THE FINER STRUCTURE OF THE GLANDULA SUBMAXILLARIS OF THE RABBIT.

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WITH 6 FIGURES.

Since the early researches of Boll, 69, the glandula submaxillaris of the rabbit has been the subject of many investigations on the part of histologists. This observer believed that the gland might well be regarded as the type of a whole important series of glands, formed on the same plan. The character of the cell, with its polygonal form, prominent nucleus and abundant cytoplasm, which after treatment with osmic acid became dark and granular, led him to consider it a pure serous gland. Later observers, notable among whom were Nussbaum, Langley, and Müller, were able to confirm his opinion as to the pure serous nature of the gland, and noted in addition that certain of the cells were loaded with granules, while others possessed a clear cytoplasm free from granulation. The weight of opinion was that these cells, unlike as to their cell contents, simply represented different physiological phases of one type of cell. The evidence presented so far to substantiate this view has not been altogether convincing, and in order to test the validity of such an opinion, a considerable number of glands, both in normal resting conditions and in various stages of activity has been studied during the present investigation in order to establish the identity or otherwise, the specificity of the different cells in this gland.

HISTORICAL.

The minute anatomy of the gl. submaxillaris of the rabbit was studied by Erik Müller, 96, in a series of investigation upon the relation of gland structure to physiological activity. He found that after sublimate fixation and staining with Heidenhain's iron hæmatoxylin, alternating transparent and darkly stained areas of gland tubules could readily be distinguished even with low magnification. By careful technique and

thin sections (2-4 micra), he was able to demonstrate certain structural differences in these two cell complexes. The cells of the darkly stained areas showed a cytoplasm filled with large, deeply stained granules. In some cases the granules filled the entire cell, while in other cells could be seen a lightly stained, fibrillar network, within the meshes of which the distinctly colored granules were held. The "transparent gland tubules" he found to consist of cells, the cell substance of which was formed of a fine network with thickened nodal points, forming regular clear spherical meshes. This second variety of cell did not fix so readily nor so well as the granule-holding cell and was frequently found shrunken and with irregular meshes, while at other times, under better conditions of fixation, the meshes appeared as only the expression of clear, round, unstained granules separated from one another by the colored network. Apart from cellular structure he believed that these two cell groups differed in no way from each other. "Only," he added, "it must be mentioned that the deeply stained tubules are found generally near an intercalated duct." To this rule, however, he claimed exceptions.

In order to explain the occurrence of these two varieties of cell forms, Müller considered two possibilities. Either the two forms of cells were of different kinds and yielded different secretions or they were identical and elaborated the same secretion, the apparent differences in structure resulting from different stages of activity. The second view appeared to him to be the correct one. There was not, he believed, a difference in kind between the cells but a difference in the state of function. Experimental evidence derived from an artificial stimulation of the gland, seemed to him to substantiate his opinion, when he found that after active secretion, the deeply colored areas of the gland parenchyma underwent a change as the granules disappeared. Here he was able to find transition stages between the light and dark cells. In case these granules might be simply artefacts in the sense of Fischer, he examined fresh tissue and found a similar and even more striking condition of differentiation between clear and dark cell areas, and furthermore that both kinds of cells contained granules, those of the dark areas being more highly refractive.

In the light of these observations, he formulated an hypothesis of the process of secretion of saliva in this gland. According to his view the cells of the clear areas contained the finished products of secretion. The unstained granules in these cells were transformed into the secretion vacuoles and these discharged their contents as saliva into the secretion

canaliculi, whence they passed into the lumen of the gland tubule. New granules were formed within the cell meshwork to replace those thus exhausted. These regenerated granules, lying within the meshes, at first small, increased in size and gave rise to the stainable granules of the dark cell areas. The latter, after attaining a certain size, lost their power of taking up stains and were transformed into the unstainable granules of the clear cells. In this manner arose the portions of the gland tubule containing clear cells.

Müller was not the first to observe that the cells of the submaxillary of the rabbit were wanting in homogeneity. Nussbaum, 77, in an investigation of the process of ferment formation in glands chose this gland as the type of a pure serous salivary gland. After treatment with osmic acid he found that certain groups of cells assumed a deep black stain which distinguished them from other groups of cells within the gland. These dark granular cells he believed to be particularly rich in ferment.

Langley, 78, also employed the submaxillary of the rabbit in his studies of ferment formation, and gave us valuable data concerning the secretory stages of this gland. He treated the gland with osmic acid, as did Nussbaum, but was unable to entirely confirm the results obtained by the latter observer. Nussbaum represented the intercalated ducts or ductules as composed of small, elongated cells, from which without intermediate forms there is a sudden change into the large cells of the alveolus. In this view he was supported by von Ebner, 72. Langley found that, while it was true that certain cells forming the part of the alveolus immediately succeeding the ductule were more deeply stained than those of the more peripheral part of the alveolus, yet there was no section in which any marked difference in coloration between the cells lying next to the ductules and the cells of the ductules proper could be discerned. In all of his sections the intralobular ducts were stained most deeply, the ductules and neighboring cells less deeply, and the distal alveolar cells least of all. His conclusion was: "It appears to me, on the contrary, that the cells composing the ductule are but slightly elongated or not at all, and graduate as to size and appearance into the alveolar cells, so that of particular ones it is difficult to say whether they belong to a ductule or an alveolus, hence I name them transitional cells."

