometimes appear to attach to the terminal bar at the central end, sometimes to a single large centriole. The chromosomes of stage *l* and to a lesser degree of stage *m*, frequently have the appearance of bands passing around the nucleus transverse to the length of the cell. Cells of this stage may be easily distinguished from the somewhat similar prophase stages *d* and *e* by their much smaller nucleus, as well as by their granular cytoplasm.

From stage *m* the nuclei pass to the interkinetic form, passing through transition stages of which figure 1 n is representative. These transitions to the interkinetic nucleus do not border immediately on the lumen of the neural tube, but they are found only in the inner part, approximately the inner fourth, of the neural tube wall. The chromosomes in them gradually lose their compact form and a reticulum develops within the nucleus, within which clumps of chromatin appear. The nuclear ground substance has again become basophilic. The basophilia begins to be noticeable in the stage at which the nuclear membrane first reappears.

In order to confirm and complete the picture of the cycle of mitotic changes just presented, measurements were made

of the dimensions and distances from the lumen of interkinetic nuclei, and of nuclei in the various stages of mitosis. The measurements were made with an eyepiece micrometer, under a magnification of 1800. The length and diameter of the nucleus, and its distance from the lumen, measured along the line of the cytoplasmic strands from the nearest point of the nucleus, was recorded. For the stages in which no formed nucleus is present, the dimensions of the cytoplasmic mass were recorded, excluding the thin peripheral cytoplasmic process when this was present. All measurements were made from the cervical region of the same pig embryo from which the drawings of plate 1 were made. These were confined to the alar plate in order that the cells measured might be a more homogeneous group, since the basal and alar plates are not in the same stage of development at the same time. Thirteen sections (consecutive, except for the omission of one damaged section) were used in the measurements. All the cells of the alar plate which showed any departure from the interkinetic stage were classified according to the stage in plate 1 which they most closely resemble, and their measurements recorded. By measuring all the nuclei in mitosis in a series of sections it was hoped to avoid any possible effect of unconscious selection. To find the dimensions of the interkinetic nuclei measurements were made, in one section, of twenty-five nuclei in the inner third of the wall of the neural tube, twenty-five in the middle third, and twenty-five in the outer third. The measurements were tabulated, the nuclei of stage *b* being separated into three groups according to their distance from the lumen. The mean measurements for each stage were calculated, and, where it would be significant, the probable error of the mean. The average measurements so obtained are presented in table 1.

It will be noted that the average size of the interkinetic nuclei is greater as they are farther from the lumen, and that the pointed nuclei of stage *b* are larger as they are situated closer to the lumen. As regards dimensions and distances from the lumen, the stages *k*, *l* and *m* in which the

nucleus is being reconstructed pass without a break into the small interkinetic nuclei near the lumen. (Nuclei resembling stage *n*, not being sufficiently clearly distinguishable from the interkinetic nuclei, were not considered a mitotic stage in making the measurements.) Similarly, the pointed nuclei form a series as they approach the lumen, which in point of nuclear size and form, and basophilia of the nuclear ground substance, passes without a break into the stages *c*, *d* and *e*

TABLE 1
Measurements, in microns, of nuclei in the neural tube of a pig embryo

STAGE, BY PLATE I	NUMBER MEASURED	AVERAGE DIAMETER —NUCLEUS	AVERAGE LENGTH —NUCLEUS	AVERAGE DISTANCE FROM LUMEN	VOLUME, CUBIC MICRONS
a ₁	25	4.44±0.064	8.68±0.145	Inner 1/3 of wall	98.7
a ₂	25	4.88±0.088	8.74±0.165	Middle 1/3 of wall	110.7
a ₃	25	5.11±0.060	9.00±0.139	Outer 1/3 of wall	127.5
b ₁	47	5.57±0.080	11.6 ±0.198	70.3	157.5
b ₂	43	5.76±0.074	12.2 ±0.171	45.9	190.1
b ₃	38	5.90±0.058	13.0 ±0.153	26.1	217
c	46	6.35±0.083	12.2 ±0.131	14.5±0.706	253
d	28	6.53±0.095	11.0 ±0.190	8.3±0.498	267
e	35	7.03±0.113	10.6 ±0.197	6.6±0.533	281
		—cytoplasm	—cytoplasm		
f	9	8.55±0.130	22.3 ±0.828		
g	44	8.83±0.095	21.2 ±0.379		
h	26	8.46±0.149	18.8 ±0.516		
i	6	9.65±0.271	15.9 ±1.22		
j	23	7.75±0.157	21.2 ±0.785		
		—nucleus	—nucleus		
k	25	3.10±0.092	5.55±0.122	4.7±0.154	31
l	24	4.05±0.124	6.12±0.124	5.3±0.253	50
m	100	4.47±0.059	7.52±0.097	7.6±0.273	84

