A HISTOCHEMICAL STUDY OF THE STOMACH AND INTESTINE OF THE CHICKEN

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INTRODUCTION

The literature concerning the microscopic anatomy of the digestive tract of the domestic fowl is voluminous, and a comprehensive review of this work with numerous excellent photomicrographs has been published recently (Calhoun, 1954). Several features, however, require further investigation. Although it has been shown that the contents of both proventriculus and gizzard are acid (Browne, 1922; Vonk, Brink & Postina, 1949), the location of acid-producing cells has not been conclusively determined. Browne (1922) states that the submucosal glands of the proventriculus secrete acid gastric juice but offers no evidence in support of this statement, whilst Bradley & Grahame (1950), on the contrary, state that there are no parietal (oxyntic) cells in the proventriculus. Batt (1924), although claiming that the submucosal gland cells do resemble the acid-producing cells of the mammalian stomach, does not detail points of resemblance and in any event appears to think that the resemblance is slight.

The information relating to pepsin-producing cells is even less specific than that relating to acid production, although Vonk *et al.* (1949) have demonstrated peptic activity in gizzard contents.

Few attempts have been made to characterize chemically the cuticular layer of the gizzard, although the majority of authors refer to it as 'horny' and appear to consider that it is a keratinous substance. Bradley & Grahame (1950), on the other hand, consider this material to be keratohyalin, but do not indicate on which reactions this claim is based. Calhoun (1954) also considers the horny material to be keratohyalin, basing this claim on the results of a single reaction. This horny material is produced by gland cells which appear to be epicrine in their mode of secretion and therefore its formation is quite distinct from that of other keratinous substances. In view of this and of the inconclusive nature of the information relating to the composition of this substance, further study is justified.

The morphology and distribution of argentaffin cells do not appear to have been studied extensively in the chicken. Dawson & Moyer (1948) found numerous argentophile cells in the proventriculus and gizzard, but were unable to demonstrate the presence of argentaffin cells in these organs; whilst Simard & van Campenhout (1932), in their study of the embryonic development of argentaffin cells in the chicken, found these cells in only one specimen of proventriculus and apparently found none in the gizzard. The comparative rarity of argentaffin cells in the stomach may be related to the structural peculiarities of this organ in the chicken and may prove helpful in elucidating problems associated with the function of argentaffin cells in general.

Simard & van Campenhout (1932) have described the development and distribution of argentaffin cells in the chicken and have suggested that at one stage in development (269 hr.) argentaffin cells migrate from the epithelium into the lamina propria. This migration does not appear to have been noted in other species. Schofield (1950, 1952, 1953) found that in the guinea-pig, mouse and man the argentaffin cells of the small and of the large intestine are functionally and morphologically distinct, those of the small intestine alone being involved in a secretory cycle culminating in the formation of a mucous goblet cell. The possibility that argentaffin cells at different gut levels may subserve different functions is obviously of great interest and demands further investigation, particularly from a comparative point of view. This author also states that the number of silver 'staining' granules in the intestinal argentaffin cells is greatest where the staining method used involves the application of an extraneous reducing agent, i.e. only some of the granules are argentaffin whilst some are argentophile. This lends support to the view expressed by Dawson & Moyer (1948) that argentaffin cells are at first argentophile, and is of particular interest in view of possible differences in function of argentaffin and argentophile cells.

MATERIALS AND METHODS

The specimens described hereafter were obtained from nine White Leghorns ranging in age from 6 weeks to 2 years, distributed as follows: 2 ♂ at 6 weeks, 1 ♀ at 10 weeks, 3 ♂ at 5 months, 3 ♀ at 18–24 months.

In each case tissue was taken from proventriculus, gizzard, gizzard-duodenal junction, caecum and large intestine. In some cases tissue was taken also from proventriculus-gizzard junction, the terminal part of the small intestine, and from the coprodaeum.

Tissue from each locality was fixed in buffered neutral formol and in Orth's fluid. Sections from all blocks were routinely stained with haematoxylin and eosin, trichrome stain (Gomori, 1950*a*), aldehyde fuchsin (Gomori, 1950*b*), mucicarmine, methylene blue, celestin blue, PAS (Gomori, 1953). In addition in appropriate instances the following methods were employed: 1 % thionin (pH 4–5—Lillie, 1954); azure A–eosin B (pH 3·75–5—Lillie, 1954); eosinophil myelin (Lillie, 1954); picrocarmine; picric acid-nigrosin; ferric ferricyanide reduction for SH groups (Lillie, 1954); azure A–eosin B following oxidation by peracetic acid (Lillie, 1954); Best's carmine; Sudan black B; Gomori's methenamine-silver (alone and combined with mucicarmine); Bodian's protargol method (original method and as modified by Dawson & Barnett, 1944); azo-coupling and indophenol reactions (Gomori, 1953).

RESULTS

Proventriculus

The mucosa of the proventriculus contains simple tubular glands lined throughout their length by a columnar epithelium the cells of which diminish in height towards the blind end of the tubule. Most of the cells of this epithelium contain granules of mucin, but these are much less numerous in the cells lining the blind end of the tubule where in fact they are sometimes entirely lacking; towards the open end of

the tubule they may again diminish and are sometimes absent from the lining epithelium of the proventriculus.