Held, 99, in a series of researches upon granules and gland protoplasm studied the granule forms in the submaxillary of the rabbit. He distinguished three forms of cells characterized by three kinds of granules.

The first form of cell was filled with very highly refractive granules ("dunkle Zellen" of Erik Müller); another kind of cell contained very transparent granules, with a refractive index about equal to that of the protoplasm ("helle Zellen" of Müller). The third form of cell occurred least frequently and contained granules of low refractive power, the so-called "ring granules." Such forms were found in fresh sections of the gland taken from a rabbit after a fast of twenty-four hours. He employed various fixing reagents for studying stained sections. With alcoholic fixation he found that all three forms of granules had disappeared. With acetic acid the clear cells were destitute of granules, while the granules in the dark cells remained undissolved. After fixation in formalin and staining with Heidenhain's iron hæmatoxylin, he was able to distinguish two kinds of cells: (1) clear cells which contained only isolated granules; (2) cells, which in addition to granules, contained filaments comparable to the vegetative fibers of Altmann.

More recently Gerhardt, 03, has observed the granule complexes of this gland. He made a study of the changes in the salivary glands which followed upon section of the secretory nerves and found these granular areas in a gland sixteen weeks after section of the sympathetic in sections hardened in sublimate and stained with iron hæmatoxylin. He noted the occurrence of groups of granule-containing cells around the intercalated ducts but offered no explanation as to their nature beyond the suggestion of a possibility of their being artefacts.

Illing, 04, in an article upon the comparative anatomy of salivary glands regarded the submaxillary of the rabbit as a pure serous gland of the tubular variety. He noted a peculiar appearance in the structure of the gland in the fact that in each cell a border and a central zone could be distinguished: "In der Randzone erscheinen die Zellen vollgepfropft mit Körnchen, während nach dem Lumen hin die Körnchen sich mehr und mehr lichten und immer sparsamer werden, bis sie nahe dem Centrum schliesslich vollständig verschwunden sind."

MATERIAL-TECHNIQUE.

In the preparation of a series of submaxillary glands, which might be regarded as representative of the different stages of physiological activity, pilocarpine was used to stimulate a flow of saliva. In each case the rabbit was deprived of food for a period of twenty-four hours prior to the administration of the drug. It was determined by experimentation upon several rabbits, that the greatest exhaustion of the gland appeared

at the end of from six to nine hours, with a dosage of 0.014 gms. per kilo of body weight of the animal. Profuse salivation commenced as a rule in from three to five minutes after the subcutaneous injection of this amount of pilocarpine, although certain of the animals manifested a marked idiosyncrasy towards the drug, as a result of which the stages of secretion as evidenced in sections of the gland revealed considerable variation at the end of the same number of hours, and with equivalent doses. In this manner a series of stages, taken at intervals of two hours was obtained, varying from the normal resting gland, through the phases of secretion, to a time twenty-four hours after the first administration of pilocarpine. At the end of this length of time it was found that the gland had again assumed a resting condition and that its microscopic appearance was that of a normal, unstimulated gland.

In each case fresh sections of the gland were examined in a medium of aqueous humor of the eye, which furnished a fluid which was at once isotonic and did not dissolve the granules within the cells. In addition to aqueous humor various reagents, referred to later, were used upon the fresh sections in order to study the conduct of the granules in different media. Small pieces of the remainder of the gland were hardened in different fixing reagents such as alcohol, formalin (10 per cent solution), Carnoy's fluid, aqueous sublimate, Kopsch's fluid, Zenker's fluid, and others.

The majority of these hardening fluids, with the exception of Kopsch's fluid, afforded little or no granular fixation. A modification of Kopsch's fluid, suggested by Dr. R. R. Bensley, consisting of a mixture of equal parts of Kopsch's fluid and saturated alcoholic sublimate, to which is added an equal volume of distilled water, gave better results than the simple Kopsch's fluid and served to fix the granules in a striking manner. A saturated solution of sublimate in normal salt solution gave good cellular fixation with scarcely any shrinkage, but did not preserve the granules. The most satisfactory results were obtained by the use of Bensley's alcoholic-bichromate-sublimate, which in addition to preservation of the granules, gave excellent cytoplasmic fixation. Even with this fluid, however, as with all of the reagents before mentioned, some shrinkage of the distal tubular cells was produced. Erik Müller, 96, has called attention to the difficulty in avoiding shrinkage in the fixation of these cells. After fixation the tissue was imbedded in paraffin and thin sections made varying from 2-5 micra in thickness.

For staining purposes Heidenhain's iron hæmatoxylin, followed by

eosin, erythrosin, or acid fuchsin and orange G. was found to give best results for the study of cytological structure. Various other special stains, referred to later, were employed for the differentiation of the cellular elements in the gland.

