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THE HISTOGENESIS OF GASTRIC GLANDS

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ON THE HISTOGENESIS OF GASTRIC GLANDS

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WITH TWENTY-SIX FIGURES

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I. INTRODUCTION

This paper represents an attempt to throw some light on the fundamental processes in the cytomorphosis of mammalian gastric glands. A study of the literature convinces one that, in spite of numerous researches, there still persists a marked lack of agreement even as to the broader outlines, which becomes, with regard to more minute details, a chaotic mass of contradiction, partially referable to the normal generic variations in the forms used, and partially, perhaps, to misinterpretation.

It was decided, on the following grounds, to limit this investigation to the study of a single species, the pig:—First, theoretical considerations of a general, embryologic nature, lead one to infer that, aside from slight generic differences, the same essential processes are probably concerned in the gastric adenogenesis

of the whole mammalian series. Second, one may disentangle from the generally confused data of the literature, certain concordant results, which confirm the validity of this assumption as to the essential unity of the process throughout mammals. Third, and very obviously, a limitation to one form means the possibility of more detailed work than is practicable in a comparative study. The present need seems to be for minute work, like that of Toldt on the cat, but utilising valuable, recently developed technique, and also one old technique, evidently very often neglected nowadays, namely the patient use of serial reconstructions in place of the easier reliance on occasional isolated sections. Finally, and herein lies the proximal motive in selecting this particular species—an abundance of fresh material of every stage has been readily procurable.

I wish to express my sincere gratitude to Dr. R. R. Bensley, at whose suggestion this work was undertaken, and under whose guidance it has been carried on. Without the aid of certain methods devised by him, much of the work on cyto-differentiation would have been technically impossible. I am indebted to Miss Katharine Hill for the accurate drawings.

II. HISTORICAL

Essentially all authors agree that the gastric epithelium is derived from the endoderm. Brand '77, Sewall, '78 and Kölliker, '84 describe a transformation of the original single endodermic layer into a stratified epithelium, which later, by some unexplained mechanism, again becomes simple. All recent writers who mention the subject at all (Toldt '81, Salivoli '90, Ross '03) agree that the epithelium remains simple from the first, although the disposition of nuclei in several planes simulates stratification; of course this does not apply to areas which become stratified and remain so, *e. g.*, the left compartment of the field mouse stomach (Töpfer '91), or the pars oesophagea.

Concerning the origin of the rudimentary gland tubules, there is great diversity of opinion. The various views may be classified under three general groups, depending on whether the tubules

are believed to develop (1) from the surface epithelium, (2) from special embryonic cells interpolated between the basal parts of the ordinary surface epithelium, or (3) from mesodermic cells, such as leucocytes.

Group 1. Of those who describe the glands as originating as downgrowths of the surface epithelium, several have considered the all-important factor in early adeno-genesis to be irregular growth of the mesoderm, first manifesting itself as upgrowths, in the form of either villi or ridges. These coalesce or intersect, thus giving rise to intervening cul-de-sacs, the rudimentary glands. Of course the surface epithelium multiplies to keep pace with the increasing area of mesoderm it must cover, but, by convention, this activity is considered secondary to that of the mesoblast. (For details *vide* Laskowsky '68, Schenk '74, Brand '77, Sewall '78, Baginsky '82, Kölliker '84, p. 360, Negrini '86.) Observations recorded in part 2 of this paper explain, we believe, the discrepancies of opinion regarding the exact nature of these mesodermic irregularities, *i. e.*, whether villi, papillæ or ridges. Kölliker ('52–'61) Toldt '81 (pylorus of cat) Patzelt '84, Griffini and Vassale '88, and Salivoli '90 believe that the epithelium displays the initial activity, and that the glands may be said to have an intra-epithelial origin, irregular growth of mesoderm later becoming a factor in bringing the glands to their definitive form. Thus, Kölliker, in '52–'61, described the downgrowth of solid plugs of epithelium, which later acquire a lumen; he discarded this view ('84), nor has it since been advocated. Griffini and Vassale describe the gland as originating from the surface epithelium, as a funnel-shaped evagination, sinking down into the mesoderm. The deepest cells, being in continual mitosis, constitute an apical growth point. Only at a comparatively late stage does unequal mesodermic growth participate. According to Salvioli ('90, rabbit), at an early stage (4 cm.), while the basement membrane is yet perfectly level, intersecting ridges of epithelial cells rise above the general level of the epithelium. The intervening depressions are the rudimentary tubules. Later (6 cm.), these epithelial ridges are reinforced from below by cores of connec-

tive tissue. Toldt ('81, cat) describes a similar process in the pyloric region. "The process is wholly confined to the epithelial layer." Patzelt ('84) describes the same process in the formation of the glands of the large intestine.

Group 2. Toldt (81 cat) working on the fundic region, and Ross (1903, pig), describes the rudiments of the glands as large, coarsely granular, eosinophile cells interpolated between the basal parts of the surface epithelium. Each divides into a cell group, which, by central liquefaction acquires a lumen, the latter secondarily coming into communication with the surface. Toldt is sure these cells are of epithelial origin, but believes they at no time reach the surface, being always shut off from the latter by the overhanging distal ends of the tall pyramidal surface epithelium; he suspects that they arise from young Ersatzzellen His Ersatzzellen have almost certainly been shown by the work of Stöhr (1882) and Bizzozero (1888) to be "Wanderzellen." Griffini and Vassale maintain that Toldt's figures and text harmonize remarkably with their own findings (V. supra), except that Toldt, through use of oblique sections, erroneously concluded that these primary gland cells do not reach the surface, and that their lumen is thus not at first continuous with the stomach lumen. Griffini and Vassale found many such groups with lumina apparently shut in on all sides, but reconstruction always demonstrated continuity with the stomach lumen from the first.

Group 3. Sewall ('78) believes that, once the original hypoblast has differentiated into ovoids (parietals) and central (chief) cells, new ovoids "*originate*" by differentiation of mesodermic corpuscles. In embryo cats (13 cm) mesoblast cells are found, presenting all transitions from connective tissue corpuscles to parietal cells (Toldt says that ~~Salvioli~~ simply cut the eccentric parietals tangentially from adjacent tubules, and hence misinterpreted them to be cells lying free in the lamina propria). The reader must be referred to the recent work of Strecker ('08), which is too elaborate to be reviewed here. His conclusions are diametrically opposed to mine. The diversity of these views as to the early formation of gland tubules make evident the need of further

investigation of the two separate problems involved: (1), from exactly what cells do the glands arise. (2), to what extent are irregular growth of mesoderm and epithelium, respectively, factors.

Compounding of the tubules is unanimously described as due to the upgrowth, at the bottom of the simple tubules, of partitions which never reach the surface. Toldt, '81, believes that these are, at first, proliferating solid ridges of epithelium, later reinforced from below by a core of mesoderm. Sewall, '78, Negrini, '86, and Ross, '05, hold that the upgrowths contain mesodermic cores from the first. According to Salvioli, '90, the process described in the original formation of the foveola is repeated at the bottom of the foveola. By mitosis, intersecting epithelial ridges arise; later these are reinforced from below by mesodermic cores.

Toldt, Salvioli, Griffini and Vassale (*opera cit.*) also describe a second method of compounding by "side-buds." Lateral diverticula appear, originating through local proliferation of a cell-group, which starts from a single parietal cell. I have confirmed this for the pig (*vide* part IV).

As to cytologic differentiation, Laskowsky, Sewall, Toldt, *et al.*, describe an early departure of the tubule cells from the surface epithelial type, the former becoming polygonal or ovoid, their cytoplasm granular, and their nuclei large and vesicular. Toldt, '81, Negrini, '86, and others demonstrate that parietals appear in the depths of the tubules by differentiation of the embryonic tubule cells. Bensley, '03, finds that mucus cells appear in the pylorus and along the lesser curve in the 6 cm. pig. Parietals appear in cardia and fundus at 7.5 cm., inter-cellular ductules being present from the first. Zymogenic (serous chief) cells appear, at about 21 cm., at the bottom of the fundic glands. A few, as Sewall, '78, and Strecker, '08, believe in a possible mesodermic origin of parietals, or of the whole gland anlagen, either from leucocytes or from mesodermic corpuscles, fibroblasts etc.

III. MATERIAL AND TECHNIQUE

The embryos were obtained in an absolutely fresh condition through the kindness of Swift & Company, of the Chicago Stock

Yards. Half a minute after the instantaneous death of the mother, by cerebral concussion, the uterus was cut out, and immediately opened. The embryos were removed, and their greatest length in the natural attitude along a straight line (Minot's System. *Vide* Minot, '03, p. 356) determined by calipers, and recorded in centimeters. The stomach was then rapidly removed, the subdiphragmatic oesophagus and also a small bit of the duodenum having been left attached in the earlier ones for orientation. The stomach was then slit open along either anterior or posterior side, and filled with fixing fluid. Stomachs from embryos of over 6 cm. length are distended with a clear, glairy, mucoid fluid, often of a greenish tinge. This must first be allowed to escape, as otherwise fixation is unsatisfactory. After being filled with fixing fluid, the stomach is immersed in it and placed in the dark. After a little practice the whole operation need take no longer than half a minute. This fixation immediately after death is of great importance in obtaining accurate pictures of intracellular conditions, especially of the zymogenic, mucus and parietal cell granules. In dealing with embryos of over 12 cm. some of the stomachs were treated as described above, others were subdivided, great care being taken to preserve the identity and orientation of the pieces. The series included embryos of 2 to 29 cm. length at intervals of ½ cm. Generally, several stomachs of each stage were used; some for sagittal sections, thro' the whole length where practicable, some for cross sections. In all cases the sections were cut serially, and this is very necessary, as will be seen later, for the correct interpretation of certain appearances.

I tried many methods of fixation, but finally settled down to the use of Bensley's fluid as giving the best general results.[1] Modified

[1] This consists of equal parts of 3 per cent aqueous K2Cr207 and sat. HgC12 in 100 per cent alcohol, mixed just before using. The cloudy precipitate is to be disregarded. The material is fixed in the dark from 30 minutes to 2 hours, according to thickness. It is then transferred to 50 per cent alcohol, in which, with frequent changes, it remains several days; then to 70, 80 and 95 per cent a day or two, with several changes in each; finally to 100 per cent and then bergamot, bergamot and paraffine, and finally paraffine. At no time until after impregnation with paraffine should the material be subjected to the action of water. Otherwise, as Bensley pointed out (1903-4) the zymogen granules and mucigen are preserved poorly or not at all.

Köpsch is a good fixative for Bensley's three color stain (see below). Controls were often made with Zenker and 5 per cent freshly distilled formalin, but these only served to emphasize the superiority of Bensley's fluid, especially for the neutral gentian and copper chrome stains. The eosinophile granules of the parietals, the zymogenic granules and the contents of mucigenous cells are especially well preserved by this fluid.

Sections were cut $\frac{1}{3}$ μ thick, and fixed to slide by the water method, or by Mayer's albumin. Demercurization was completed by immersion of slides in iodine-alcohol.