leading up to division. These facts would seem to indicate that as the nuclei grow after a division they are migrating, first away from the lumen, and later toward the lumen. The simple interkinetic nuclei of stage *a* are passing away from the lumen as they increase in size. When they are about to undergo mitosis they are drawn back to the lumen, assuming the pointed form of stage *b*, and continuing to increase in size. They then pass through the mitosis and again recede from the lumen during the next interkinetic stage.

The stages of division and the writer's concept of the accompanying migration are shown diagrammatically in figure 8. In this plate all dimensions of nuclei and distances from the lumen are drawn to scale, according to the average measurements given in table 1.

The measurements of the linear dimensions of the nuclei, in table 1, and the drawings in figure 8 do not show adequately the changes in volume of the nucleus, since the volume varies as the cube of the linear dimensions. In order to determine the volume changes, models were made of the nuclei of the various stages in plasteline, with dimensions 5000 times the mean dimensions given in table 1. These models were weighed and the weights reduced to volumes of the nuclei, in cubic microns. These volumes are given in the last column of table 1. The models are illustrated in figure 9. It will be seen from the table that the nucleus which first reforms after a division, stage *k*, increases in volume more than sevenfold before it passes into the early prophase, stage *c*, and that just before the nuclear membrane disappears, stage *e*, the nucleus has reached a volume more than nine times that of stage *k*. It is likely that the increase largely represents an absorption of water.

MITOTIC STAGES IN THE NEURAL TUBE OF THE CHICK

The mitotic stages in the neural tube of the chick were studied from preparations of chicks of $3\frac{1}{2}$ and 5 days. The drawings in figure 5 and the measurements in table 2 are from a $3\frac{1}{2}$ -day specimen. Except in minor points, the process of division follows that described for the pig embryo, and so can be described more briefly.

The structure in which the cells terminate at the lumen of the neural tube is the same in the chick as in the pig. In sagittal sections there can be seen the same polygonal areas bounded by terminal bars, with a diplosome in the end of most of the cells. In sagittal sections, however, the diplosomes always appear as a single body, since their two elements are placed in a line at right angles to the epithelial surface.

In transverse sections the diplosomes show as two granules. The cut ends of the terminal bars, the terminal web, and the projection of the cytoplasm beyond the terminal bar net appear as in the pig embryo.

The stages of mitosis in the chick neural tube are shown in figure 5. Stage *a* shows the nucleus, with short sections only of the external and internal cytoplasmic strands of the interkinetic stage. Stage *b* is the pointed type of nucleus, which in the chick has a less smoothly tapering point than in the pig embryo. The metaphase spindle is nearly always parallel to the epithelial surface, as in the pig embryo, and the division of the cytoplasm appears invariably to be made by a division furrow which cuts into the cytoplasmic mass from the surface farthest from the lumen, as is the case in the pig. The beginning of the cytoplasmic division is shown in stage *j*, and stage *k* shows it nearly completed. The spindle fibers are crowded toward the lumen and the dark staining centers of the fibers are crowded together against the lumen surface as division proceeds. The centers of the fibers appear to go into the formation of the terminal bar between the two new cells.