THE STRUCTURE OF THE NORMAL RESTING GLAND.

If a thin, fresh section of the normal resting gland be examined in aqueous humor, with low magnification, the two cell groups described by Müller can readily be discerned. Many cells, occurring always in groups

FIG. 1. A section of the submaxillary gland of a rabbit after a fast of twenty-four hours. From a preparation, hardened in Bensley's alcoholic-bichromate-sublimite, and stained in hematoxylin and eosin. $\times 70$.

are found to contain highly refractive granules, while other less conspicuous groups of cells are filled with granules of a low refractive index. Light and dark areas in the gland are thus mapped out with sharp distinctness, even with slight magnification. An examination under high magnification reveals the fact that all of the cells of both groups are loaded with granules. From this it can be at once inferred that the granules found in sections of the fixed gland are not artefacts produced by the precipitating action of the hardening fluid, but are in reality pre-existent in the cells. In the fresh preparation, a careful examination

discloses the fact that the cells of the dark groups occur invariably in close relation to the ductules. Repeated observations, both in fresh and hardened sections, fails to show any exception to this rule.

The granules seen in the dark cells in a fresh section appear to be smaller in size than those in the light cells. Considerable variation is to be noticed in the size of the individual granules in the dark cells. The nuclei in both kinds of cells, in a resting gland, are obscured by the great abundance of granules. The action of certain reagents on these unfixed granules gives striking evidence of the ready solubility of the granules in both kinds of cells. Those found in the clear cells are especially soluble in most reagents. With isotonic salt solution the granules are not preserved but slowly dissolve. If placed in a one per cent solution of acetic acid, the section at once becomes opaque and the granules cease to be spherical, although remaining visible, while the two cell groups can be no longer distinguished. Glycerine, in concentrated solution, also dissolves the granules. After treatment with osmic acid (1 per cent solution), the lumina of the ducts are seen to be crowded with dark brown granules, while the granules in the dark cells assume a brownish black coloration. When treated with alcohol, or with aqueous sublimate, the granules are readily dissolved. Solger, 96, moreover, has called attention to their ready solubility in dilute solutions of chromic acid, potassium bichromate, acetic acid, and pure water.

In a thin section (2-3 micra) of a resting gland, fixed in Bensley's fluid, and stained with hæmatoxylin and eosin, there can be readily observed, even with low magnification, in the portion of the gland near an intercalated duct, a group of cells loaded with granules, which stain intensely with eosin. (Fig. I.) These centrally disposed cells are followed by a second group of cells, the cytoplasm of which stains indifferently with eosin and is destitute of granules. At no time does this second variety of cell lie in close relation to an intercalated duct, while the granule-holding cell, upon repeated observations, is found always opening directly into a ductule. After staining with Heidenhain's iron hæmatoxylin the granules assume a deep blue-black color and are seen to be crowded towards the lumen of the tubule. The nuclei, when visible among the mass of granules, appear oval in form and are situated towards the base of the cell. This proximally disposed group of granular cells is of constant occurrence throughout the lobule and has a variable diameter of from 85-200 micra. The individual cells of the group have an average diameter of 24 micra, while the diameter of the nucleus averages about 11 micra.

The clear cells of the distal group present a well-marked cytoplasmic net-work, forming large meshes. (Fig. II.) The cytoplasm stains feebly with most of the ordinary stains. The nuclei of these cells are

FIG. II. A portion of the same gland shown in Fig. I more highly magnified. Preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

on the whole smaller and less spherical than those of the granule cells, and are located nearer to the base of the cell than in the other variety of cell. These cells, even after the shrinkage with which their fixation is always attended, possess a somewhat larger diameter than the granule

cell. Their average diameter is about 28 micra, while that of the nucleus is 9 micra. Secretory canaliculi occur abundantly in connection with these outer cells, and are seen to ramify between the cells. (Fig. III.)

The evidence adduced by a study of sections of the normal resting gland, stained with certain selective dyes, is most convincing of the specificity of these two kind of cells. Sections hardened in Bensley's fluid, and stained with iron hæmatoxylin and orange G., show the cytoplasm of the granule-holding cells deeply stained by the orange G., while the cytoplasm of the clear cells takes on a bluish tint from the hæmatoxylin. The granules stain a deep blue-black. With Bensley's neutral gentian method the granules are colored an intense violet. A sharp differentiation between the two kinds of cells is afforded by means of Mann's methyl-blue eosin, the granules and cytoplasm, of the granular

FIG. III. A section through a group of distal alveolar cells, showing secretion canaliculi. From a preparation stained in iron-alum hæmatoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

and duct cells staining a deep red, while the cytoplasm of the clear cells is colored by the methyl blue. With Nissl's methylene blue the intralobular duct and granular cells stain metachromatically, a reddish violet, and the clear cells are stained blue. Dahlia (1 per cent aqueous solution) also stains the cytoplasm of the granular cells metachromatically; the granules assume a decided reddish color. With this stain the clear cells and nuclei are blue, and the cytoplasm of the intralobular duct cells takes on a violet color. By means of iron hæmatoxylin and erythrosin, a clear differentiation can be established. The cytoplasm of the granular cells stains a deep pink in contrast with the black granules, while the cytoplasm of the clear cells is stained a feeble pink by the erythrosin. Orcein stains both kinds of cells, the cytoplasm of the intralobular duct cells taking up the stain intensely, while the granular cells also stain strongly; the cytoplasm of the clear cells displays much less affinity for

the stain. Unna's polychrome methylene blue stains the clear cells blue and leaves the granular and duct cells with scarcely any coloration.

