# XVI. *On the Histology and Physiology of Pepsin-forming Glands.*

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THE following paper contains an account of observations upon *Rana temporaria, Bufo vulgaris, Triton tæniatus, Triton cristatus, and Coluber natrix.* In these animals I have examined the structure of the resting stomach and noted the alterations which occur in it during secretion. I have also estimated the relative amounts of pepsin contained by different portions of the stomach, and the amount of pepsin contained by a definite weight of the gastric mucous membrane in the resting and in the active state. I have further attempted to ascertain whether pepsin exists as such, or in a combined form, in the gland-cells.

I do not propose to give a complete account of the structure of the resting stomach in each animal, although certain points in which my observations differ from or extend those of previous observers I may have to treat somewhat fully.

I shall first describe the individual peculiarities which occur, and shall then discuss them with a view of drawing some general conclusions.

## RANA TEMPORARIA.

### STRUCTURE OF ŒSOPHAGEAL AND GASTRIC GLANDS.

*The œsophageal glands.*—These glands have been described by SWIECICKI,* NUSSBAUM,† and PARTSCH.‡ The glands are of the complex tubular type;§ amongst the proper secreting cells are mucous cells, these occur in smallest number in the final dilatations of the ducts. In the ducts ciliated cells are sometimes, though rarely, to be seen. The secretory cells are cylindrical or conical and are smaller than the gastric gland-cells. NUSSBAUM‖ has shown that they contain in the fresh state conspicuous granules; in a teased-out fresh preparation many of these granules are seen floating in the fluid: they are three to five times as large as the granules seen on teasing out similarly the gastric glands; they are even larger than the granules of the pancreas.

The granules have the following reactions:—They dissolve readily in hydrochloric acid 0·4 per cent., less readily in weak alkalies. Bile dissolves them almost instantaneously. Alcohol, varying in strength from 50 per cent. to absolute, dissolves them in part but not entirely; with each granule an undissolved residue is left. I conclude that the solution is real and not simply caused by the extraction of water, since a like effect is not produced by glycerine or saturated solution of sugar. On adding alcohol the granules sometimes run together before the partial solution takes place. Thus, in one

* SWIECICKI, PFLÜGER'S Archiv., Bd. xiii., s. 444, 1876.

† NUSSBAUM, MAX SCHULTZE'S Arch., Bd. xiii., 1877.

‡ PARTSCH, MAX SCHULTZE'S Arch., Bd. xiv., s. 179, 1877.

§ I apply the term "simple tubular" to such glands as consist of one tube; when several tubes are given off by one duct, I call the glands "compound" tubular; when the tube or tubes arising from a duct divide, I call the gland a "complex" tubular gland. KLEIN describes the œsophageal glands as acinous glands (STRICKER'S 'Handbook,' vol. i., p. 538).

‖ *Op. cit.*, s. 748.

instance, I watched three granules lying close together; first one ran into its neighbour, then this into the remaining granule, the whole forming one large granule; in it several brighter spots appeared; later, the greater part suddenly vanished leaving four or five rather bright particles arranged so as to produce the appearance of a fragment of a small-meshed network. I have little doubt that the apparent network seen in the cells in alcohol specimens has its origin from these residual particles.

I may mention that the zymogen granules of the pancreas behave in a similar manner with alcohol, so that in alcohol specimens the granules of the inner zone are only the representatives of the actual zymogen granules.

The granules are not obviously affected by irrigation with a 5 per cent. solution of ammonium chromate or bichromate; they disappear however from the gland-cells when a piece of the œsophagus is left for one or two days in either of these fluids, the nuclei then show distinctly a network or a tangle of fibres.

According to HEIDENHAIN* the gland cells of the pancreas after a two to three days' stay in 5 per cent. ammonium chromate show a marked striation in their outer portions. In similarly prepared specimens of the œsophageal glands I have not been able to observe a similar structure, although in osmic acid specimens the outer zone not infrequently has a faint striation.

It was shown by NUSSBAUM† that the œsophageal gland granules are preserved by osmic acid. In treating glands with this reagent I usually use the following method. The tissue is placed in a 1 per cent. solution for twenty-four hours, removed to 50 per cent. alcohol for fifteen minutes, and then transferred to 75 per cent. alcohol. Sections are cut on the following day. In sections so prepared the granules are stained not very deeply and have a yellow-brown tint. The sections as a whole are less stained than similarly prepared sections of the stomach. The tint of staining of the gastric gland granules tends to be brown-black rather than yellow-brown.

SEWALL and myself‡ found that the œsophageal gland-cells occasionally showed clumps of highly refractive granules in their peripheral portions. To distinguish these from the proper granules of the cells, we called them "border" granules. Further investigation has shown me that these are really fat globules. Their position is very constant and they give a striking appearance to osmic acid specimens. Quite similar fat globules are occasionally to be seen in the pancreatic gland-cells. They occur also in the gastric gland cells of the Frog, Toad, and Newt (see Plate 78, fig. 7), although their arrangement is not quite so regular. In a subsequent paper I hope to discuss the causes which influence the appearance of fat globules in the above and in other secretory gland-cells.

*The oxyntic glands.*—I propose to use the term oxyntic glands (ὀξύνειν—to make

* HEIDENHAIN, PFLÜGER'S Archiv., Bd. x., s. 561, 1875.

† *Op. cit.*

‡ LANGLEY and SEWALL, Proc. Roy. Soc., Oct., 1879, p. 383; Jour. of Physiol., vol. ii., p. 283, 1879.

sour, to acidulate) for those glands in the stomach which are differently called by different observers "fundus," "peptic," or "rennet" glands. It is only after great hesitation that I venture to employ a new term, but without a new term I find myself reduced to circumlocution or inaccuracy. That the present nomenclature is unsatisfactory scarcely needs to be pointed out. In the Rat there are no glands in the fundus of the stomach; in the Rabbit the glands of the fundus proper differ in some important points from those of the greater curvature, yet both are called fundus glands. The terms peptic and rennet glands are inappropriate, since the pyloric glands also secrete the peptic and rennet ferments. The terms "simple" and "compound" glands suggested by EBSTEIN* are applicable only to the gastric glands of Mammals, for it is only in Mammals that compound glands, *i.e.*, glands possessing both border- and chief-cells, occur.

The one characteristic point of the "fundus," "peptic," or "rennet" glands in all animals is the secretion of an acid fluid. This characteristic is suggested by the word "oxyntic."

In the Frog, then, the glands which produce the secretion active in gastric digestion are

- The œsophageal glands.
- The gastric glands.
  - The oxyntic glands.
  - The pyloric glands.

The oxyntic glands have been most fully described by PARTSCH.† The epithelium on the surface of the mucous membrane and that in the mouths of the glands consists of long cylindrical cells, which in their outer portions contain mucigen. Each cell is prolonged into a fine process. In the necks of the glands are found, in the upper portion, nearly cubical cells, in the lower portion two or three very marked mucous cells. In the body of the gland are the proper secretory cells; they are rather irregular in form, but have a tendency to be ellipsoidal. When the cells are partially isolated after treatment with neutral ammonium chromate 5 per cent. they are frequently seen to possess a short prolongation corresponding to the process of a mucous cell of the surface. The cells on the opposite sides of a gland-tube are usually so arranged that the nucleus of a cell on one side faces the junction line of two cells on the other. (See Plate 77, fig. 10.)

* EBSTEIN, MAX SCHULTZE's Archiv., Bd. vi., s. 538, 1870. The words "simple" and "compound" are so commonly used to describe the form of glands, that it would probably lead to some confusion to use them for glands consisting respectively of one or of two kinds of cells.

† *Op. cit.* The earliest account I have met with is that of HEIDENHAIN, MAX SCHULTZE's Archiv., Bd. vi., s. 394, 1870.

When the muscular coat is removed from a fresh stomach and the mucous membrane pinned out with the muscularis mucosæ uppermost, the glands do not as a rule show distinct granules, but present a ground-glass appearance. When the mucous membrane is thin and the light good, small granules of scarcely greater refractive power than the cell-substance in which they lie can be seen with ZEISS' obj. D, oc. 2. The cells are not filled with obvious granules, as are the œsophageal gland-cells, but, on the other hand, they are not clear and transparent like the cells of the pyloric region.

The small granules come out distinctly on teasing a portion of the mucous membrane in salt solution* (0·6 per cent.) or, better, in water. The small size of the granules and their slight refractive power make it difficult to observe the action of reagents on the individual granules. The reagents, however, mentioned above as dissolving the œsophageal gland granules—viz: bile, dilute acids, and alkalies—soon make the cells transparent, leaving in them little or no trace of the granules which previously crowded them. It would appear, then, that the granules of the œsophageal and oxyntic glands resemble one another in certain general characteristics.

On treatment with neutral ammonium chromate (5 per cent.) the nuclei of the oxyntic gland-cells show a network like, but less distinct, than that described by KLEIN† in the similar cells of the Newt.

*The glands in the junction of the œsophagus and stomach.*—The characteristic œsophageal and oxyntic glands just described are separated from one another by intermediate forms. The last two or three millimetres of the œsophagus and the first one or two millimetres of the stomach contain many transition-forms between the two. PARTSCH has mentioned that near the stomach the œsophageal glands lose their complex tubular form and pass into the simple tubular gastric glands. They do not, however, regularly and in succession become more and more simple; there are many irregularities. Here and there may occur what is little more than a depression of the surface epithelium, or there may be a return to the complex gland. The mucous membrane in this intermediate region is thinner than that either above or below it. SWIECICKI, from the examination of hardened specimens, described the œsophageal glands as stretching into the cardia. What we, in fact, see when the fresh mucous membrane is stretched out is that in the intermediate region the glands are fairly equally scattered throughout, and are not arranged in packets with intervening spaces as in the œsophagus, but that, nevertheless, the first part retains the characteristic œsophageal gland granules. When this intermediate region is treated with osmic acid, and subsequently with alcohol, we find that the first simple tubular glands which occur have rather large yellow-brown-stained œsophageal granules (cp. Plate 77, figs. 1, 2, and 3), whilst farther backwards these granules begin to be replaced in some of the gland cells by the small brown-black-stained oxyntic-cell granules. We have, then,

* Salt solution makes the glands at first more cloudy; then the cloudiness disappears and the granules become obvious.

† KLEIN, Quar. Jour. Mic. Soc., vol. xviii. (new ser.), July, 1878, p. 315, *et seq.*

glands with some cells resembling in the main the œsophageal, and others resembling the oxyntic gland cells. Farther from the cardia the œsophageal granules diminish still more in size, so that they are scarcely or not at all larger than the oxyntic-cell granules. In some cases the granules can be referred without much difficulty to one type or the other; in other cases they cannot. Occasionally one or more cells with large "œsophageal" granules occur in oxyntic glands at some distance from the cardia. The granules in the anterior oxyntic glands are, as a rule, rather larger than those in the posterior.

*The pyloric glands.*—The pyloric region of the stomach forms about one-fifth to one-fourth of the length of the whole stomach; it is recognised under the microscope by the transparency of its cells. PARTSCH* compared the pyloric gland to the mouth and neck of an oxyntic gland. The comparison is, I think, just. The cylindrical cells of the surface of the pyloric mucous membrane become shorter and shorter, and pass without any break into the sub-cubical cells which form the greater part of the glands; below these are usually, though not always, one, two, or more distinct mucous cells. When the glands between the oxyntic and pyloric glands are examined it is seen that the mucous cells at the lower part of the pyloric glands correspond to the mucous cells at the lower part of the neck of the oxyntic glands.

If the stomach of a hungry Frog is hardened in alcohol, and sections cut, it is seen that the sub-cubical cells of the oxyntic and pyloric glands closely resemble one another, and further that both closely resemble the cylindrical cells.

The outer portion of the cylindrical cell consists, as we know, mainly of mucigen. In alcohol specimens this mucigen portion is transparent and sharply marked off from the protoplasm which forms the rest of the cell. Now, in alcohol specimens the outer portion of the sub-cubical cells, both in the oxyntic and pyloric glands, is similarly marked off; that is, the outer portion of the sub-cubical cells also consists mainly of mucigen. The two kinds of cells, then, resemble one another in having a protoplasmic inner portion and a mucigenous outer portion. They differ somewhat in shape: the one is usually a four-sided wedge, tapering to a fine point; the other more approaches a cube in shape, with a process from the base which bends round and overlaps the cell next below it. If the process were straightened and the cell elongated a little we should have a "cylindrical" cell. The position of the processes of the sub-cubical cells is exactly similar to the position of the processes of the mucous cells; probably, indeed, these cells differ from one another chiefly in the extent to which they form mucigen. In the above description I have added but little to the account given by HEIDENHAIN, PARTSCH, and NUSSBAUM.

Osmic acid specimens prepared as above described (p. 665) do not show the resemblance of the cylindrical and sub-cubical cells equally clearly. The former have the mucigen border fairly well marked, but the latter are much more equally stained throughout: the mucigen border is only shown by a somewhat lighter yellow-brown

* *Loc. cit.*

coloration.* Neither show any granules. The distinction comes out, however, on keeping the specimens in glycerine (Plate 77, fig. 2), partly by the protoplasmic portion becoming darker, and partly, I think, by the mucigen portion becoming lighter. The distinction is also clear if the osmic-hardened stomach is left in alcohol a week or more before sections are made. In the sub-cubical cells the nucleus is placed in the outer portion of the cell and takes up nearly the whole of its transverse diameter.

*The glands in the intermediate zone.*—In the intermediate zone between the oxyntic and pyloric glands, the glands become shorter, the ellipsoidal cells of the oxyntic glands become fewer, and some of them are replaced by cells similarly shaped but containing few or no oxyntic gland granules; amongst these glands are found simple pyloric glands, which increase in number towards the pylorus until they form the sole constituent. Close to the intestine the glands are very irregular in form, and become more and more simple depressions of the surface epithelium.

## THE CHANGES WHICH OCCUR IN THE ŒSOPHAGEAL GLANDS DURING DIGESTION.

SEWALL and myself† have previously given some account of the most striking event of secretory activity, viz.: the using up of the cell granules. We found that in the normal hungry Frog the cells were granular throughout and that very soon after feeding the animal the granules began to disappear, and continued to disappear until about the sixth hour; at some later period which we left undetermined the granules began to increase, and increased steadily until the cells were again granular throughout.

To this account I would make one or two additions.

When a Frog is fed, the œsophageal glands near the stomach show greater signs of secretory activity than glands more remote. This is the case, at any rate, when only a moderate amount of food is given. The glands which are nearest the stomach are the first to show a clear zone, then those just above, and so on to the beginning of the œsophagus.

Generally speaking, the smaller the amount of food given the more is the obvious effect confined to the œsophageal glands that are near the stomach, and within certain limits the more digestible the food and the greater its amount the more simultaneous is the change taking place in the glands.

Hence, in comparing the state of the glands at different times after food has been given, it is important to take a strip down the whole length of the œsophagus; and in comparing the amount of pepsin in different stages of digestion it is important to take pieces of the œsophagus from a corresponding region.

As the outer zone increases the granules in the inner zone become smaller, the

* The mucous cells are also fairly equally stained in specimens treated with osmic acid only. This probably explains how it is that BLEYER (quoted by PARTSCH) failed to observe mucous cells in osmic acid specimens.

† *Op. cit.*

diminution in the size of the granules is very marked in cells in which the outer zone takes up the larger part of the cell. After great activity there is also an obvious diminution in the size of the cells, and although it is difficult to be certain of the changes which take place in the first stages of activity, I have little doubt that the diminution in the size of the cell begins with the development of the clear outer zone.

In specimens treated with osmic acid there is another effect of activity to be seen, viz. : the cell-substance stains more deeply than during rest. In the œsophageal glands the tint of staining is not in so constant a relation to the amount of secretion produced as in some other glands, the extent to which the tint deepens seems to vary as the stimulation is produced by digestible food or by mechanical stimulation. These and some other apparent causes of variation have not been closely determined, but the main fact of an increase in the depth of staining has always been obvious.

It was stated by SEWALL and myself* that absolute alcohol added to the fresh teased-out gland altered the normal appearances; and I have said above that the granules are in part dissolved. Nevertheless, alcohol specimens of œsophageal glands taken during digestion show the two zones in the gland-cells; the non-granular zone stains with carmine, and thus specimens can be obtained (Plate 77, fig. 8), which, except for the smaller size of the cells, closely resemble similarly prepared specimens of the pancreas. It is almost unnecessary to remark that the "granules" of the inner zone in alcohol specimens are not the granules present in the fresh gland. With ZEISS', oc. 2, obj. E or F, the granular zone appears as a fine network.

