THE DEVELOPMENT OF THE RETICULATED BASEMENT MEMBRANES IN THE SUBMAXILLARY GLAND.

BY

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WITH 9 TEXT FIGURES.

Mall's¹ recent paper on the development of the connective tissue has done not a little to clarify our ideas concerning the origin of these perplexing products of the mesoderm. Their relation and ancestry he has traced back to a primitive syncytium from which they are all derived. To explain in his own words: "The network of fibrils which forms Wharton's tissue, to employ the best known example, is composed of a mass of anastomosing cells, a syncytium from which the connective tissues arise. In very early embryos the mesenchyme is composed of individual cells which increase rapidly in protoplasm and then unite to form a dense syncytium. The protoplasm of the syncytium grows more rapidly than the nuclei divide, so that in a short time we have an extensive syncytium with a relatively small number of nuclei. In its form the syncytium appears as large bands of protoplasm with spaces between them filled at times with cells and at other times with fluid. The second condition we have in the umbilical cord of young human embryos. About this time the protoplasm of the syncytium differentiates into a fibrillar part, which forms the main portion of the syncytium—the exoplasm—and a granular part, which surrounds the nucleus—the endoplasm. The fibrils of the exoplasm are very delicate and anastomose freely." From this period and these two differentiating products of the syncytium, Mall traces the development of cartilage, white fibrous tissue, reticulum, the cornea, and elastic tissue.

In describing the syncytium of the tadpole Malls says: "The point I wish to leave open is whether the mesenchyme was ever composed of individual cells. Was it not a syncytium throughout its development? At any rate, it is quite evident that the earlier syncytium if it exists

¹Am. Jour. of Anatomy, Vol. I, No. 3, 1902.

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is a very incomplete one with very loose protoplasma bridges, easily broken and easily united to allow the cells to wander in all directions during the earlier stages of development. So it may be that the syncytium as seen in the tadpole 3 mm. long has existed ever since the appearance of the mesenchyme." The apparent discrepancy between these two statements concerning the primitive condition of the mesenchyme may be explained from the fact that Mall's problem is not to solve that question but simply to trace the development of the connective tissues from the syncytial stage. The other problem is still unsettled, but, at the same time, not the least suggestive work is that of His which is quoted by Mall.

Now in tracing the development of the connective tissues Mall considered not only their simpler and more elemental relations, but also in a general way the manner in which they enter into the formation of certain organs, as, for example, the intestines and the framework of lymph glands. Reticulum he regards as distinct from white fibrous tissue, but concludes that it represents simply an embryonic form of that tissue. Together with reticulum, white fibrous tissue, the framework of the cornea, bone, and cartilage, must all be classed as collagenous. While it may be true that the framework of the lymph glands resembles a more embryonic type of white fibrous tissue, nevertheless, in other places reticulum shows peculiar, highly specialized adaptations to the needs of organs of which it forms the framework, suggesting the probability that morphologically, at least, it may represent the highest development of any of the fibrillated products of the syncytium. Such adaptations, for example, are beautifully shown in the framework of the submaxillary gland and many other organs.

It will be the province of a later paper to discuss the development of the exoplasmic fibrils in the submaxillary of the pig from the simple syncytial stage to the complex relations which they show in the adult, indicating at the same time some of the physical factors that may be involved in bringing these relations about. The specific point of the present communication, however, is to show how the reticulated basement membranes are formed from the primitive syncytial products. Throughout the literature and throughout the history of the development of the basement membrane idea, the question has been raised with considerable discussion whether or not these structures were homogeneous or fibrillar. Many investigators have maintained from the first that basement membranes in general were homogeneous, others showed with apparent conclusiveness by numerous digestive and precipitative methods that they were composed of reticulated fibrils. More

recently Mall² who has contributed extensively to the subject stated that a definite, homogeneous membrane surrounds some of the tubules of the kidney, which reacts, in many respects, not like reticulum or white fibrous tissue, but quite like yellow elastic tissue. These lie within the fibrillated basket-work he had previously found by digesting frozen sections of the kidney. This means that we must reclassify these structures and consider the entire group of basement membranes as consisting of two types, namely, the homogeneous and reticulated. For, while it is by no means certain that all the cell complexes of secreting glands are surrounded by homogeneous membranes, it is probable from the results of digestion experiments, that most secreting alveoli, acini, ducts and cell groups are embraced and supported by a membrane made up of a delicate interlacing meshwork of reticulum. We may find that many, if not most, of the glandular structures are embraced by homogeneous as well as the reticulated membranes, but the exact distribution of the homogeneous membranes can only be determined by a series of special investigations. Some method must be devised for the study of the origin of the homogeneous membranes, but the development of the reticulated fibrillar basket-work which embraces most glandular structures is readily followed in a series of embryos by Mallory's aniline blue fuchsin connective tissue stain,³ either used according to the original directions of Mallory or by the modifications more recently suggested by Dr. Sabin and quoted by Mall.