Hematoxylin and eosin stains were used in each stage for comparison, but the main reliance was placed upon the four stains named below, serial sections of each stage being stained with each of these:

1. Bensley's three color stain as described by Klein ('06) p. 323. The granules of parietal cells are stained a vivid cherry-red and the intercellular ductules are brought out well. This has proved the best all around stain for stomach material.

2. Neutral gentian (Bensley '00) in 20 per cent alcoholic solution was invaluable in determining the appearance of zymogenic granules. It is also, as far as my experience goes, the best cement line stain, and is thus of great value in the study of the parietal ductules, and of cell boundaries. The material should never be brought into water, as the zymogen granules then disappear. I stained two to four days.

3. Bensley's Copper chrome hematoxylin as described by Harvey ('07, p. 209). The granules of the parietals are stained a steel blue or deep blue black. The chromatin stains black, but is more readily destained than are the parietal granules.

4. Mayer's Muchematin, as modified by Bensley ('03, p. 11) for the detection of mucigen. It is best to avoid the water and heat method in fastening sections to slide, as the granular form of the mucigen is thereby altered.

HISTOGENESIS OF THE GASTRIC GLANDS OF THE PIG

1. The early stages

The stomach of a 2 cm. pig is about 2 mm. long. The cephalad part just left of the oesophagus has already been partially folded off as the secondary cardiac pouch (Coecum) being now separated from the main lumen by a fold which isolates it.[2] This ridge is an infolding of all the layers, including the tunica muscularis, the latter and the connective tissue coat being also thickened at this point.

A definite basement membrane separates epithelium from mesoblastic coats. This stains densely with Rubin S, and has a fibrous structure.

The mesoblast has already differentiated into the connective tissue layer, the tunica muscularis and the serosa. Only the first concerns us. It is a typical mesenchyme, with numerous stellate and spindle cells, anastomosing by their processes. The nuclei are spherical, ovoid or elongated and many are in mitosis. The ground substance is transparent and gelatinous. Already this connective tissue coat has definitely differentiated into two strata (fig. 1).

The cells and nuclei of the inner layer, just beneath the basement membrane, are very closely set, almost touching. Mitoses are very numerous. There is here but little of the mucoid, intercellular matrix, and it is dense, staining somewhat reddish or purplish with the three color or H & E. The blood vessels are of capillary size only. The endothelial cells of the vessel walls are very frequently mitotic. This layer is the young lamina propria mucosae.

Outside this is a broader zone with cells and nuclei sparsely distributed, and a corresponding predominance of intercellular substance, the latter staining hardly at all, and being very clear and transparent. Mitoses occur, but are rare. Blood vessels are very numerous and many of them are of large size. This coat

[2] For the topography of the adult stomach, reference may be made to Greenwood's accurate diagram, Fig. 252, Oppel, 1896.

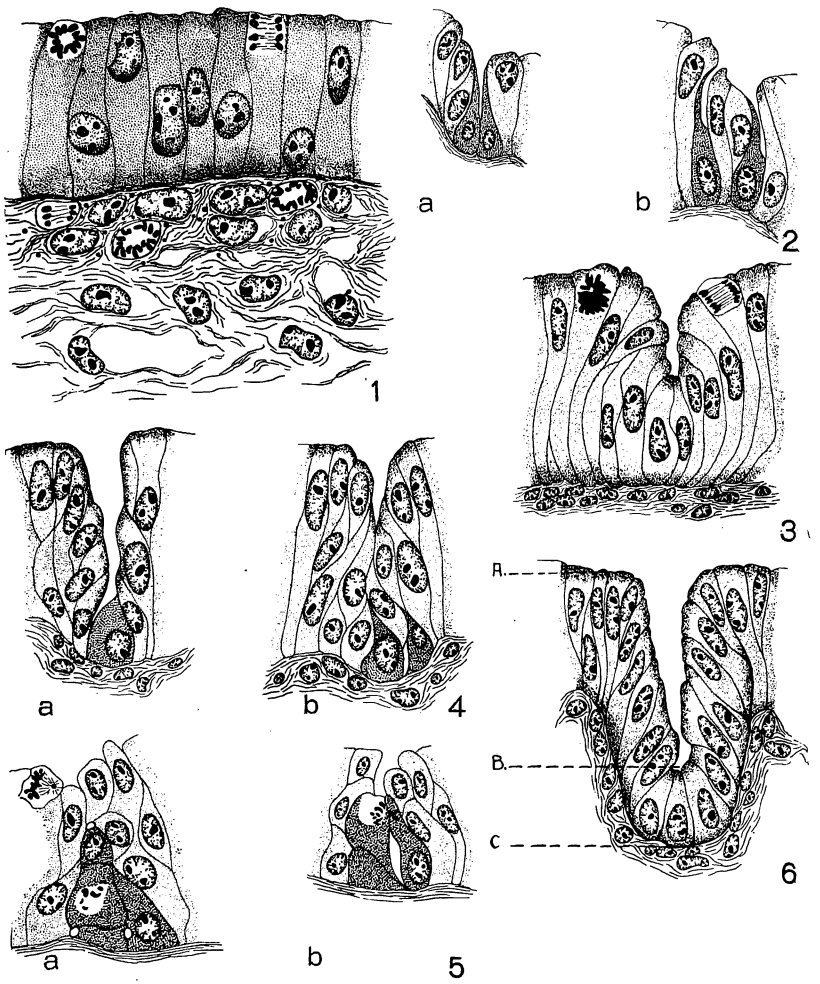


FIG. 1. Earliest or preglandular stage. Epithelium and underlying mesoblast 6 cm. embryo. Three color stain.

FIG. 2. Young parietal cells, showing intercellular ductules. 6 cm. Fundus. Three color.

FIG. 3. Intra-epithelial gland stage. 6 cm. fundus. Three color.

FIGS. 4 and 5. Young glands cut obliquely. 6 cm. fundus. Three color. 5a and 5b represent adjacent sections; similarly 6a and 6b. These illustrate the true nature of the interbasal groups of Toldt and Ross.

FIG. 6. Fundus gland. 6 cm. parietal cells were present in other sections of this same tubule. Three color.

is the young tela submucosa. The boundary between these two layers, while not absolutely sharp, is quite well marked, being definable within the limit of one or two cells breadths.

There is yet no trace of a muscularis mucosae, this first appearing about 9 or 10 cm. at the boundary between lamina propria and tela submucosa. It originates through the elongation of mesenchyme nuclei to a torpedo shape, and the condensation of the protoplasm about each nucleus, as a highly eosinophile substance. The primitive syncitial anastomoses are retained, the new muscle cells being thus joined *inter se* by delicate prolongations of the finely fibrous stroma.[3] In some stomachs isolated muscle fibres apparently appear somewhat earlier (8-½, 9 cm.) At 15-16 cm. a definite, fairly compact muscularis mucosae is present. The fibres of both coats of the tunica muscularis are well defined at 2 cm.

The diversity of opinion as to the part taken in the inception of the glands by unequal mesodermic growth, and as to the nature of the first mesodermic irregularities, is partially referable, no doubt, to differences in the forms used, but there has often been the lack of a definite criterion of distinction between such upgrowths as simply minister to an increase of surface, and such as have a definite rôle in glandular development. By reference to the adult structure, it is seen that *only such mesodermic elevations can have taken an active part in adenogenesis as are constituted by local thickenings of the lamina propria alone.* At 2 cm. the later is of uniform thickness throughout, the basement membrane presenting no irregularities of contour, and the epithelium being everywhere of the same height. But the tela submucosa has thickened in linear ridges, so as to produce folds or rugae, several of which run almost the length of the stomach.

The epithelium is a single layer of high columnar cells, as shown in fig. 1 of the 6 cm. stage The nuclei are arranged in several rows; this, in connection with the small diameter of the cells, readily gives rise, in even slightly oblique sections, to a stratified

[3] This confirms McGill, 1907.

appearance. At no stage does the epithelium acquire stratification except in the pars oesophagea.[4]

The nuclei really occur at all heights in the cells, but, may, for convenience, be arbitrarily grouped into those occupying the distal middle and basal parts. At 2-3 cm. almost every nucleus of the distal row is in mitosis, and all stages of this process are, of course, represented. The nuclei lying at deeper levels are all resting. Whenever the mitosis is in such a stage that its direction may be determined, its axis is invariably parallel to the surface of the epithelium. The nuclei are oval, saccular, and so broad as to almost touch the sides of the cell. They have a moderate amount of chromatin, arranged as a net, and with nodal karyosomes. There are usually two nucleoli, metachromatic or slightly acidophile. The cytoplasm is very finely granular in the epithelium of the coecum, cardia and cardiac part of the fundus; in the plyorus, pyloric part of the fundus and pars oesophagea it is clear and transparent. Aside from this, no cyto-differentiation is discoverable by the techniques employed.

At 2½ cm. irregular thickenings of the tela submucosa in the coecal pouch begin to give rise to the characteristic grosser ridges folds and papillae of that region.

About 2½-3 cm. the surface line of the epithelium, hitherto level, becomes, in some parts of the stomach (pylorus and fundus along the greater curve near the pyloric region), undulatory, displaying alternate very slight elevations and depressions. The basement membrane shows no corresponding waviness, and the lamina prop. no irregularities. On reconstruction, the elevations are found to be short ridges, intersecting in all directions, the depressions thus representing a slight pit bounded on all sides by the ridges. It is readily determined that the elevations consist of cells slightly taller and narrower than those of the depressions (fig. 3), with the nuclei in the distal end; the cells of the depressions have central or basal nuclei, and have retained their primitive height and breadth, or, sometimes increased slightly in breadth. Mitoses are very frequent in the ridge cells. *All the cells reach from basement membrane to surface.* These slight intraepi-

[4] This finding agrees with those of all the later investigators, as Toldt, Salvioli, Ross.

thelial depressions, due to irregularity in height of the epithelial cells, represent the beginnings of the glands, as I have satisfied myself by tracing, in unbroken series, all steps from the adult glands back to these. It will be convenient, hereafter, to refer to this as the *intra-epithelial stage of* gland formation.

Nevertheless, it is hardly possible to say that the mesoderm plays no part in even the initial stages, for these epithelial ridges have scarcely appeared before mesodermic buds begin to push up into them (fig. 4). These latter are constituted by local thickenings involving only the lam. propria, and are true mesodermic gland processes.[5] This latter condition is found rarely at 2½ cm. but often at 3 cm. in the precocious regions. As soon as mesodermic cores appear, the basement membrane is seen to be sharper in outline, and more compact, beneath the pit or gland cells than beneath the ridge cells, being frayed out in the latter locality into fibres which penetrate beween the bases of the cells. Fig. 6, indicates this same condition in a later stage. Possibly this has some influence on the supply of nutrition to the epithelium, and consequently on local differentiation of the latter(?).

The process just described is, in essence, the same as Salvioli's finding in the rabbit, but there are minor differences:

1. In the pig the cells of elevation and depression do not diverge quite so widely in form, in these earliest stages. But at the stage represented in fig. 3 the form divergence seems quite as great.

2. In the pig, there is yet no differentiation, as to the cytoplasm into two types of cells, as Salvioli's clear cells of the elevations, and granular cells of the depressions. Instead all the cells in the areas of gland formation, possess, as yet, a finely granular, slightly acidophile cytoplasm. The cells of the fundus and pylorus were non-granular at 2 cm. but, shortly before the appearance of the intra-epithelial gland anlagen, have acquired the fine cytoplasmic granulation.