Nothing very definite can be said as to the time after feeding at which the changes in the œsophageal glands occur. When Frogs are taken as nearly as possible alike, and they are treated in the same way, then the results obtained correspond very closely, but when such results are compared with those obtained from Frogs at a different season of the year, with those obtained from Frogs which are older or younger, more or less healthy, or when different amounts of food are given, then considerable divergences occur.

The changes occurring are in each case of the same nature, but the extent to which these changes take place varies largely. Hence any estimation made of the time taken for the first appearance of a clear zone, for its maximum development, and so on, can only be approximate.

During the first hour or hour and a half after feeding, no distinct change is to be seen. After this period a diminution in the number of the granules in the outer half of the cell becomes obvious. Usually this is first seen in the glands close to the stomach. The disappearance of granules in the outer portion of the cell goes on, so that a clear zone is formed. The clear zone steadily increases until the sixth to twelfth hour, or even later, the time varying with the state of the animal and the amount of food given. The glands then begin to become more granular; the time of complete recovery varies enormously; in some cases the glands are throughout granular

* *Op. cit.*, p. 283.

in twenty-four hours from the time of feeding the animal, in others they do not become so for several days. It will be noticed that the granules begin to increase before the food has left the stomach.

### THE CHANGES WHICH OCCUR IN THE ŒSOPHAGEAL GLANDS WHEN THE ANIMAL IS FED WITH SPONGE.

Hitherto I have spoken of the effect of feeding with worm, *i.e.*, with a readily digestible substance; having in mind HEIDENHAIN'S experiments on the isolated fundus in Dogs I was anxious to see what would be the effect on the œsophageal glands of mechanical stimulation of the gastric mucous membrane. To this end a number of Frogs were fed with sponge.

If the piece of sponge is small so that it can pass the pylorus a slight effect only is produced: usually a thinning of granules in outer portion of the cells of the anterior œsophageal glands, and a small zone in the posterior œsophageal gland-cells.

When the piece of sponge is too large to pass the pylorus, it serves as a continual stimulus to the œsophageal glands. The extent of the change produced is within certain limits the greater the larger the piece of sponge; it varies, too, widely with the condition of the Frog. The changes produced are like those produced by feeding with worm, but go on very much more slowly. The first distinct thinning of granules is usually not seen for three or four hours, and may not be obvious till even later. The glands near the stomach are first affected. The disappearance of granules then goes steadily on.

It will be remembered that in the worm-fed animal the granules begin to increase in six to twelve hours. After feeding with sponge no such increase occurs until at any rate some days.

The extent to which the disappearance of granules proceeds varies in different cases: in many cases two days after feeding* with a rather large piece of sponge, occasionally in a less time, scarcely any granules are left; and in some glands not a granule is to be seen (Plate 77, fig. 6 (*a*), 6 (*b*)).

When the clear zone is largely developed there are usually to be seen in it, often in rows, fine granules much smaller than those which form the remains of the granular zone (see Plate 77, fig. 6 (*b*)).

At this stage the diminution, both in the size of the cells and in the size of the granules, is very striking. Moreover a very characteristic appearance is imparted to many of the glands by the large size of the lumen. The diameter of the lumen varies considerably in neighbouring glands; in some it is more than half the length of the cells; when granules remain they form a kind of ragged fringe to it (Plate 77, fig. 5).

* NUSSBAUM (*op. cit.*, s. 749) made some observations upon the *direct* stimulation of the œsophageal mucous membrane, the cardia being ligatured before the animal was fed with sponge. He found under such circumstances that the granules entirely disappeared from the cells in three to five hours. SEWALL and myself (*op. cit.*, p. 285) were unable to observe any such rapid action.

If at any time the sponge is vomited by the Frog, or if the sponge be removed, the glands begin at once, or almost at once, to return to the normal state; and in one to two days the lumen is no longer visible, and the cells are crowded with large granules.

We know from the experiments of HEIDENHAIN that in Mammals the mechanical stimulation of one part of the stomach causes only a temporary secretion from the glands of other parts. In the Frog the case is different, the mechanical stimulation of the stomach causes a considerable secretion from the œsophageal glands—a secretion which lasts several days at least.

### ON SOME DISPUTED POINTS IN THE HISTOLOGY OF THE ŒSOPHAGEAL GLANDS.

In the account of the changes in the œsophageal glands given by SEWALL and myself there were two points which clashed with the earlier observations which NUSSBAUM made on osmic acid specimens. He described the gland-cells of the normal hungry Frog as having a large clear zone, and found that on feeding the animal the granules increased so that in three to five hours a clear zone was no longer to be seen.

GRÜTZNER'S* results suggest an explanation of the divergence between the account of NUSSBAUM and that of SEWALL and myself. He finds that in the normally hungry Frog the œsophageal glands are granular throughout and diminish in granularity during digestion; but finds also that if a Frog is kept longer than usual without food a clear zone is then formed in the œsophageal glands and that on feeding there is at first an increase of granules. Thus according to GRÜTZNER, NUSSBAUM'S results would represent what occurs in a pathological and not what occurs in a normal condition.

During the last year and a half I have made a considerable number of observations with the view of determining the points at issue. As regards the state of the glands in the normal hungry Frog I have seen no reason to alter my first-formed opinion.

In some Frogs a large clear zone in the œsophageal glands does occur, namely, in those in which there are signs of general inflammation. In nearly all cases in which I have found a marked clear zone in the gland-cells of a hungry Frog, the animal had some mark or other of an ill state of health. I have frequently selected lively, active Frogs, and sluggish, unhealthy ones from a batch brought to the laboratory, and in a few days examined the œsophageal glands. The gland-cells in the former were granular throughout; those of the latter had almost always a clear zone.

There is one condition in which I have found apparently healthy summer Frogs, kept without food for three or four days, show a clear zone in the œsophageal glands, viz.: when they have remaining in the stomach some piece of stick or leaf, or other undigested substance; such undigested material causes a continuous secretion from the œsophageal glands (see action of sponge, p. 671).

* GRÜTZNER, PFLÜGER'S Arch., Bd. xx., s. 395, 1879.

During prolonged fasting a diminution of granules occurs. The amount of diminution varies in different Frogs, and varies with the time of year: it is less in winter than in summer. In most cases the granules only become fewer at the outer borders of the cells; in others a clear zone is formed. Thinning of granules, however, rather than the formation of a clear zone, seems to me to be the normal effect; for in all the *perfectly healthy* December Frogs I have examined, the granules, though fewer at the outer part of the cells, still extended to the periphery. Some diminution in the size of the cells also takes place.

I conclude, then, that in a healthy Frog during the winter months fasting does not necessarily produce a distinct non-granular zone in the œsophageal glands. In other months when the tissue change is more rapid, fasting may produce a non-granular zone, but I think the most usual and effective cause of such a zone is some alteration in the general condition of the body, independent of fasting, by which the normal equilibrium in the gland-cells is disturbed.

My experiments have only been made on *Rana temporaria;* in other species of Frogs, fasting may have a greater effect, just as the effect is greater in *Triton tæniatus* than in *Triton cristatus.*

It remains to consider how far an increase of granules takes place immediately after feeding. NUSSBAUM considered that the granules always increase in the first hours of digestion, but it is to be remembered that his observations were made on Frogs in which the œsophageal glands had a non-granular zone before feeding. GRÜTZNER only found an increase of granules in the first hours of digestion when a clear zone had been previously developed in the œsophageal glands by long fasting. In both cases, then, the increase is only described as taking place in glands having a non-granular zone to start with.

The method of experimenting contains an obvious source of error. We do not know with certainty what is the state of the glands before feeding, and therefore cannot say positively whether an increase or decrease of granules takes place. My own observations lead me to conclude that in a normal hungry Frog no increase of granules takes place in the first hours of digestion, and that in abnormal Frogs, *i.e.*, in those having already zones in the œsophageal glands, an increase may or may not take place. I have seen only one instance of apparent distinct increase, and I am by no means certain that it was not simply apparent.

I am not prepared to deny that a *slight* increase may not take place in all cases, for I think our present methods do not allow us to detect slight differences in the amount of granules contained by gland-cells. Further, I am strongly of opinion that a formation of granules goes on during the whole digestive period, and I can readily conceive that under certain circumstances the formative might overbalance the excretory processes in the first hours, as they certainly do in the last hours of digestion.

## THE CHANGES WHICH OCCUR IN THE OXYNTIC GLANDS.

PARTSCH * is the only observer who has described any alteration in the gastric glands during activity. His observations were made upon alcohol-hardened specimens. According to him, when a Frog is fed the cells of the gastric glands increase in size for twelve to eighteen hours, and subsequently to this diminish, so that in about twenty-four hours they have returned to their normal condition. This account I cannot in any point confirm.

I will first briefly state what the changes are which I find do occur. Some are like those which occur in the œsophageal glands, viz.: the cells become smaller; the granules become smaller and less distinct; the lumina become apparent; there is an increase in the cell-protoplasm of substance staining with osmic acid. These are the only changes which normally occur in those glands which immediately follow the intermediate region between the œsophagus and stomach. The number of these glands is not constant; within certain limits the larger the meal the fewer glands there are which do not undergo the changes to be presently described.

In the remainder of the oxyntic glands, forming a considerable majority, there are other additional changes which strongly contrast with those which take place in the œsophageal glands; we have seen that in the œsophageal glands the granules disappear from the outer portion of the cells during activity, *in the majority of the oxyntic glands, on the contrary, the granules disappear from the* inner *portion of the cells during activity.* The other and less important differences which exist will be considered in the course of the following description of the glands.

*Examination of fresh specimens.*—Several of the changes mentioned above cannot be satisfactorily seen in fresh specimens of the oxyntic glands; in the pinned-out mucous membrane it will be remembered that the glands do not show distinct granules, so that any alteration in their number and size cannot be well observed. The active glands are, however, much more transparent than the resting; this is in part no doubt due to the cells having become smaller and the membrane consequently thinner; but it is also due to a diminution in number and size of the granules, for a distinct difference in granularity is seen in the two specimens on adding salt solution 0·6 per cent.

During strong activity the oxyntic glands near the pyloric region may become almost as transparent as the pyloric glands; nevertheless, they can still be fairly readily distinguished; they are more refractive than the pyloric glands, and with a not too bright light have a very faint yellowish tinge. The oxyntic and pyloric glands of the Newt and Snake show in similar circumstances similar differences.

The increase in the size of the lumen can be seen; it is usually most obvious in the gastric glands near the pyloric region. It is only at the height of digestion after a

* *Op. cit.*, s. 193.

very heavy meal, or in certain abnormal circumstances, that the lumen becomes large and conspicuous.

The disappearance of granules from the inner portion of the cells is little or not at all marked in the fresh specimen. No distinct zones, such as occur in the œsophageal glands, are formed.

*Examination of specimens treated with osmic acid.*—In order to make out more in detail the changes which take place, the stomach is pinned out in osmic acid for twenty-four hours and then put into alcohol. The best preparations are obtained when the tissue is left in alcohol for several days before sections are made, so that the cells are stained of a black instead of a yellow-brown tint.

In the resting state the glands show no lumina, the cells are crowded with fine granules, and through the granules the nuclei appear as clearer spots, the cell outlines being very faintly marked (Plate 77, fig. 9); the cell-substance is almost unstained.

It will be convenient to consider first the changes which take place in a healthy summer Frog fed with a worm small enough for the stomach to have emptied itself completely in twenty-four hours.

In one to two hours after feeding, the lumina begin to be obvious, and the granules to disappear from the inner borders of the cells. This causes the glands to assume a very characteristic appearance. When examined with obj. C or D (ZEISS) a clearer line is seen to run down the middle of each gland; it is usually of a zig-zag or corkscrew form; the form being naturally dependent upon the arrangement of the cells in the gland-tube (Plate 77, fig. 10).

Up to about the fifth hour these changes become more and more pronounced, and at the same time the cells and the remaining granules they contain become distinctly smaller, and the cell-substance stains more deeply.

It is noteworthy that the granules do not disappear simply from that part of the cell which immediately borders the lumen, but to some extent also at the sides of the cells where they are in contact with one another (see Plate 77, fig. 11). The disappearance of granules at the sides of the cells does not extend to the basement membrane.

At the period of maximum change the nucleus is much larger compared with the cell-substance than it is during rest; it is still surrounded by finely granular protoplasm and is sometimes placed near the outer border of the cell, in this differing from the nuclei of gland-cells hitherto observed. Very frequently the granules appear to be most numerous in the cell-substance immediately on the inner side of the nuclei.

The return to the normal appearances begins about the fifth hour; so that during the greater part of the digestive period the formative processes go on whilst the excretory are still active. In twenty-four hours the glands have nearly or altogether returned to the hungry condition.

The above I regard as the normal round of changes in the oxyntic glands of the Frog during digestion.

In the oxyntic glands, however, as in the œsophageal, the times and extent of the changes vary enormously with the amount of food given and the general condition of the Frog.

If a Frog is fed with several worms so that the stomach is much distended with digestible food, the changes are greater and persist for a much longer time. The diminution in the size of the gland-cells makes itself obvious in a diminution in the length of the glands. In twenty-four hours the glands instead of having returned to the hungry state are still small and consist of somewhat small cells with a more or less distinct inner non-granular border; the lumina are frequently large. These points will be seen in Plate 77, fig. 11, taken from a Frog twenty-four hours after feeding with four worms. In such specimens we are better able to observe the increase in size of the lumen. Generally speaking, as the cells become smaller the lumen becomes larger, but we have to reckon not only with the size of the cells but with the pressure of the surrounding tissue. It is probably due to local variation in this respect that the glands in different regions of the same stomach have lumina of very different size. Usually the lumina are most conspicuous at the base of the glands.

In Frogs to which an excess of food has been given, the non-granular inner zone is usually most obvious about the eighteenth or twentieth hour after feeding. The cells then have increased and are still increasing in size; the greater clearness with which the non-granular zone can be seen is then probably due to the nett increase in the cell-granules taking place more slowly than the increase in the cell-protoplasm. A slower nett increase of cell-granules might clearly result either from granules being used up more quickly or from granules being formed more slowly.

The effect of fasting is not very great in winter Frogs which are subjected to the ordinary winter temperature. The glands and their cells become somewhat smaller, but the granules do not ordinarily disappear from the inner part of the cells.

But in Frogs at other times of the year, or winter Frogs which are kept in the warm, fasting produces a fairly marked secretory appearance; the glands are small, the cells of the posterior gastric region have a small non-granular inner portion, and the lumen is to be seen. When Frogs in this condition are fed the ordinary secretory changes set in, except that in twenty-four hours the glands have not or have scarcely recovered their initial condition. They still differ considerably from normal glands.

We have seen that in the œsophageal glands the maximum change is produced by feeding the Frog with sponge. It is not so with the oxyntic glands; in these feeding with readily digestible substance, as worm, is the most effective means of producing a change. When the oxyntic glands are examined twenty-four to forty-eight hours after feeding the Frog with sponge, no very great divergence from the normal hungry state is to be observed. The gland-cells are smaller, but there is very little sign of a non-granular inner zone, and the lumen is seldom obvious. The amount of acid contained by the sponge shows that the secretory processes have been going on.

If however a Frog that has been fed with sponge two days previously be fed with

worm, very marked signs of secretory activity result. The chief feature is the diminution in the size of the gland-cells; the nuclei are usually oval, frequently lie close to the basement membrane, and are surrounded by only a small amount of darkly staining cell substance (see Plate 78, fig. 1). It is to be noted that this gland is much more highly magnified than the rest). The recovery, too, is long delayed, and an inner non-granular zone is visible in the posterior oxyntic glands for some time after the cells have begun to increase in size. The amount of change produced here—and in the case of feeding fasting summer Frogs—*seems* to be greater than that produced by a like treatment of normal hungry Frogs. I say the change *seems* to be greater, since it is hazardous to institute a comparison between the amount of change produced in glands which start secreting in different states. Assuming, however, that the comparison is just, a not unlikely conclusion is suggested, viz.: that the formative processes require certain elaborated material, and that during the stimulation of the glands of the fasting Frog with sponge, the elaborated material is largely used up, so that the rapid waste brought by the presence of digestible food cannot be made good so quickly as normally.