It may be well to call attention to the fact, however, that the stain has to be modified somewhat for each set of preparations. Owing, perhaps, to chemical differences in the cells or exoplasm at different ages, varying pictures are obtained, even when the stain is allowed to act under precisely the same conditions. Moreover, it is also true that the blue is very easily washed out of many of the finer fibrils, often giving rather unequal pictures unless the duration of the action of the stain is rigidly controlled.

In the preparations from which the following description of the development of the reticulated membranes is taken, the question of the origin and relations of the demilunes of Giannuzzi is clearly settled, but these facts will be discussed later in another place.

In a pig's embryo, then, 3 cm. in length, the submaxillary gland consists of a single tube with simple terminal arborizations lying below and medial to the ossifying mandible. At this time the gland is clearly defined from the adjacent structures because the dendritic branching

² Mall. Bulletin of the Johns Hopkins Hospital, Vol. XII, 1901.

³ Mallory. Journal of Experimental Medicine, Vol. V.

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forming the organ, while probably not exceeding divisions of the first and second orders, consists of solid columns of cells lying in a rather dense syncytial connective tissue, from which they are sharply differentiated. The syncytium is not yet completely separated into exoplasm and endoplasm, to use Mall's terms for the fibrillar and cytoplasmic portions of the embryonic framework of the organ. Immediately surrounding the tubes is a dense blue staining line which has the same reaction as the exoplasm, while just outside of this, and, indeed, resting directly upon it, is a mass of branching and anastomosing syncytial cells which form, with the other cellular elements of the syncytium, a direct protoplasmic continuum. Still further external a somewhat clearer

FIG. 1. Terminal bud of Submaxillary arborescence showing the developing basement membrane and the adjacent syncytium. Exoplasm just differentiating from the embryonic connective tissue. Pig 3 cm. Fixed in Zenker's fluid. Magnified 900 diameters.

differentiation of the developing connective tissue into exoplasm and endoplasm is obtained. Short, branching anastomosing fibrils of varying caliber are seen scattered here and there in the syncytium; this exoplasm in many places appears as dots which represent often simply fibrils cut in cross-section. At a great many points exoplasmic fibrils extend from the surrounding tissues to the basement membrane and here and there one sees deeply-staining fibrils apparently in the endoplasm of the cells immediately surrounding the cell columns. Now, whether the basement membranes up to this point are formed solely by the deposition of fibrils or whether the syncytial protoplasm differentiates into exoplasmic structures just about the developing glandular cells, it is very difficult to say, but in all probability the basement mem-

branes in the early stages are formed in both ways, namely, by a primary deposition of exoplasm from the syncytium and later augmented by increments of millions of fibrils from the general syncytial exoplasm. Following the formation of the membranes beneath the epithelium of the buccal cavity or in the skin in these preparations does not throw definite light on this question for in a pig 3 cm. in length the membranes when viewed tangentially appear to be made up partly of exoplasmic fibrils and partly of a simple granular substance in the meshes. The problem is complicated by the fact that in the earlier embryos, the endoplasm is diffusely tinged by the blue element of the dye. This

much, however, is certain; the formation of the membranes about the growing apices of the cell columns which are increasing constantly in length and circumference is chiefly by successive deposits of fibrils so the layer of syncytium around the growing ducts and alveoli forms what may well be termed the deposition zone. In this region the nuclei in the earlier stages at least are very numerous. Somewhat further out there is a noticeable diminution in their number and in the exoplasm and endoplasm are many clear spaces which are apparently filled with fluid. The nuclei of the syncytial cells are oval or round, contain a very indistinct nucleolus and are in general about the same size as the nuclei in the developing cell columns. The membrane itself under the highest powers is more or less irregular and, while looking somewhat homogeneous, still bears in some places definite evidences of fibrillation. There, as in the buccal cavity, the staining of the endoplasm destroys to some extent the sharpness of the picture.