The chromosomes at the end of anaphase lie approximately in a plane, so that the appearance of the cells of stage *k* varies, as one sees this group of chromosomes from the surface or the edge of the plane. In the pair shown in figure 5 *k* one is so turned that the surface view of the chromosome group is shown, while in the other cell the group of chromosomes is seen from the edge. Further variation in the appearance of the cell is due to the fact that when the chromosomes have reached the stage shown in figure 5 *k* the extension of the cytoplasm into the peripheral cytoplasmic strand and the recession of the nucleus from the lumen may have proceeded to varying degrees. In some cases the chromosomes will still be discrete bodies, while the cytoplasm has flowed out into the peripheral strand to such an extent that little is left about the chromosomes. The chromosomes then appear as a closely grouped pencil-like mass. Cells of this stage rup-

tured into the lumen such as those discussed for the corresponding stage j of the pig embryo have not been observed. Their absence may be due to the fact that chick embryos can be secured with less exposure to physical manipulation than is the case with pig embryos from a packing plant.

Measurements were made of the dimensions and distances from the lumen of nuclei in various stages of mitosis in the chick embryo, in the same manner as that described for the

TABLE 2
Measurements, in microns, of nuclei in the neural tube of a chick embryo

STAGE, BY PLATE 2	NUMBER MEASURED	AVERAGE DIAMETER —NUCLEUS	AVERAGE LENGTH —NUCLEUS	AVERAGE DISTANCE FROM LUMEN	VOLUME, CUBIC MICRONS
a ₁	25	4.04±0.17	5.83±0.21	12.75	46
a ₂	25	4.60±0.12	6.65±0.22	32.90	74
a ₃	25	4.68±0.10	6.73±0.27	47.60	80
b ₁	21	4.74±0.067	8.80±0.098	37.6	90
b ₂	26	4.43±0.088	9.63±0.113	16.1	99
c	28	4.81±0.082	9.18±0.185	8.36±0.055	117
d	28	4.86±0.083	9.35±0.149	6.52±0.391	122
e	40	4.65±0.165	9.60±0.159	5.92±0.448	135
		—cytoplasm	—cytoplasm		
f	12	6.46±0.417	9.74±1.80		
g	10	6.07±0.160	9.48±0.308		
h	67	6.93±0.088	9.20±0.088		
i	13	8.46±0.252	9.54±0.139		
j	21	6.50±0.098	8.54±0.108		
k	47	6.33±0.103	8.25±0.098		
		—nucleus	—nucleus		
l	107	3.13±0.045	5.10±0.067	3.66±0.103	22
m	81	3.52±0.038	5.88±0.062	6.01±0.155	37

pig embryo. The results, which are essentially similar to those for the pig, are presented in table 2. Models of the nuclei were made to average dimensions, at a magnification of 5000, as for the pig embryo. These models are shown in figure 10, and the nuclear volumes determined from them are given in table 2.

The columnar form of the cells of the neural tube is especially clearly shown in the roof plate and floor plate where the cells have not departed so greatly from the condition

present in the neural plate. Figures 6 and 7 show the floor plate cells of the chick embryo as seen in transverse and sagittal sections. The development of fibers passing from side to side through the floor plate is accompanied by an alteration of the cytoplasmic columns external to the nucleus, by which these columns have flattened into ribbons whose surface is parallel to the course of the fibers. In transverse sections these cytoplasmic columns show as faint broad bands. In sagittal sections their edge is seen, and they appear as sharp, thin lines. Central to the nuclei the cytoplasmic columns of the floor plate cells usually contain one or two large vacuoles.

It has been claimed by Stough ('31) that in the chick embryo the number of mitotic figures present seem insufficient to account for the growth of the embryo, and that mitosis "is replaced by a shortened and simplified process, which is not amitosis, but is probably a modified form of mitosis." As this would, if correct, have an important bearing on the subject under consideration, it has been given attention.

One cannot determine from the percentage of nuclei in mitosis whether or not the number of mitoses present is sufficient to account for a given rate of cell multiplication. It is necessary to know also the length of time required for a mitosis, since rapid cell multiplication by mitosis might take place with few mitotic figures present at a given time, provided the mitotic stages were passed through rapidly. It is possible, from the percentage of cells in mitosis, to determine how long an interval of time must be assumed to be occupied in mitosis in order to account for a given rate of cell multiplication. If this interval of time were found to be shorter than is reasonably likely, then one might be forced to assume that the cells multiply by some means other than mitosis.