It is worth remark that the oxyntic and œsophageal glands do not necessarily show a parallelism in the amount of change they respectively undergo in digestion. Under special circumstances the one or the other may be most affected.

Lastly, we have to consider whether on feeding a Frog there is a preliminary increase in the size of the oxyntic cells or in the number or size of the granules contained by them, before the decrease sets in. From what I have already said, it will be seen that, as far as my observations go, the preliminary increase, if it takes place, can only last a short time. Even of such brief increase I have seen no instance. I am not, however, inclined to deny that it might under certain circumstances take place.

## Changes occurring in the Pyloric Glands and in the Necks of the Oxyntic Glands during Digestion.

The changes are of a like nature in both, but usually more strongly marked in the former, and, as might be expected, the changes do not run a parallel course in the two portions of the stomach. My observations have been made upon osmic acid specimens. Of the early stages of digestion I cannot speak with any confidence. The mucigen border in all cells frequently appears to be larger and to bulge more at its free surface; this I am inclined to attribute rather to a swelling of some constituent of the outer part of the cells than to an increase by metabolism. However this may be, there is, at the height of digestion with a heavy meal, a very considerable diminution in the amount of mucigen in all the cells.* The cylinder cells of the surface are affected in a similar manner although to a less degree. All the cells, too, are smaller. The inner portion of the sub-cubical cells in osmic acid specimens instead

* Sewall and myself found similar changes take place in the mucous cells of the œsophagus and of the œsophageal glands during digestion. (Journal of Physiol., vol. ii, p. 284, 1879.)

of appearing homogeneous, shows a network with inter-fibrillar substance. The inter-fibrillar substance takes the form of granules. In sections cut the day after the tissue has been placed in alcohol, the granules are only slightly stained, but if the tissue be left in alcohol for several weeks before sections are made, the granules then stain deeply and are very distinct (Plate 77, fig. 4). The network with its contained granules makes its first appearance at the junction of the protoplasmic and mucigenous portions of the cells (see Plate 78, fig. 1), and as secretion goes on encroaches more and more on the mucigen. A similar network is seen during digestion in the cylindrical cells of the surface of the mucous membrane, but it is usually confined to the junction of the protoplasm of the cell with the mucigen.

The changes which take place in these cells differ somewhat, but not essentially, from the changes which take place during secretion in the mucous salivary glands of Mammals. The protoplasm around the nucleus and the protoplasmic network throughout the cell grow; the growth is more rapid in the peripheral protoplasm. It is not quite clear, however, why the inter-fibrillar substance increases in power of staining with osmic acid.

We have seen that during fasting the granules in the pepsin-forming cells slowly diminish, in the distinct mucin-forming cells the amount of mucigen goes on increasing for some time after the granules of the pepsin-forming cells have begun to decrease. The maximum amount of mucigen is contained by the pyloric and similar gland-cells after a moderately prolonged fast. The minimum amount of mucigen is contained by these cells twelve to eighteen hours after a heavy meal; it is then only with difficulty that the mucous can be distinguished from the sub-cubical cells.

Similar changes to those above described are seen in the stomach of a Frog one to two days after feeding with sponge; the main difference is that the tint of staining is usually lighter than after feeding with worm or other digestible substance.

It is worth remark that although the mucous cells of the necks of the glands in alcohol specimens closely resemble the mucous cells of such glands as the sub-maxillary of the Dog, yet in fresh specimens they differ in one important respect. In the sub-maxillary gland of the Dog the cells are in life densely crowded with distinct granules, whilst the mucous cells of the stomach and, generally speaking, of the alimentary canal are transparent and show no trace of granules.

## THE PEPSIN-CONTENT OF THE ŒSOPHAGEAL AND GASTRIC GLANDS.

SWIECICKI concluded from his observations that the œsophageal glands are at any rate the chief source of pepsin, and that the gastric glands produce little, perhaps indeed none.*

* He says: "Alle diese Thatsachen sprechen hiernach dafür dass bei den Fröschen die Pepsinbildung vorzugsweise, ja vielleicht nur allein in dem Œsophagus von statten geht, während der die Belegzellen führende Magen die Saüre bildet" (s. 452).

NUSSBAUM suggested that the pepsin arose from the œsophageal gland granules. He found a correspondence between the number of granules in the gland-cells and the amount of ferment contained by the glands.

SEWALL and myself, whilst differing from NUSSBAUM as to the times of increase and decrease of granules, came nevertheless to the conclusion that the granules were connected with the formation of ferment. To this conclusion we came partly on general grounds, partly by comparing GRÜTZNER'S results on the times of increase and decrease of pepsin with our own on the times of increase and decrease of granules.

I have made some experiments to determine this point. Since the œsophageal glands are affected during digestion to such different degrees in different Frogs, I have paid no especial attention to the alteration in pepsin-content of the œsophagus which occurs in successive hours after feeding.

I proceeded in the following manner: hungry Frogs and Frogs fed with worm or sponge were taken, and (1) the granularity of the gland-cells observed; (2) the relative amount of pepsin contained by equal weights of dried œsophagus estimated.

The Frogs were taken as much as possible alike in size and general condition. Part of the œsophagus was observed fresh, part after treatment with osmic acid and alcohol—the rest of the mucous membrane of the œsophagus was dried and a definite quantity weighed out, this was treated with HCl. 0·2 per cent.—3 cub. centims. for 0·01 grm.—for twenty-four hours. The filtrate from this was tested for pepsin in the ordinary manner by GRÜTZNER'S colorimetric method. Only those specimens which were analysed for pepsin on the same day were compared.

In some cases of slight difference in amount of granules the results were not constant, this I attribute to the imperfection of the method; equal weights of dried œsophagus do not necessarily contain equal weights of secretory gland-cells.

In all cases where there was a marked difference in the amount of granules there was also a marked difference in the amount of pepsin.

Hence, then, the greater the amount of granules the greater is the amount of pepsin to be obtained from the glands. I think we can fairly conclude that the granules give rise to the ferment.

We can now consider the question, Do the oxyntic glands form pepsin? I have suggested above that SWIECICKI was inclined to attribute the pepsin found in the gastric mucous membrane to an absorption of the pepsin secreted by the œsophageal glands. In this I cannot agree with him. The amount of ferment found in the mucous membrane of the stomach is far too great to allow of any explanation except that it is formed by the gastric glands. It is true that when *equal weights* of the mucous membrane of the œsophagus and stomach are taken and their relative pepsin-content compared, the amount is found to be considerably greater in the former than in the latter, yet the latter contains a not inconsiderable amount. Thus in the experiment given below an acid extract of the gastric mucous membrane is made in the proportion of 1 grm. of dried tissue to 2,000 cub. centims. of hydrochloric acid—0·2 per cent. Of this acid-extract 18 cub. centims. are added to 2 cub. centims. of swollen

carmine-fibrin. Without warming a coloration =VI. of GRÜTZNER'S scale is produced in eight minutes, and $1\frac{1}{2}$ cub. centims. of the fibrin is dissolved in three-quarters of an hour.

Moreover, if the pepsin were simply absorbed, how could we explain the enormous difference in pepsin-content which exists between the latter part of the mucous membrane with oxyntic glands, and the adjoining mucous membrane with pyloric glands?

For whilst the acid-extract of the mucous membrane of the median portion of the stomach produces a coloration =VI. of the scale in eight minutes, a similar extract of the pyloric mucous membrane does not produce a trace of coloration with 2 cub. centims. of carmine-fibrin in three-quarters of an hour.

In favour of the view that the gastric glands do not produce pepsin, SWIECICKI adduces the following experiment:—He ligatured the œsophagus, and introduced into the stomach bits of flesh through an opening in the duodenum; in twenty-four hours the flesh was not digested, and contained only traces of pepsin.

The experiment seems to me to contain many sources of error; the ligaturing of the œsophagus seriously interferes with the peristaltic movements of the stomach; nothing is said of collecting the jelly-like masses of mucin which are secreted after such an operation, and which contain the greater part of the pepsin.

I have not repeated this experiment, because of the difficulty of removing completely the contents of the stomach and the consequent impossibility of deducing anything from a positive result were it obtained. But the following experiments, I think, show clearly that the gastric glands do form pepsin. Having destroyed the fore-brain in a Frog, I lay bare the stomach, and ligature it a little below the cardia, then cut open the stomach longitudinally, and remove all mucus and fluid from the mucous membrane. On the mucous membrane a piece of sponge is then placed. Several Frogs are treated in the same manner. In two, four, six, and eight hours respectively the sponge and the mucus that has been secreted by the stomach is tested for pepsin. Within certain limits the longer the sponge has been left in contact with the mucous membrane the greater is the amount of pepsin found.

The oxyntic glands, then, form pepsin. We have seen that the oxyntic glands contain granules, although smaller than those contained by the œsophageal glands. Have the granules in the former a connexion similar to that which exists in the latter? There seems to me to be little doubt that they have. Reasons exactly similar to those which lead us to refer the ferment produced by the pancreatic and œsophageal gland-cells to the granules contained by them lead us also to refer to the granules of the oxyntic gland-cells the ferment produced by the oxyntic glands. As in the œsophagus so in the stomach—the fewer and smaller the granules contained by the cells the less is the amount of ferment contained by a definite weight of dried mucous membrane.

There is another point that deserves mention. We have seen that the anterior oxyntic glands contain, as a rule, somewhat larger granules than the posterior, and,

further, that in some of the former, granules like those of the œsophageal glands not infrequently occur. If, then, the granules are connected with the formation of ferment, we should expect a definite weight of the anterior gastric region to contain more ferment than an equal weight of the median gastric region. This, in fact, is the case (see experiment given below); part of the difference in amount is, however, probably due to the difference in the size of the glands in the two regions.

Lastly, we can consider the pyloric glands. The acid extract of the dried mucous membrane does contain some pepsin, for it dissolves carmine-fibrin more quickly than hydrochloric acid alone; but the amount is very small, and we know that a small amount of pepsin is found in nearly every tissue. I have not compared the ferment-content of the pyloric region of the stomach with that of other tissues, since it seemed to me that even if any pepsin is produced by the pyloric glands the amount must be so small as to be unimportant in digestion. There seemed to me to be no reason for ascribing any special function of producing pepsin to the pyloric glands in the Frog, and consequently no reason for ascribing any such function to the cells of the necks of the oxyntic glands.

The following experiment will show the method I have used in determining the pepsin-content of the œsophagus and different portions of the stomach :—

*Experiment.*—Frog killed by destroying brain and spinal cord. Œsophagus and stomach removed, cut open longitudinally, and pinned down with the mucous membrane uppermost. A moist sponge is passed once over it, starting from the pyloric end, and thus the greater part of the mucus or other stomach contents removed. To remove the remaining fluid or mucus the surface of the mucous membrane is carefully pressed with blotting paper, then moistened with salt solution, and again pressed with blotting paper.

The œsophagus and stomach are then spread out on a glass slide with the mucous membrane downwards and the muscular coat removed. The character of the glands is examined under the microscope and the intermediate regions between the different kinds of glands are cut away—*i.e.*, the junction of the œsophageal and gastric glands is removed, and that region which contains both oxyntic glands and pyloric glands. The oxyntic gland region is then cut through transversely into two, as nearly as possible, equal portions. Thus we obtain the mucous membrane of

(1) Œsophagus.
(2) First portion of stomach.
(3) Second portion of stomach, containing no pyloric glands.
(4) Third portion of stomach, the pyloric region containing no oxyntic glands.

The glass slide with these four pieces of mucous membrane is put in a warm chamber at about 33° C. for one day, and then kept till required in a bell jar over strong sulphuric acid.

The œsophagus and stomach of several Frogs are treated in the same way. Of (1), (2), and (3), 0·03 grm. is weighed out; of (4) 0·015 grm.*

To each of (1), (2), and (3) 3 cub. centims. of hydrochloric acid (0·2 per cent.) is added, to (4) 1·5 cub. centim. They are then put in small stoppered bottles, and left for twenty-four hours at 33° C.

To (1), (2), and (3) 9 cub. centims. hydrochloric acid (0·2 per cent.) is added, to (4) 4·5 cub. centims. Each is well shaken up and filtered.

* Of the Frogs I have used, two give about 0·03 grm. of (1), three give about the same weight of (2) and (3), nine to twelve give about the same weight of (4).

Four equal-sized test tubes are taken; in each is placed 15 cub. centims. of hydrochloric acid (0·2 per cent.), together with 2 cub. centims. of carmine-stained fibrin swollen to a jelly by hydrochloric acid (0·2 per cent.). To these 3 cub. centims. of the filtered fluid from (1), (2), (3), and (4) respectively are added; in five minutes the test tubes are shaken well, and the fibrin allowed to settle. In eight minutes the coloration is compared with GRÜTZNER'S scale freshly made.

(1) tint considerably deeper than x. of scale.
(2) =viii.
(3) =vi.
(4) =0.

There is an obvious difference in the amount of fibrin undissolved in (2) and (3).

In three-quarters of an hour the following is the state of things:—

(1) Mere trace of fibrin undissolved.
(2) Small quantity fibrin undissolved.
(3) About twice as much fibrin left as in (2).
(4) No trace of coloration.

This shows clearly enough the difference in pepsin-content of (3) and (4). In order to bring out more clearly the difference in (1), (2), and (3), a smaller quantity of the acid extract must be taken.

3.20.—One cub. centim. of (1), (2), (3), and (4) is added respectively to four test tubes, each of which contains 15 cub. centims. hydrochloric acid (0·2) and 2 cub. centims. swollen carmine-stained fibrin.

3.27.—(1)=v.
(2)=i.
(3)=0.
(4)=0.

3.37.—(1)=vii–viii.
(2)=iii.
(3)=0.
(4)=0.

3.45.—(1) Nearly all fibrin dissolved.
(2) About half fibrin dissolved.
(3) Small amount dissolved.
(4) No fibrin dissolved.

At 4.45 (4) still shows no coloration. On the next day, however, only a trace of fibrin is left. Under similar circumstances hydrochloric acid (0·2 per cent.) alone takes two days to produce a like effect.

SWIECICKI gives the following table (s. 450):—

*Rana temporaria;* hungry; winter time.

| Time | 2.35. | 2.45. | 2.50. | 2.55. | 3.0. |
|---|---|---|---|---|---|
| Œsophagus | >I. | I.–II. | III. | IV.-V. | V. |
| Cardiac region | 0. | I. | I. | II. | <II. |
| Pylorus | 0. | 0. | >I. | I. | II. |

So that in twenty-five minutes the pyloric region has dissolved very nearly as much fibrin as the cardiac region, and the cardiac region about half as much as the œsophagus; and yet SWIECICKI concludes that the stomach forms no pepsin. It will be seen that my experiments show much wider differences in the pepsin-content of the various portions, in great part, I think, since I have been careful to obtain each gland form without any admixture of the neighbouring gland forms. It is possible, too, that the winter-frog used by SWIECICKI contained very few granules in the œsophageal glands—*i.e.*, that the pepsin-content was in these glands below normal.

## BUFO VARIABILIS.

### Histology of the Œsophageal and Gastric Glands.

Much less attention has been given to the structure and arrangement of the pepsin-forming glands in the Toad than to their structure and arrangement in the Frog. Swiecicki* apparently considers that in both these points the two are alike. He treats the question, however, very briefly, simply mentioning at the end of his paper on the œsophageal and gastric glands in the Frog, that as regards the estimation of pepsin, he obtained in the Toad results similar to those obtained in the Frog.

Partsch† denies that any glands resembling the œsophageal glands of the Frog are to be found in the œsophagus of the Toad. He found in the latter no pepsin-forming, but only mucous glands.

My own observations lead me to take up an intermediate position between these observers. Of the œsophageal glands of the Toad a very considerable number are, it is true, mucous glands, but pepsin-forming glands also occur; these, however, differ in many points from the pepsin-forming glands of the œsophagus of the Frog.

In the Toad it is less easy than in the Frog to tell exactly where the œsophagus ends and the stomach begins, for there is no constant constriction between the two regions, nor is there any change in the character of the glands sufficiently abrupt to enable a distinction of œsophageal and gastric regions to be drawn, and, as was observed by Partsch, the cylindrical cells of the surface of the œsophageal mucous membrane are devoid of cilia.