In the submaxillary gland of a pig 4 cm. in length there has been a radical change in the syncytium. While it has not entirely differentiated into endoplasm and exoplasm, the exoplasmic fibrils have become extensively anastomotic although somewhat short, irregular and ill-defined. Here and there they seem matted together by some interfibrillar substance which on coagulation has become slightly tinged with the stain and thus destroys to some extent the sharp contour of the fibrils. This happens often in the best preparations. Numerous granules are also found deposited on the fibrils or at their nodes. It is also possible that these may be displaced particles of endoplasm that have become detached from the cells and remained entangled in the exoplasmic network. The endoplasm is gathered as a small cellular mass about the nucleus forming bipolar or multipolar cells. In the area just around the apices of the growing gland, that is to say, in the deposition zone, the arrangement of the cells is such that, in general, the long axis of the nuclei is parallel with the basement membrane, while the cells in parts more distant have no such definite arrangement. This seems to indicate that the tension caused by the

FIG. 2. Growing tips of submaxillary tree with the adjacent deposition zone from pig's embryo 4 cm. long. Exoplasm and endoplasm well differentiated. Fixed in Zenker's fluid. Stained by Mallory's method. Magnified 900 diameters.

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tips of the growing tree is having a certain influence on the course and direction of the adjacent fibrils, and they, in turn, have influenced the position of the cells that lie either at their nodes or along their course. Relative to the entire syncytial mass, the growing tree forms a comparatively small proportion of the embryonic gland, so that either the force exerted by the growing columns is lost in the plastic mass or the exoplasm has not developed a sufficient strength to transmit it any distance from the foci where it is exerted. In the syncytium are numerous spaces which are quite clear and are probably filled with fluid. The basement membrane is more distinct at this period than in a pig 3 cm. in length, but the presence of so many syncytial cells immediately about the growing columns is no longer so frequently observed. Now an occasional nucleus with its endoplasm rests directly on the membrane and it is noteworthy that there is a marked diminution in the quantity of endoplasm about the nuclei. From this structure extending into the adjacent syncytium and forming with it a direct fibrillar continuum are

FIG. 3. Alveolus of growing submaxillary of pig 8 cm. long showing formation of mucous cells from the inner cellular layer of the alveolus. Basement membrane and syncytium of the deposition zone show the enormous numbers of fibrils that are being laid on the surface of the alveolus as it grows out into the glandular framework. Fixed in Zenker's fluid. Stained by Mallory's method. Magnified 900 diameters.

the exoplasmic fibrils which are so numerous in this region, that, as the columns grow out into the general syncytium, it is impossible that fibrils should not be laid down by thousands on the surface of the advancing columns.

In a pig 8 cm. in length the cell columns show at their apices the beginning of a differentiation destined to mark the future alveoli. In the terminal buds a lumen has appeared and the cells are divided into two general, but more or less indefinite layers. Within the cells of the inner layer globules of mucus begin to appear making it a very simple matter to distinguish the alveoli from other portions of the gland by the presence of these characteristic mucous cells. The basement membrane is now somewhat thicker and more definite. Thousands of distinct fibrils run from the adjacent syncytium to lose themselves upon the growing membrane. In this, as in the earlier embryos, the deposition zone is quite as clearly marked out and consists now entirely of distinct, sharply-defined, exoplasmic fibrils, containing a few nuclei lying immediately adjacent to the alveolus and surrounded by a little granular endoplasm. The nu-

merous exoplasmic fibrils, containing a few nuclei lying immediately adjacent to the alveolus and surrounded by a little granular endoplasm. The nu-

clei of the syncytium are less numerous but still show the concentric arrangement about the growing buds, suggesting the continuance of the stress which, exerted in the younger stages, undoubtedly caused them to take this position. In the general syncytium the amount of exoplasm has markedly increased. The fibrils are branched and distinct, but of unequal size, the latter characteristic being most obvious in those bounding the little lacunae in the interstices of the syncytium.

In a pig $12\frac{1}{2}$ cm. in length the basement membranes are very sharply and deeply stained and show numerous fibrillar connections with the

FIG. 4.