In sections stained with hæmatoxylin and Congo red, the cytoplasm of the duct and granular cells is strongly colored by the Congo red, while the clear cells take up the blue of the hæmatoxylin. By means of the Ehrlich-Biondi stain, the intralobular duct cells are dyed an orange-yellow, the granules and cytoplasm of the granular cells an intense red, and the clear cells a violet-blue. This stain was employed by Krause, 95, in his study of the glandula submaxillaris of the hedgehog (*erinaceus europæus*) and the results obtained bore a striking similarity to the conditions found in the same gland in the rabbit with the use of the same stain. With toluidine blue the granular cells and granules stain a bluish-green, while the clear cells take on a fairly strong blue color. After fixation in Kopsch's fluid, and staining with a mixture of lichtgrün and safranin, the clear cells and all of the nuclei take up the brilliant red of the safranin, and the granular cells, as well as the intralobular duct cells, assume a violet blue. With Ehrlich's neutral solution the granules stain an intense red, as does also the "rodged" cytoplasm of the cells of the intralobular ducts; the cytoplasm of the clear cells stains blue.

By the use of the so-called specific mucin stain of Krause, thionin, followed by potassium ferrocyanide, the clear cells stain metachromatically, giving the red color characteristic for mucous cells. The granular and duct cells stain with the thionin and remain unchanged after treatment with the potassium ferrocyanide. A similar and suggestive result may be obtained by staining sections hardened in Kopsch's fluid with Mayer's muchæmatein, as modified by Bensley, 93. The clear cells when thus treated give a well-marked "mucous" reaction, while the granule and duct cells remain unstained. With other ordinary methods of fixation, this so-called mucous reaction cannot be obtained in so striking a manner. After formalin fixation, however, the clear cells give a feeble reaction with muchæmatein.

In order to study the relation of the cells of the granular areas to the intercalated ducts, a number of Golgi preparations of the gland were made. With the resulting impregnation, the granular cell complexes appeared as dark brown masses, while the clear cells were stained a light yellow. The injected secretion canaliculi were found present in both varieties of cells. In the cells of the granular areas the canals appeared short, broad and unbranched, a condition which can readily be observed in sections of the gland, hardened in aqueous sublimate, and stained with

iron hæmatoxylin. In these Golgi preparations the cells of the granular area were found invariably lying next to an intercalated duct. They, in turn, were constantly succeeded by a group of clear, non-granular cells, occupying a distal position in the tubule.

The intralobular ducts of this gland are lined by a single layer of epithelial cells of a high columnar type. This layer rests upon the basement membrane, outside of which there is a scanty amount of connective tissue. The nuclei of the cells are large and spherical and occupy a position central in the cell. A well-marked nucleolus is present, but the chromatin is less abundant than in the nuclei of the gland cells. The cytoplasm is abundant and stains readily with acid dyes. Towards the base of the cells it is arranged in the form of longitudinal striations,

FIG. IV. A gland alveolus, with its accompanying intercalated duct leading into an intralobular duct. Preparation stained in iron-alum hæmatoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

giving rise to the "rodde" variety of cell constituting the "salivary tubes," described by Pflüger, who believed that this form of cell was actively concerned in the secretory processes of the gland. In an exhausted gland, after prolonged stimulation, the cytoplasm is observed to stain more feebly than in the resting gland. Large secretion granules are never found in these cells, but the cytoplasm itself manifests a finely granular appearance.

The ductules, or intercalary ducts, open into the intralobular ducts, obliquely or at right angles, with an abrupt change from the low, flat cubical cell of the ductule to the high columnar, rodde cell of the intralobular duct. (Fig. IV.) The cells are elongated in form, contain but little protoplasm and are devoid of granules. The reaction of the