The submucosal glands of the proventriculus are arranged in lobules, each consisting of numerous uncoiled tubules radiating from a central cavity into which they discharge. Except near their open ends these tubules are lined by a simple epithelium consisting of cells which only make contact with adjacent cells towards their bases and consequently the epithelium has a dentate appearance (Pl. 1, fig. 1). Towards the open end of the tubule, however, the epithelium is composed of tall, columnar cells which are closely applied to one another and therefore do not exhibit this dentate appearance. A similar epithelium lines the central cavity. The latter is drained by a duct with an irregular lumen with tubular evaginations extending into the adjacent tissue. This duct leads through the mucosa to the free surface.

The epithelial cells lining the main excretory duct and the central cavity exhibit staining reactions identical with those characteristic of the epithelium of the mucosal glands, but as the distance from the mucosa increases, the number of secretory granules in the cells diminishes and they may be completely absent from the excretory ducts of individual gland tubules.

In sections stained with Gomori's trichrome, however, red granules are found scattered throughout the cytoplasm of the secretory cells of the submucosal glands, often appearing in similar concentration in both the basal and luminal cytoplasm, although when particularly abundant they tend to accumulate in the luminal cytoplasm (Pl. 1, fig. 2). The granules are large, rounded and are reddish in colour, though variations in staining reaction are noted in different cells, some showing a particularly intense red. Cells showing this intense reaction may form the entire lining of a particular gland tubule or they may be scattered singly or in small groups amongst less intensely stained cells. Fixation in buffered neutral formalin does not affect the degree of granulation which is then just as distinct as in preparations fixed in Orth's fluid.

In sections stained by methylene blue or thionin at pH 4–5 these cytoplasmic granules remain unstained and therefore the cells have a vacuolated or foamy appearance. The intervening cytoplasm exhibits varying degrees of basophilia and in the more intensely stained preparations may give the cell a finely granular appearance, although it is reasonably certain that the cells do not contain any granules other than those mentioned above. The reaction to pyronin paralleled that noted in the methylene blue and thionin preparations. In no case, however, was the basophilia or pyronin reaction intense.

In preparations stained with azure A–eosin B the cytoplasm was coloured light blue whilst the granules were either unstained or stained a very faint pink colour. No variations in staining reaction were noted between Orth fixed and formalin fixed preparations or as a result of varying the pH from approximately 3·75 to 5.

Argentaffin cells occur very infrequently in the mucosa and are entirely absent from most sections. They are not present in the submucosal glands.

Gizzard

In the gizzard the glands are simple uncoiled tubules lined throughout the greater part of their length by low cuboidal cells. Towards the open end of the tubule

however the cells become taller and in the upper part of the glands and over the free surface are columnar. The gland lumen is frequently filled with a secretion which accumulates on the mucosal surface of the gizzard to form a thick layer of 'horny' material (Pl. 1, fig. 3).

The columnar cells covering the free surface and lining the upper part of the gland tubule contain granules which stain with mucicarmine, PAS and aldehyde fuchsin and which are metachromatic with celestin blue and methylene blue (Pl. 1, fig. 4). In some instances the secreted material at the mouths of the glands exhibits similar reactions and the secretion which accumulates on the surface of the mucosa occasionally exhibits a horizontal striation, due to layers of material which give the above reactions alternating with layers of non-reactive substance (Pl. 1, fig. 3).

The cuboidal cells which line the greater part of the gland tubule on the other hand do not give the above-mentioned reactions. In preparations stained by the Gomori trichrome technique these cells are found to contain very fine red granules which are concentrated particularly in the luminal cytoplasm. These are not readily distinguished in sections stained with Lillie's eosinophil myelin stain but may sometimes be seen and are then dark blue or brown in colour. They remain uncoloured in sections stained with Delafield's haematoxylin, azure A–eosin B, picrocarmine, Sudan black B, PAS or picric acid–nigrosin, and are not argentophile.

The secreted material within the gland tubules and covering the free surface is also coloured red by the Gomori trichrome technique and a very dark blue or dark brown with Lillie's eosinophil myelin stain, both colours in some instances being present in the same section. This material is PAS positive even after treatment with saliva, but the reaction is most pronounced in the gland tubules, the reaction of the material on the surface, although varying somewhat from section to section, being generally much weaker and occasionally negative except for vertical streaks opposite the mouths of the glands (Pl. 1, fig. 3). A similar, though perhaps slightly less intense reaction, is noted on staining with Schiff without pretreatment with periodic acid. (Sections of mammalian skin stained simultaneously with sections of gizzard give negative results with the PAS technique both with regard to hair cortex and stratum corneum.) The secretion, both on the surface and in the gland tubules, is coloured yellow in sections stained with picric acid–nigrosin or picrocarmine. In sections stained with azure eosin or thionin following oxidation with peracetic acid, the secretion is generally coloured varying shades of blue, the material within the gland lumen generally being a dark blue whilst that on the surface is a light-greenish blue streaked perpendicularly with lines of a darker blue corresponding to the mouths of the glands (Pl. 2, fig. 5). (Both these colours may be observed in mammalian hair cortex at hypodermal levels when stained by this method.) In some instances, however, the secretion in the lumen of some of the glands in a section stains a very faint pinkish tinge. This is not comparable to the red characteristic of stratum granulosum or stratum corneum of mammalian skin, in both of which a much more distinctive red is obtained on staining by this method.