There is, however, one means of distinguishing the œsophagus from the stomach, viz., by observing the closeness of attachment of the muscular and mucous coats. In the stomach the two coats can be much more easily separated than in the œsophagus. It is by the use of this method that I have judged where the œsophagus ends and the stomach begins. It seems to me that if this method is rejected there is no alternative but to consider all the glands, mucous glands included, as occurring in the stomach, for there is no abrupt change in structure in passing from mucous to pepsin-forming glands.

The question is, however, not an important one, the important question being, Is there any differentiation in the structure and function of the glands, such, for instance, as occurs in the Frog?

In examining sections of the mucous membrane which has been treated with osmic acid, proceeding from the beginning of the œsophagus onwards, we first find short simple mucous glands as described by Partsch, then in the mucous glands are found one or two cells containing a few large granules; further on these cells become more frequent and contain more granules, until we come to the regular oxyntic glands; in these the body is usually long, and frequently coils or branches at its end; the mucous cells are confined to the necks of the glands. The granules contained by any one cell

* *Op. cit.*

† *Op. cit.*

vary not inconsiderably in size; but apart from this there is a considerable diminution in the size of the granules in passing from the beginning to the end of the oxyntic gland region. The latter portion of the œsophagus contains the majority of the glands with large granules. The difference in the size of the granules in the anterior and posterior pepsin-forming regions is much less in some Toads than in others; there is also some variation in the extent to which the glands with granular cells stretch into the œsophagus. The cell-substance of the glands in the resting state stains very slightly with osmic acid. The pyloric glands resemble the pyloric glands of the Frog; the fully-formed mucous cells are, however, usually more numerous.

It is not so easy in the Toad as it is in the Frog to observe the glands in the pinned-out mucous membrane owing to the greater amount of sub-mucous tissue. In a small Toad, however, it can be seen that the latter œsophageal and anterior oxyntic glands contain in the fresh state obvious granules; they are less conspicuous than the granules in the œsophageal glands of the Frog, but much more conspicuous than those in the oxyntic glands of the Frog. Towards the pyloric region the glands become less granular and more transparent, and resemble fairly closely the Frog's oxyntic glands; the lumen, however, is frequently distinct. The pyloric glands need no special notice.

## Changes which occur in the Œsophageal and Gastric Glands.

During digestion the changes which are common to the œsophageal and oxyntic glands of the Frog occur also in all the granular pepsin-forming glands of the Toad; the granules become smaller and diminish in number and size, the cells become smaller, and the cell-substance stains more deeply with osmic acid. Apart from this, there is in the Toad a slight gradual alteration in the character of the changes in passing from one end of the pepsin-forming region to the other.

In the pepsin-forming œsophageal glands we find a very feeble picture of what happens in the œsophageal glands of the Frog. During activity the outer portions of the cells become more sparsely granular, though very seldom showing an outer clear zone.

In the anterior oxyntic glands of the stomach the gland-cells are as a rule equally affected throughout; there is no alteration in the relative distribution of the granules.

In the posterior oxyntic glands the granules disappear rather more from the inner than from the outer part of the cells. An inner non-granular border is however seldom so distinctly seen as it is in the corresponding glands of the Frog.

In the Toad then the changes which the cells of the different parts of the pepsin-forming region undergo in digestion are much less divergent than they are in the Frog; moreover, in the Toad there is a gradual alteration in the character of the changes from the beginning to the end of the pepsin-forming region, whilst in the Frog the intermediate forms are largely confined to the junction of the œsophagus and stomach.

During the latter part of the digestive period there is in the Toad as in the Frog, a more or less complete restoration of the normal quiescent state of the gland-cells. The restoration, however, does not take the same course in all animals; usually as the cell increases in size there is a corresponding increase in the number of the granules; this is not always the case, for in some Toads, especially in the gastric oxyntic glands, the formation of granules does not keep pace with the growth of protoplasm.

One other variation on the normal course of events is worth mention: occasionally there is no perceptible change in the anterior gastric glands during the first three or four hours of digestion; since it can scarcely be doubted that the glands secrete during this time, we are led to infer (cp. below, p. 704) that the apparent absence of change is due to the formative keeping pace with the excretory processes.

Fasting causes changes like those which occur in the first stage of digestion, with the exception that the mucous cells of the necks of the glands become more instead of less prominent. This is perhaps only the case when the fasting is not too prolonged. The posterior oxyntic glands are usually more affected than the anterior.

Feeding with sponge does not cause very great changes in the œsophageal or gastric glands, although a very acid secretion with a high peptic power is obtained: the cell-granules become smaller, but I have not observed that they are more affected in one part of the cell than in another.

## The Pepsin-Content of the Œsophageal and Gastric Glands.

As in the Frog so in the Toad, the amount of pepsin contained by a definite weight of dried mucous membrane is less as the cells become less and less granular during digestion. I have made no experiments to determine the pepsin-content of the mucous membrane in successive hours after feeding, but have analysed for pepsin only such cases in which I could be certain of a difference in the amount of granules contained by the respective gland-cells.

I have also compared the amounts of pepsin contained by the different parts of the œsophagus and stomach. The method of proceeding was like that described above in the case of the Frog. The amount of pepsin contained by the parts was found to vary directly with the amount of granules contained by them. The latter part of the œsophagus and first part of the stomach contains most pepsin; the amount of pepsin is rather less in the succeeding part of the stomach; the posterior gastric region in which these glands are not markedly granular in life contains considerably less pepsin than the preceding; the pyloric region contains only a very trifling quantity.

## TRITON TÆNIATUS.

### Histology of the Gastric Glands.

The pepsin-forming glands of the Newt may be conveniently divided into anterior oxyntic glands, posterior oxyntic glands, and pyloric glands. There is no abrupt transition in passing from the anterior to the posterior oxyntic glands, although the extreme forms of the two differ not inconsiderably.

The oxyntic glands are of the compound tubular type, several secreting tubes coming off from a common neck. This form is most pronounced in the anteriorly-placed glands. Klein* described a ring of acinous glands as occurring just above the cardia; this was confirmed by Partsch.† These "acinous" glands are the most anterior of the oxyntic glands in which the compound tubular form is most developed; they occur, as was mentioned by Sewall and myself,‡ under a ciliated epithelium.

The glands of the anterior oxyntic region occur in groups separated from one another by a considerable amount of connective tissue; this is most marked at the cardia, and becomes less and less towards the posterior oxyntic region, where the glands are not obviously arranged in groups, and are separated by only a small amount of connective tissue. In passing from the anterior to the posterior region, the mucous cells diminish in number, and the diameter of the glands decreases; the glands are longest in the first part of the posterior oxyntic region.

All the oxyntic glands both in the fresh condition and when treated with osmic acid are granular throughout. In osmic acid specimens the nuclei of the cells here and there appear as a clearer spot through the granules, but they do not form a marked feature in the glands; the cell-substance is scarcely at all stained (Plate 78, fig. 2). The posterior oxyntic glands stain somewhat darker with osmic acid than the anterior; the cell-granules contained by the former are smaller than those contained by the latter (Plate 3, fig. 3). The granules behave with reagents much as do the granules of the œsophageal and gastric glands of the Frog. Hydrochloric acid 0·4 per cent. causes them to disappear suddenly without any progressing diminution in size. When treated with bile they become smaller and smaller until they disappear. Alcohol, even 50 per cent., partly dissolves them, leaving a small slightly refractive mass behind. Some of the granules are much more readily acted on by the above reagents than others. Salt solution up to 20 per cent. leaves them unaffected.

The pyloric glands seldom contain mucous cells. They are transparent in the living condition; treated with osmic acid they remain homogeneous and stain yellow-brown. The nuclei are large compared with those of similar cells, and show when treated with appropriate reagents—as do all the gastric gland cells of the Newt (Klein)—a very

* Klein, Stricker's Hdb., vol. i., p. 542.

† *Op. cit.*, s. 198.

‡ *Op. cit.* p. 290.

distinct network. During digestion the cells of the pyloric glands frequently bulge in a very marked manner at their outer border; but from the inconstancy with which the bulging occurs I am not inclined to consider it a genuine result of secretion.

## Changes which occur in the Oxyntic Glands.

The *effect of fasting* is more marked in *Triton tæniatus* than in any other animal which I have examined. In the anterior oxyntic glands a more or less distinct outer non-granular zone is formed: in the posterior oxyntic glands the clear zone is less distinct, but other changes are obvious; the cells are smaller, the lumen usually distinct, and the cell-substance stains more deeply with osmic acid. The mucous cells in the necks of the glands increase in mucigen—at any rate up to the time when the outer zone begins to be distinct in the anterior oxyntic glands.

*Changes during digestion.*—The diminution of granules which takes place in the living glands of a Newt after feeding it, has been described by SEWALL and myself.* This diminution can also be seen in osmic acid specimens of the gastric mucous membrane, in such specimens some other changes can be seen which are less obvious in the fresh tissue. The anterior differ somewhat from the posterior oxyntic glands in the changes they undergo; in the former the outer clear zone is usually more distinct during digestion than in the latter, and in the latter the increase of the lumina and decrease in the size of the cells and granules is usually more distinct than in the former. These changes go on in all the glands, but to an unequal degree in the two glandular regions.

When the living glands are examined in the first or second hour of digestion the granules frequently appear at first sight larger than normal;† this is, I think, only caused by many of the granules having disappeared, so that instead of a confused granular mass, the separate granules can be distinctly seen. Osmic acid specimens show that at any rate about the second hour the granules are smaller. It is noteworthy that as the granules become smaller during cell activity they vary much more in size than do the granules of the resting gland (see Plate 78, figs. 3 and 4, 2 and 6). The extent and time of the changes vary widely under varying circumstances. In a normal hungry Newt fed with a small worm the following is the ordinary course of events as followed in successive osmic acid preparations.

A thinning of granules at the outer border of the cells is visible in half-an-hour to one hour; the nucleus and cell-substance begin to stain slightly. Usually at the end of an hour a decrease in the size of the cells and the cell-granules is to be seen.

These changes then proceed rapidly, the time of maximum change being three to four and a-half hours from the beginning of digestion. The cells and cell-granules are then very distinctly smaller (see Plate 78, figs. 3 and 4), and a more or less distinct

* *Op. cit.*

† They were so described by SEWALL and myself from the examination of fresh specimens.

outer clear zone is present. Under ordinary circumstances the granules of the inner zone are not massed around the lumen but spread out through the inner two-thirds or even more of the cells; different glands vary widely in this respect. The small size of the granules at the time of maximum change often makes in the living glands the outer zone appear larger than it really is.

In about four and a-half hours the cells begin to return to their normal state. The changes above described disappear with astonishing rapidity, and in six to eight hours the granules are large and stretch to the outer border of the cells. The anterior glands, indeed, then differ very slightly from normal "hungry" glands. What difference still exists gradually disappears during the remainder of the digestive period. The recovery of the normal condition proceeds more slowly in the posterior than in the anterior oxyntic glands.

Since it takes twelve to twenty-four hours for the stomach to empty itself, it is obvious that the regenerative processes go on very actively at a period when the secretory processes are also still active.

The time and the extent of the several changes are very greatly affected by the condition of the animal and the amount of food given to it.

Thus, if it is fed with several worms instead of with one, not only are the changes more pronounced, but they also continue much longer; so that in twenty-four hours from the beginning of digestion the gland-cells may be somewhat small and darkly stained and possess comparatively few and small granules.

A like alteration in the extent and duration of the changes is produced by feeding a Newt which has long fasted. At the time of maximum change after such treatment the alteration from the normal "hungry" state is most remarkable. The great majority of the glands are devoid of granules and devoid of mucous cells as such; the gland-cells are small, the greater part of the cell being taken up by the nucleus; the cell-substance stains deeply with osmic acid. Plate 78, fig. 5, represents an anterior oxyntic gland in this condition. The amount of change which takes place here suggests that in the fasting animal the formative processes go on more slowly than normally (see Frog, p. 677).

Another interesting variation commonly occurs when the food for some reason or other is digested with unwonted slowness. The rapid diminution in the size of the cells during the first four hours of digestion does not take place, but the cells diminish in size and the granules in size and number for many hours, the former more slowly than the latter; it is under such circumstances that the distinction of the outer and inner zone is most plainly seen.

The question of an increase of granules during the first hours of digestion is in much the same state as the similar question with regard to the œsophageal glands of the Frog (see p. 673). Apparently in some Newts, the oxyntic glands of which are already less granular than normal, a preliminary increase takes place, but in the normal hungry Newt I have failed to observe any such increase. It will be noticed,

too, that the time during which it can take place is shorter even than in the Frog, for in the Newt a distinct decrease is generally obvious in half an hour to an hour. Moreover, in some cases of fasting Newts, *i.e.*, Newts with gastric glands diminished in size and containing granules diminished in number, I have observed a distinct diminution in a quarter of an hour which steadily progressed up to six hours.

The *effect of sponge feeding* on the oxyntic glands is much more marked in the Newt than in the Frog. There are wide variations in the extent and duration of the changes depending on the condition of the animal. But apart from this, the character of the change produced tends towards one of two types: the first, a diminution in the size of the cells and in the number and size of the granules without a very distinct formation of inner and outer zones; in the second, a distinct formation of zones without any very great diminution in the size of cells and with only a moderate diminution in the size of the granules. It will be observed that these types correspond respectively to (1) the type of change in normal rapid digestion; (2) the type of change with abnormal slow digestion. That which chiefly affects the character of the changes in the glands is, so far as I have observed, the amount of sponge given,* *i.e.*, the strength of the stimulus. In both cases the changes proceed very slowly, the first effect being obvious in about four hours, and steadily progress for one to two days; about this time the glands begin slowly to recover. Sometimes after feeding a Newt with a relatively large quantity of sponge the glands lose all or nearly all their granules and approximate in appearance to fig. 5, Plate 78. This is especially the case if the Newt has fasted for some time previously. When the sponge is removed the glands recover, although not very rapidly, their normal appearance. Glands in which the first type of change has occurred form small granules which soon stretch throughout the cells; the cells and their granules then together become larger. Plate 78, fig. 7, shows the first stage of this return; the granules are small, and though absent from the outer part of the cell do not form a dense zone around the lumen. (Dark clumps of fat globules are seen at the periphery, cp. p. 670.) Glands in which the second type of change has occurred continue to show distinct zones for some hours after the granules have begun to increase. Plate 78, fig. 6, shows the condition of the anterior oxyntic glands in a Newt which had been fed for eighteen hours with sponge, and the stomach of which was put in osmic acid six hours after the removal of the sponge.

## The Pepsin-content of the Gastric Glands.

In the same manner as that given above for the stomach of the Frog I have analysed the amount of pepsin contained by the anterior oxyntic, the posterior oxyntic, and the pyloric glands in *Triton tæniatus*.

The amount of pepsin contained by the pyloric glands is very small; in both oxyntic

* I may mention that the most marked instances of the second type of change I obtained in February, 1880; of the first type of change in the autumn of 1880.

regions a considerable amount is found. It will be remembered that in the oxyntic glands alone obvious granules occur in the fresh state; hence, then, pepsin is found in quantity only where obvious granules occur.

A definite weight of gastric mucous membrane taken from the posterior oxyntic region contains a somewhat greater quantity of pepsin than an equal weight of mucous membrane taken from the anterior oxyntic region. We have seen that in the latter the glands are shallower and are separated from one another by much more connective tissue than in the former, or that a definite weight of mucous membrane taken from the anterior region contains a less amount of granules than an equal weight taken from the posterior region. Hence probably the difference in the pepsin-content of the two parts.

In the Newt as in the Frog and Toad a diminution in the cell-granules, such as occurs in fasting or during digestion, is accompanied by a diminution in the amount of pepsin contained by a definite weight of mucous membrane.