cytoplasm towards stains is similar to that of the cells of the ducts and of the granular areas. The nuclei are oval and are situated towards the central part of the cell. The change from the elongated, non-granular ductule cell to that of the large cubical, granule-holding cell of the granular area is also an abrupt one. Langley, 78, working from a physiological point of view, believed that there was a gradual transition from the cells of the ductule to the cells of the alveolus. He treated sections with osmic acid, which affords at best a poor protoplasmic stain. Nussbaum, 77, represented the ductules as formed of small, elongated cells, from which without intermediate forms, there is a sudden change to the large alveolar cell. By the use of osmic acid he found them to contain granules. Von Ebner, 72, had previously supported the view of a sudden change from the low, cubical cell of the ductule into the large alveolar cell. Lastly Klein, 82, has noted that the flattened, elongated, epithelial cells of the ductules pass directly into the columnar secreting cells of the large alveoli. An examination of preparations of thin sections, stained with iron hæmatoxylin, confirms the correctness of the observations of Nussbaum, von Ebner, and Klein. One hesitates to dispute an opinion, entertained by so distinguished an observer as Langley, but his technique was for physiological, not cytological purposes. In a later paper, 79, his opinion in regard to a graduation in form and size of cell was not so positive; he says, "Some of the cells, which, from their shape, certainly would be called ductule cells, certainly contain granules, though they are, I am inclined to believe, absent from the ductule cells, springing immediately from the ducts. The absence of cell outlines and the difficulty of obtaining thin sections of the fresh gland, makes a decision on this point difficult to arrive at."

The question of the existence of a differentiated, tubular portion of the gl. submaxillaris, designated by the name of Bermann's gland, has been the subject of much controversy among investigators of salivary glands. Bermann, 78, described in connection with the submaxillary gland of the rabbit, a small tubular portion of the gland enclosed in the same connective capsule. This part of the gland he believed to be an "organ sui generis." In the new born rabbit he found it to be very small and lying in close proximity to the hilus of the gland. According to him a similar tubular portion occurs in the submaxillary glands of the guinea pig, bat, dog, cat, and fox.

A number of other authors have confirmed, in the main, the observations of Bermann. Langley, 79, found that sections of the gl. submaxillaris of the rabbit, made near the entry of the ductus Whartonianus.

usually displayed a lobule or a portion of a lobule, consisting of branching tubules in place of the ordinary alveoli or ducts. W. Krause, 84, believed that this gland was undoubtedly a morphologically and functionally differentiated portion of the submaxillary gland, tubular in form, which represented a rudiment of the gl. sublingualis, the connection of which with the ductus submaxillaris was altogether secondary.

The mass of evidence, brought by the majority of investigators, is opposed to the existence of a clearly differentiated portion of this gland in the sense of Bermann. Beyer, 79, after a series of researches upon this subject, maintained that Bermann's gland was none other than a gl. sublingualis. Von Ebner, 99, as a result of experimentation, regarded Bermann's gland as being produced by a stasis of secretion in the broad lumina of the gland tubules. Illing, 04, after a comparative study of the gl. submaxillares of the domestic animals, could not confirm the presence of this gland: "Eine sog. Bermannsche Drüse, wie sie von Bermann als eine deutlich differenzierte, besondere, zusammengesetzte tubulöse Drüse in der submaxillaris beschrieben wurde, habe ich weder bei Hunde und Katze noch beim Kaninchen oder irgend einem anderen Tiere konstatieren können." In order to substantiate his opinion, he sectioned the entire submaxillary glands of several rabbits, and reconstructed models of the same. In no case did he find a differentiated tubular portion of the gland. He concurs with the view, expressed by a number of other observers, that Bermann's gland is simply a portion of the gl. sublingualis (polystomatica), which frequently lies in such close proximity to the gl. submaxillaris as to be sectioned along with it. S. Mayer, 94, in his Gland Studies, has noted that forms conforming with Bermann's description ("rein tubulösen Drüse") may be found in the gl. submaxillaris and parotis of the rabbit, and in the gl. parotis of the dog. Among the structures occurring in the region of the submaxillary gland, presenting sources of errors for observers, he mentions the "Winterschalldrüse," found in many animals in this region. In the female rat he has noted the presence of mammary gland tissue extending into the submaxillary region, and he suggests the possibility of Bermann having mistaken degenerated mammary gland tissue for a differentiated portion of the submaxillary: "Es ist leicht möglich, dass Bermann in den von ihm geschilderten, in der Nachbarschaft der Submaxillardrüse gelegen 'rein tubulösen Drüsen,' auch rückgebildete Milchdrüsen läppchen vor sich hat."

In a study of many glands during the present investigation a specially differentiated part of the gland, as described by Bermann, was in no instance observed.

THE STAGES OF PHYSIOLOGICAL ACTIVITY.

The microscopic appearances of the hardened and stained sections of the gland, taken at any stage of activity, simulated closely the conditions found in the preparation of the fresh gland of the corresponding stage, with this notable exception, that while in fresh sections of the gland, granules were found present in both varieties of cells, in the fixed sections they occurred only in the group of cells lying next to an intercalated duct. No reagent so far employed has had the property of fixing, to any extent, the granules in the clear distal group of cells. Always associated with this lack of granule fixation, there was present more or less shrinkage of the cell cytoplasm. It was, moreover, to be observed, that the newly formed granules, in a gland recovering from exhaustion, were more readily soluble than the granules of a resting gland, for which reason, while in the fresh tissue abundant granules might be present, the corresponding fixed specimen might display scanty granulation.