[5] This term of Sewall has the priority. His gland processes undoubtedly correspond to these, and despite Ross' criticism, certainly play an integral part in gland development.

3. In the pig, the mesodermic cores appear relatively much earlier—in fact so early that it was only after careful reconstructions that the appearance of epithelial ridges slightly in advance was established. Toldt's description for the pylorus is very similar. He admits that in this region there is no independent origin of the gland cells, with, later, secondary communications between these and the lumen.

From 3 cm. on, the ridges of lamina propria grow rapidly up into the epithelial ridges (figs. 4 and 6), causing a correspondingly rapid heightening of the latter, and deepening of the glands. For the correct interpretation of certain papilloid structures seen in all stages of the gastric development up to about 23-27 cm., it is essential to understand the exact way in which the irregularity of mesoblastic growth manifests itself. As has been indicated, the early, intra-epithelial ridges intersect in all directions. They are not straight, nor do they intersect often at right angles; rather they are short, very irregular, curved or angular, and they intersect at all angles. Now, when the mesoblastic ridges begin to reinforce them from below, the mesoblast pushes up much more rapidly at the nodal points of intersection of the ridges than along the intervening parts. The result is that in a sagittal section taken (fig. 7A) through the line a, b, c, d, the appearance seen in fig. 7B results. That is, we have in addition to the ridges the papilloid elevations a, c, and d, situated at the intersections of the ridges, and higher than the rest of the interglandular ridge. In cross or oblique section of the upper level of the mucosa, they are, of course very numerous, and appear like true papillae. One might readily gain the impressions, as did Brandt and others, that in the early stages there are no gland processes, but simply papillae, the depressed areas between them communicating with each other. Such a condition would be represented by fig. 7A, with all elevations except the nodal points effaced. But this is very different from the condition actually found—namely, that ridges, not so high, it is true, as the papillae, but still high in comparison with the depressed areas, run across from the base of each papilla to the adjacent one. This papilloid condition is marked from the

time of the appearance of the mesoblastic reinforcements to the 20-26. cm. stage, but all this time there is a very gradual increase in height of intervening ridges, until finally at 24–28 cm. the definitive condition is reached, in which there are no longer any papilliform elevations, all the ridges having grown up to the same height as the papillae.

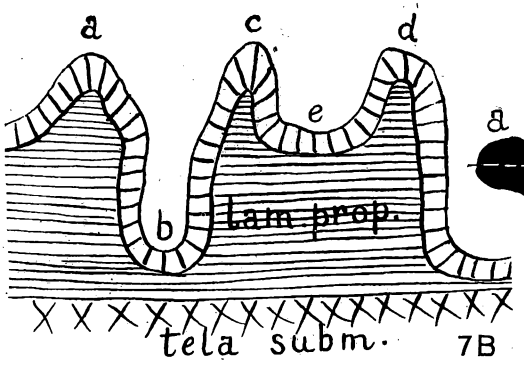
The nodal papillae are often expanded or club-shaped at the top, resembling greatly intestinal villi, but of course shorter. By reference to fig. 7B, it will be seen that in any section through the mucosa the elevations and depressions must necessarily present great irregularities, as *e. g.*, the epithelial line may dip into a gland pit, then perhaps ascend to the top of a nodal elevation, then descend to the level of an interglandular ridge.

Moreover, these ridges and pits cannot be interpreted as mere folds of the mucosa, having no permanent significance, nor any relationship to the adult gland. Once established, these pits indeed deepen and compound at the bottom, many of them even divide into two glands; but their continuity with the adult gland is established by unbroken series. Moreover, we shall see that soon after this formation, certain cells in their depths differentiate into recognizable elements of the adult gland. Finally, the mesoblastic cores of the ridges are, as shown, true thickenings of the lamina propria, not mere foldings of a l. p. of uniform thickness.

It is now easy to understand the many contradictory accounts of the nature of the primary gland processes. Brandt described papillæ or villæ, which later fuse at their bases. Sewall, Salvioli and many others described only the interlacing ridges, and failed to explain the very frequent appearance of villoid cross sections.

As growth proceeds, all the epithelial cells increase in absolute size. Those on the ridges become very high, inverted pyramidal in shape, the proximal part being compressed into a narrow, caudal process, which attaches to the basement membrane (figs. 6 and 11). The nuclei are elongated and laterally compressed. The cells in the pits, or embryonic gland cells[6] increase in breadth, but

[6] I retain Sewall's term, as it has priority, and is self-explanatory. Toldt calls these "adelomorphs," but applies this term at a later stage to the mucus and serous chief cells.



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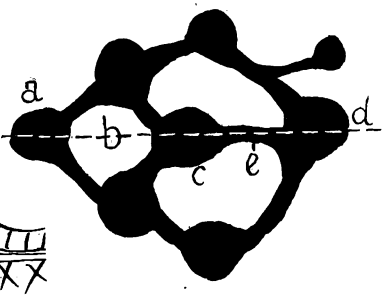

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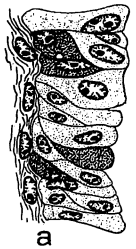

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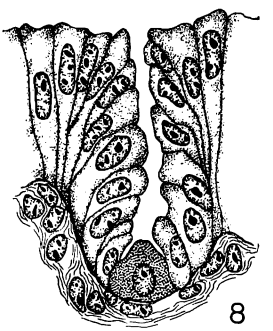

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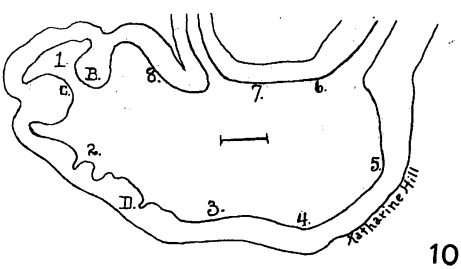

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FIG. 7. A and B. Diagrams (vide text).

FIG. 8. Fundus gland. 7 cm., showing one parietal cell with ductules. The other cells are "Embryonic gland cells," *i.e.*, undifferentiated; at least they present none of the characteristic staining-reactions of any of the adult cell-types.

FIG. 9. a and b. Fundus epithelium 7 cm. Copper chrome hematoxylin.

FIG. 10. Tracing of mesial sagittal section through 7 cm. stomach enlarged x 8.6. B to C, Caecal pouch. C to 4, Cardia and fundus. 4 to pyloric aperture, and latter to 7, pyloric gland area. 7 to B, pars oesophagea. B, Caecal "Wulst" or ridge.

not in height, so that they become thick, short and cylindric, or pyramidal, with broad, basal ends. The nuclei are ovoid, or often almost spherical and moderately chromatic. In glands of the stage indicated in figs. 6 and 11, the gland cells average 16μ in length, 8μ breadth; the ridge cells, 32μ height, 3.2μ breadth at middle, 6.4μ breadth at the broadest part or distal end. Up to the stage of fig. 3 the only distinction between the epithelial cells of the pits and ridges, are those just described, of size and of contour of cell and nucleus. The cytoplasm of all these cells is quite refractile owing to the presence of very fine, slightly acidophile, granules. Up to this stage, then, we have found two factors coöperating in the deepening of the primitive gland tubules. (1) Upgrowth of the mesodermic gland processes, (2) increased height of the ridge epithelial cells, which are now over twice as high as the gland cells.

But as the mesoblastic buds reinforce the epithelial gland processes, mitoses become relatively fewer in the ridge cells, and appear in the pit cells, and it soon becomes apparent that the gland tubules elongate by interstitial growth, i. e., by mitoses of the gland cells. Thus the epithelial part keeps pace with the growth of the lamina propia.

The chronological priority, however slight, of the epithelial ridges to the mesoblastic cores, seems, to indicate that, from the first, certain epithelial cells possess an inherently different potentiality from others. For the primary ridges and depressions are so minute that it is inconceivable that differences in the distribution of vascular supply in the underlying mesoblast could account for this appearance. But, waiving this, at present, fruitless discussion of the predetermination of the cells, it is obvious that the grosser differences in the external form of the ridge and pit-cells are largely a function (in the mathematical sense) of the formation of ridges and pits, i. e.,—the inverted pyriform contour of the ridge cells is largely the mechanical resultant of the compressive forces exerted at their base end and of tensile force exerted at their distal end; and the short, pyramidal form of the gland cells, of compressive forces exerted at their distal end, and of tensile force applied at the base. And these

compressive and tensile forces are obviously referable, in great measure to the uneven mesoblastic growth.

However, we shall see below that other differentiative forces, intrinsic to the cell, and of a metabolic nature, are at work, as is indicated by the early departure of certain of the gland cells from the primitive embryonic type.

These glands appear earliest in the following localities:

(1) The pyloric portion of the greater curve; (2) the part of the fundus adjacent to (1); (3) the caecal pouch; (4) a narrow, peripheral zone of the pars oesophagea. There is some doubt as to whether these latter become true glands.

From these primary regions they extend to all other regions, with the exception of the major part of the pars oesophagea, i.e., that epithelial territory which later becomes stratified squamous. Thus, gland development is most retarded in the cardiac end, over the facies anterior and posterior, and especially over the caecal ridge. Moreover, apart from this general, regional precocity or retardation, several slightly different stages of development may be represented in each region.

At 4–5 cm. the pyloric glands are mostly in the stage of fig. 4 the processes having cores of mesoblast. Some are advanced almost to the stage of fig. 6. Those of the fundus are of the stage of fig. 3, while the cardia displays a slightly undulating epithelium, with numerous mitoses in the upper row of nuclei of the elevations. There is, as yet, no sharp boundary between fundus and pylorus, as to size and form of the glands, but we shall see below that already, through cyto-differentiation, these two regions are marked off from each other.

Between cardia and fundus, no definite boundary of any sort is discoverable, since the change from the shallower (younger) glands of the cardia to the deeper (older) of the fundus is very gradual.

The developmental retardation, as intimated above, finds its extreme expression over the coecal ridge, where the epithelium is at this stage, level, with very numerous mitoses in the distal row of nuclei.