Neither the gland cells nor the secreted material give a positive reaction with the ferric ferricyanide test for sulphydril groups except at the free surface of the secretion mass lying on the mucosa where a line of reactive substance may be noted.

Variable amounts of pyronin-positive material are found in the cuboidal cells lining the gland tubule, this material extending throughout the greater part of the cytoplasm in some cells, whereas in others it is present only in small quantity and sometimes is completely absent. When present, it tends to be concentrated particularly in the basal cytoplasm.

Argentaffin cells appear to be absent from the gizzard.

Gizzard-duodenal junction

Between the gizzard and the duodenum there is a transitional zone measuring approximately 0·5 cm in length. This zone is readily distinguished from the gizzard by the villous character of the mucosa and by some degree of coiling of the glands which are lined by an epithelium which is composed of tall columnar cells and therefore unlike that of the glands of the gizzard proper. Intermingled with the columnar cells are large rounded cells the cytoplasm of which is usually unstained or lightly stained in most preparations.

On the other hand, this transitional zone is readily distinguished from the duodenum by the fact that all the epithelial cells covering the surface of the villi and the free surface of the mucosa are mucus secreting, whereas in the duodenum proper this function is restricted to goblet cells scattered singly amongst columnar cells with a striated border. The latter are absent from the junctional zone. In the duodenum, as in all parts of the intestine, the lower parts of the gland tubules are lined by mucus secreting cells, thus further distinguishing the duodenum from the transitional zone in which a mucus secreting function is largely confined to the superficial cells and to the upper parts of the gland, the number of PAS positive granules diminishing rapidly as the distance from the surface increases.

A further feature by which the transitional zone is distinguished from the duodenum is that argentaffin cells are absent except in its terminal part immediately adjacent to the duodenum where in some specimens argentaffin cells may be found. They are much less numerous in this locality, however, than in the immediately succeeding section or the duodenum where they are more numerous than anywhere else in the gastro-intestinal tract (Pl. 2, fig. 7).

In sections stained by the Gomori trichrome technique, it is possible to distinguish granular and agranular cells in the glandular epithelium. In some cases the granular cells are identical with the large poorly staining cells mentioned above, but this is not constantly the case. The granules are very fine and are not a prominent feature. They remain unstained in sections stained with thionin at pH 4–5 and in azure A–eosin B preparations.

In the transitional zone the 'keratinous' layer which covers the mucosa of the gizzard gives way to a layer of mucus of similar thickness. This stops abruptly at the beginning of the duodenum.

Glands resembling mammalian Brunner's glands are not present in the transitional zone nor in the adjacent part of the duodenum.

Intestine

In both small and large intestine the surface epithelium is simple columnar and consists of goblet cells scattered amongst columnar cells with a striated border

(Pl. 2, fig. 8), the former apparently increasing in number as the gut is traced caudally. The glands are simple tubules slightly coiled and are lined by stratified or pseudo stratified columnar epithelium.

The goblet cells and all the cells lining the blind ends of the glands contain granules of mucin. Brunner's glands are apparently lacking and Paneth cells are not present in either small or large intestine; eosinophil leucocytes also appear to be absent from the intestinal mucosa.

Argentaffin cells are present at all levels of the intestine including the coprodaeum. They are most numerous in the duodenum in a narrow zone at its origin, a feature particularly marked in younger birds in which this zone is very narrow and is succeeded by one in which lymphatic tissue is particularly abundant and gland tubules in consequence are widely separated from one another. In the latter zone the number of argentaffin cells per field is greatly reduced. This reduction, however, is not accounted for entirely by the reduction in the number of gland tubules, there being also a reduction in the number of cells per tubule. In older birds the amount of lymphatic tissue in this area is considerably less. Nevertheless, here also there is a reduction in the number of cells per field although this is not so spectacular as in the younger birds, in which in the abundant zone there may be as many as 26 cells per field, whereas in the adjoining zone the number falls to some 4–6·8 per field. (The term 'field' implies, in this instance, a microscopic field using a $\times 45$ fluorite oil-immersion objective and a $\times 10$ eyepiece.) Thereafter the number of argentaffin cells per field is fairly constant throughout the small intestine. Argentaffin cells are also found in the caecum and large instestine and are more abundant in the latter than in the small intestine other than in the area immediately following the transitional zone.

The argentaffin cells may be located mainly in the blind ends of the glands but sometimes are found to be equally numerous at all mucosal levels, this being particularly a feature of younger birds (Pl. 2, fig. 9). These cells are invariably located in the epithelium and never in the connective tissue and usually are in contact with the basement membrane, although occasionally cells may be found apparently migrating towards the free surface (Pl. 3, fig. 10). Rarely the argentaffin cells are arranged in small groups of two to three contiguous cells.