After a period of three hours' stimulation with pilocarpine a thin, fresh section of the gland, examined in aqueous humor, showed all of the cells still loaded with granules. In the cells of the dark areas, close to the ductules, the granules varied much in size and occurred in irregular masses. The cells of the clear areas were still filled with granules. The nuclei were discernible with difficulty. In a fixed preparation of the gland, stained with Heidenhain's iron hæmatoxylin and eosin, the cells were seen to be in a condition of partial exhaustion. There were fewer granules present in the cells of the dark areas and the cytoplasm of these cells was clearly differentiated from the more lightly stained cytoplasm of the cells of the clear areas. With eosin the cytoplasm of the granular cells was stained a lively red, while that of the distal, clear cells was feebly colored by this stain. The nuclei of the granular cells were now more readily visible than in the resting gland, and showed a tendency to assume a position more central in the cell. Secretion canaliculi could now be seen in both kinds of cells, but were observed to be more numerous in the clear cells. In the granule-holding cell they were found to be short and broad without any apparent branching.

At the end of four hours in fresh sections, the blood vessels of the gland were seen to be much congested, and both cell complexes showed fewer granules. It was to be noted that the granules in the cells of the dark areas appeared to persist longer under stimulation than those in the cells of the clear areas. After seven hours the gland was found shrunken, with the blood vessels engorged. The two cell complexes could

be differentiated only with difficulty, while all of the cells appeared exhausted and almost destitute of granules. In the cells of the dark areas some few small granules were still present, and in some cells border fat granules could be seen. The gland tubules appeared shrunken while the nuclei were readily visible and were found to be round and small. The hardened and stained specimens of the gland taken from these stages revealed conditions which corresponded closely to those of the fresh sections.

The stage of most complete exhaustion was obtained from an animal killed nine hours after stimulation. The gland here appeared pale

FIG. V. A section of an exhausted gland of a rabbit, after a fast of twenty-four hours and subsequent stimulation with pilocarpine for a period of nine hours. From a preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

and shrunken. In fresh sections, examined in aqueous humor, the cells were found to be free from granules, with the exception of the dark cells in which a few still persisted. At no time did the granules entirely disappear from these cells, a fact that accords with the observations of Langley, 78. The few granules remaining in the dark cells were irregular in form and of unequal size. The cells bore evidence of shrinkage and the nuclei had lost their rotundity. In a fixed and stained specimen of this stage (Fig. V), the distinction between the two kinds of cells was less characteristic than in the resting gland. The clear cells appeared even

more shrunken than in the normal gland. The protoplasmic network was formed of larger and more irregular meshes, which did not stain so readily as in the resting gland. The nucleus was observed to be large with no apparent shrinkage, and with nuclear membrane and nucleolus well marked. The chromatin, on the other hand, was not so abundant. The location of the nucleus was more central in the cell and further removed from the basement membrane, than in the resting gland, while its form was more spherical with less apparent distortion.

The cells of the granular areas in the exhausted gland were recognized with greater difficulty in stained sections on account of the loss of secretion granules through active stimulation. Even here, however, a few granules still persisted. Those remaining were smaller than the granules found in a normal gland and were found wholly in the distal part of the cell, lying in close proximity to the lumen of the tubule. The cytoplasm was diminished in amount and revealed a reticular meshwork. It stained feebly in marked contrast to the strongly staining character of the cytoplasm of the resting granular cell. The nuclei were located towards the center of the cell and showed little evidence of shrinkage. They were observed in this stage, as in the resting gland, to be slightly larger than the nuclei of the clear cells, and to be poor in chromatin.

A section of the gland in the exhausted stage, stained with iron hæmatoxylin, afforded a clear view of the secretion canaliculi. The lumen of such a canal, as well as the lumen of a tubule, appeared broader than in the resting gland. Ramifications of the canaliculi could plainly be observed among the clear cells but were absent from the granular cells. The cells of the intercalated and intralobular ducts were somewhat shrunken and fallen away from the basement membrane; the protoplasmic striations of the "rodged" duct cells were found to stain less intensely than in the resting gland. The blood-vessel in the interlobular connective tissue were engorged with red corpuscles.

The next stage was taken from a gland twelve hours after stimulation, when both kinds of cells were found to again contain granules in a fresh section. In the cells of the dark areas many granules were present, while the clear cells were seen to contain fewer and smaller granules. It was, moreover, quite obvious that the dark cell groups occurred in close relation to the ductules, in the same position as in the resting gland. After a period of twenty hours, the gland, although somewhat shrunken, had again assumed in a large degree, the appearance of a normal resting

gland. In fresh sections the cells and nuclei were found to be but little shrunken. Both areas could be distinguished but not so readily as in the normal unstimulated gland. The tubules surrounding the ductules contained many granules, none of which were of great size. In a hardened and stained section of this stage, the cells of the dark areas were found to again contain many granules, the nuclei being for the most part obscured. The clear cells were now less shrunken, and the cyto-

FIG. VI. A section of the gland of a rabbit killed twenty-four hours after stimulation with pilocarpine. Preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

plasm stained more vigorously. The nuclei gave the appearance of again being displaced towards the base of the cell by the abundant formation of secretion, and were more or less irregular in contour. It was, moreover, to be noticed in stained, just as in fresh preparations, of this gland, returning to a normal resting condition, that the granule cell-complex was again intermediate in position and situated, in every case, in close relation to an intercalated duct.