All the epithelial cells, both ridge and gland, with the exception of those of the pars oesophagea, present at the first appearance of the glands, the same type of cytoplasm,—highly refractile from the inclusion of a very fine, closely set, slightly acidophile granulation. These embryonic gland cells do not all remain undifferentiated very long after the appearance of the gland anlagen. At 3 cm. some of the glands of the stage of fig. 3 or even slightly earlier, situated in the fundus, along the greater curve, display one or two cells, which have acquired a cytoplasmic granulation somewhat coarser than that of the primitive cells, and very highly eosinophile. These cells are at first of the same size and contour as the others (figs. 4 and 9) but they soon enlarge somewhat, the basal end becoming broader and rounded, while the distal end narrows, often becoming caudate (fig. 2). Later, the narrow distal end is lost (retracted?), but at all times, up to 24 cm. the cells reach the surface (fig. 8, etc.). The nucleus, at first ovoid, later rounds up, and approaches the spheroid form. These cells are the earliest parietals. Even at their first appearance, one often finds, in relation to them, minute intercellular ductules, always continuous with the lumen of the gland, or, in the more shallow glands, opening directly into the stomach lumen (figs. 2, 4, 5). These pass down between the parietal and the adjacent primitive cells. The almost absolute constancy with which these early parietals display the ductules is striking. As already intimated, parietals sometimes appear in insinkings so slight that they seem almost to have differentiated in level epithelium. This occasionally occurs as late as 7–8 cm. (fig. 9) in small patches of epithelium which remain almost level in the midst of active gland formation on all sides, as also in the extreme cardia at a stage when the glands are just appearing. Nevertheless, even these parietals are probably always situated within the territory of a gland-to-be, for after the 10 cm. stage, parietals are found only in bona fide glands, and no primary gland-pits appear later than this stage. Transitional forms between embryonic gland cells and parietals are also found, their cytoplasmic granules being intermediate in size and avidity for acid stain. These occur not only in the early stages, but even

at 11–12 cm. and probably up to 19 cm. A point which I wish to emphasize is that the glands are largely constituted from the very first, of adelomorphs.[7] Some glands have parietals almost from the first; some do not develop any for some time. Thus, as late as 6–7 cm. glands occur, especially in the cardia, but also in the fundus, in which all the cells are undifferentiated. And by the side of these will be found glands of the same age (size) but with one or more parietals. I am able to confirm Toldt's statement that parietals do not appear in the pylorus at any stage. However, once or twice,—so very rarely that even to mention it is to give an exaggerated idea of the importance,—I have found a single parietal in a young gland from the undoubted regio pylorica; never, however, a group of parietals. Also, while in later stages, the boundary between fundus and pylorus is very sharp on the greater curve, there is, at some points on the facies anterior and posterior a zone, two or three tubules wide, in which occur mucus chief and parietal cells, but no zymogenics.

Thus, with the differentiation of parietals, we have at once a rough divergence of left gastric region, corresponding to caecal pouch, cardia and fundus, where they appear, and right gastric region, the pylorus, where they never appear. Merely a rough divergence at first, because, in the earliest stages, not all fundic tubules have parietals. Thus the parietals arise at first, by the differentiation of certain of the adelomorphs; but, as early as 4–5 cm. they increase also by mitosis (fig. 5). This method of division in parietals has been denied or doubted by many observers, but I can say positively that it frequently occurs up to rather late stages of glandular development, certainly as late as 16 cm. The eosinophile granules remain intact, but a clear zone, free from them, surrounds the chromatic figure. As soon as groups of 2 or 3 have thus arisen the ductules are formed not only between the parietals and adjacent embryonic cells, but also between adjacent parietals (Figs. 2, 4, 5). Thus, by mitotic division of preëxisting ones, and by differentiation of new ones

[7] This convenient term, used by Toldt in the same connection, may, for brevity, be used interchangeably with primitive gland cell, care being taken not to apply it, as did Toldt, to the specialized mucous and serous chief cells.

from adelomorphs, larger and larger groups and rows appear in later stages. I do not believe that new ones develop from adelomorphs after 13–14 cm. and it seems almost certain that this does not occur after 19–20 cm., as all the adelomorphs have at tnat time differentiated into other types, mucus or zymogenic. Thus, in the latest stages, the parietals probably arise only by division of parietal predecessors, unless there is a genetic relation between parietals and zymogenic or mucus cells, of which I found no evidences.

If the section of the early gland be even slightly oblique the parietals, whether isolated or in small groups, appear as conspicuous, large, granular cells, lying apparently between the bases of the surrounding adelomorphs and often as if far removed from the surface. If cut obliquely, the cells appear of ovoid contour, if cut transversely, of circular outline, hence sometimes interpreted as referable to a spherical shape (Ross, '03). Figs. 4b, 5a, and 9a illustrate this condition, as do Ross' ('03) figs. 25, 26, and 24. *By reconstruction it is found that they reach the surface. Vide* figs. 4a representing the section adjacent to 4b, 5b adjacent to 5a; 9b where obliquely cut parietals are shown, including the distal end of one. These obliquely cut parietals are much more conspicuous objects than are the obliquely cut bases of the surrounding adelomorphs, owing to their larger size, granulation and deep red color. This probably accounts for the inconsistency of the interpretations of Ross, whose figures show the obliquely cut bases of parietal cells surrounded on all sides by undifferentiated cells, likewise cu^{+} obliquely. The parietal cell bases are then described as basal gland anlagen, which have never yet approached the surface of the epithelium; curiously, this interpretation is not extended to the obliquely cut bases of the adelomorphs. Ross' figs. 28 and 29 are especially significant as showing the true condition, and are in perfect accord with my findings. The little depressions mentioned by Toldt and Ross (Ross '03, fig. 24d) and interpreted as surface insinkings which later communicate with the fundus of the gland, are shown in my fig. 4b etc., but a glance at adjacent sections (as 4a) always shows that they are merely part of the shallow lumen of the gland,

i. e.; have been from the first in communication with the lumen of the gland fundus. Toldt and Ross have both spoken of the appearance of a minute vacuole between the cells of a group; thus Ross: "A central lumen, small, to be sure, but still a lumen" (fig. 26). This is said to have arisen independently within the group, and to have secondarily communicated with the surface. They figure and describe very accurately the appearance of the intercellular parietal ductules, as seen in cross or oblique sections (*Vide* my figs. 2, 4, 5).

Salvioli, too, ('90) has pointed out the source of Toldt's error ('81)—namely, oblique sections, with no reconstruction control—but this warning was evidently overlooked or disregarded by Ross ('03). Ross pictures similar groups of basal cells for Amblystoma and the pig but the two are different in nature. The basal group figured for Amblystoma are undoubtedly cells of the fundus segment of the glands, the latter possessing but one type of cell,—namely, those which become, in the adult, zymogen cells. But Ross homologises with these groups, those of somewhat similar appearance in the pig; and the latter are, as I have shown,—simply certain very conspicuous ones of the fundic segment cells,—namely, the newly differentiated parietals.

How may we know that these are really young parietals? Because they can be traced, in unbroken lineage, from the 3 cm. stage to the stage just before birth (29 cm.), long before which they are definitive in size, position and all morphologic characters; because from their first appearance, they exhibit the slight enlargement as compared with the adelomorphs, the conspicuous intercellular ductules and the cytoplasmic granules, the latter, at all stages, showing a characteristic affinity for Rubin S and Eosin, and staining black or steel blue with copper chrome hematoxylin, and copper red with neutral gentian. Certain other characteristics are not so marked until the later stages; such are the polygonal, spherical or lenticular shape, which succeeds the earlier piriform shape. The nucleus of the early parietal is ovoid, as are those of all the gland cells, but by 6 cm. many of the older parietals have spheroidal nuclei. This latter, however, is not a constant character, as, even in the adult, parietals are found with ovoid nuclei.

We have seen, then, that all the cells of the gland tubule, are, from the first, on the same morphologic level; all reach the surface and the basement membrane in the early stages; there are no "basal cells" in the sense of cells which are shut off from the stomach lumen by higher cells. Moreover, the parietals are the first of the adult gastric cell-types to be differentiated. We shall find that, in the earlier stages,[8] they appear only in the lower parts of the gland tubule.

While the epithelium of all other parts of the stomach has become granular and refractile, that of the pars oesophagea has remained absolutely clear and transparent. As had been anticipated, no glands appear in the major portion of this territory, which at 4 cm. yet presents a simple columnar epithelium. But a small peripheral one has already developed glands, by the same general process described for other regions. Their cells, like those of the rest of the pars oesophagea, are absolutely clear, and the distinction from adjacent regions is very sharp, so abrupt that one is able to compare the last transparent cell with the adjacent finely granular cell of cardia, fundus or pylorus.

The caecal ridge is covered by simple, granular epithelium which displays the slight waviness and the numerous distal mitoses seen in the 2 cm. stage of the fundus and cardia.

In the caecal pouch, development is exceedingly erratic. At 2–3 cm. slight irregularities occur; some of these elevations are broad and being due to unequal growth of the submucosa, correspond, when linear, to the gross rugae, and, when circumscribed, to the gross papillae or villi, of the adult caecum; others are due to a thickening of the lamina propria alone, but are very broad, or very high, and manifestly have no direct relation to gland formation, helping to increase the surface area. Other stretches are yet level and present numerous mitoses in the distal row of nuclei. At other places, the first, minute, intraepithelial glandular depressions are appearing. At 4 cm. some of these glands are in the stage of fig. 3, some of fig. 6. They are rather sparsely distributed. Thus, the small caecal pouch shows a developmental

[8] Thus up to about 7 cm., in the fundus, and about 12 cm. in the cardia.

picture as varied as that of all other parts of the stomach together, including within its limits all types of glandular development found at these stages in the stomach. It is thus defined by its gross boundaries, rather than by any well marked histologic or glandular type. By 7 cm. the rugae and papillae have by the successive appearance of smaller ones on the larger, acquired complex, fantastical shapes, like those seen in a papilloma. The comparatively few glands appear both on these rugae and in the depressions between them. Some of them have progressed to the stages of Figs. 6 and 11, and hence resemble the pyloric glands in size and form, but have developed some parietal cells.

Mucus cells appear, for the first, in the 6–6.5 cm. stomach, at which time all the cells in the lesser curve (pyloric) area stain very definitely and deeply with muchematin. In general, the surface cells and those near the top of the gland have but a shallow, distal rim of mucus, the rest of the cytoplasm being of the ordinary, granular type. In the deep cells of the tubules, the cytoplasm distal to the ovoid nucleus is of alveolar structure, the alveoli being filled with mucin. If care is taken to preserve the tissue from the action of water, the mucus occurs in the form of spherules, one in each alveolus (fig. 12), and enclosed by the slender alveolar wall. With the three color, or hematoxylin and eosin stain, the cytoplasmic part of these cells, surrounding the nucleus, and in the first mentioned type, occupying the whole cell except the distal rim, presents the ordinary, finely granular, slightly reddish appearance of the ordinary embryonic cells; the mucus part appears clear, and has an alveolar network. In the other parts of the pylorus (greater curve and facies anterior and posterior) no mucus cells have appeared, all the cells being of the primitive type. As to size and form, the glands are uniform in all parts of the pylorus.

At 7 cm. the region from C to 4 (fig. 10) represents the territory of the adult cardia and fundus. There is a gradual and progressive increase from C to 4 in the age of the glands. Those toward C are in the intraepithelial stage (fig. 3). They occur clear up to the caecal ridge, where the epithelium becomes level. Toward 4 the glands approach the type of fig. 7, but are

not quite so deep, the mesoblastic cores of the processes being smaller. Many glands in the whole area C to 4 possess parietals, which in the part toward C are isolated, groups being often found toward 4. Thus cardia and fundus merge insensibly as yet, the fundus being as always, more precocious. The fundic glands, from about D to 4, present at 7 cm., the following measurements: The lumen depth[9] averages 25μ; the process height, 35–40μ. Mitoses now occur in the tubules, mostly about one quarter the distance from the surface, i.e., in the region corresponding to the adult neck.