The number of argentaffin granules per cell varies widely. In a few only a small number of granules are present and are then mainly located in the basal cytoplasm close to the basement membrane (Pl. 3, fig. 11), or very occasionally are arranged as a halo around the nucleus, but generally the granules are much more abundant than this, frequently completely filling the basal cytoplasm, obscuring the nucleus and usually extending to a variable extent into the luminal cytoplasm. In the latter case the cell may be extended towards the lumen of the gland or surface of the intestine in the form of a slender process containing a few discrete granules or, more rarely, as a thicker process filled with densely packed argentaffin material in which the individual granules can rarely be distinguished (Pl. 3, fig. 12). Intermediate forms between these extreme types are also found which are regularly triangular with the base resting on the basement membrane and the apex directed towards the free surface which it may reach. In this type the granules in the luminal cytoplasm are abundant but discrete (Pl. 3, figs. 13, 14). The nucleus is generally

located in the cytoplasm near the basal surface, but in occasional cells it is displaced towards the free surface and in these the extended basal cytoplasm is so deeply stained that individual granules cannot be distinguished (Pl. 3, fig. 14). In occasional cells the basal cytoplasm is extended laterally and these therefore are wider than average at this point. Occasionally this extension appears as a short process running laterally for a short distance between the overlying cells, and the basement membrane. Very rarely the basal cytoplasm is vacuolated (Pl. 3, fig. 13), and even more rarely intracellular fibrils appear to be present. It is possible, however, that the latter appearance is due to linearly disposed granules, the concentration of the latter in cells in which this appearance is noted being too great to permit accurate observation.

Every type of argentaffin cell found in the small intestine is duplicated in the large intestine and the reverse is also true. Furthermore, no differences in cell types or in the number or disposition of argentaffin granules in individual cells are noted between specimens stained by the methenamine silver technique (without subsequent reduction) and those stained by the modified Bodian technique with subsequent reduction.

The granules in chick argentaffin cells are very strongly eosinophilic, stain bright red with the Gomori trichrome stain, give diazo and indophenol reactions and are also positive with the ferric ferricyanide reduction test (Pl. 3, fig. 11). (This response was noted incidentally when examining sections of gizzard-duodenal junction for SH groups at the presumed site of keratin production in the gizzard. In such specimens the argentaffin granules in cells in the duodenum were strongly stained and almost as readily distinguished as in specimens stained by the silver techniques. This has been noted by Laskey & Greco, 1948 (quoted by Lillie, 1954).

In sections stained with Southgate's mucicarmine after hexamine silver the argentaffin cells remain uncoloured. This is true even of those cells in which the basal cytoplasm contains no argentaffin granules.

DISCUSSION

Proventriculus

Batt (1924) has stated that there are 'no marked acidic or pepsin-producing cells' in the submucosal glands of the proventriculus, but also claims that in their staining reactions the cells of these glands 'resemble somewhat the acid-producing or parietal cells of the mammalian stomach'. Browne (1922) states that the submucosal glands secrete acid gastric juice, but does not offer any evidence that the submucosal gland cells are in fact responsible for acid production. Vonk *et al.* (1949) found that the secretion of the proventriculus was acid, but that it was less so than the contents of the gizzard. Bradley & Grahame (1950), on the other hand, state that there are no parietal (oxyntic) cells in the proventriculus.

A reaction similar to that noted in the submucosal gland cells in sections stained by the Gomori trichrome technique is noted also in the parietal cells in sections of mammalian stomach (dog, cat, rat and sheep) stained by this technique. This reaction, however, is not specific for hydrochloric acid secreting cells, and although in the circumstances the response is suggestive, the fact remains that the submucosal

gland cells do not exhibit the degree of eosinophilia noted in mammalian parietal cells. Whether the variations in the intensity of the reaction noted in the trichrome preparations indicate different functional states of the same cell type is uncertain, but this seems likely since similar variations are noted in parietal cells in mammalian stomach stained by this method.

The evidence with regard to the production of pepsin by the submucosal gland cells is more conclusive in that the failure to stain secretion granules with thionin at pH 4–5 strongly suggests that these cells are not zymogenic, and although occasionally a very faint pink colour is noted in the cytoplasmic granules in azure eosin preparations, this is of doubtful significance, particularly as it is not intensified on varying the pH of the staining fluid. Again, the basophilia in the submucosal gland cells is never pronounced, nor do these cells contain significant quantities of pyronin positive material in their basal cytoplasm as might be expected if they were zymogenic. These observations apply equally well to Orth-fixed and formalin-fixed preparations, and therefore it is unlikely that the failure to demonstrate pepsinogen granules is due to imperfect fixation. Furthermore, the cytoplasmic granules noted in trichrome preparations are equally prominent in formalin-fixed and Orth-fixed material which suggests that they are not pepsinogen since, at least in mammals, pepsinogen granules are poorly preserved after formalin fixation but well preserved in Orth-fixed tissue (Lillie, 1954).

Gizzard

Various authors have applied the term 'horny' to the secretion of the glands of the gizzard, but only Calhoun (1954) appears to have attempted to characterize this material on a chemical basis. Bradley & Grahame (1950) state that this substance is keratohyalin, but do not indicate which tests were employed to establish this claim. Calhoun (1954) also considered the horny substance to be keratohyalin and in sections stained by Pasini's method was able to demonstrate secretion granules in the adjacent cells. However, Opdyke (1952) states that the granules of keratohyalin in the stratum granulosum of stratified squamous epithelium stain beautifully with picrocarmine, have an intense affinity for all haematoxylin stains, stain metachromatically with toluidine blue and are argentophile, whereas the granules in the gland cells of the gizzard exhibit none of these reactions. In sections stained with Lillie's eosinophil myelin some staining of cytoplasmic granules does occur but their colour when they responded to this technique is indicative of keratin rather than keratohyalin (Lillie, 1954). Sections stained by this method are, however, difficult to interpret as far as cytoplasmic granules are concerned and this conclusion should be confirmed before it is accepted that the granules are keratinous, particularly as they do not respond to other stains known to stain keratin. Failure to stain with the azure A–eosin B technique indicates that the granules are not trichohyalin.