The final stage taken for the study of secretion phases, was from a

gland stimulated twenty-four hours previously. Here the appearance was found to be that of a normal resting gland. In fresh sections all of the cells were found loaded with granules. The dark cell areas could be readily detected, and were seen to occur regularly around the ductules, just as in the unstimulated resting gland. The cells of these areas were filled with granules, much larger than those in the cells of the clear areas. These latter cells were loaded with small granules of low refractive power, and while some of the cells were completely filled, in others the granules were found only towards the lumen of the tubule. The nuclei in most of the cells were not visible, owing to the mass of granules. When distinguishable, they were found close to the base of the cell, and were spherical in form. There was no evidence in the gland of degenerative processes. In a fixed and stained specimen, taken from this stage (Fig. VI), there could be observed little or no deviation from the appearance of a normal resting gland. The cells of the dark areas, occurring around the ductules, were found loaded with granules, while the granules in the cells of the clear areas remained unfixed, just as in the normal gland.

DISCUSSION.

After an examination of the different physiological phases of the gl. submaxillaris of the rabbit, conducted in the manner described, and a study of the morphology and microchemical reactions of the cells of the gland, one is forced to abandon the view held by some earlier observers, that it is a pure serous gland formed of cells of one and the same type. The following observed facts justify us in regarding it as a composite gland with at least two kinds of cells in the secreting tubules, and furthermore, that these two varieties of cells do not represent different secretion phases of the same cell but are morphologically and physiologically distinct:

(1). In fresh sections of the gland, two distinct areas can be observed, each composed of a group of cells containing granules with a refractive index different from that of the granules in the cells of the other group, indicating a difference in chemical composition. The granules in the cells of the two groups behave in a different manner towards various reagents.

(2). The cells forming the "dark" areas in hardened sections always contain granules and are always associated in close relation with an intercalated duct. In such fixed and stained specimens the distal group of cells, composing the "clear" areas are always destitute of granules.

(3). An examination of the secretion phases, observed in fresh sections, reveals the fact that under stimulation, the granules of the clear cells disappear earlier than those of the dark cells, while in a gland recovering from exhaustion, those in the clear cells are the later to reappear.

(4). In stained preparations of stimulated glands, the granules are found to have disappeared from the cells of the dark proximal group, and to have reappeared in a gland recovering from exhaustion, in one and the same region, around an intercalated duct. The cells of the distal clear group, in a section prepared after fixation in any of the fluids employed, are never found to contain stainable granules during any phase of secretion.

(5). The cytoplasm of the two kinds of cells exhibits different staining properties and affords distinctly different microchemical reactions. The proximally disposed granule-holding cell conforms to the classic description of a serous cell, while the clear distal cell resembles in some degree the appearance of a mucous cell.

The significance of the presence of these two types of cells in this gland is as yet doubtful, and must remain so until our knowledge of the chemistry of the submaxillary saliva of the rabbit is more exact and the microchemical methods at our disposal further developed. The rôle of each cell in the production of the secretion is unknown and, indeed, even the presence of a diastatic ferment in this saliva is still a debated question. Many suggestive, but by no means convincing, results were obtained concerning the nature of these cells through their staining and microchemical properties.

The cytoplasm of the cells afforded many valuable criteria for differentiating the two types. In the clear cells it is less abundant than in the granular cells and contains a basophilic reticulum. It possesses a substance which has a marked affinity for hæmatoxylin and basic stains. With acid stains it does not stain so readily as the cytoplasm of the granular cell. With P. Mayer's muchæmatein, as modified by Bensley, **03**, in sections fixed with Kopsch's fluid, a typical "mucous" reaction was obtained in these clear cells. With Krause's so-called mucin stain (thionin followed by potassium ferrocyanide) these cells also behaved as mucous cells and gave a metachromatic red color. Neither of these stains, however, can be regarded as specific for mucin. According to Hoyer, **90**, a metachromatic color with thionin is a positive indication of mucin or a mucin-like body. Krause, **95**, tested the validity of this assertion by successfully staining sections of hyaline cartilage and of

the epithelium of the gall bladder, and concluded that the reaction is not specific for mucin. Although such reactions, along with the accompanying morphological structure of a large, clear cell, of indifferent staining power, and a somewhat flattened nucleus lying in the proximal zone of the cell, is suggestive of the mucous nature of these distal tubular cells of the rabbit's submaxillary, we are not warranted in interpreting them as such, at least in the light of our present meager knowledge of the chemistry of the mucins. The researches of Langley, 86, and Hammarsten, 85, have shown that the elaboration of mucins by the secreting cells is a process involving several stages. Mayer, 97, constructed a graded series of mucins, ranging from those which stained with difficulty in muchæmatein to mucin which stain readily and deeply with the same dye. Conversely he was able to obtain a typical mucin reaction in the cells of the gl. submaxillaris of the hedgehog, a gland which, according to Krause, does not secrete mucin. Bensley, 02, in a study of the cardiac glands of mammals has called attention to the fact that it is unreasonable to expect that the different mucins and different stages of the elaboration of mucin would present always the same staining properties. It is possible that future researches may prove that these cells secrete a mucin-like substance.