To determine the percentage of cells in mitosis, counts were made of resting nuclei and nuclei in mitosis in the neural tube of a 3½-day chick embryo. The counts were made for strips through the alar plate, extending from lumen to periphery, and 20 μ wide. Such counts were made for ten successive

sections, with the result that 4.25 per cent of the nuclei were found to be in mitosis. For this purpose, only those nuclei in stages of division from the beginning of prophase to the separation of the daughter nuclei were counted as in mitosis, the stages of reconstruction of the nucleus being included with the resting nuclei. The reason for this procedure is that a count on this basis makes possible a simple calculation of the time required for mitosis at any assumed rate of cell multiplication. Each time a nucleus goes through the series of stages leading up to the separation of the nuclei, the number of nuclei present is increased by one. If 4.25 per cent of the nuclei of the neural tube are in such stages of division at one time, then in the average length of time necessary for a cell to go through these stages, the number of nuclei present will be increased by 4.25 per cent. In the next similar interval of time the number will again be increased by 4.25 per cent, and so on, in the manner of compound interest. A number which is successively increased by 4.25 per cent of itself will be a little more than doubled by seventeen such increases. If the number of nuclei in the neural tube were doubled by the growth that takes place in 24 hours, it would follow that the length of time required for a division would be one-seventeenth of 24 hours, or approximately $1\frac{1}{2}$ hours. Similarly, a quadrupling of the number of nuclei in 24 hours would require that the division take place in 45 minutes, and an eightfold increase in 24 hours would be explainable if the division required only about 20 minutes.

It is evident from these considerations that the number of mitoses present in the chick neural tube is sufficient to account for a far more rapid rate of growth than that which actually takes place without one being driven to make an improbable assumption as to the time required for a division. It thus appears that one need not assume any method of cell division other than mitosis to account for the growth of the chick neural tube, but it is, of course, still possible that multiplication by some other method might take place. I have searched carefully for the stages of modified mitosis such as Stough

describes. The nuclei in which the chromatin is principally present in one clump, those in which it is partially separated into two main clumps, and those which contain two separate clumps are abundantly present, as well as nuclei which contain three or more clumps of nearly equal size. But these alone, as Stough points out, do not demonstrate division by a modified mitosis. The critical stages are those in which Stough describes a membrane as forming across the nucleus, separating it into two parts, and these I have been unable to find, either in the neural tube or the mesenchyme of the chick.

DISCUSSION

The most important phase of this study, in the writer's opinion, is the concept of migration of nuclei to and from the lumen at each division, and the bearing that this migration has on the differentiation of the cells of the neural tube. It appears that the radially arranged columnar cells, designated by His (1889) as spongioblasts, and the rounded cells in stages of mitosis near the lumen, which His named germinal cells, are not two types of cell, but are the interkinetic and mitotic stages of the same cell. It is difficult to see how any other interpretation can be made to fit the data herein presented. The germinal cells are obviously a series of mitotic stages, yet taken by themselves they do not form a closed series of mitotic changes. The series begins, so far as the germinal cells are concerned, with a relatively large nucleus in prophase, and it ends with a small nucleus in the early reconstruction stage. If only the germinal cells are taken into account there is no connection between the end and the beginning of the series. The stages of the process of formation of the resting nucleus, its subsequent growth, and passage into prophase are apparent if one looks for them among the spongioblasts, and as one finds such stages the apparent difference between the spongioblasts and the germinal cells disappears—there is no natural dividing line between them. The transitions from the simple spongioblast nuclei to the pointed nuclei which pass into the prophase appear

to be present only among the more peripheral of the radially arranged nuclei. The interkinetic nuclei, therefore, must be conceived of as migrating from the lumen to the more peripheral part of the neural tube wall after mitosis, and toward the lumen again as they prepare to undergo the next division.

To the question as to what forces bring about the migration to and from the nucleus, no final answer can be given. The migration to the lumen is evidently associated with the facts that the cells assume a rounded form in mitosis, and that they are attached to each other at the lumen by terminal bars. A cell which is to change from a columnar to a rounded form, and at the same time to maintain its attachment to the terminal bar net, must obviously move the mass of its cytoplasm, together with the nucleus, to the region of the lumen. The simple explanation that the phenomenon is due to turgidity is, however, inadmissible. It is apparent that the cytoplasm does become more turgid in mitosis, as is shown by the fact that cells in mitotic stages are able to push aside the surrounding cytoplasmic columns of the cells not in mitosis, but the nucleus may complete its migration to the lumen and in some cases pass as far as the metaphase before the peripheral cytoplasmic strand is all drawn in. If an increased turgidity of the cytoplasm were the sole explanation of the phenomenon, the nucleus would be drawn to the lumen only when the peripheral cytoplasmic strand had been drawn into a rounded mass.