The secreted material in the gland lumina and on the free surface on the other hand gives many of the reactions characteristic of keratin. Thus it is stained yellow with picric acid–nigrosin and picrocarmine, and in sections stained with azure A–eosin B or thionin after oxidation with peracetic acid it gives reactions indicative of a substance containing cystine. The reaction obtained with the latter technique is similar in most cases to that of the hypodermal portion of mammalian hair root

which exhibits a peripheral dark blue portion and a central greenish blue part. In the gizzard the more darkly stained material is that within the gland lumen and extending as narrow vertical streaks from the mouths of the glands towards the free surface, and therefore the most recently formed secretion in the gizzard gives the most intense reaction whereas in hair the older free portion tends to be more intensely stained than the hypodermal part. The faint pinkish tinge noted occasionally with this technique does not correspond to the colour noted in keratinous structures in the epidermis in which a more decided red was generally characteristic of stratum granulosum (keratohyalin) and stratum corneum, although sometimes the outermost layers of the stratum corneum did give a somewhat similar reaction.

According to Lillie (1954) the keratin of stratum corneum yields a light to dark brown colour when stained by his eosinophil myelin, whilst hair cortex stains varying shades of blue. The bulk of the secreted material in the gizzard thus resembles hair cortex in yielding a dark blue colour when stained by this method. Occasionally, however, patches of brownish material suggestive of stratum corneum are present.

From the foregoing, therefore, it seems reasonable to conclude that the material secreted by the glands of the gizzard is a form of hard keratin and there appears to be little support for Calhoun's (1954) contention that its production is preceded by the formation of granules of keratohyalin. This material most closely resembles the keratin of hair in its staining reactions, but differs in some respects, particularly in its response to PAS or to Schiff without preliminary oxidation by periodic acid. Again, in their study of keratin production in epidermis and its derivatives Giroud & Leblond (1951) demonstrated the constant presence of sulphur as sulphydril groups at the site of keratin production. The absence of such groups in the gizzard indicates that here the method of keratin production is different from that in skin and its derivatives, and this may be related to the fact that in the gizzard the keratinous material is discharged by the cells which are apparently epicrine in type.

The response to the Schiff reagent, even without preliminary oxidation with periodic acid, is not understood, but clearly it is not due to glycogen since it occurs even after treatment with diastase and is negative with Best's carmine. It is interesting, however, that a corresponding reaction is not noted in the gland cells and that it is much reduced in the secretion layer on the surface suggesting the possibility of a progressive chemical change beginning immediately after secretion.

The mucus secreted by the cells lining the gland tubules near the free surface and covering the latter may serve a protective or adhesive purpose. Horizontal streaks of faintly metachromatic material in the overlying keratinous mass suggest that this mucous secretion is gradually incorporated into the latter.

Gizzard–duodenal junction and intestine

Calhoun (1954), in her review of the literature relating to the intestine of the chick, states that 'all authors with the exception of Kaupp (1918) are agreed that Brunner's glands are lacking', although in fact Bradley & Grahame (1950) are at variance with this view. In the specimens examined in this work no glands comparable in any way to mammalian Brunner's glands were found. It is possible, however, that the absence of these glands and the absence of a structure comparable to the mammalian pylorus is compensated from a functional point of view by the abundance

of mucus-secreting cells in the surface epithelium and superficial parts of the glands in the transitional zone interposed between the gizzard and duodenum.

Whether the large granular cells and the small agranular cells in the glands in the transitional zone represent different functional states of the same cell type has not been determined, nor has it been possible to characterize them beyond noting their reaction in preparations stained by the Gomori technique. No zymogenic cells have been demonstrated in this zone.

The goblet cells of the avian intestine have been studied by Cloeta (1893) and Ackert, Edgar & Frink (1939). Zietschmann (1911) described cells with a cuticular border, and Moog (1950) demonstrated alkaline phosphatase in the striated cuticular border in the epithelial cells of the chick duodenum. The striated border of the epithelial cells is comparatively prominent in the chick and the goblet cells, which are scattered singly amongst the cells with striated borders, appear to be most numerous in the colon and least numerous in the caecum. The latter feature may be related to the fluid nature of the contents of these tubes (Browne, 1922). In the blind end of the glands at all intestinal levels all the cells whose cytoplasm reaches the free surface are mucus-secreting.

Looper & Looper (1929) and Calhoun (1954) found numerous eosinophils in the caecum of the chick, but this apparently is not a constant feature. Denke (1954) noted the absence of eosinophils from the intestinal mucosa of the turkey. The statement by Bradley & Grahame (1950) that Paneth cells are present at all levels in the chick has not been confirmed, and it is suggested that the argentaffin cells may have been mistaken for Paneth cells. These are unusually eosinophilic in the chick and readily distinguished even in haematoxylin and eosin preparations. According to Calhoun (1954), Cloetta (1893) doubted the presence of Paneth cells in the intestine of the chick, whilst Greschick (1922) and Clara (1926, 1927) agree with the view expressed by Bradley & Grahame (1950).