At point 4 an abrupt change occurs, the glands from 4 to the pyloric aperture being almost twice as deep as the fundic glands, the average lumen depth being 40–50μ, the process height, 50–70μ. The gland processes are broader (32–40μ) than in the fundic glands. These pyloric glands also occupy the lesser curve, from the pylorus to point 7, where the pars oesophagea abruptly begins. The pyloric glands and processes are strikingly symmetrical, the former having the form of simple tubes. Many mitoses are present at all depths, not so many on the surface.

It must not be forgotten that in the cardia, fundus and pylorus longitudinal rugae occur, some running the whole length of the stomach. They are not so large as those of the caecum, but are readily visible with a hand lens. In the cardia, they are especially large ($\frac{1}{8}$–$\frac{1}{4}$ mm. in diameter), and often papilliform or highly irregular. The caecal ridges are very high.

Parietals now occur in caecum, cardia and fundus; in the latter, groups of them. We have seen that the first to appear, came to occupy only the deeper parts of the tubule. But now, in the deeper tubules (from 3 to 4, fig. 10) new ones are differentiated farther up along the sides, at first as single cells, which, by mitosis, soon give rise to groups in this new position. They never appear in the upper quarter of the tubule.

At 7 cm. the mucous cells have spread down to point 5 on the greater curve, the pyloric glands from 5 to 4, yet containing no trace of mucin. The boundary between the mucous and non-

[9] For brevity the distance, fig. 6, from A to B will be called "lumen depth;" from A to C "process height."

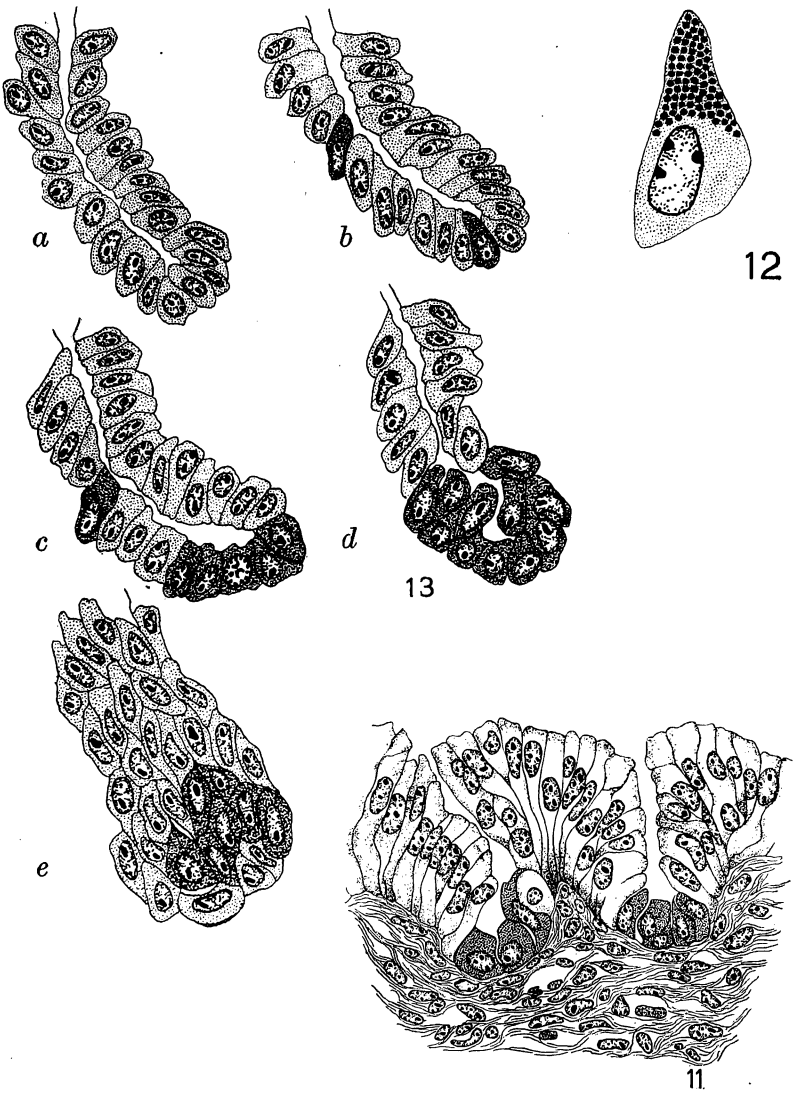


FIG. 11. Tubules of cardia near fundus. 7 cm. Three color.

FIG. 12. Mucous chief cell from bottom of a pyloric tubule. 10 cm. Muchaematin.

FIG. 13. Adjacent serial sections through a tubule of fundus region. 10 cm. Three color.

mucous pyloric tubules is absolutely sharp. Many of the mucous cells in the gland tubules are mitotic, as are also the embryonic cells. The transition, then, between fundus and pylorus is absolute (point 4) and marked by the sudden difference in size of tubules, and by absence of parietals in the pylorus. Rarely, the surface fundic cells show a very slight line of mucous stain, very inconstant, and probably absorbed from the general stomach content.

From now on, mitotic activity is incessant at all depths of all the gland tubules. Surface mitoses also occur, but are much rarer.

In the pylorus of 7–8 cm., and fundus of 9–10 cm., secondary gland processes appear at the bottoms of the primary tubules. They are of the same general nature as the primary processes, being intra epithelial for a short time, but being very soon reinforced by mesoblast. By this upgrowth they at first render the lower portion of the tubule compound, but as they progress until they reach the surface, the result is not a compound tubule, but two independent glands. This mode of increase of the glands is very common in fundus and pylorus, in these earlier stages. In the cardia, the same process occurs later (14–15cm.). These upgrowths are of the same nature as the primary ones, being intra-epithelial for a short time, but being speedily reinforced by mesoblast.

The true compounding occurs somewhat later, with the failure of these secondary processes to reach the surface. This begins in the pylorus about 17 cm., in the fundus about 19–20 cm., in the cardia about 21–22 cm. During this compounding, there is enormous proliferative activity in the depths of the tubules, as evinced by the numerous mitoses, and by the rapid downward elongation of the glands. This applies especially to fundus and pylorus, but many mitoses are found even in the cardia.[10]

After the gland-compounding has been largely completed (25–29 cm.), the mitoses are somewhat less frequent, but are still present in all parts of the tubule, especially in the elongating fundus segments.

[10] In the compounding of the cardiac tubules we shall find a complicating factor, the evagination of parietal tubules.

It was deemed best to deal with the stomach as a whole in the discussion of these earlier stages, up to and inclusive of 7 cm. But inasmuch as certain definite gastric regions have now differentiated, the development of these will, in the interest of clearness, be followed separately.

2. Pylorus

At 9 cm. all the pyloric epithelium, both surface and glandular, consists of mucous cells. The deeper tubule cells are of the mucous chief type, those higher in the tubule have shallow thecae, while the surface cells display a mere distal rim of mucus. No parietals are present, except in two or three tubules next to the fundic zone, and in these only a very few scattered ones. Now, as earlier, the fundo-pyloric boundary is very definite. If, as seems justifiable in the light of the adult structure, our criterion of a pyloric tubule be the mucous chief cell-lining of the deep segment, then the fundo-pyloric boundary is absolutely sharp. For, if adjacent sections stained with muchematin and three color be compared, we find that the fundic segment of the fundus glands, even the last, has no mucous cells, while, the fundus of the pyloric tubules is lined exclusively by mucous chief cells, except for the scattered parietal or two in the last two or three tubules toward the fundus.

The pyloric processes are, in general, broader (40–54μ) than those of the fundus (25–40μ), but resemble the latter in often possessing expanded or clubbed tops. The pyloric tubules usually present a very symmetrical test-tube form. Their appearance at 9 cm. is indicated by fig. 21, for although the latter is taken from a much older embryo (19 cm.) yet the change in the pyloric glands, after 9 cm. are mainly those of size and of the upgrowth of the enclosing ridges rather than any fundamental alterations in form and cytology. In the pyloric tubules, as in those of the fundic region, there are from now on, many mitoses, and at all depths. They also occur in the ridge cells, although not so frequently.

At 10–11 cm., the deep cells are so packed with mucus that the nuclei are often flattened; the goblets of surface and foveola cells are also becoming deeper.

At 15 cm., many of the surface cells have goblets reaching clear to the nucleus, while some show merely a superficial rim, there being no regularity in the distribution of these types.

At 18 cm., the mucous chief cells line the lower third or quarter of the tubule, the goblets the upper $\frac{2}{3}$ or $\frac{3}{4}$, the deeper in position having, as hitherto, the shallower goblets. At 26 cm., all the surface and foveolar cells have very deep goblets.

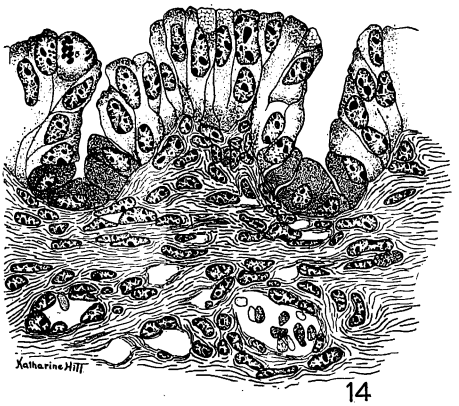
Thus cyto-differentiation in the pylorus is practically complete at 9 cm. The size of the cells, the depth of the goblets and other details alter somewhat later, but, microchemically and presumably, metabolically, the cells have then reached their definitive condition. I do not mean to imply that they have reached a terminus of potential differentiation. Harvey's work ('07) shows that very probably even the adult gastric cells, highly specialized as we are accustomed to consider them, may under changed conditions, pathologic or experimental, assume other forms and functions. But this seems not to occur in normal, embryological cyto-differentiation in the pylorus.

Measurements were taken of glands from each region at all stages but inasmuch as such figures have no general embryological value, I quote only a few, and these for the purpose of comparing the rapidity of growth in these regions. Such figures are necessarily only approximate, as there are considerable differences between those from embryos of the same length, and also between adjacent tubules.

	9cm.	16cm.	19cm	23cm.	29cm.
Cardia	35–50μ	80μ	105μ	112μ	160μ
Fundus	70μ	110μ	140μ	165μ	210μ
Pylorus	80–110μ	130μ	160μ	208μ	240μ

3. Fundus

From the first we have found considerable difference between cardiac and fundic tubules, but the two districts have always merged insensibly and the distinctions have been those of size and age, depending on the general precocity of the right and retarda-



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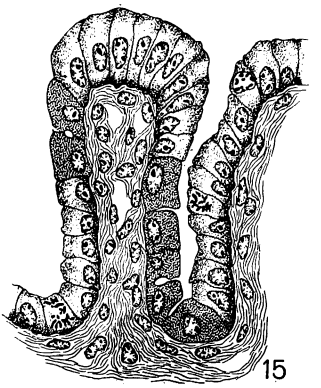

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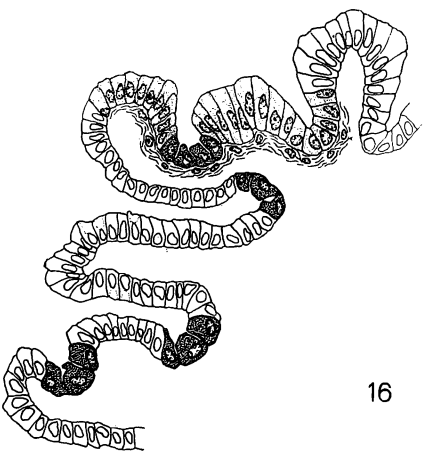

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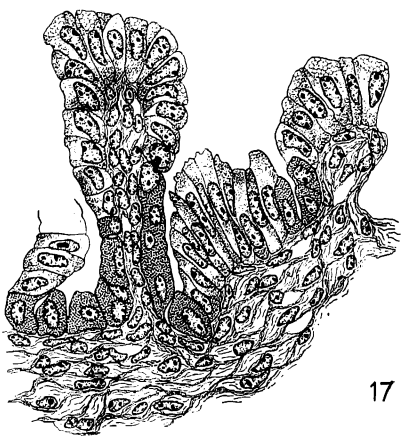

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FIG. 14. Cardia. 15 cm. Three color.