## TRITON CRISTATUS.

The gastric glands of *Triton cristatus* are in the resting state very like the resting gastric glands of *Triton tæniatus*. There is, however, much less difference between the glands found in the anterior and posterior oxyntic regions in the former than in the latter. The anterior oxyntic glands of *Triton cristatus* are in groups and consist of several tubes coming off from a single duct, but the granules contained by the cells are as a rule not much larger than those contained by the cells of the posterior oxyntic glands. Further, the digestive changes in the glands are much less marked in *Triton cristatus* than in *Triton tæniatus*. Even at the period of maximum change with worm- or sponge-feeding it is rarely that an outer non-granular zone occurs. In one or two glands, usually either at the beginning or at the end of the oxyntic region, the outer portion of the cells does not contain granules, but even then the line between the granular and the non-granular zones is not a sharp one. Both in the fresh and in osmic acid specimens fewer granules are found towards the outer portion of the cells during digestion; there is a diminution in the size of the cells and some increase in the diameter of the lumina. In the latter period of digestion these changes are more or less completely repaired.

We have seen that during fasting a clear zone gradually appears in the oxyntic glands of *Triton tæniatus*, in the glands of *Triton cristatus* I have not seen a corresponding change under similar circumstances.

The time of maximum change with sponge-feeding is in the animals I have observed about twenty hours. Osmic acid specimens then show that the cells are much smaller than normal; the cell-substance stains much darker; the granules in the cells are also fewer and smaller; the granules are most numerous in the inner portions of the cells but are seldom absent from the outer portion. The lumen is enlarged often con-

siderably. Of the pyloric glands little need be said; like the pyloric glands of *Triton tæniatus,* they do not contain any definite mucous cells.

*Pepsin-content of the gastric glands.*—What has been said (p. 689) above on the pepsin-content of the gastric mucous membrane of *Triton tæniatus* holds also for that of *Triton cristatus,* with one or two slight modifications.

The difference in the pepsin-content of the anterior and posterior oxyntic regions is greater in the large than in the small Newt. This is, as before, in correspondence with the relative amount of granules contained by the two parts. It will be remembered that in *Triton tæniatus* the granules are considerably larger in the anterior than in the posterior oxyntic glands, and that in *Triton cristatus* the difference in the size of the granules in the two regions is only slight. The less frequency of the glands in the anterior region is, then, more compensated in the small than in the large Newt.

We have seen that there is less disappearance of granules during digestion in *Triton tæniatus* than in *Triton cristatus;* we find also that in the latter there is a less difference in pepsin-content in hunger in digestion than in the former.

## COLUBER NATRIX.

### Histology of the Gastric Glands in Hunger and Digestion.

The end of the œsophagus and the beginning of the stomach can be readily distinguished; in passing from one to the other the mucous membrane becomes suddenly thicker and more opaque; the junction line of the two is not a circle, but an oval, the glands occurring first on the ventral side of the alimentary canal.

The epithelium of the œsophagus near the stomach consists of long and unusually thin cylindrical cells, which have at their free ends suffered some amount of mucous metamorphosis. Partsch* described the epithelium near the stomach as consisting of ciliated and goblet cells; I have not observed either of these. There are no proper œsophageal glands; there are some few dippings down of the surface epithelium, but the cells do not markedly change in character.

The most anterior glands are, as in the Newt, separated by more connective tissue than are those in the remainder of the stomach. They are arranged in groups, and several tubes are connected with each neck; the tubes, moreover, usually divide in their course. In passing towards the pylorus the glands consist of a smaller number of tubes, but remain, as a rule, complex glands.

In the fresh state all the oxyntic glands are densely granular, and little difference is to be seen in them except that the most anterior are larger in cross section. The pyloric glands are transparent and non-granular; the intermediate region is comparatively large.

If three Snakes are fed each with a Frog, and the gastric glands are examined in

* *Op. cit.*

the fresh state in one, two, and three days respectively, it is seen that the changes which take place in the gland-cells are first obvious in the posterior oxyntic region, and steadily progress towards the anterior region. No zones are at any time distinctly formed, but the granules contained by the cells become fewer and less obvious; so that the posteriorly-placed oxyntic glands appear in the fresh state nearly as devoid of granules as the pyloric glands. The two gland-forms can be, however, still distinguished; they have a different general look. Compared with the pyloric glands, the oxyntic glands have a faint yellow tinge and almost oily appearance; sometimes, too, the rounded outline of their cells can be made out.

The greatest change that I have observed was in a Snake examined sixty hours after feeding. The glands in the latter third of the oxyntic region showed scarcely any granules; in the middle third the glands contained many less granules than normally; they contained, however, more and more in passing towards the anterior oxyntic region, where the glands were densely crowded with granules. That is, the changes increase in intensity in passing from the beginning to the end of the oxyntic region. This is very similar to the manner in which the glands of the Rabbit's stomach are affected during digestion.

In osmic acid specimens some other points can be made out: The glands become longer and narrower in passing from the beginning to the median portion of the stomach; thence to the pyloric region they become shorter—at first gradually, then more rapidly. In the Snakes which I have examined the pyloric glands are simply mucous glands; the sub-cubical cells which form the sole constituent of the pyloric glands in Newts and a partial constituent of the pyloric glands in Frogs are absent in the Snake. PARTSCH,* however, found exactly the contrary: sub-cubical cells he observed, but no mucous cells.

Osmic acid specimens of the gastric mucous membrane of a hungry animal show no great difference in the characters of the cells throughout the oxyntic gland region; all contain granules fairly equally distributed throughout their substance. The granules vary in size; the larger ones closely resemble those in the œsophageal glands of the Frog or in the anterior oxyntic glands of the Newt. The granules are, as a whole, largest in the most anterior oxyntic glands, and, generally speaking, the farther the glands are from the beginning of the stomach the smaller are the granules they contain. The cell-substance stains very slightly, but more in the posterior than in the anterior oxyntic glands. The necks of the glands, especially of those in the median gastric region, differ from the necks of the gastric glands in other animals; sub-cubical cells are usually absent.

When osmic acid specimens of a digesting stomach (sixty hours) are examined there is little or no change to be seen in the anterior portion; the granules are large and the cell-substance unstained (Plate 78, fig. 8). In passing towards the latter third of the gastric gland region certain changes become more and more obvious: the granules

* *Op. cit.*, s. 200.

become smaller and fewer, the cell-substance begins to stain, the cells are smaller, and the lumen often visible (see Plate 78, figs. 9, 10). These changes are most pronounced in the latter third of the oxyntic gland region. The lumen is never large; it may be either straight or zig-zag, depending on the position of the cells in the gland tube. It is to be remarked that osmic acid brings out a number of granules in cells which in the fresh condition showed scarcely any. In this respect osmic acid specimens show a less striking difference between the anterior and posterior oxyntic glands than is shown by the fresh specimens.

I have not noticed any great change from normal in the glands of the fasting Snake. The cells become somewhat smaller; this is marked in the posterior gastric gland region by an increase of the lumen; the cell-substance, too, especially in the posterior gastric region, stains more deeply with osmic acid, and contains smaller granules.

In the posterior oxyntic region during digestion the granules disappear somewhat more at their inner than at their outer portion (see Plate 78, fig. 9); this is, however, so far as I have observed, much less markedly the case than in the Frog.

### The Pepsin-content of the successive Regions of the Stomach of the Snake.

Having observed that in the digesting stomach the diminution in the number of the granules became more and more marked in passing from the anterior to the posterior region, I made some experiments to determine whether there was a corresponding diminution in the amount of pepsin. I divided the mucous membrane containing gastric glands into four parts, throwing away a strip between each part. The four pieces were then put aside to dry in a bell-jar containing sulphuric acid. A definite weight of the dried mucous membrane of each part was taken and treated with dilute hydrochloric acid for twenty-four hours. The details of the method of proceeding were in the main like those given above (p. 681) in the account of the pepsin-content of the stomach of the Frog.

The first experiment was on a Snake that had been fed with a Frog twenty-four hours previously. Only that part of the Frog which was in the posterior portion of the stomach had been much digested. The glands of the posterior portion of the stomach were distinctly granular, although they were, as a rule, rather more thinly granular at their outer portion. The analysis showed a steady diminution in the amount of ferment from the beginning to the end of the stomach, the first part containing more than the second, the second than the third, the third than the fourth. The fourth part contained very much more than an equal weight of the mucous membrane of the pyloric region.

The second experiment was made on a Snake sixty hours after feeding with a Frog. The legs of the Frog, which were in the anterior part of the stomach, were still nearly intact. Examined in the fresh state, the first part of the stomach showed many granules; the second part showed less than the first, but still a considerable number; the third part few granules; the last part very few.

The analysis showed that the diminution in the amount of ferment in passing from the beginning to the end of the stomach was much more rapid than in the previous case. In parts three and four a comparatively feeble pepsin action was found—in three, rather more than in four; the difference in rapidity of action between the second part and the fourth was considerably greater than between the fourth and the pyloric region. In the normal hungry Snake the amount of pepsin contained by a definite weight of dried mucous membrane also diminishes from the anterior to the posterior portion, but to a much less extent than in the digesting animal. The amount of pepsin contained by the pyloric region is very small, although, as in the Frog, Newt, and Toad, quite appreciable. In the Snake I compared the amount ot pepsin contained by equal weights of œsophagus and pyloric gland region; from the former I obtained no pepsin reaction.

It will be noticed that in all cases the amount of pepsin contained by a definite weight of mucous membrane varied directly with the granularity of the gland-cells.

In the above experiments the dried mucous membrane was extracted with dilute hydrochloric acid; quite similar results are, however, obtained if the dried mucous membrane be extracted with glycerine, and the glycerine extract tested for pepsin.

I have made some experiments to determine whether the gastric glands of the Snake contain pepsinogen. Since I propose considering in detail in a later paper the question of the formation of pepsinogen in gastric glands, I will here only mention that the stomach of the Snake contains a very considerable amount of a substance insoluble in glycerine, but which when treated with dilute hydrochloric acid gives rise to pepsin, and that this substance is in greatest quantity in the anterior region of the stomach, and diminishes in amount in passing towards the posterior region.

## General Conclusions on the Secretory Processes in Pepsin-forming Glands.

From the preceding details some general conclusions can, I think, be drawn.

*Pepsin is formed from the granules seen in the gland-cells in the living state.*

An account has been given above of the pepsin-content of the stomach and of various parts of it in *Rana temporaria, Bufo vulgaris, Triton tæniatus, Triton cristatus,* and *Coluber natrix.* By the method used, viz.: extraction of the dried tissue with hydrochloric acid 0·2 per cent., the total amount of free and combined pepsin* present in the tissue was obtained.

* Grützner (Neue Unters. ü. d. Bildung u. Ausscheidung d. Pepsins § 26, 1875) has pointed out that this method only gives accurate results under certain conditions, viz.: (1) when a relatively large quantity of hydrochloric acid is used for extraction; (2) when the extraction proceeds for at least ten hours; (3) when the amount of tissue is not too great compared with the amount of pepsin. The first two conditions were complied with in all my experiments. With regard to the last, it may result that the amount of pepsin contained by the pyloric glands is greater than that which I have found.

In each of the animals studied we have seen that the amount of pepsin contained by any part of the stomach (or of the pepsin-forming region) is directly proportional to the amount of granules contained by the gland-cells of that part.

In each of the animals studied we have seen that the cell-granules diminish in number and size during digestion. GRÜTZNER found in the animals* investigated by him a diminution, during digestion, in the amount of pepsin contained by a definite weight of the stomach. Without examining in detail the amount of pepsin contained by the gland-cells at different digestive stages, we have seen above in each of the animals considered that a definite weight of gastric mucous membrane taken at a stage of digestion when the granules are markedly diminished contains a markedly diminished amount of pepsin.

Further, we have seen that during fasting the number of granules contained by the gland-cells diminishes, and we know from GRÜTZNER'S† experiments that during fasting the amount of pepsin contained by a definite weight of gastric mucous membrane diminishes.

Lastly, it has been shown by SEWALL and myself‡ that in the Rabbit the amount of pepsin contained by any portion of the stomach is in direct proportion to the number of granules contained by the "chief" cells of that portion in the living state.

Hence, then, in all the cases which have been investigated the amount of pepsin is in direct proportion to the amount of granules. From this, I think, we may fairly conclude that the granules in the gland-cells give rise to pepsin. We have at least as much reason to conclude this as we have to conclude that granules in the pancreatic gland-cells give rise to trypsin.

It may, however, be said that since the cells diminish in size during secretion, the diminution in the amount of ferment might be caused by a diminution in the cell-substance and not in the granules. The following consideration will, I think, show that this cannot be the case. Although each gland-cell becomes smaller, there is in most cases no obvious diminution, and in some there seems to be an actual increase in the amount of cell-substance. The removal of the granules alone is sufficient to cause the diminution observed in the size of the cell. Further, since the glands are smaller, to obtain an equal weight of resting and of digesting mucous membrane, a larger area of the latter, *i.e.*, a greater number of glands, must be taken.

* GRÜTZNER, Neue Unters. u. s. w., § 36 *et seq.*, Dog, Pig, Rabbit, and Cat. PFLÜGER'S Archiv. Bd. xvi., 1878, § 118, Dog; § 120, Œsophagus of Frog.

† GRÜTZNER, Neue Unters. u. s. w., § 52, Rabbit; § 61, Dog, Cat. PFLÜGER'S Archiv., Bd. xx., 1879, § 407 and 408, Œsophagus of Frog.

‡ Journal of Physiol., vol. ii., p. 291, *et seq.*, 1879. I may mention that the arrangement of the gastric glands in the Guinea-pig is similar in main points to the arrangement described by SEWALL and myself as existing in the Rabbit.

*The gland-cells do not store up pepsin as such, but store up zymogen, out of which pepsin arises when the cell secretes.*

The researches of HEIDENHAIN* have shown that the cells of the pancreas do not store up trypsin but store up a substance which under certain conditions can give rise to trypsin.

Since it had been shown by EBSTEIN and GRÜTZNER† that the gastric glands of Mammals contain a certain quantity of a substance capable, when acted on by sodium chloride or by dilute hydrochloric acid, of giving rise to pepsin, HEIDENHAIN introduced the word "zymogen" to include the antecedent of the ferment in both these and in any later found cases.

In the pancreas the conspicuous granules in the cells consist in part or wholly of zymogen. The zymogen can then in this instance be distinguished from the rest of the cell-substance.

When we compare in the Frog the pancreatic with the œsophageal gland-cells, and observe the close resemblance which exists between the two, especially as regards the granules they contain, it seems in the highest degree improbable that they should differ so widely as that one should store up zymogen and the other store up ferment.

We have seen that the gastric glands of the Newt closely resemble the œsophageal glands of the Frog as regards the reaction and behaviour of the granules they contain: the other gastric glands we have investigated above, differ in some points from both of these glands, but in the important point of the behaviour of their granules show a general similarity to them. In these glands then, too, the microscopical appearances seem to me to render it far more probable that the granules stored up should consist of zymogen, than that they should consist of ferment or other ready-formed secretory product.

I know of no satisfactory instance in any gland in which the substances found in the secretion are found stored up in the cells. In the liver-cells none of the characteristic constituents of bile are found. It may be objected that in these no substance is stored up, and that the cell protoplasm in activity forms straightway and excretes the constituents of bile. From many observations carried on during the past twelve months on the liver of the Frog, Toad, Newt, and Snake, I have been able to satisfy myself that in these cases also granules are stored to be used during secretion.

In mucous glands the evidence is against the substance stored by the cells being actually the mucin found in the fluid secreted. It is probably a pre-product closely related to mucin.

In the serous glands the facts known are not sufficient to be of much use for or against.

* HEIDENHAIN, PFLÜGER'S Archiv., Bd. x., s. 557, 1875.

† EBSTEIN and GRÜTZNER, PFLÜGER'S Archiv., Bd. viii., s. 122, 1874.

When the secretion produced by a gland contains undissolved particles, these particles may be stored up by the gland, thus fat globules are apparently stored up by the cells of the Harderian and Mammary glands.

I do not overlook the fact that a glycerine extract of the fresh Mammalian gastric mucous membrane contains a good deal of pepsin, but I believe this to be due simply to the zymogen being more easily split up in these glands than in others. It will be remembered that the granules of the "chief" cells which probably consist in part or wholly of zymogen are not preserved by osmic acid; *i.e.*, these zymogen granules split up with osmic acid, whilst the other zymogen granules we know of do not. Further, as mentioned above, EBSTEIN and GRÜTZNER have shown that the gastric glands of Mammals do contain some zymogen.