FIG. 4. Alveolus from submaxillary gland of an embryo pig $12\frac{1}{2}$ cm. long, showing the gathering of the exoplasm into fasciculi and increase in the size and number of the lacunar spaces. The dots represent partly fibrils cut in cross-section and partly granules of endoplasm situated on the fibrils. Fixed in Zenker's fluid. Stained by Mallory's method. Magnified 900 diameters.

FIG. 5.

FIG. 5. Alveoli from submaxillary gland of pig's embryo 16 cm. long, showing the fasciculi and the irregular direction of the exoplasm and nuclei caused by the different forces exerted by the growing alveoli. Stained by Mallory's method, after Zenker's fluid. Magnified 900 diameters.

adjacent syncytium of the deposition zone. The anastomosing mass of fibrils now stands out with greater distinctness owing to the compact fascicular arrangement, a fact emphasized by interstitial lacunae which increase proportionally as the exoplasmic fibrils are gathered into bundles or fasciculi that bound these spaces. To this fact may be due the apparent diminution in the relative number of fibrils. The changes that occur between the ages represented in pigs $12\frac{1}{2}$ and 16 cm. in length are of some importance. Besides a general clarification and increase in the distinctness and definition of the fibrils, they may now be

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observed running in very distinct fasciculi which bound still larger spaces. In some places there are groups of three or four nuclei surrounded by a common protoplasmic mass embedded in a dense fibrillar meshwork while at certain other nodal points of the fasciculi some nuclei have either little or no endoplasm at all. The basement membranes now have a much sharper outline and the fibrils of exoplasm that connect them with the general network do not appear so numerous because they are gathered into fasciculi. Many nuclei with a slight amount of endoplasm are observed flattened against the growing membranes with their long axis parallel to that structure. Now as the further development of the organ proceeds and as the alveoli grow closer together it must be obvious that the increments of exoplasm are in the form of fasciculi instead of individual fibrils. This, indeed, is in accordance with the appearances seen in digested specimens of the adult where a uniform network will be seen crossed by heavier strands of reticulum. In such cases, of course, this network represents the products of fibrillar deposits, while the strands, on the other hand, indicate fascicular deposition. As long as the alveoli are extending out into the general syncytium, sufficiently isolated to exert only the stress caused by their own growth, the nuclei and fibrils take the direction mentioned above, but, in the latter stages, where, owing to their great numerical increase, the alveoli begin to encroach on each other, we have the stress exerted by one alveolus transferred through the syncytium to another so that excepting those immediately touching the basement membranes, the nuclei and cells occupy no definite position. Of course, even while this is so, the exoplasm embracing larger units of the growing organ may show by its direction the lines of stress and strain exerted by the more differentiated structural complexes.

Pari passu with the changes that have been occurring in the developing organs, a constant relative diminution in the quantity of syncytium and gland substance has taken place. Whether this is absolute or simply a relative difference it is difficult to say, but the gathering of the fibrils into fasciculi during the later periods of embryonic life has, undoubtedly, much to do with this appearance. However, to a certain point the syncytium, particularly the exoplasm, seems to increase and from that time on there is a constant apparent diminution until it is, finally, all changed into basement membranes or intervalveolar framework.

In a pig 19 cm. in length the developing alveoli are now rather closely pressed together so that between them there is quite a good deal of embryonic connective tissue. The basement membranes are

gradually becoming firmer and more definite and the exoplasmic fibrils that run out into the adjacent syncytium are confined into smaller sharper bundles. These, with the progressing age of the embryo, have more definite and distinct characteristics. The fibrils are of unequal size and often run so that a definite cross hatch is seen along course of the fasciculi. In the syncytium there is very little granular debris, the dotted or granular appearance noted along fibril bundles or at their nodes being due to the intersecting strands of exoplasm cut in cross-section.

FIG. 6. Mucous alveoli of the submaxillary gland of pig's embryo 19 cm. long. Many of the basement membranes are now in apposition, although their identity is still distinct. Stained by Mallory's method, after Zenker's fluid. Magnified 900 diameters.