FIG. 15. Fundus tubules. 15 cm. Note ductules of the parietal cells and "lateral parietal rows." Three color.

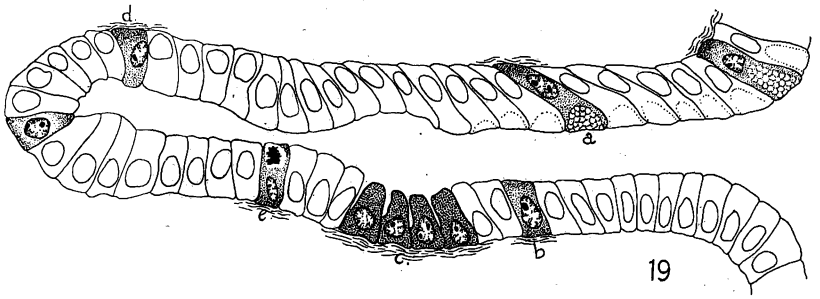
FIG. 16. Cardiac tubules. 18 cm. Three color.

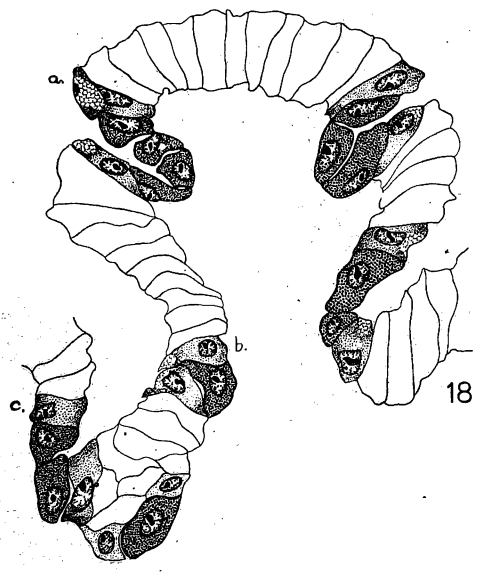
FIG. 17. Cardia. 19 cm. Three color.

tion of the left region, rather than on any true, differential divergence. Nor, indeed, shall we expect to find, at any time, a sharp boundary comparable to that between the fundus and pylorus—for such does not exist in the adult pig. (*Vide* Greenwood '85; Bensley '02, p. 128) Hence, the somewhat arbitrary nature of the separation for descriptive purposes, of these two regions, should be constantly borne in mind.

After 7 cm., the parietals are no longer confined to the deepest part of the fundic tubule, but invade all but the upper quarter. Some groups contain 10–12 cells. Especially characteristic from this time on is what we may call the "lateral parietal row." This is a row of parietals, one or two abreast, extending, often, from a point $\frac{3}{4}$ up the tubule, either to the very bottom, or only part way down (fig. 15). The occurrence as a row is, of course, determined only by reconstruction and by cross sections, and has been often verified thus. In cross section such a tubule will show one or two parietals, all the others being adelomorphs. These lateral rows occur even in the last stages before birth. However, the haphazard distribution of parietals ordinarily described is also found, isolated parietals, or groups of 2–3, or even 10–12, occurring from now on, not only in rows, but also in superficies. Fig. 13 represents serial sections through a gland-tubule of 10 cm., and illustrates the *partial* differentiation of the fundic elements into parietals, staining a brilliant red in fuchsin S.

The ductules in relation to the parietal cells are present at all subsequent stages, and hereafter, in this paper, their presence will be taken for granted. Often a ductule ramifies, sending branches between three or four parietals, or two branches between the same two parietals. These intercellular ductules are limited by cement lines, as shown beautifully by neutral gentian. Most of the parietals, at 8 cm. and later, display clear vacuoles, sometimes as clear areas surrounding the nucleus, but often lying in the peripheral cytoplasm. Harvey ('07) Hamburger and others describe these in the adult parietal (*vide* figs. 18, 20). In general, they become more constant, numerous and large in the later stages. Of course the obvious inference is that they represent droplets of secretion to be poured into the intercellular ductules. I have never





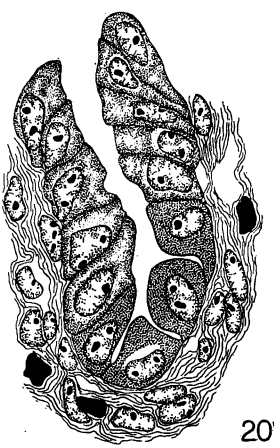


FIG. 18. Cardia. 19 cm. Three color. The cells represented in outline are surface mucous cells.

FIG. 19. Fundus. 19 cm. A complete tubule. Three color.

FIG. 20. Fundus. 19 cm. Bottom of a tubule, showing parietals and adelomorph cells. Copper chrome hematoxylin.

been able, by Golgi impregnation, although I have tried many times, to demonstrate, even in the latest embryonic stages, any continuity between these vacuoles and the ductules.

After 7 cm. mitoses are very frequent in the fundus tubules, occurring at all depths, but especially at the very bottom and at the juncture of the first (upper) and second quarter, *i. e.*, in the territory corresponding in the adult, to the lower part of the foveola. Most are in the adelomorphs, nevertheless parietals are often caught in mitosis. At 8–9 cm., every tubule of the fundus region has acquired some parietal cells. At 9 cm., and later, parietals are found with constricted nucleus. These I interpreted as examples of amitosis. Possibly they presage a cessation of cytoplasmic division. In later stages (20–29 cm.) multinucleate parietals appear. After 10–11 cm. the actual and relative increase of parietals in the cervical region, and relative decrease in the bottom of the tubule is marked. The latter is due to the proliferative activity of the undifferentiated gland cells, by which the tube is constantly lengthening downward. Mitoses also occur on the processes but not so frequently.

At 9–10 cm., no mucus is present in any fundic cell. At 12-13 cm., this is yet true for the large part of the fundus, but, near the pylorus, along the greater curve, very shallow, distal goblets of mucus appear in the cells of the surface and upper quarter of the tubule. At 15 cm. in this part of the fundus, the surface cells have acquired quite deep goblets, while in the upper third of the tubule are shallower goblets, and a few mucous chief cells. In the stages 15–20 cm. this mucous differentiation of surface and foveolar cells spreads rather gradually to the other parts of the fundus.[11]

Thus, even at 17 cm. we find a condition not far advanced, in this respect, over that of 13 cm.,—the surface and foveolar cells of some areas presenting but narrow rims of mucus, and the neck chief cells staining but faintly, or not at all, in muchematin.

[11] This occurs with striking slowness as compared with its rapidity in the pylorus. Moreover, in the latter, the gland cells at the very bottom were the first to manufacture mucin, the pyloric surface cells of some embryos showing no mucus as late as 12–13 cm, but the gland mucus chief cells staining heavily.

Fig. 15 represents the appearance of an average fundic gland of 12 to 16 cm., but it must be remembered that reconstruction shows a preponderance of parietals in the 2d and 3d quarters, over the number in the deepest fourth, also that in some parts of the fundus the surface and upper tubule cells may have acquired shallow goblets, which appear clear and transparent in the three color stain. It will be seen that the tubule has been lengthened by upgrowth of the gland processes. The latter are often clubbed or expanded at the tops and sometimes irregular. The upper $\frac{1}{4}$ or $\frac{1}{3}$ of the tubule devoid of parietals, and with cells already sometimes bordered with mucus, represents the future foveola. The rest of the tubule contains the cells from which will be derived all the epithelium of the adult cervix and fundus. Already (12–16 cm.) the parietals are preponderating in the middle third of the tubule, while the embryonic gland cells, in the deep $\frac{1}{3}$, are multiplying rapidly mitotically, thus deepening the tubule, and incidentally furnishing the material from which, as we shall see later, the zymogenic cells are to differentiate. For the present, these adelomorphs are, in every way, as far as the staining methods at my disposal show, the same as the primitive, embryonic gland cells. They have become however, somewhat more definitely cubic or low cylindric.

This description of tubules applies to the large part of the fundus, including those parts near the pylorus. In the fundic areas toward the cardia, we find that after 11–12 cm. not only are the foveola and surface mucous cells present, but occasionally cells in the deeper parts of the tubule tinge definitely with muchematin. At 14–15 cm. some of the tubules are lined to the bottom by mucous and parietal cells, the mucus cells in the depths being of the mucous chief type, those in the upper third shallow or deeper goblet cells. Interspersed with such tubules are some with three cell types—mucous, parietal and adelomorphs; and these tubules predominate more and more in progressing toward the right, until at last the typical tubules are reached. These left fundic tubules have as far as cytodifferentiation is concerned, reached the definitive condition. They constitute in embryos as in adult, a zone transitional, in all respects, between cardia and fundus.

In embyro and adult there is found, in passing through this zone from left to right, a progressive decrease in mucous chief cells, an increase of embryonic gland cells (or, in the adult, of serous chief cells) a gradual lengthening of the whole gland, and a gradual relative shortening of the foveola and lengthening of the fundus segment.

Thus at 17–19 cm., in the typical fundus area, the bottom of the tubule is lined by adelomorphs and parietals, the former preponderating. The former do not tinge with muchematin, up to the time of their final conversion into zymogen cells, for such will soon be their fate. In other words, those cells destined to become the serous chief cells, do not pass through a mucous stage, but pass directly from the embryonic form to the complete zymogenic cell. Up to 19 cm., neutral gentian, aside from tinting the cytoplasm of all the cells a rather faint, diffuse violet or blue, stains no granules, except the moderately course ones of the parietal cells. At 19 cm. granules of a new type appear in the distal zone of those cells of the fundic segment of the fundic glands, which have hitherto preserved the embryonic type. These granules stain, from the first, a dense blue. At the time of their appearance they are slightly larger than the parietal cell granules. They do not appear simultaneously in all the adelomorphs, but at first in scattered ones, spreading rapidly to the other cells of this type, so that, at 25 cm. no embryonic gland cells remain in the fundus, all having differentiated into serous chief or zymogenic cells. The granules increase rapidly in size, so that they are soon much larger than those of the parietals (fig. 22). In some cells, from the first, they are not limited to the distal zone, but occur throughout that part of the cytoplasm distal to the nucleus. The cytodifferentiation of the fundic glands seems now complete.