Argentaffin cells

The distribution of argentaffin cells in the chick intestine is generally similar to that characteristic of the intestine of some mammals, e.g. mouse (Schofield, 1952), dog, cat, rat (personal observation) in which a high concentration in the upper duodenum is followed by a fall which in turn is succeeded by a rather smaller rise in the colon. Denke (1954) recorded a similar distribution in turkey poults, and although Simard & van Campenhout (1932) reported that 2 days after hatching argentaffin cells were more numerous in the large intestine than in the duodenum it is possible that this apparent contradiction is due to the fact that the zone in the duodenum in which argentaffin cells are particularly abundant is very narrow and may have been overlooked by these authors. In man, however, the distribution of argentaffin cells appears to differ slightly from that noted in the chick, the fall in concentration in the small intestine not being reversed in the colon (Schofield, 1953).

The occurrence of numerous argentaffin cells in the surface epithelium and in the glands towards their open ends, however, is somewhat unusual, these cells in the mammal generally being located mainly in the proximal one-third of the glands. In older birds the distribution may be similar to that noted in the mammal, but the number of birds examined is not sufficient to justify an assumption that dis-

tribution in the chick varies with age, although this is clearly a possibility. It is interesting, however, that one of Simard & van Campenhout's (1932) photographs showing a section of large intestine from an 18-day embryo demonstrates a distribution similar to that noted above in younger birds.

'Migrating' cells apparently moving towards the gland lumen are a feature of other species and do not appear to be particularly frequent in the chick. Simard & van Campenhout (1932), however, observed argentaffin cells in the lamina propria of the embryonic chick intestine and concluded that they had migrated from the epithelium. They were able to demonstrate such migration only at approximately 269 hr. of development, however, and although they are not explicit on this point argentaffin cells do not appear to be demonstrable as such in the lamina propria after this time. Certainly they are not present in birds varying in age from 6 weeks to 18 months, and must therefore either migrate beyond the gut wall or lose their argentaffin granules during development. The intraepithelial location of argentaffin cells in man has been recorded by Schofield (1953).

Schofield (1952, 1953) described morphological differences between the argentaffin cells of the small and large intestines in mouse and man, and advanced the hypothesis that these differences are associated with functional differences. In this respect the chick differs sharply from man and mouse, there being no morphological distinction between argentaffin cells at different intestinal levels in this species, a feature which may be related to the close similarity in structure between these two parts of the intestine.

In his study of argentaffin cells in man Schofield found that the number of reactive cells remained the same whether or not a reducing agent was employed (i.e. the cells are argentaffin rather than argentophile), but that the number of granules in the individual cell was greater when the silver bath was followed by reduction and that only in such circumstances were granules located in the luminal cytoplasm. In the chick also the number of reactive cells is not affected by the use of a reducing agent, but in this species reactive granules are found in the luminal cytoplasm even in methenamine silver preparations. The number of argentaffin granules in this location varies from a very few to such numbers that they completely fill the cell and they may be so closely packed that individual granules cannot be distinguished. It seems unlikely therefore that in the chick the granules pass through a preliminary argentophile phase as suggested by Schofield in man. In this species, therefore, as in man, guinea-pig and mouse (Schofield, 1953, 1950, 1952), there does appear to be a gradual accumulation of granules suggesting the possibility that a secretory cycle is involved, and this is supported to some extent by the fact that occasional cells are found containing only a few granules, distributed throughout the distal cytoplasm from the nucleus to the free border and suggestive of a cell in which a discharge of granules is taking place. There is, however, no direct evidence other than this of exocrine secretion by the argentaffin cells.

Schofield (1950) has described a transformation of the supranuclear granules of the argentaffin cells of the small intestine of the guinea-pig into an argentaffin network containing a mucicarmine-positive substance in its meshes, and he has also described a series of transformations between this cell and the typical goblet cell. This author also found evidence of the transformation of argentaffin cells into

goblet cells in the small intestine of man and mouse (Schofield, 1953, 1952). In the chick the argentaffin cells do not contain mucicarmine-positive material at any stage in development, nor is there any evidence in this species of the reciprocity between the number of argentaffin cells and goblet cells noted by Schofield in man, mouse and guinea-pig. Again in the chick argentaffin cells occasionally occur in small groups of 2–3 cells and are not invariably isolated from one another as in man, mouse and guinea-pig. It is reasonable to suppose, therefore, that in the chick the argentaffin cells are not the source of goblet cells.

Schofield (1953) also described vacuolation of the proximal cytoplasm in argentaffin cells in man and has suggested this may be due to post-mortem change consequent on delay in penetration of the fixative. In the chick intestine, cells showing a slight degree of vacuolation of the proximal cytoplasm were noted occasionally, but were very rare indeed and this may be related to the fact that the maximum delay between death of the bird and fixation was less than 30 min. in every case and generally only a few minutes. Moreover, fixation was carried out in a processor with constant agitation of fixing solution.

Argentaffin cells have rarely been found in the stomach of the chick, but Simard & van Campenhout (1932) did find some in the proventriculus in one case. Dawson & Moyer (1948), on the other hand, found these cells to be constantly absent from the gizzard and proventriculus. It seems unlikely, therefore, that the occasional argentaffin cells found in this situation are functionally significant.