The general course of reasoning given above, led me to make some direct experiments on the subject. The result completely confirmed the justice of the deductions drawn from microscopical examination. Since I propose to discuss in a later paper the whole question of the formation of pre-products by gland-cells, I will here only briefly mention the main facts which show that zymogen and not ferment is stored up by the glands we are considering.

If the œsophagus or stomach of a Frog be placed in glycerine as rapidly as possible after removal from the body, the glycerine extract has only a weak peptic power.

If the œsophagus or stomach of a Frog be kept moist for twenty-four hours before it is placed in glycerine, the glycerine extract has a very much greater peptic power.

If the œsophagus and stomach which has been extracted with, say, 5 cub. centims. of glycerine for a week be washed free of glycerine and treated with 5 cub. centims. of dilute hydrochloric acid, then an enormously greater amount of pepsin is found in the acid than is found in the glycerine extract.

The glycerine extracts increase somewhat, although only slightly, in peptic power when treated with dilute acid for twenty-four hours.

Since in testing a glycerine extract for pepsin, hydrochloric acid is added, it is possible that even the small amount of pepsin found in it may arise from the splitting up by the acid of combined pepsin.

These facts, I think, show that both the œsophageal and gastric glands form zymogen which under certain conditions, and particularly when acted on by dilute acids, give rise to pepsin. The zymogen is only slightly soluble in glycerine.

Similar observations made on the gastric glands of *Triton tæniatus* give similar results. In the Snake, lack of animals has prevented me from comparing the pepsin-content of glycerine extract of the fresh with glycerine extract of the exposed stomach, but the experiment mentioned above (p. 694) shows that the gastric glands of the Snake also contain zymogen.

Since the pepsin of the œsophageal and gastric glands which we have considered arise from granules which are visible in life, the fact that the pyloric glands show no granules in life and yet apparently form pepsin may seem to require explanation.

We know that there are many cells which consist of protoplasm* and inter-proto-

* I use the word protoplasm for all living substance. I take the network which KLEIN and others have shown exists in so many gland-cells to be protoplasm, and the interfibrillar substance to be chiefly at any rate stored up material.

plasmic substance, *i.e.*, of protoplasm and of the products of its metabolism which are kept imbedded in the cell; and that further in a great number of such cells the protoplasmic and inter-protoplasmic portions are not optically distinguishable from one another in the living cells. Whether they are distinguishable or no depends mainly upon the refractive index of the inter-protoplasmic substance. The antecedent of pepsin in the above-mentioned gastric glands differs from the protoplasm sufficiently in refractive power to be visible in life.

In the pyloric glands the inter-protoplasmic substance is almost altogether mucigen, having much the same refractive power as the protoplasm which formed it. It seems, then, not unnatural that the small amount of the antecedent of ferment contained by the pyloric gland-cells should be unable to render the inter-protoplasmic substance obvious in life.

Since the pepsin of different animals differs somewhat in its properties,* it is probable that both pepsin and its antecedent differ in different animals somewhat in chemical constitution. I can then readily imagine that the gastric gland-cells of some animals *may* form an inter-protoplasmic substance giving rise to a large quantity of pepsin, and yet having so nearly the same refractive index as the cell-protoplasm as to be very slightly or not at all visible without the action of reagents.

Since the inter-protoplasmic substance gives rise to pepsin, it is in the highest degree improbable that the cell should form pepsin in any other way than through the medium of the inter-protoplasmic substance. This means in the gastric glands we are considering, that pepsin is formed in the cells only through the medium of granules. *If, then, two gastric glands secrete an equal amount of pepsin, they will have used up an equal, or, allowing for a slight difference in the chemical composition of the granules, a nearly equal, amount of granules.* This deduction we shall have occasion to allude to later.

*During secretion the three chief phenomena which can be recognised in gland-cells, viz.:—(a.) a using up of granules, (b.) a fresh formation of granules, (c.) a growth of protoplasm—go on simultaneously. The different aspects of the gland-cells depend upon the relative activity of these three processes.*

This view has been already put forward by HEIDENHAIN to account for the changes which take place in the pancreas. He says:—†

"In the cells a continuous change takes place; a using up of substance in the

* See HOPPE-SEYLER, Physiol. Chemie., s. 218, 1878.

† "An den Zellen findet ein fortwährender Wandel statt; Stoffverbrauch innen, Stoffansatz aussen. Innen Umwandlung der Körnchen in Secretbestandtheile, aussen Verwendung des Ernährungsmaterials zur Bildung homogener Substanz, die sich ihrerseits wiederum in körniges Material umsetzt. Das Gesammtbild der Zelle hängt von der relativen Geschwindigkeit ab, mit welcher sich diese Processe vollziehen. Die

inner portion, an addition of substance to the outer portion. In the inner portion, change of the granules into secretory constituents; in the outer portion, employment of the nutritive material for the formation of homogeneous substance, which on its part is changed into granular material. The appearance of the cell as a whole depends upon the relative rapidity with which these processes proceed. The first digestive period is characterised by a rapid using up in the inner portion and a rapid addition to the outer portion. In the second digestive period the most active changes proceed at the junction of the inner- and outer-zone, inasmuch as the substance of the latter is converted into the substance of the former."

HEIDENHAIN* draws a just analogy between the changes which take place in the pancreas and those which take place in the gastric and in the serous and mucous glands; since we now know† that in these latter the cells form granules to be used up in activity, the analogy is even closer than that drawn by HEIDENHAIN.

The changes described by HEIDENHAIN as occurring in the gastric glands differ somewhat from those which he describes as occurring in the pancreas. The differences mainly arise from his conclusions about the gastric glands having been made on alcohol hardened instead of on fresh specimens. Thus instead of speaking of the granules of the chief cells he speaks of the non-staining substance, and instead of the homogeneous protoplasm of the living cell he speaks of the cloudy or finely granular protoplasm which is seen in the cells of alcohol specimens. He says:‡ "In the earlier secretory period the in-come is as a rule in excess of the out-go in the chief cells of the glands of the fundus, hence ensues an increase in the size of the cells. At the same time however there is an active formation of non-stainable substances (pepsinogen and pepsin) from the protoplasm, hence the cloudiness of the cells is for the time only slight. As digestion proceeds the out-go becomes gradually in excess of the in-come, hence the cells diminish in size; at the same time the change of the albuminous substance still continued to be taken up by the protoplasm goes on more slowly, hence the cells become more cloudy, richer in protoplasm, and more deeply stainable."

erste Verdauungsperiode charakterisirt sich durch schnellen Verbrauch innen und schnellen Ansatz aussen. In der zweiten Periode vollziehen sich die lebhaftesten Veränderungen an der Grenze der Innen- und Aussenzone, indem die Substanz der letzteren sich in die Substanz der ersteren umwandelt."—(PFLÜGER'S Arch., Bd. x., s. 569, 1875, and HERMANN'S 'Handbuch d. Physiol.,' Bd. v., s. 202, 1880.)

* HERMANN'S 'Handbuch,' s. 147, 1880.

† Journal of Physiol., vol. ii., p. 261, *et seq.*, 1879.

‡ "Beim Beginne der Absonderung überwiegt in den Hauptzellen der Fundusdrüsen in der Regel die Aufnahme über die Abgabe, deshalb tritt Vergrösserung der Zellen ein. Gleichzeitig aber findet noch lebhafte Bildung nicht färbbarer Substanzen (Pepsinogen und Pepsin) aus dem Protoplasma statt, deshalb wird die Trübung der Zelle vorläufig eine nur geringe. Beim Fortgange der Verdauung wird allmählich die Abgabe vorherrschend über die Aufnahme, deshalb schwellen die Zellen ab; gleichzeitig geschicht die Umwandlung der immer noch von dem Protoplasma aufgenommenen Albuminate langsamer, deshalb werden die Zellen trüber, protoplasmareicher, und stärker färbbar."—(HERMANN'S 'Handbuch d. Physiol.,' Bd. v., s. 146, 1880.)

He points out* two differences between the changes which occur in the pancreatic and gastric glands. First, that the regeneration of the used-up material takes place in the pancreas whilst the gland is still secreting, but does not in the gastric glands. Secondly, that in the pancreas the granules are formed out of the homogeneous outer zone, and in the gastric glands out of the finely granular cell protoplasm. As to the first point of difference, we have seen that in the Frog, Newt, and Toad the used-up material is regenerated more or less completely whilst secretion is still going on. The second point of difference chiefly rests on the assumption that the protoplasm of the fresh cell is finely granular, since in alcohol specimens an increase of protoplasm in the cell is marked by an increased cloudiness.

The differences which exist with regard to the formation of granules in those cells which acquire an outer non-granular zone in activity and those which do not we shall discuss later. We have first to consider what facts we have to support the view given above as to the changes which take place in the pepsin-forming glands during secretion.

We have to show that (*a*) the using up of granules, (*b*) the formation of granules, and (*c*) the growth of protoplasm all go on from the beginning to the end of secretion.

(*a*.) Since it has been shown that the granules give rise to pepsin, and since we know of no other origin for pepsin, we may conclude that as long as pepsin is formed, *i.e.*, as long as the secretion goes on, granules are used up.

(*b*.) We have seen that in all cases in the latter half to two-thirds of the digestive period the granules increase. There can be no doubt that during this time a secretion is going on; this is indeed shown by feeding a Frog or Newt with sponge. It is then seen that the simple mechanical stimulation caused by the presence of a foreign body in the stomach is sufficient to cause a secretion lasting for two or more days. HEIDENHAIN, too, has shown that in the Mammal secretion of gastric juice goes on as long as food remains in the stomach. But since a secretion means a using up of granules, there is during a large part of the digestive period a using up and formation of granules going on at the same time.

We have also seen that in certain circumstances an increase of granules occurs in the first hours of digestion. During this time there is also a using up of granules, that is, the two processes go on together, sometimes at any rate in the first hours of digestion.

Now, when we consider that the using up of granules is continually going on, and that the formation of granules certainly takes place in the latter digestive period and sometimes in the first digestive period, and further consider that the formation of granules can only become obvious in the glands when it takes place at a faster rate than the using up, the conclusion seems irresistible that there is a formation of granules during the whole digestive period, but that during the first five to ten hours it is not sufficiently rapid to cover the loss from the using up of granules. In certain circum-

* HERMANN'S 'Handbuch d. Physiol.,' Bd. v., s. 202, 1880.

stances the loss may be, though it is not normally, covered during the first one to three hours. The number of granules contained by any gland-cell at a given time during secretion depends then upon the relative rate of formation and using up of granules.

(*c.*) There is, I think, also sufficient evidence to justify us in believing that there is a continuous though not uniform growth of protoplasm during activity. The organic substances in the fluid secreted are formed, for the most part at any rate, by the cell-protoplasm. A growth of cell-protoplasm sufficient to cover the loss of the organic substances secreted must then, at some time or other, take place. Does this growth take place in "rest" or "activity"? The following consideration will, I think, show that although there may be a slight growth in rest, the growth takes place, in the main, during activity. With the exception, perhaps, of the oxyntic glands of the Snake, all the glands above considered have normally nearly returned to their hungry condition by the end of the digestive period, *i.e.*, at the end of twelve to twenty hours of secretion. The protoplasm which has been continuously diminishing, owing to the continuous formation of zymogen, is hardly appreciably in less quantity than at the beginning. The protoplasm, then, has grown during secretion. I know of nothing which tends to show that the growth of protoplasm is limited to any particular period of digestion; there are, on the other hand, several facts which go far to prove, if indeed they do not prove, that the growth is continuous during digestion.

In the second stage of digestion the cells increase in size, since we have no reason to imagine that the stored-up substances, the zymogen and so forth, occupy a greater space than the protoplasm from which they are formed, we may, I think, fairly refer the greater part at any rate of this increase in the size of the cells to a growth of the protoplasm; further, since the cells are during this period still losing substances to the fluid secreted, the total growth of protoplasm must be very considerable.

We have seen that under certain circumstances it may happen that there is a similar increase in the size of the cells, *i.e.*, a similar growth of protoplasm in the first one or two hours of digestion; we might then adopt a course of reasoning like that adopted in considering the formation of granules, and conclude that the protoplasm is growing also in the first stage of digestion when the cells are diminishing in size. We have, however, more direct evidence to show that this is the case.

It is true that the cells diminish in size during this stage of digestion, but they do not diminish sufficiently in size to warrant us in supposing that there is no growth of protoplasm. The mere extraction of the granules which have disappeared would leave the cells very much smaller; this fact becomes the more striking when we reflect that in the meantime more protoplasm has been used up to form granules. The changes which take place in the œsophageal glands of the Frog after the animal has been fed with sponge will serve as an instance. When the granules have entirely disappeared from the cell, is it to be supposed that the cell is simply as it was at the beginning of secretion, except that it has lost all its granules and a part of its protoplasm, viz.: that

which has been converted into granules. Clearly were this the case the cell would be very much smaller than it is.

Further, in the first stage of digestion the cells increase in power of staining with carmine and other similar reagents. This is only what has been shown to take place by HEIDENHAIN and others in a great number of gland-cells. The increased staining power is usually considered a proof of the increase of protoplasm. From this, too, then we should conclude that the protoplasm is growing, although the cells are diminishing in size.

There can, I think, be little doubt that these three processes also go on simultaneously in the serous glands during secretion. The increased staining power of the cell with carmine indicates that fresh protoplasm has been formed. I have previously shown that a using up of granules takes place. If we grant, as from analogy I think we may, that the granules give rise to the organic substances in the secretion, it would, as in the gastric glands, appear unlikely that these substances should be formed from the cell-protoplasm, otherwise than through the intermediate step of granules. But from this I think it follows that the granules are formed during secretion as well as in rest, for with a corresponding amount of solids excreted, the visible diminution in the number of granules is less in the sub-maxillary than in the parotid of the Rabbit, so that more new granules must have been formed by the sub-maxillary gland-cells than in the parotid. Further, the total amount of organic solids obtainable by protracted stimulation of the secretory nerves of the parotid seems to me too great to allow them to be referred to the granules present to start with.

*Some remarks on the difference of rate with which the above-mentioned three processes go on in the gastric glands, according as the animal is fed with digestible or with non-digestible food.*

If we compare the granular content of the gastric gland-cells at different periods after an animal has been fed with worm, with the granular content at different periods after an animal has been fed with sponge, we find that the main point of difference is that in the latter case the diminution in the number of granules lasts much less time, but is, during that time, much more rapid.

The explanation of the appearances which accompany sponge-feeding seems simple; the stimulation causes a slow nett using up of granules; after about twenty-four hours the stomach becomes accustomed to the presence of the sponge, and the stimulation becomes less and less; as the stimulation becomes less the tendency of the cells to form granules gets the upper hand of the using up of the granules, and they slowly increase.

In the worm-fed animal we have to account for the more rapid using up during the first hours of secretion, and the more rapid formation during the last hours of secretion.

HEIDENHAIN has shown that in the Mammal the presence of digestible food causes a more rapid flow of gastric juice than the presence of non-digestible food. He referred this to a chemical stimulation caused by the absorbed products of digestion. The same explanation will probably serve here. It seemed not unlikely that the peptone formed might be the particular product causing increased secretion. I have made some experiments upon the effect of injecting peptone into the lymph-sac and

into the stomach of the Frog; but the results, although on the whole favourable to the view that peptone causes a secretion, yet present some exceptions which prevent my forming any decided opinion on the subject.

The increase of granules and the growth of the cell during the last hours of digestion is in all probability due to the presence of the assimilated products of digestion.

In a worm-fed Frog, from the eighth to the eighteenth hour the secretion is going on at least as rapidly as in a sponge-fed animal, and yet the granules increase in number in the cells. We have seen reason to think that the first absorbed products cause an increased using up of granules, so that during the eighth to the eighteenth hour of digestion there must be some additional factor causing the formation of granules. It seems to me most likely that this additional factor is the presence of some of the digestive products which have been converted into a fit state to be assimilated by the gland-cells.

The effect of digestible substances in increasing the rate of growth of the cells and the formation of granules is strikingly shown in such an experiment as the following.

Three Frogs are fed with sponge. In twenty-four hours the sponge is removed from two of these Frogs; one of the two is fed with worm. All three are killed in twenty-four hours more, and the œsophageal glands examined. We have, then, specimens of glands from Frogs all forty-eight hours from the time of feeding with sponge, but (1) has been stimulated by the sponge during the whole time, (2) has been stimulated during the first twenty-four hours only, (3) during the first twenty-four hours with sponge, during the last twenty-four hours with worm.