The remaining changes are merely such growth processes and shifts in the relative size and position of parts, as are necessary to bring about the definitive form and positions. As these undoubtedly display much generic, or even specific variation, they can have no general developmental significance, such as possessed by the details of cytodifferentiation, and will, therefore be described but briefly.

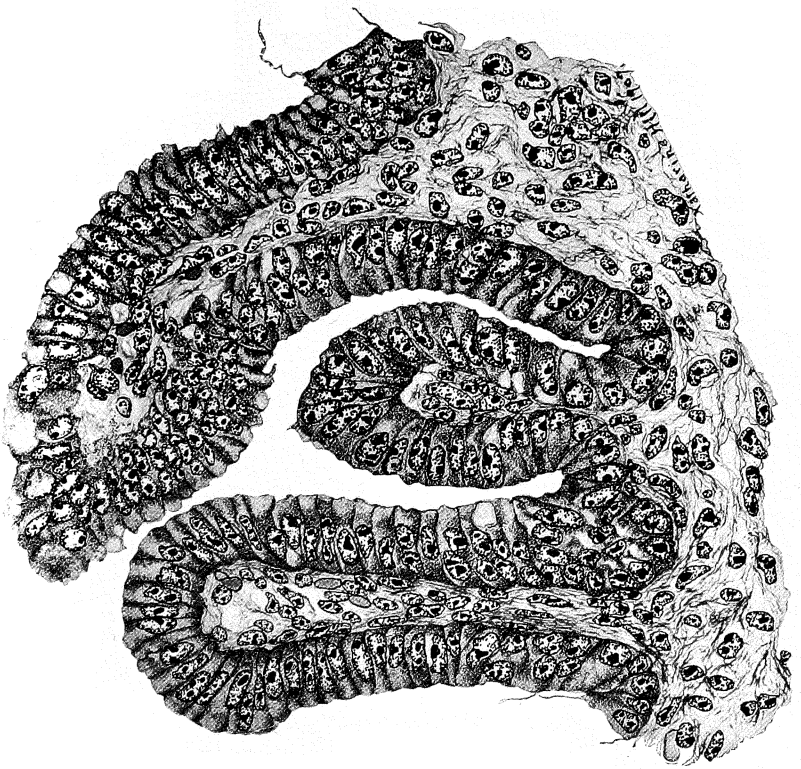


FIG. 21. Pylorus. 19 cm. Three color.

In cross sections of tubules at 19 cm. most of the parietals are shown to be in line with the other cells (fig. 23) but here and there a few are assuming the true parietal position. At all stages of this process, the ductules preserve their direct continuity with the gland lumen. The parietals here, as in the cardia, are, by the 24 cm. stage, much larger than the chief cells and of a rounded or ovoid form.

At 19–20 cm., by the progressive upgrowth of the gland process the foveola (part above the region of parietals) has lengthened very perceptibly, so that now it constitutes about one-half the total tubule length (fig. 19). The deep goblets of the foveola (a, fig. 19 are replaced below, in the cervical region, with mucous chief cells (b), with which parietals (c) are interspersed. The lower third is lined by serous chief cells, adelomorphs and a few parietals. The adelomorphs and serous chief cells are not distinguishable in the three color stain of fig. 19. Both are in frequent mitoses.

After 19 cm. by elongation of the tubule part (cervix and fundus segments) the proportions of tubule length and foveolar length are rapidly altered so that at 25 cm. the foveola : tubule : 1: 2. This proportion is preserved up to the time of birth.

4. *Cardia and caecum*

At 7–9 cm. the cardiac glands are mostly either in the intra-epithelial stage or in stages between those of figs. 3 and 6. Almost all, except the very youngest, have one or two parietals. The cardiac glands have now extended to the summit of the caecal swelling, being here of the intra-epithelial type. At 7 cm. all the cells are either adelomorphs or parietals. The later are at first, as in the fundus, confined to the lower part of the tubule (fig. 11) but at 10–11 cm. appear higher up, as in the fundic tubules of 7–8 cm. Lateral parietal rows soon become very common (13 cm.)

At 11–12 cm. and thereafter, differentiation of the surface cells into mucous cells occurs. Some acquire a mere distal rim of mucus, some a deep goblet. At 13–14 cm. the mucous change

extends into some of the tubules, sometimes to the very bottom. These deep mucous cells are then of the mucous chief type, the whole cytoplasm distal to the nucleus being infiltrated by the stainable substance. Thus, mucous differentiation occurs much later than in the pylorus, and somewhat earlier than in the fundus. However, the process lacks uniformity, for, as late as 14–15 cm. the cardia of some embryos exhibits mucous cells only on the surface, and a short distance into the tubule, with only an occasional one in the deeper parts of tubules.

The parietals, are, of course, unstained in muchematin, but are, as always, readily distinguishable (as, indeed, even in unstained specimens, fixed in Bensley's fluid) by high refractility, absolute opacity, and glistening shiny appearance. Moreover the ductules are very apparent. The adelomorphs are clear and transparent, though very finely granular.

At 17–18 cm. all the cardic cells are either mucous or parietal. The cells of the surface, for a little way into the tubule, possess shallow to deep goblets. The deep cells, aside from parietals are all of the mucuos chief type. Fig. 14 illustrates the typical surface goblet cell (a) and the shallow goblets (b), which go over by gradual transitions into the deeper mucous chief cells (c). In some tubules the goblet cells extend quite to the bottom but it should be noted that, in embryonic stages, the distinction between mucous chief and goblet cells is often not so sharp as in adult life, for, in the surface and upper tubule cells, the mucus often seems not to be homogeneous, but separated into minute goblets by a cytoplasmic mesh. Karyokineses are frequent in the mucous chief and goblet cells. Even the deep surface goblet cells are found in mitosis frequently.

From 14 cm. on, a marked form-divergence is added to the difference in size between cardiac and fundic tubules, the former now becoming shallower (wider relative to the depth), while the fundic tubules preserve their accustomed narrower, deeper form, so that, after 14 cm. it is easily possible to distinguish the two under low power (compare figs. 16, cardia, with 15 and 22, fundus). The cardiac epithelium has, from now on, a curious tendency to shrink away from the underlying mucosa, surface

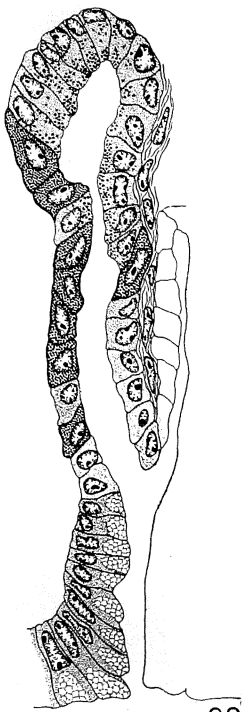
epithelium and tubules cohering so that the whole epithelial area loosens en masse. This tendency is observable from 14 to about 20 cm. in almost all stomachs, and is doubtless referable to the shallowness of the pits, taken in conjunction of course, with the fixation shrinkage.

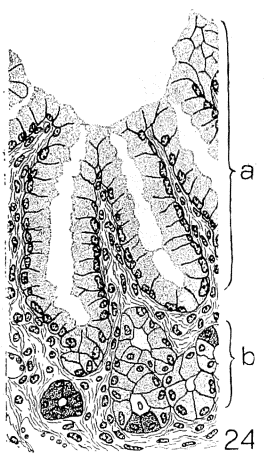
The upper $\frac{1}{3}$ or $\frac{1}{4}$ of the primary cardiac tubule, devoid of parietals and lined by goblet cells, represents the adult foveola, for the lineage may be traced, without any break of continuity, to the 29 cm. stage, long before which all the definitive parts of the tubule are readily distinguished. The lower $\frac{2}{3}$ or $\frac{3}{4}$, lined by groups of parietals, interspersed with a large number of mucous cells, gives rise later (20–22 cm.) to the tubules proper.

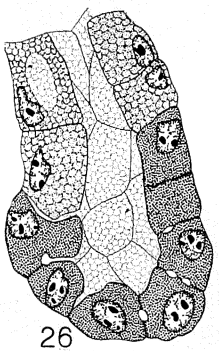
At 15 cm. certain of the groups of parietals begin to push outward slightly (fig. 16). These invaginations, at first shallow, may occur wherever groups of parietals occur,—namely, in the depths of the tubule, and in all but the upper quarter. At all stages they consist entirely of parietals. They appear with increasing frequency in the later stages, and become definite, secondary tubules (figs. 17 and 18) by 18–19 cm. Those from the sides of the primary tubule are directed outward and downward; those from the bottom, straight downward. These secondary tubules are narrower than the primary ones, and at 19 cm. vary from the merest incipient outpouchings to complete, though short, tubules. No similar process occurs in the fundus or pylorus at any stage.[12]

About 20 cm., the compounding of the lower part of the primary tubule occurs in the usual way, so that, at 22 cm., several tubules open into a foveola. Some of these tubules are purely of parietal cells; these were partially derived from the parietal evaginations. Some are purely of mucus cells. Many display both types, and in all proportions. These three types of cardiac tubules persist up to the time of birth (29 cm). I do not believe that the formation of these parietal tubules differs in essential mechanism from the or-

[12] This unexpected finding agrees absolutely with Toldt's observation in the cat, that some tubules arise as lateral buds, which grow out and downward. They arise at a parietal cell, and the latter by division goes over into the new tubule.







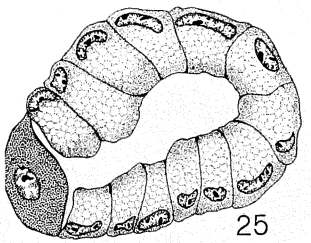


FIG. 22. Complete tubule of fundic region. 23 cm. Neutral gentian. Cyto-differentiation is practically complete. (29 cm is about the birth-length).

FIG. 23. Cross section of fundic tubule. 23 cm. Three color.

FIG. 24. Cardia. 25 cm. *a.* Foveolae. Mucous chief and goblet cells. *b.* Tubules. Mucous chief and parietal cells.

FIGS. 25 and 26. Cross and longitudinal sections taken at the bottom of a cardiac tubule. 29 cm. (just before birth). This shows the parietals, which later, by the third week of post-uterine life, disappear or become converted into mucous cells.

dinary process of compounding, found in fundus, pylorus and also in the cardia. In each type, the epithelium seems to display the initial irregularity, but soon mesoblastic growth seems to become the active factor. I wish to emphasize the point that there are never, at this time, any transitions between the parietals and the mucous cells, each of which are, in the cardiac tubules, sharply distinguishable by the criteria mentioned above.

While the lower part of the tubule lined by mucous chief and parietals, is being compounded, a very rapid growth of the gland processes elongates greatly the wide, upper part lined by goblet *i.e.*, the foveola (19–25 cm.) Thus at 18 cm. (fig. 16) the foveola constitutes only the upper third or fourth. At 22.5 cm. the measurements are, foveola 48μ, tubule 64μ, total 112μ or foveolar length to tubule length as 3 to 4. At 25 cm. the foveolar length is to the tubular length as 5 to 3, and the total length is 140μ, so that the tubule portion has scarcely lengthened since 22 cm. Thus the definitive form and proportions are practically reached at 25 cm. (fig 24) and from now on, foveola and tubules grow with about equal rapidity.