SUMMARY

1. The secreting cells of the submucosal glands of the proventriculus present features which suggest that they are concerned with HCl production.

2. The secretion of the glands of the gizzard gives histochemical reactions which suggest that it is a form of hard keratin. It presents certain features, however, which distinguish it from the other keratinous structures of the body.

3. A transitional zone with distinctive features situated between the gizzard and duodenum has been described. It is suggested that this bears some functional resemblance to the mammalian pylorus.

4. The distribution and morphology of the argentaffin cells in the chick have been described. These cells appear to be morphologically similar in small and large intestine, a feature which may be related to the general similarity between these two parts of the intestine in the chick. There does not appear to be any connexion between the argentaffin cells and the intestinal goblet cells in the chick.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Submucosal glands of proventriculus. Gomori's trichrome. × 120.

Fig. 2. Proventriculus. Secreting cells of a submucosal gland. Gomori's trichrome. × 1100.

Fig. 3. Gizzard. PAS. × 120.

Fig. 4. Gizzard to show reaction of cells lining the gland tubule towards its open end.

PLATE 2

Fig. 5. Gizzard. Azure A–eosin B after oxidation by peracetic acid. × 120.

Fig. 6. Gizzard–duodenal junction. Southgate's mucicarmine. × 120.

Fig. 7. Gizzard–duodenal junction. Argentaffin cells are particularly abundant in the duodenum which lies in the upper half of the photograph. Gomori's methenamine-silver. × 120.

Fig. 8. Duodenum. Southgate's mucicarmine. × 120.

Fig. 9. Small intestine. Numerous argentaffin cells can be distinguished in the surface epithelium. Gomori's methenamine-silver. × 100.

PLATE 3

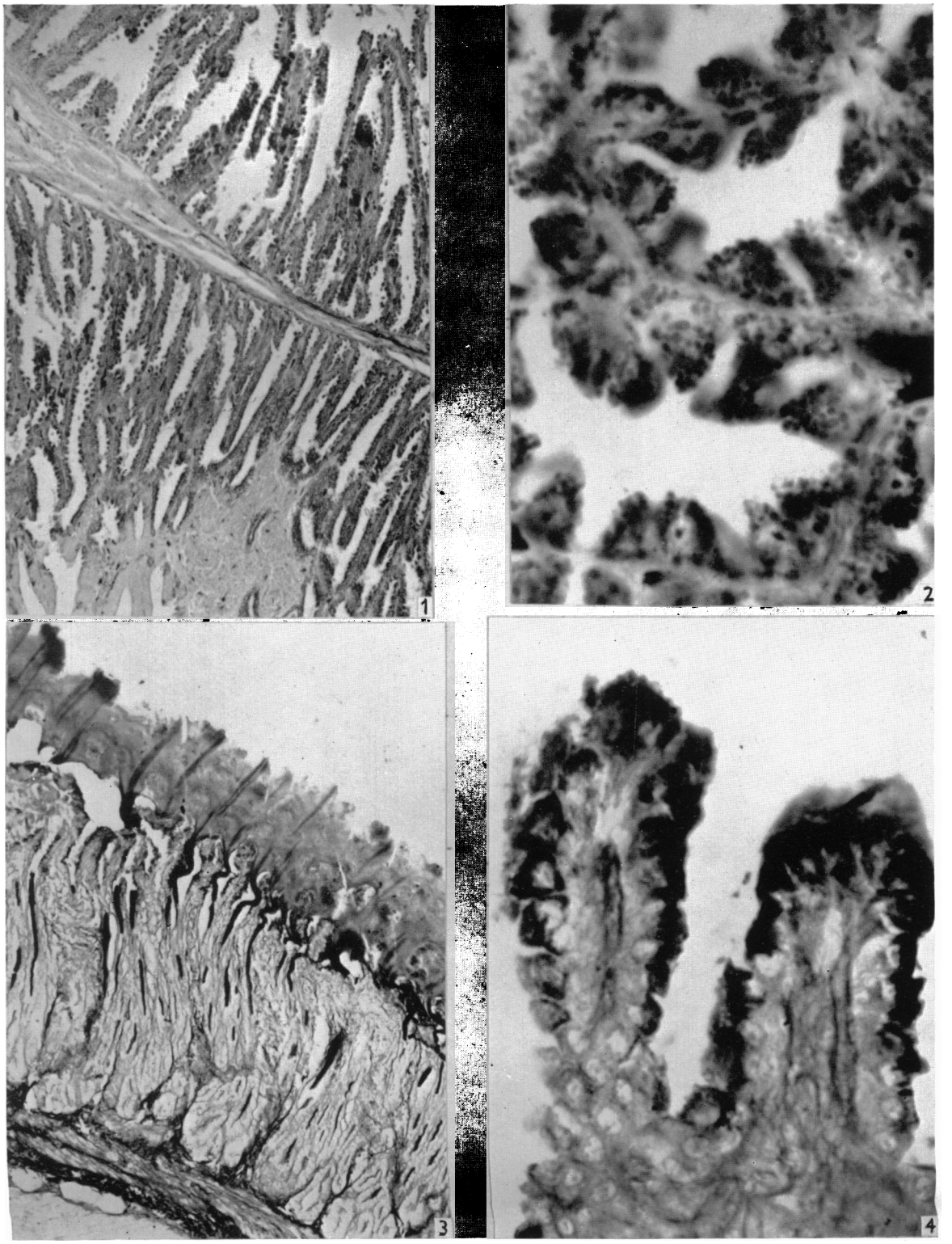
Fig. 10. Small intestine. To show 'migrating' argentaffin cell. Gomori's methenamine-silver. × 1000.