The fibrils which are frequently seen in fixed material attaching to the pointed ends of the migrating nuclei seem suggestive of cytoplasmic forces drawing the nucleus toward the lumen. The fact that they are not invariably seen, however, warns one that the fibrils must not be too hastily taken to be a definite organelle of the cell. The writer is inclined to regard the fibrils as fixation pictures, perhaps indicative of a tension in the cytoplasm.

The migration of the nucleus away from the lumen after division appears to be a slow one, as the growth of the nucleus before it nears the periphery shows that this stage occupies

most of the interkinetic period. It is possible that the nuclei may be crowded back from the lumen by other nuclei being drawn in for later divisions.

The concept of migration of nuclei makes clear points otherwise difficult of explanation. It removes at once the difficulty in which His (1889) found himself of accounting for the multiplication of the spongioblasts, among which there appeared to be no mitotic figures. His pointed out that possibly the spongioblasts might increase in number by a transformation of the rapidly proliferating germinal cells into spongioblasts. The writer's explanation would be that such a change of form does occur, but that it is not a one-way process, and is followed by a change of form by which the spongioblast again becomes one of His' germinal cells preceding the next division.

The concept of migration of nuclei explains the otherwise puzzling fact that mitotic figures are confined to the region bordering the lumen. In order to explain this fact, Schaper (1897) assumed a lesser mechanical resistance to mitosis near the lumen, and a greater supply of nourishment to cells near the lumen from a store supposed to exist within the lumen. The first of these reasons seems unconvincing when one considers that in mitosis the cells round up, and that the region near the lumen where the columnar cells converge is the very place where there is least room for cells to round up. If the supply of nourishment were a determining factor, then when the blood vessels invade the neural tube the region of mitosis should shift to the neighborhood of these blood vessels. Some mitoses do, in fact, occur near the blood vessels at a later stage than that with which the writer has been mainly concerned, but in pig embryos of about 10 mm. and in chick embryos of a corresponding stage, blood vessels are present in the outer part of the neural tube wall, yet the mitoses are confined to the region near the lumen. In the writer's opinion, the mitoses are confined to the region of the lumen not because only nuclei of that region divide, but because a nucleus that is about to divide moves to the region of the

lumen to do so. This explanation, I believe, holds good not only for the neural tube, but for all the columnar epithelia of embryos. Altmann (quoted by Merk) and Merk (1886) have pointed out that in all these epithelia mitoses are found only at the surface farthest from the mesenchyme.

The acceptance of the germinal cells and spongioblasts as mitotic and interkinetic stages of the same cell would necessitate some revision of the usual account of the early differentiation of cells in the neural tube. The separate derivation of spongioblasts from medullary plate epithelial cells and of nerve cells from germinal cells, as proposed by His (1889), still appears to be generally accepted, in spite of the contrary views of Kölliker, Schaper, and Ramón y Cajal. Schaper (1897) believed that the germinal cells and the epithelial cells of the medullary plate were a single cell type in its interkinetic and mitotic stages. The spongioblasts, according to Schaper, are derived from indifferent cells, which in turn are derived from the medullary plate epithelium. Kölliker (1896) and Ramón y Cajal ('09) expressed similar views concerning the neural plate epithelium. Ramón y Cajal appears to indicate that the neural plate epithelium, with its mitotic divisions, remains in some forms close to the lumen, and that the spongioblasts are produced by differentiation from this layer. He describes and pictures the resting stages of cells of this continuation of the neural plate epithelium as rounded cells near the lumen. Kölliker's brief statement appears to indicate a similar view.