As in all earlier stages, there is, at 25 to 29 cm., a gradual transition from undoubted cardiac to undoubted fundic tubules, the foveolae, in approaching the fundic region, becoming relatively and absolutely shorter, but the tubules so much longer that the whole gland length is greater.

At 29 cm. the cardic tubule measures 160μ in length, the undoubted fundic 210μ, and the pyloric 240μ.

In stages 26–29 cm. the parietals are just as numerous and just as well defined (figs. 25 and 26) as ever, staining with normal brilliancy, and possessing ductules and all the characteristic structures. But the cardiac glands of the adult pig contain no parietals, only mucous chief and thecal cells. Thus the parietals must either degenerate, or become transformed into mucous cells, during the intervening period. I have been unable to obtain stomachs of young pigs. I suspect, from the results of Cade and Harvey ('07) that the parietals may transform into mucus cells. I have to report on this in a subsequent paper.

One more point in the cyto differentiation of the cardia should

be mentioned. The neutral gentian stain was used on every stage of cardiac development. This was done purely as a matter of routine, as I never anticipated finding anything in the cardiac tubules staining specifically with it, except the copper red granules of the parietals. But at 25 cm. and thereabouts granules resembling zymogenic granules appear in the deeper cells of undoubted cardiac tubules. Adjacent sections stained with muchematin show these same cells to be mucous chief cells of the ordinary type, so that the granules lie within the mucous parts of the cell. These granules are found intracellularly for one or two (cm.) stages, and after that disappear. I did not find them in the extreme left cardiac region.

At birth, the cytodifferentiation of the cardia is incomplete, or, rather, that involution required to bring about the adult cytologic status, with its single type of gland cell, has not yet begun.[13]

Caecum. At 10 cm. the glands are rather sparsely distributed. Many are as deep as the pyloric tubules, but contain few or many parietals. By 15 cm. the glands are almost as thickly distributed as in cardia or fundus, the parietals are abundant, and the tubules have the general characters of those of cardia and fundus, approaching more the deeper form of the fundic tubules. In later stages this region undergoes the same change as the general cardia.

5. *Pars oesophagea*

The greater part of this region gradually becomes stratified during the 6 to 10 cm. stages, the cells still remaining clear and transparent, except those of the deepest layer, which have acquired a finely granular cytoplasm of the ordinary type. The clear cells of the superficial strata have already begun to flatten out. At the periphery of the stratified area, the columnar cells constituting the deepest layer, are continuous with the simple columnar epithelium of the surrounding zone. We have seen that at 4–6 cm. a narrow, peripheral zone of this region has developed gland

[13] Negrini, 1886, noted that parietals are present in the foetal, but absent in the adult cardia; also Bensley, 1903.

tubules. These glands display the same form-development as the others. They are more precocious than the cardiac tubules, and deeper progressively, keeping pace with the pyloric tubules in development. At 11 cm. they still consist of clear, transparent cells. At this stage, then, we find the major and central portion of the pars oesophagea to be of stratified squamous epithelium; outside this is a zone of simple columnar epithelium which displays the glands at the very periphery. At 11 cm. the boundary between the clear cells of pars oesophagea and granular cells of adjacent cardia, fundus and pylorus is sharp to a cell. I have not been able to settle definitely the fate of this glandular zone in later stages. It seems, by differentiation of the cells, to merge with the adjacent cardia.

6. *Conclusions and theoretical deductions*

a. Homologies and phylogeny of the cardia

We have seen that fundic and cardiac glands display an absolutely parallel development in the earlier stages, the cardia, however, lagging behind, from the first. In each the parietals appear at a corresponding stage. It is impossible to distinguish an isolated 8 cm. fundic from a 12–13 cm. cardiac gland. After 13–15 cm. however, development in the cardia is retarded; while the fundic tubules elongate rapidly and become narrow, the cardiac tubules lengthen but slowly, and assume a shallow contour.

At the same time a significant parallelism appears in the cyto-differentiation. The foveolar region of each, with goblet cells, appear to be, at all stages, homologous. The lower part of the 16–17 cm. cardiac tubules corresponds, cytologically, to the neck region of the young fundus tubule, each being composed of mucous chief and parietal cells. The length of the whole cardiac tubule at this stage, is about that of foveola plus the neck of the fundus gland.

In the fundic gland of 20 cm. the body and neck segements become compound. In the cardiac gland of 25 cm. the body segment is evidently absent, barring the very temporary appearance

of a few zymogenic cells in the deepest part, but the neck segment, like the corresponding part of the fundic gland, has become split up into several tubules, which display the two cells characteristic of the neck of the fundic gland. While the aberrant development of zymogen granules is confirmatory, I lay most emphasis on the early parallelism in form-development, and especially on the presence, distribution and remarkable persistence of parietals in the cardiac tubules, as demonstrating their affinity with the fundic tubules.

By all these findings we are inevitably brought to one conclusion with reference to the genetic relation of cardia and fundus. *The cardia represents a part of the fundus which has undergone partial involution.* The regressive changes have manifested themselves first in the general chronologic retardation of ontogenetic development in the left part of the old cardio-fundic region; second, in the phylogenetic disappearance of the fundic segment of the tubule with its zymogenic cells,—the curious, delayed appearance of zymogenic granules at one stage of the developing cardia representing, probably, the last vestige of this segment; third, in the ontogenetic transformation of the cervix of the old fundus gland, with its mucous and parietal cells, into the tubule of the adult cardia with its mucous chief cell. This last process I have not seen, but it must needs occur, for we have the first and last terms of the series before us.

The zone of fundic tubules, lying between undoubted cardia and the ordinary fundic zone, represents an intermediate stage in the regressive process.

The facts of development, then, show that the cardiac glands are not primitive, but regressive structures. For the early stages do not resemble, in any way, the condition found in lower vertebrates, where, for example, even the fundus glands possess no definitely identified parietals. Moreover, no fact in the developmental history so much as suggests a possible derivation from the oesophagus.

Thus, our embryological findings seem to confirm strongly the conclusion as to the origin of the cardiac glands reached by Bensley ('02), working from the standpoint of adult comparative

anatomy and histology. It has been pointed out that general, mucous differentiation occurs slightly earlier in the cardia than in the fundus, although it is less uniform. The cardiac glands, in their whole development, display a very decided mucous character, as manifested in the discarding of the deep, zymogenic segment of the fundic tubule, in the involution of parietal cell, and in the slight acceleration of the mucous differentiation. This must be a response to a functional demand. Bensley has already suggested ('02 p. 147) that in these retrogressive glands, the cellular types disappear in the order of their specialization,—zymogenics first, parietals next. We find that not only is there no tendency on the part of the mucous cells to disappear, but that they even differentiate at a slightly earlier stage. Probably the altered food, or the other conditions which have brought about the involution of fundic to cardiac glands, have also demanded an increased mucus secretion.

It will be recalled that, while part of the caecal glands were as retarded as those of the cardia proper, others kept pace with the pyloric tubules, both types, however, developing parietals. Later, all the caecal glands, whether precocious or retarded, shared the fate of the cardiac tubules. This would seem to indicate that seclusion in the secondary pouch has tended to partially protect these glands from the regressive changes which have attacked the old left fundic area.

Bensley's comparative work on the mammalian stomach (Op. cit. '02) led him to the same conclusion,

b. Cell specificity

At 2 cm. all the cells are of the type described as "embryonic gland cells." From 3 cm. on, some of these are constantly differentiating into parietals, others retaining their undifferentiated character for a shorter or longer period. Both types multiply mitotically.

From 6 cm. on, certain of the adelomorphs differentiate into mucous cells, both mucous chief and goblet. New parietals and new mucous cells arise, differentiating from adelomorphs. The

residual adelomorphs of the deepest part of the fundic tubules differentiate at 19–20 cm. into zymogenic or serous chief cells, and then multiply mitotically.

Thus the parietals are the first of the adult cell types to appear. Since the completion of this work, Dr. Bensley has pointed out that we have here a very remarkable and extreme instance of the throwing back of a coenogenetic character (parietals are not definitely identifiable in lower vertebrates) into the earliest ontogenetic stage,—a process called by Cope and Hyatt "acceleration."

Phylogenetically, zymogen cells (*vide* Amphibia), and especially mucous cells, are much more primitive, yet they here appear in ontogeny later than the parietals. Dr. Bensley has suggested that the early appearance of the intracellular ductules perhaps points to a functional explanation of this otherwise puzzling ontogenetic anticipation.

Taken per se, the facts of cytodifferentiation, as recounted above, seem to throw no definite light on the problem of cell specificity. But studied in conjunction with Harvey's findings ('07) they point, I believe, very definitely to certain conclusions.

The cytodifferentiation proceeds, as if the cells all started with the same potentialities or character complexes. These might be represented by *a*, *b*, and *c*. In some cells those metabolic character complexes represented by *a* become, as development proceeds, dominant, while *b* and *c* are dormant. In other cells, character complex *b* may dominate, and so on. But the dormant character may, under experimentally (Harvey) or pathologically (Cade, etc., cited by Harvey) altered conditions, become the dominant ones.

The results of the present work seem to indicate that this latter process does not occur in the normal course of histogenesis. The embryonic cells differentiate directly into the definitive types which then give rise, by division, to cell generations of the same specialized type. Thus, if the zymogenic characters have become dominant in a given cell, during the cytodifferentiation, then the off-spring of this cell are all zymogenic, but, nevertheless, the dormant parietal and mucus characters are transmitted to

each new generation and are available, if altered conditions make it desirable or imperative that they should become dominant. This view of the morphologic flexibility of "specialized" cells, under changed conditions will be recognized as O. Hertwig's doctrine of the nature of cell specialization, under a slightly different guise. But this conception is also expressable in terms of Weismann's Keim-plasma theory, provided sufficient emphasis be laid on the accessory Keim-plasms.

It seems probable that the mucigenic character of the large proportion of cardiac mucus cells,—namely, those derived from the mucus cells of the foveolar and cervical segments of the old left fundus glands, is palingenetic. On the other hand, it is probable that some of the deeply situated mucous cells are coenogenetically mucous, having passed, in race history, through the zymogenic or parietal stages.

The temporary occurrence of zymogen in the deeper mucous cells seems to indicate this, and to suggest that here the reserve zymogenic characters have not yielded, without a struggle, to the mucigenic, the old dominance of the zymogenic having become through the habit of ages, too strongly impressed on the cell metabolism to be rapidly effaced by the coenogenetic dominance of the mucous characters.

Bensley ('00) found, in the developing of gastric glands of Amblystoma, cells which contained for a brief period, both zymogenic and mucigenic granules. Thus it seems that sometimes, in the embryonic and possibly in the adult stages,—the cell metabolism may be controlled by two distinct sets of determinants,—through the manifestation of either of which we are ordinarily inclined to consider a cell as "specialized." Such instances seem to be uncommon, and it is probable that sooner or later,—one metabolic complex takes a subordinate place,—at least in the metazoa.

The fate of the cardiac parietals, after birth, promises to be of the greatest interest in this connection, as there are only two possibilities open to these cells —degeneration, or conversion into mucous cells.

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