In a pig 22 cm. in length the alveoli are very closely approximated and the strands of exoplasm bridging the spaces between them are very sharp and take a deep clear blue stain. In places, the membranes which are now sharp and distinct are in close apposition with those of adjacent alveoli, although in some parts of the lobule, particularly the central region, there is still a considerable amount of general syncytium. Between the alveoli are bridges of exoplasm, in the mesh-work of which the connective tissue cells now lie. This portion of the syncytium becomes the interalveolar framework. These have differentiated and show several types, some round and some branched. At points where the alveoli are in apposition the nuclei are flattened out between them so that they now form in these situations elongated lanceolate-shaped nuclei which stain with the orange element of the dye and show an extremely granular structure. Those out in the looser syncytium still retain their spherical or ovoid contour and still show a poorly-marked nucleolus.

In the submaxillary of a pig two days old the alveoli are now almost entirely in apposition with each other. Here and there, one may find slight chinks or clefts where the basement membranes of

FIG. 7. Submaxillary gland of pig's embryo 22 cm. long. Stained by Mallory's method, Zenker's fluid. Magnified 900 diameters.

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adjacent alveoli are not contiguous. The membranes stain an intense deep blue, like fine, delicate lines and are slightly thicker at the points where two adjacent membranes have fused than at the boundaries of the small interalveolar spaces. The fibrillar nature of the membrane is shown in places where the alveolus and its membrane are cut tangentially. Now the interalveolar syncytium has almost entirely fused with the basement membranes, but here and there oval or lanceolate nuclei rest in close approximation to the developing membrana propria. These nuclei, however, are provided with very little protoplasm. From now on it is a very simple matter to follow the changes that occur in the

FIG. 8. Reticulated basement membranes about the mucous alveoli of the submaxillary gland of a pig two days old. Stained by Mallory's method, after Zenker's fluid. Magnified 900 diameters.

transformation of the organ as it appears at birth to the conditions of adult life. In the adult gland there is between the alveoli an almost complete disappearance even of the small interalveolar spaces and the basement membranes of adjacent alveoli now lie fused together by the fibrils so that they form a single sharp distinct blue line which is separated here and there by the elongated nuclei of the connective tissue cells. At many nodal points the blood-vessels can be distinctly seen. In some places there is still a considerable amount of framework left especially in the regions about the lobular ducts and those near the membrana limitans of the lobule. Even under the highest powers of the microscope these membranes appear as a homogeneous blue line except where the direction of the sec-

tion yields a tangential view, and thus betrays to a certain extent their fibrillar nature. This, of course, is shown absolutely by the digestion methods. To recapitulate, then, we see that the basement membranes are laid down almost simultaneously with the column of cells which springs from the buccal cavity and later forms by its dendronal branching the gl. submaxillaris. This membrane is deposited partly in the syncytium in which it lies and partly by increments derived from the exoplasmic fibrils of the embryonic connective tissue. As the ramification of the columns proceeds and the terminal buds of the growing gland come into closer approximation, the amount of syncytium between them greatly diminishes and gradually takes on a more definite fibrillated structure until finally the two fuse and form a single mem-

brane which is absolutely inseparable by any of the methods at our disposal.

The alveoli of the submaxillary of the adult pig have no elastic tissue either around them or in the interalveolar spaces. In studying, therefore, the development of the lobules through a series of embryos by means of elastic tissue methods, no reaction is obtained while the developing reticulated membranes are easily demonstrated by Mallory's method. Specimens of the embryonic glands that have been macerated for some time in a saturated solution of bicarbonates of soda and subsequently shaken to loosen and remove the cells show the fibrillated membranes but none of the homogeneous type. This is true of the ducts as well as the alveoli. If these preparations are treated with 0.5% HCl or 0.1% KOH the reticulated membranes swell and become clear and the individual fibrils can usually be seen with the immersion lens.

In this description there has been a somewhat loose use of the term syncytium, for even where the cells of the embryonic connective tissue have become separated and obtained their individuality, the word has in many cases been employed. It is very difficult, however, to draw a clear dividing line, because cellular independence is not, as a rule, granted to all of the elements in an organ at one time. Strictly speaking, of course, the syncytial stage ceases when the cells take up their individual existence, but to avoid confusion it is perhaps best to use the same term throughout, provided, it is understood that the tissue so designated is undergoing constant changes.

FIG. 9. Mucous alveoli of adult pig's submaxillary showing reticulating basement membranes. Stained by Mallory's method after Zenker's fluid. Attention should be directed to the fact that the scale of this drawing is just one-half that of the balance of the series. Magnified 450 diameters.