The writer agrees entirely with the authors cited above that in the neural plate epithelium the germinal cells are simply mitotic stages of the epithelial cells, and believes that this condition continues into the neural tube. With the increasing thickness of the neural epithelium which occurs as the neural plate passes into the neural tube and the further increase in thickness in the early neural tube stage, the columnar cells of the neural epithelium become much elongated, but there is no change in their relation to the germinal cells. The elongated epithelial cells, now the spongioblasts of His'

terminology, still assume the rounded form and draw toward the lumen to divide, and in division appear as the germinal cells of His. With the increase in thickness of the epithelium there is a lengthening of the path over which the nucleus must migrate in going to and from the lumen.

SUMMARY

1. The spongioblasts and germinal cells of the neural tube are elements of a columnar epithelium. The spongioblasts are interkinetic stages, and the germinal cells the mitotic stages, of the same cell.

2. Cells of this epithelium, when about to divide, undergo a change of form by which the nucleus migrates to the lumen and the cytoplasm assumes a rounded form. After division the nucleus migrates away from the lumen.

3. The elements of the epithelium are attached to each other at the surface bordering the lumen by a terminal bar net. There is no internal limiting membrane other than the cytoplasmic membranes of the cells.

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EXPLANATION OF PLATES

The drawings of cells in plates 1 and 2 are of material fixed in Bouin's fluid, sectioned 6μ thick, and stained with Heidenhain's iron hematoxylin. The outlines and main structures of the cells were drawn with the camera lucida and the details filled in free hand. The original magnification of the drawings of cells was 3000, reduced for reproduction to 1500.

PLATE 1

EXPLANATION OF FIGURES

Figures 1 a to 1 n show cells of the neural tube of a 10-mm. pig embryo in successive stages, leading up to mitosis, in mitosis, and in the reconstruction of the nucleus. The upper part of the cell is in each case the end toward the lumen.

2 Cell in telophase, apparently losing its connection to the terminal bar net. From the neural tube of a 10-mm. pig embryo.