The second type of cell is found in the group of granule-containing cells in the region of an intercalated duct and presents the well-known picture of a serous cell. The cytoplasm is abundant and stains readily and deeply with ordinary dyes. The nucleus is prominent, lies towards the center of the cell, and is spherical in form. If a ferment were elaborated by this gland, it would be natural to conclude that the cells of this second type gave rise to it, and that the granules found within them were zymogenic in character. From the fact that the granules stained black with osmic acid Nussbaum, 77, concluded that these cells were particularly rich in an amylolytic ferment and that the distal tubular cells were not concerned in its formation. Langley, 78, and Grützner, 78, were unable to confirm his observations and believed that an amylolytic ferment was absent from this gland. With Macallum's, 95, microchemical test for iron, a negative result was obtained. The presence of iron either in the granules or in the cytoplasm could not be demonstrated. The nitro-molybdate reaction, employed by Macallum, 98, to detect phosphorus microchemically in the tissues, also gave a negative result. Prozymogen, either in a diffused form in the proximal zone of the cell, or irregularly distributed in the basal cytoplasm in the form of "basal filaments" of Solger was accordingly regarded as absent.

A comparison of the cellular structure of this gland with the gl. submaxillaris of the hedgehog (*Erinaceus europæus*) discloses a similarity of structure which is at once both striking and suggestive. This gland in the hedgehog was first described by Kultschizky, 85, and later by Krause, 95. In the tubules of the gland are found granular and non-granular cells. With Biondi's stain the granules assume an intensely red color, as does also the meshwork found within these cells. The granule-containing cell in the rabbit's submaxillary react similarly with Biondi's stain. The cells destitute of granules, which occur in the peripheral part of the tubule in the hedgehog's gland are stained blue, while the clear, non-granular cells of the rabbit's submaxillary are colored a violet-blue with this stain. The cytoplasm of these outer peripheral cells possesses a well-marked network, within which granules are never found. The nucleus is often irregular and lies close to the basement membrane. Kultschizky described the granular cells as serous, and the cells lying outside of the latter and not containing granules as mucinoid. He was able to show that the two kinds of cells in this gland were arranged in regular groups and that the serous cells did not appear in the form of demilunes, as in the mixed salivary glands of other animals but occupied once in a while a great part of the gland lumen.

Krause also called attention to the similarity existing between the submaxillary glands of the rabbit and hedgehog: "Es lag natürlich sehr nahe der Submaxillaris des Kaninchens, in welchem wie Nussbaum zuerst beschrieben hat, die Endstücke der Ausführungsgänge zahlreich sich in Osmium säure intensiv schwärzende Granula enthalten, welche der erwähnte Autor für Ferment Körnchen halt."

The relation of the two kinds of cells to each other in this gland of the hedgehog, while quite simple does not display the constancy nor regularity of position found in the corresponding gland of the rabbit, where the granule-containing cells always occupy a position intermediate between an intercalated duct and the group of clear or non-granular cells. In the hedgehog the tubule of granular cells branches simply, and its end piece is covered with these mucinoid cells. The latter are related in such a way to the former that mostly the middle of a small lobule contains only granular cells, which are surrounded by a garland of non-granular cells. This relationship Krause verified by means of Berlin blue injection.

The similarity in the behavior of these cells of the two glands towards stains and in their microchemical reactions is very marked. In both

glands the granule-containing cells are oxyphilic and the non-granular basophilic. When fresh sections are placed in a one per cent solution of pyrogallie acid in both glands the roddeed epithelium of the ducts and the granule-containing cells are stained a lively brown, while the second kind of cell manifests no reaction. Merkel, 83, employed pyrogallie acid to demonstrate the presence of calcium salts microchemically in the salivary glands. Hence Krause concluded that the granule-containing cells secrete the bulk of the lime salts and albumin of the submaxillary saliva of the hedgehog. On the other hand sections of the rabbit's submaxillary when treated with a solution of ammonium purpurate for the detection of calcium salts failed to show the presence of calcium either in the cells of the ducts or of the granular areas. A brown coloration with pyrogallie acid cannot be regarded as a test for the presence of calcium.

That both glands when treated with Krause's mucin stain give a similar reaction has been already mentioned in the present paper. In the hedgehog the granular (serous) cells stain a weak blue and the non-granular mucinoid cells take on the simple metachromatic red color characteristic of mucin. The submaxillary of the rabbit when treated with this stain gave a similar result; the granular cells stained blue and the clear cells a metachromatic red. Although this stain, as before pointed out, is not specific for mucin, it nevertheless serves to indicate a similarity in the chemical composition of the cytoplasm in the corresponding cells of these two glands.

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