Now, it is found that the gland-cells of (1) are small and contain very few granules, whilst those of (3) are large and contain many granules; yet the gland-cells of (3) have poured forth at least as much and probably more secretion than those of (1), *i.e.*, at least as many granules have been used up in (3) as in (1), and yet (3) contains very many more granules.

Further, the size of the cells and the number of the granules is not very different in (2) and (3), *i.e.*, the gland-cells of (2) which have used up no granules during the twenty-four hours before examination are not more granular than those of (3), in which a very considerable using up of granules has taken place.

We see from (2) that when the stimulus is removed the cells form granules, although no digestive products have been absorbed; the more rapid formation of granules which takes place when digestible substance is given I attribute to an increased supply of those substances which serve as food to the cells.

The same conclusion results from comparing the effects of sponge and of worm-feeding on *Triton tæniatus*. The details of such experiments are given above; it is unnecessary to discuss them here.

*The differences in the changes produced by secretion in different gland-cells is due partly to variations in the relative rates with which the using up of granules, the growth of protoplasm, and the formation of granules go on both in each cell as a whole and in various parts of it; partly also to variations in the power of the gland-cells to move the granules towards the lumen.*

We have seen that in any one form of cell the three processes go on at different rates at different periods of secretion. We should be *à priori* inclined to conclude that they would go on at different relative rates in the gland-cells of different animals. A comparison of the extent of the changes during activity presented by the gastric glands of *Triton tæniatus* and those of *Triton cristatus* makes this conclusion, I think, necessary.

If a Newt of each species be taken and each fed with an amount of worm sufficient to moderately distend the stomach, the apparent using up of granules is much greater in the gastric glands of *Triton tæniatus* than in those of *Triton cristatus.* But we have no reason to think that one has secreted proportionately more gastric juice than the other, that is to say (see above, p. 698), we have no reason to think that one has used up more granules than the other.

Since, however, we observe a marked difference in the total loss, and know further that in both the loss as it goes on is partially replaced, the conclusion naturally follows that in one the replacement is more active than in the other, *i.e.*, that the comparatively slight change observed in the gland-cells of *Triton cristatus* is due to the formative processes going on at a rate closely corresponding with that of the excretory processes. The effect of sponge feeding in the two species of Newts also leads to the same conclusion. In both cases an acid secretion of good digestive power is obtained, but the observable diminution in the number of granules in the cells is very small in the one compared with the diminution observed in the other.

But although variations of this kind may serve to explain certain of the differences which we find in the behaviour of the gland-cells of different animals, they still leave a great deal to be explained. Why do some cells form a non-granular zone at their outer border, others at their inner border, whilst others again form no non-granular zone at all?

We may attempt to explain these differences, still having recourse to the three main changes which we have seen take place in all the cells. We may suppose that these three processes proceed at unequal rates in the outer and inner portions of the cells. Obviously a non-granular region might be formed in any part of a cell either by a more rapid using up of granules, or by a more rapid growth of protoplasm in that part.

I shall not here discuss the cause of the non-granular border which is formed in the *inner* portion of cells, such as those of the gastric glands of Frogs. For although

I think that it is due to the granules being used up in the one part of the cell faster than in the other, I am unable to offer any satisfactory proof that it is so.

On the cause of the *outer* non-granular zone which is formed in the œsophageal glands of the Frog, I would, however, offer a few remarks. I take the œsophageal glands only as a convenient example, my remarks will equally apply to the gastric glands of the Newt and other glands in which an outer non-granular zone is formed during secretion.

In the œsophageal glands we have seen that the cell-granules diminish in size during activity; if, then, in any part of the cells the granules are being used up more quickly than in another the granules will be smaller in that part than elsewhere. The diminution in the size of the granules takes place, however, equally in all parts of the cell, consequently we may conclude that the non-granular zone is not caused by any variation in the rate of using up granules in the inner and outer portions of the cells.

We have then to fall back on local variations in the rate of growth of the cell-protoplasm to explain the appearances. Will the assumption of a rapid growth of protoplasm at the outer part of the cell suffice to explain the formation of the outer non-granular zone? I think not, for several reasons. It seems to me necessary to assume that there is another factor in the result, viz.: a translation of the granules towards the lumen by the cell.

In the œsophageal and other similar glands the first sign of the subsequent zones is a diminution in the number of granules in the outer portion of the cell. If this is due to a growth of protoplasm the protoplasm must grow in the outer one-third to one-half of the cell. How, then, can the further increase of protoplasm give rise to a non-granular outer zone and a densely granular inner one at all sharply marked off the one from the other? In passing from the granular to the non-granular zone there should be a very obvious region where the granules become fewer and fewer.

In certain circumstances the œsophageal glands of the Frog contain comparatively few granules, these being scattered throughout the cells (see Plate 77, fig. 7). During activity such glands show distinct zones. How could zones in such a case be produced by a growth of protoplasm unaccompanied by a direct movement of the granules in the cells?

Again, in glands when the granules have completely disappeared, can we suppose that the protoplasm has completely regrown from the periphery?

An observation of HEIDENHAIN's* upon the pancreas of the Rabbit is, I think, in favour of the view that the granules are moved towards the lumen. He found that when a piece of fresh pancreas was warmed to about 50° C. the granules moved outwards into the clear zone, and returned to their original position when the temperature fell. He, too, pointed out† that cells which were granular throughout, on being removed from the body gradually developed an outer non-granular zone as they died.

* PFLÜGER's Archiv., Bd. x., s. 563, 1875.

† *Op. cit.*, s. 559.

It may no doubt be objected that no one has seen any such movement in the living cells; but then it is to be remarked that observations on living cells have not been numerous, and that no particular cell-granule has been observed to disappear although numbers do disappear.

*In the cell-protoplasm there is during secretion an increase of substance capable of reducing osmic acid.*

In all the cases investigated above we have seen that the protoplasm of the "quiescent" gland-cells stains very slightly with osmic acid, whilst the protoplasm of the active cells stains more or less deeply.

This is opposed to the observations of NUSSBAUM, but NUSSBAUM, I think, directed his attention so much to the granules contained by the cells that he overlooked the cell-protoplasm.

I have not been able to satisfy myself of the meaning of this increase in staining-power of the protoplasm. Since the granules split up during activity, we might imagine that the cells do not at once cast out the whole of the substances formed, but retain a certain proportion, and that this diffused throughout the cell causes it to stain more than normally with osmic acid.

There is, however, another way of looking at the fact. Generally speaking, the formation of granules goes hand-in-hand with a diminution in the power of staining of the cell-substance. In the latter period of digestion, when the granules are increasing the cell-substance stains less and less with osmic acid. In *Triton tæniatus* during the rapid increase in the granules which takes place from the fourth to the eighth hour of digestion there is a rapid diminution in the staining power of the cell.

This would indicate that the protoplasm of the cells, in passing through those changes which result in the formation of granules, uses up the substance stainable with osmic acid. This substance might be either taken up by the cell during secretion to be further assimilated, or it might be an integral part of the cell-protoplasm.

The facts we have at present are, I think, insufficient to allow any satisfactory conclusion to be drawn on this point; but whatever the cause of it may be, it is, I think, a very general phenomena of cells to stain more deeply with osmic acid during active secretion than during rest. I have on a former occasion* pointed out that this is the case in the parotid, sub-maxillary, lachrymal, and infra-orbital glands of the Rabbit.

In parotid and sub-maxillary glands the resting cells treated with osmic acid show lightly-stained cell-substance with darker-stained granules; when the cells are similarly treated after a period of secretion the cell-substance is distinctly more deeply stained, the depth of staining increasing within certain limits with the length of time during which the cells have secreted.

In the lachrymal and infra-orbital glands the resting cells treated with osmic acid

* Proc. Royal Soc., vol. xxix., p. 377, 1879; Jour. of Physiol., vol. ii., p. 261, 1879.

show a networked appearance, from the presence of a lightly-stained protoplasmic with a darker interprotoplasmic portion. When the cells are similarly treated after a period of secretion the networked appearance disappears from the outer portion of the cells; it now is homogeneous—the protoplasm stains deeper than before. After prolonged secretion the cells stain equal and fairly darkly throughout.

In all these glands, however, the increase of substance in the cell-protoplasm capable of staining with osmic acid, although distinct, is not so marked as it is in the gastric glands.

GRÜTZNER* has independently come to a somewhat similar conclusion. He found in all the salivary and gastric glands investigated by him a difference of tint in osmic acid specimens of the resting and of the active glands. The former he found to be grey-green, the latter a dirty (*schmutzig*) brown. This, indeed, represents not unfairly the general difference in tint of the glands in the respective states when examined with not too high a power. I should prefer to call the tint of the one yellow-brown and that of the other brown-black. GRÜTZNER applied his description to the cells as a whole; the yellow-brown tint is, however, in the main due to the staining of the cell-granules, the brown-black tint in the main to the staining of the cell-protoplasm.

It will be noticed that my views on the processes taking place during secretion are opposed to the lately-advanced views of STRICKER and SPINA, and on the whole similar to those of HEIDENHAIN. The theory of STRICKER and SPINA† is based upon some interesting facts observed by them in the glands of the web and nictitating membrane of the Frog. The theory so deduced holds, they think, for all secretory glands; they attempt to show that it holds for the salivary glands.

I may be pardoned for not entering into a discussion of the question as long as the objections raised by HEIDENHAIN‡ remain unanswered. I will only remark that in my observations on serous and mucous salivary glands, on gastric and pancreatic glands, and on the liver, I have seen nothing in favour of the theory of STRICKER and SPINA, but many things against it.

The observations, an account of which has been given in this paper, were made in the Physiological Laboratory of the University of Cambridge. I cannot too much thank Dr. FOSTER for the kindness and generosity with which he has placed at my disposal all the material and apparatus belonging to him.

* GRÜTZNER, PFLÜGER'S Arch., Bd. xx., s. 399, 1879.

† STRICKER and SPINA, Sitzb. d. k. Akad der Wissensch. z. Jena, Bd. lxxx., abt. iii., 1879.

‡ HEIDENHAIN, HERMANN'S 'Hdb. d. Physiol.,' Bd. v., Th. i., s. 414, 1880.

## DESCRIPTION OF FIGURES.

Unless otherwise stated the specimens from which these drawings were made were prepared in the following manner. The tissue was placed for twenty-four hours in osmic acid 1 per cent.; removed from the osmic acid and washed for ten minutes in 50 per cent. alcohol, then placed for twenty-four hours in 75 per cent. alcohol. The sections were mounted in a mixture of one part glycerine to one part water; they undergo after mounting certain slow changes; the protoplasmic portions become darker, the mucigen portions more obvious, the outlines of the cells, nuclei and nucleoli, come more or less distinctly into view. It is almost unnecessary to point out that parts of several of the figures are drawn in outline only.

## PLATE 77.

### Œsophageal and Gastric Glands of *Rana temporaria* (Plate 77, figs. 1 to 11, and Plate 78, fig. 1).

Figs. 1, 2, 3. To illustrate the transition from the œsophageal to the oxyntic glands.

Fig. 1. × 360. Oblique section of end-tube of œsophageal gland. The granules are large and vary somewhat in size. The outlines of the cells are indicated; in the specimen they were only with difficulty to be made out.

Fig. 2. × 250. Gland of the intermediate region. The form of the gland resembles the form of the oxyntic rather than that of the œsophageal glands; the granules are larger than the oxyntic gland granules, but smaller than those of the œsophageal glands; they resemble closely the œsophageal gland granules.

Some characters of the resting cylindrical and sub-cubical cells are also shown here.

Fig. 3. × 370. Oxyntic gland, from latter part of anterior oxyntic region. The cells contain many granules, which are, however, much smaller than those in the cells of figs. 1 and 2.

The above three specimens were taken from a Frog (February, 1879) which had been fed with a large amount of food (0·5 grm. worm) forty-six hours previously. The last remnants of the food were still in the stomach. All the glands have slight signs of activity, especially the glands of the intermediate region, in which a small outer zone is seen.

Fig. 4. × 410. Longitudinal section of neck of oxyntic gland; the specimen illustrates also the changes which take place in the sub-cubical cells of the pyloric glands. Specimen prepared from a Frog (April, 1879) which was killed twenty-five hours after a heavy meal (four worms). The tissue was allowed to stay a month in alcohol before sections were cut, hence the distinctness of the interfibrillar substance (granules) in the mucigen portion. An earlier stage of this change in the mucigen portion of the cell can be seen in Plate 78, fig. 1.

Fig. 5. × 290. End-tubes of œsophageal gland. From Frog (September, 1879) forty-five hours after feeding with large piece of sponge. The granules are much diminished in size, and form only a narrow zone around the enlarged lumen. Several end-tubes in this specimen had lost all their granules.

Fig. 6 (*a*). × 330. End-tubes of œsophageal gland. The granules have disappeared from the cells, except at one place where a few small granules border the lumen. From Frog (September, 1879), twenty-five and a half hours after feeding with sponge. In no other Frog which I have observed did the granules disappear in so short a time. In this and the preceding figure the striation of the cells is *much* too distinct.

Fig. 6 (*b*). × 410. Same specimen as preceding after three months' stay in dilute glycerine. The striation and the very fine granules which are often seen in the clear zone are here brought out.

Fig. 7. × 350. End-tube of œsophageal gland. Frog (September, 1879) fed with sponge; after twenty-two hours sponge removed; 1·5 cub. centim. strong aqueous solution of peptone injected under skin of back; animal killed twenty-six hours after this. The granules are comparatively few, and although more numerous in the inner portion of the cells, occur also in their outer portion.

Fig. 8. × 340. End-tubes of œsophageal gland. Tissue placed in absolute alcohol for twenty-four hours, then in dilute carmine for twenty-four hours. Shows stained non-granular and unstained granular zones. Frog (September, 1879) fed with sponge thirty-five hours; sponge then vomited; animal killed ten hours after.

Fig. 9. × 410. Anterior oxyntic gland. Hungry state. The cell outlines are scarcely or not at all to be made out; the granules are distinct, numerous, and scattered throughout the cells. The nuclei and cell-substance is very slightly stained. Frog (July, 1879) killed five days after a good meal.

Fig. 10. × 580. Posterior oxyntic gland. Three hours' digestion. The granules are smaller than normal, and have disappeared from the inner border of the cells, leaving a small homogeneous zone. The nuclei and cell-substance stain darker. The nuclei are placed in the outer portion of the cells, in this specimen indeed more so than usual. The corkscrew-shaped lumen is obvious. Frog (April, 1879) several days' hunger, then fed with worm, killed in three hours.

Fig. 11. × 370. Posterior oxyntic gland. Twenty-five hours after heavy meal. Frog (April, 1879) several days' hunger, fed with four worms; killed twenty-five hours after; stomach still distended with debris of food, although a considerable amount fills out the intestine. The cells bulge out into the large lumen; the homogeneous inner zone is distinct.

## PLATE 78.

Fig. 1. × 640. Oxyntic gland, middle region of stomach. Frog (September, 1879) fed with sponge forty-six hours, then with worm; killed in three hours. There is a small inner non-granular zone; the cells are much diminished in size (note the amount of magnification), the granules are small and comparatively few. The sub-cubical and cylindrical cells also show marked secretory signs.

In the actual specimens the non-granular inner zone is more distinct than in the above figures, since in the figures (owing to the method used to reproduce the drawings) the inner zone appears very finely granular instead of homogeneous; further, in the actual specimens the granules disappear more from the sides of the cells than is here represented.

### Gastric Glands of *Triton tæniatus* (Figs. 2 to 7 inclusive).

Fig. 2. × 420. Anterior oxyntic gland. Resting state. Granules conspicuous, extend throughout the cells; here and there nuclei show through them. Nuclei and cell-substance very slightly stained. Mucous cells in necks of the gland tube are shown; they are less obvious than this in the freshly mounted specimen. Newt (February, 1879), several days' hunger.

Fig. 3. × 420. Posterior oxyntic gland. Resting state. The granules are smaller than those in the anterior glands. (Newt, September, 1879.) The granules, which largely hide the nuclei in the actual specimen, are not drawn here.

Fig. 4. × 420. Posterior oxyntic gland. Four hours after feeding with worm. (Newt, September, 1879.) The granules are fewer, smaller, and vary more in size; there is a small, ill-defined outer clear zone; the nuclei and cell-substance stain darker. The cells are smaller.