3 Cell ends bordering the lumen of a 10-mm. pig embryo, in transverse section.

4 The same, in sagittal section, showing the terminal bar net and diplosomes.

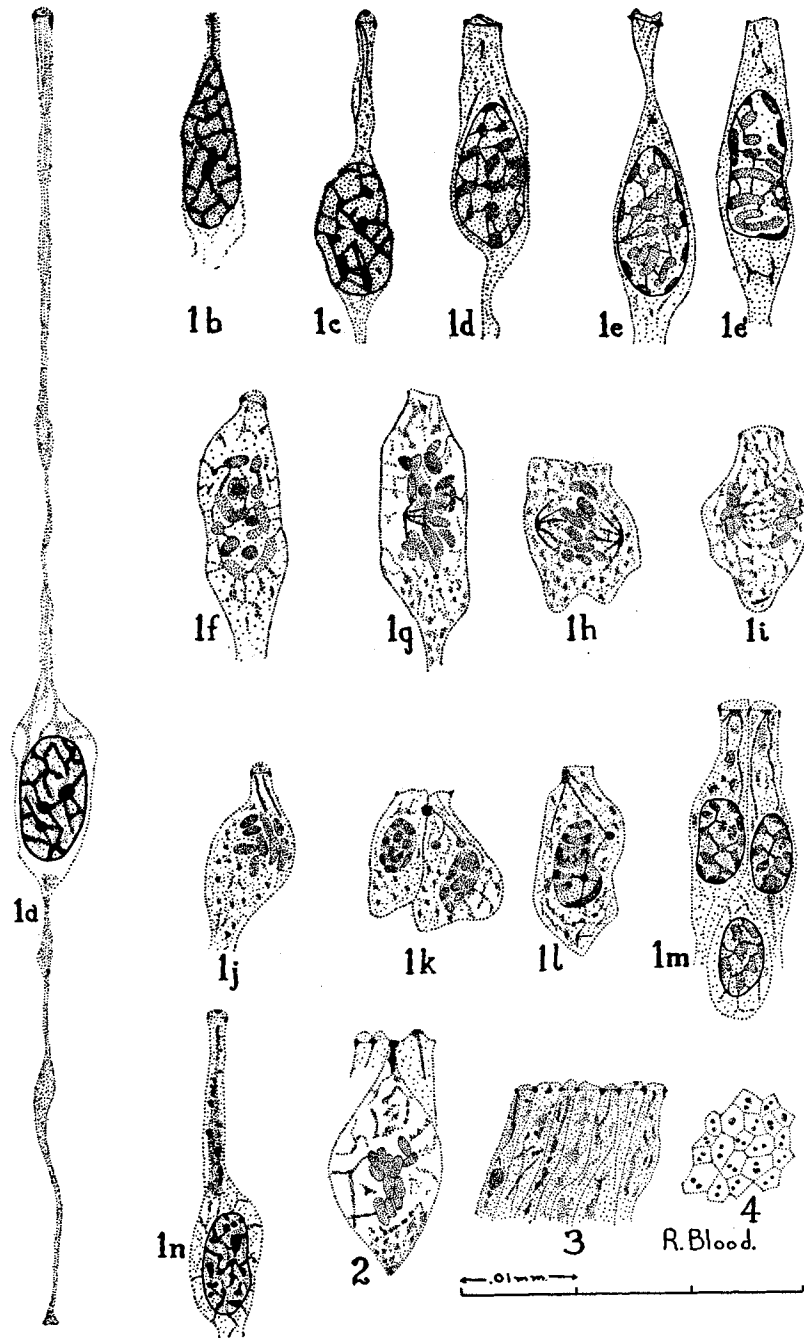


PLATE 2

EXPLANATION OF FIGURES

5 a to 5 m Cells in successive stages of mitosis and of the stages immediately preceding and following mitosis, in the neural tube of a $3\frac{1}{2}$ -day chick embryo. The upper part of the cell is the end toward the lumen in each case.

6 Cells of the floor plate of a 6-day chick embryo, in transverse section, showing the epithelial structure.

7 The same in sagittal section.

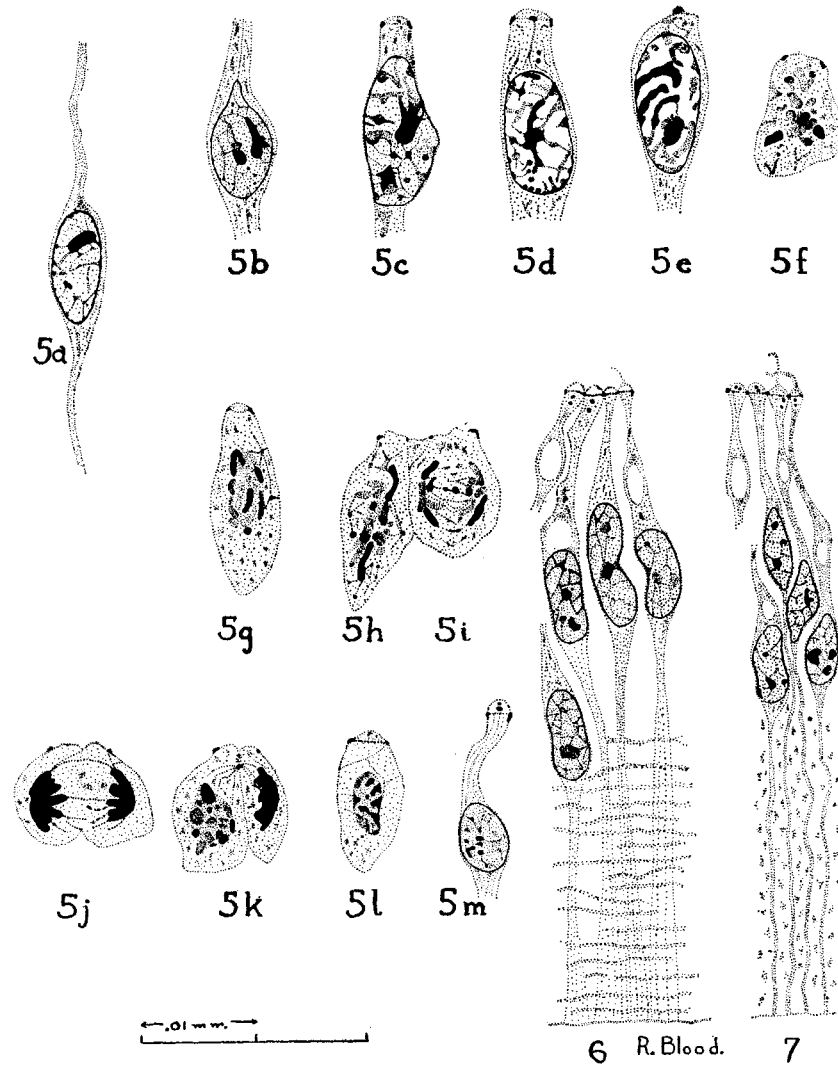


PLATE 3

EXPLANATION OF FIGURE

8 A diagram of the alar plate of a 10-mm. pig embryo. Nuclei in the various stages of interkinesis and mitosis are shown with dark outlines, with the average dimensions and at the average distances from the lumen, as shown in table 1. The arrangement of the cells, in the order of the series of mitotic changes, from the upper to the lower part of the diagram, is arbitrary.

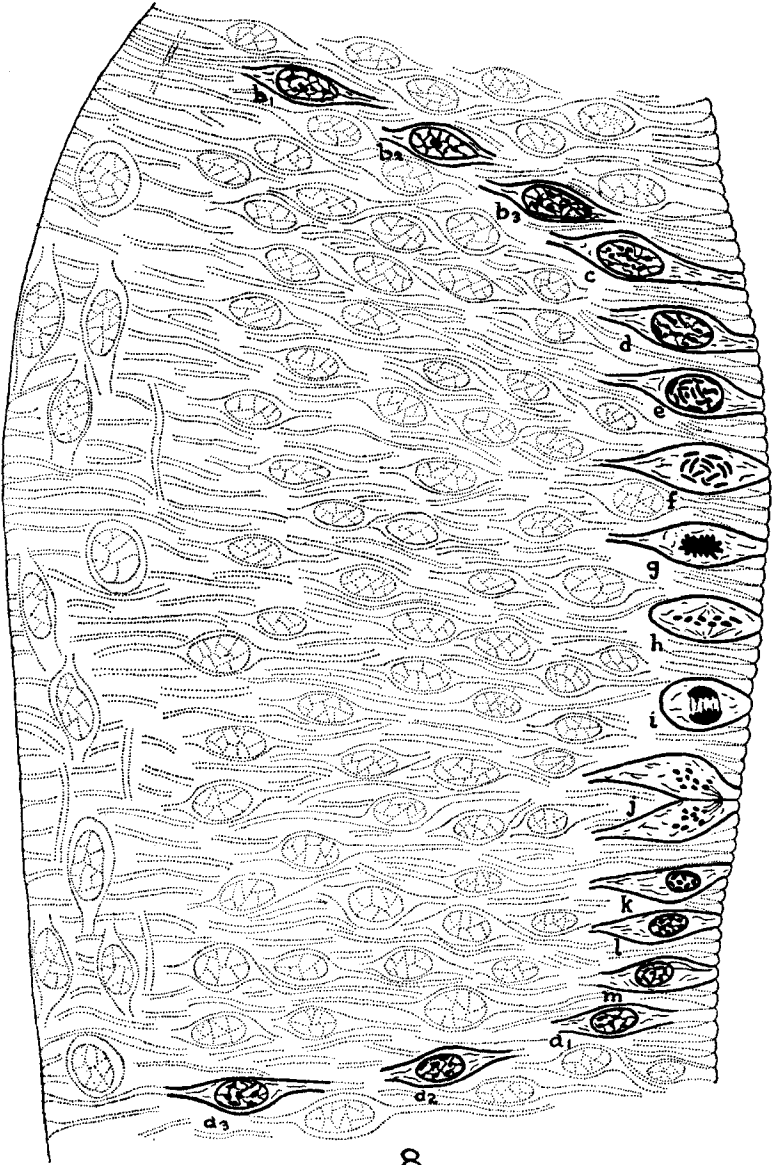


PLATE 4

EXPLANATION OF FIGURES

9 Drawings of models of the nuclei of the neural tube of a 10-mm. pig embryo, made to average dimensions for the various mitotic stages, as shown in table 1. The magnification is 2500 times the actual nuclear dimensions.

10 The same for the nuclei of a $3\frac{1}{2}$ -day chick, as shown in table 2.