Fig. 5. × 440. Oxyntic gland, latter part anterior region, six hours after feeding Newt (July, 1879), which had long fasted. The cells have become much smaller, and lost all their granules; the cell-substance stains deeply. In the most anterior glands in this specimen a few granules were left.

Fig. 6. × 325. Anterior oxyntic gland. Newt (July, 1879) fed with sponge; in eighteen hours sponge vomited; killed six hours later. The zones are sharply marked the one from the other. (2nd type, see p. 689.) The cells are nearly of normal size, the granules somewhat smaller than in the normal hungry animal. In the actual specimen they vary more in size than is here figured.

Fig. 7. × 280. Posterior oxyntic gland. Newt (December, 1879) fed with sponge forty-eight hours. The granules are small, and although they are absent from the outer region of the cells, yet do not form a distinct non-granular zone. (1st type, see p. 689.) This is an early stage of recovery. Deeply stained fat-globules are seen in groups in the outer portions of the cells (see p. 655).

### Gastric Glands of Snake.

Figs. 8, 9, 10 are drawn from different regions of the same stomach, viz.: that of a Snake which was killed sixty hours after having eaten a Frog. A considerable part of the legs of the Frog were still nearly intact.

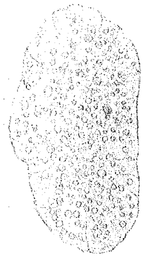
Fig. 8. × 380. Oxyntic gland from anterior region of stomach.

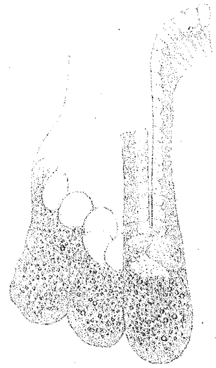
Fig. 9. × 380. Oxyntic gland from front part of the middle region of the stomach. Digestive changes are obvious. Only the lower part of the gland is drawn.

Fig. 10. × 420. Posterior oxyntic gland. The digestive changes are here very marked; in this, as in preceding glands, the tubes usually branch; they are not drawn here.

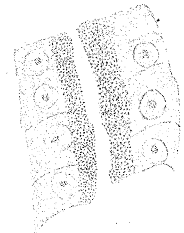
Fig. 11. × 280. Oxyntic gland, middle region of stomach. Hungry Snake; only the lower part is drawn. This represents fairly closely the general condition of the glands drawn in figs. 9 and 10, before digestive changes set in.

In this figure the protoplasm of the cells is made rather too dark, and most of the granules rather too small.

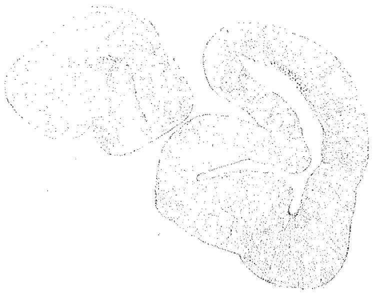

1. × 360

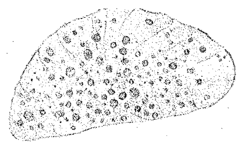

2. × 250.

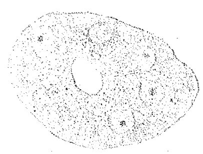

3. × 370.

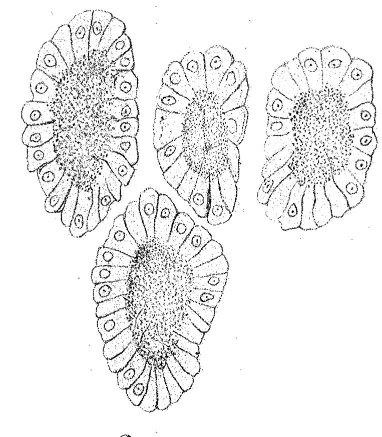

4. × 410


5. × 290

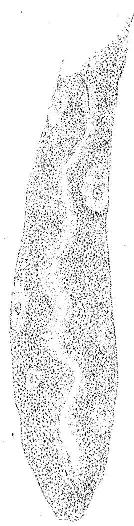

6(a) × 330


7. × 350


6(b) × 410

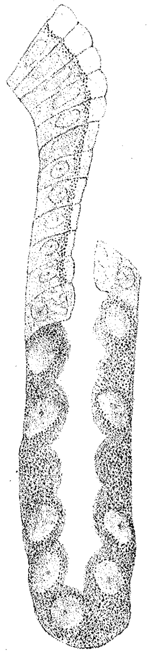

8. × 340


9. × 410.

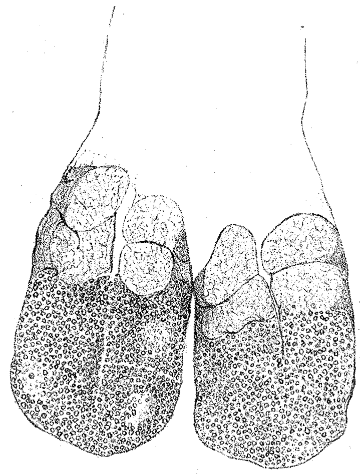

10. × 580


11. × 370

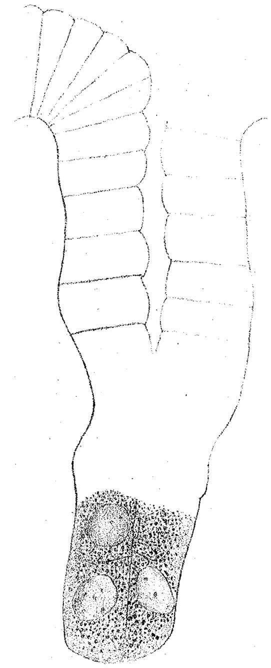
West, Newman & Co. lith.



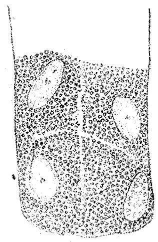
1. × 640



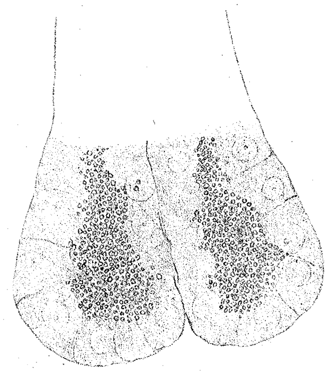
2. × 420



4. × 420

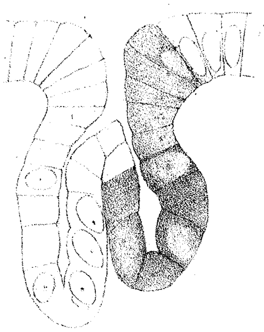


3. × 420

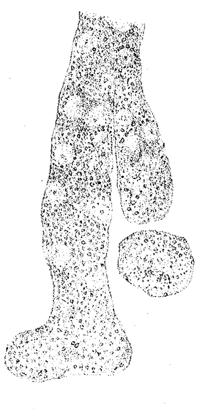


6. × 325.

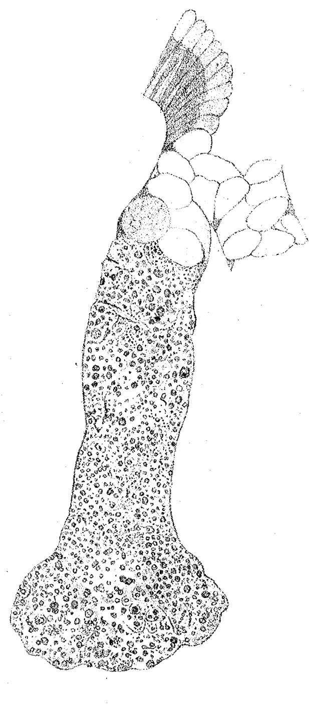
7. × 280



5. × 440.

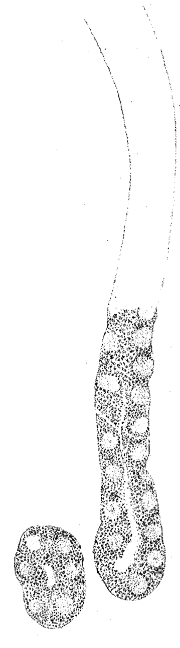


9. × 380



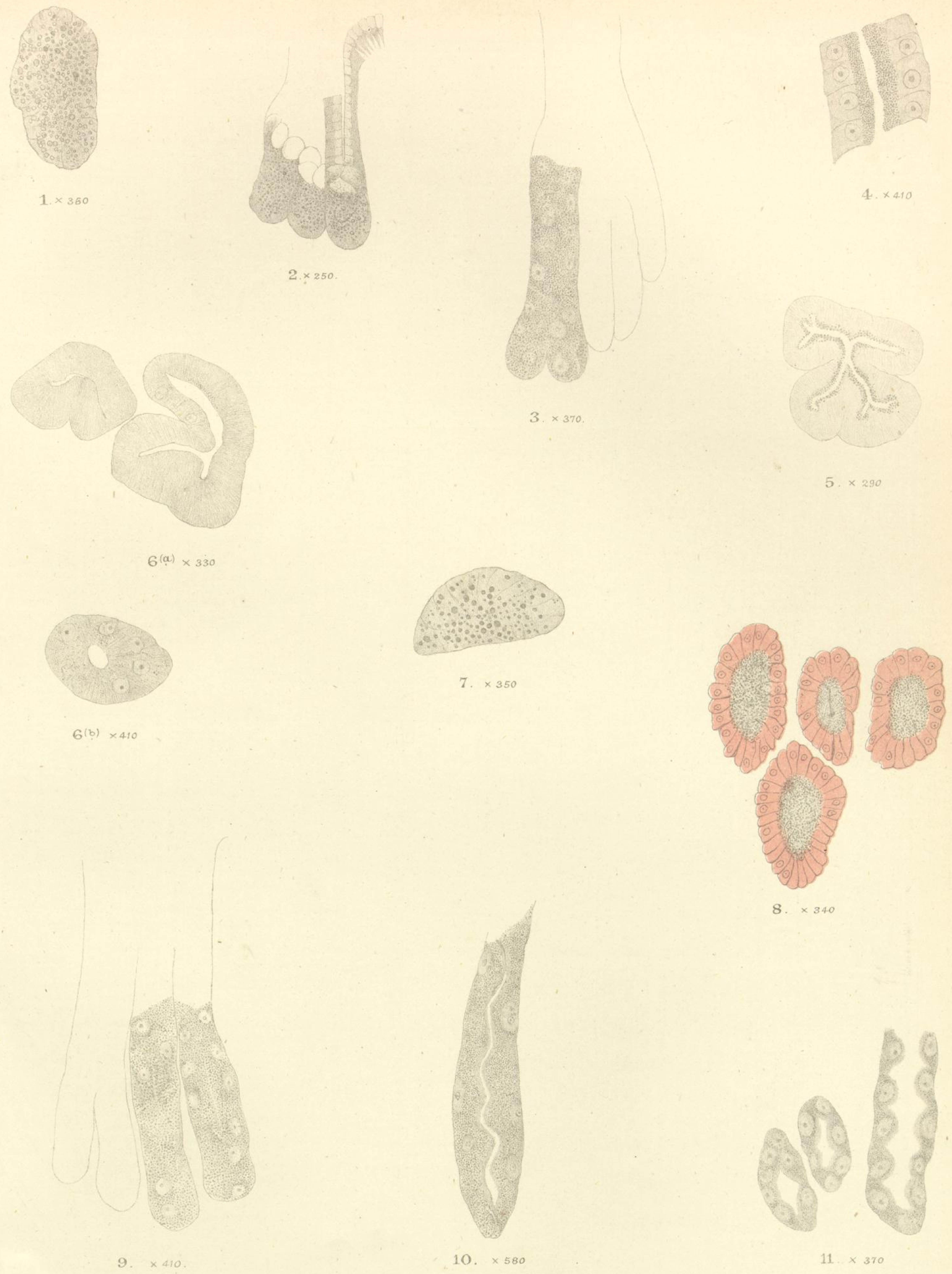
8. × 380

10. × 420



11. × 280.

West Newman & C° lith.




## PLATE 77.

ŒSOPHAGEAL AND GASTRIC GLANDS OF *Rana temporaria*
(Plate 77, figs. 1 to 11, and Plate 78, fig. 1).

Figs. 1, 2, 3. To illustrate the transition from the œsophageal to the oxyntic glands.

Fig. 1. × 360. Oblique section of end-tube of œsophageal gland. The granules are large and vary somewhat in size. The outlines of the cells are indicated; in the specimen they were only with difficulty to be made out.

Fig. 2. × 250. Gland of the intermediate region. The form of the gland resembles the form of the oxyntic rather than that of the œsophageal glands; the granules are larger than the oxyntic gland granules, but smaller than those of the œsophageal glands; they resemble closely the œsophageal gland granules.
Some characters of the resting cylindrical and sub-cubical cells are also shown here.

Fig. 3. × 370. Oxyntic gland, from latter part of anterior oxyntic region. The cells contain many granules, which are, however, much smaller than those in the cells of figs. 1 and 2.

The above three specimens were taken from a Frog (February, 1879) which had been fed with a large amount of food (0·5 grm. worm) forty-six hours previously. The last remnants of the food were still in the stomach. All the glands have slight signs of activity, especially the glands of the intermediate region, in which a small outer zone is seen.

Fig. 4. × 410. Longitudinal section of neck of oxyntic gland; the specimen illustrates also the changes which take place in the sub-cubical cells of the pyloric glands. Specimen prepared from a Frog (April, 1879) which was killed twenty-five hours after a heavy meal (four worms). The tissue was allowed to stay a month in alcohol before sections were cut, hence the distinctness of the interfibrillar substance (granules) in the mucigen portion. An earlier stage of this change in the mucigen portion of the cell can be seen in Plate 78, fig. 1.

Fig. 5. × 290. End-tubes of œsophageal gland. From Frog (September, 1879) forty-five hours after feeding with large piece of sponge. The granules are much diminished in size, and form only a narrow zone around the enlarged lumen. Several end-tubes in this specimen had lost all their granules.

Fig. 6 (*a*). × 330. End-tubes of œsophageal gland. The granules have disappeared from the cells, except at one place where a few small granules border the lumen. From Frog (September, 1879), twenty-five and a half hours after feeding with sponge. In no other Frog which I have observed did the granules disappear in so short a time. In this and the preceding figure the striation of the cells is *much* too distinct.

Fig. 6 (*b*). × 410. Same specimen as preceding after three months' stay in dilute glycerine. The striation and the very fine granules which are often seen in the clear zone are here brought out.

Fig. 7. × 350. End-tube of œsophageal gland. Frog (September, 1879) fed with sponge; after twenty-two hours sponge removed; 1·5 cub. centim. strong aqueous solution of peptone injected under skin of back; animal killed twenty-six hours after this. The granules are comparatively few, and although more numerous in the inner portion of the cells, occur also in their outer portion.

Fig. 8. × 340. End-tubes of œsophageal gland. Tissue placed in absolute alcohol for twenty-four hours, then in dilute carmine for twenty-four hours. Shows stained non-granular and unstained granular zones. Frog (September, 1879) fed with sponge thirty-five hours; sponge then vomited; animal killed ten hours after.

Fig. 9. × 410. Anterior oxyntic gland. Hungry state. The cell outlines are scarcely or not at all to be made out; the granules are distinct, numerous, and scattered throughout the cells. The nuclei and cell-substance is very slightly stained. Frog (July, 1879) killed five days after a good meal.

Fig. 10. × 580. Posterior oxyntic gland. Three hours' digestion. The granules are smaller than normal, and have disappeared from the inner border of the cells, leaving a small homogeneous zone. The nuclei and cell-substance stain darker. The nuclei are placed in the outer portion of the cells, in this specimen indeed more so than usual. The corkscrew-shaped lumen is obvious. Frog (April, 1879) several days' hunger, then fed with worm, killed in three hours.

Fig. 11. × 370. Posterior oxyntic gland. Twenty-five hours after heavy meal. Frog (April, 1879) several days' hunger, fed with four worms; killed twenty-five hours after; stomach still distended with debris of food, although a considerable amount fills out the intestine. The cells bulge out into the large lumen; the homogeneous inner zone is distinct.