Fig. 11. Small intestine. Ferric ferricyanide reduction. × 1600.

Fig. 12. Large intestine. Gomori's methenamine-silver. × 1600.

Fig. 13. Large intestine. Gomori's methenamine-silver. × 1600.

Fig. 14. Caecum. Gomori's methenamine-silver. × 1600.



AITKEN—HISTOCHEMICAL STUDY OF STOMACH AND INTESTINE OF CHICKEN

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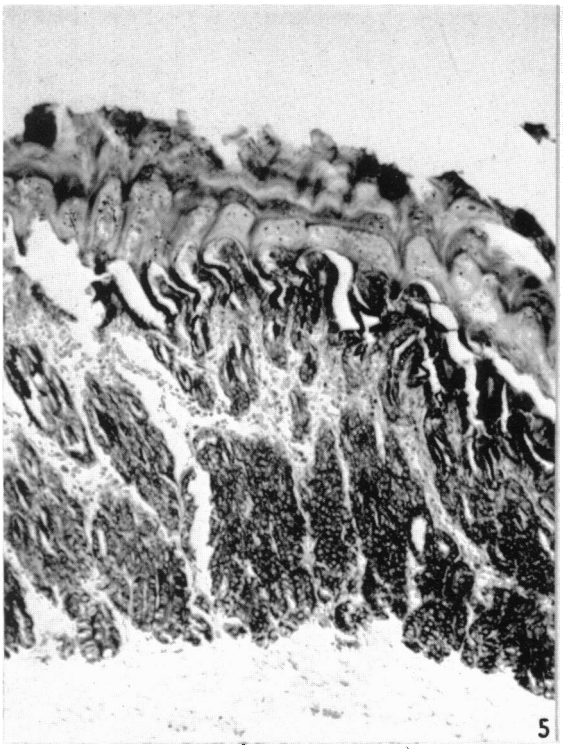
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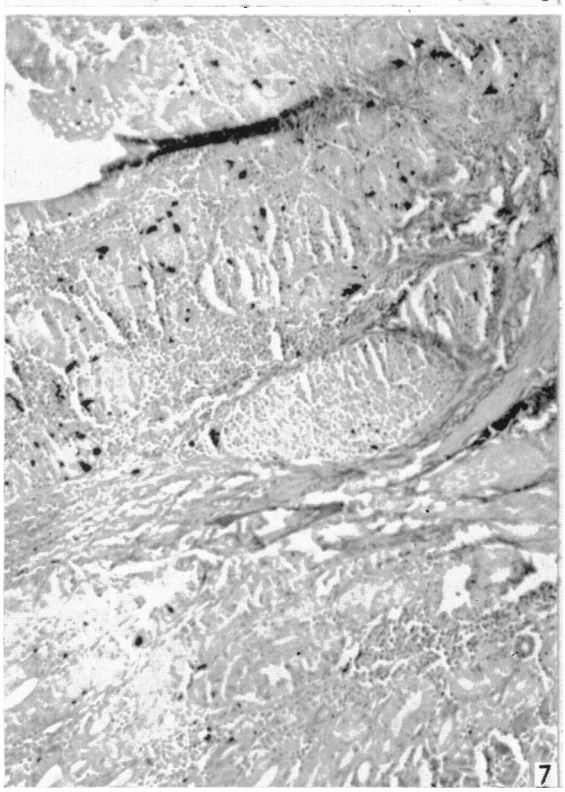
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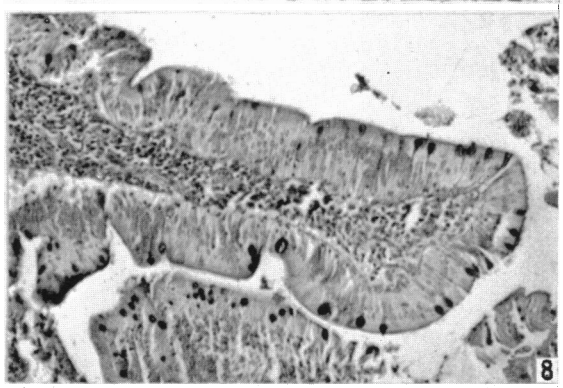
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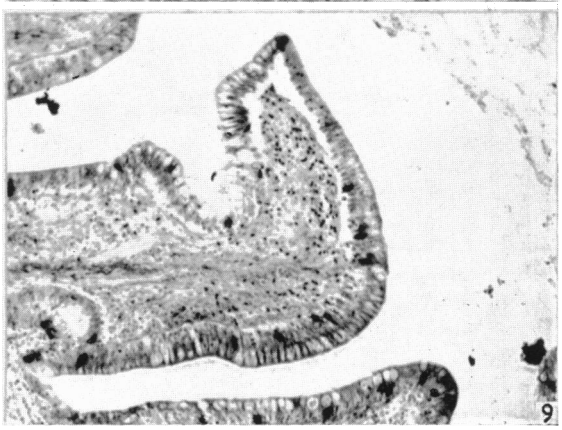
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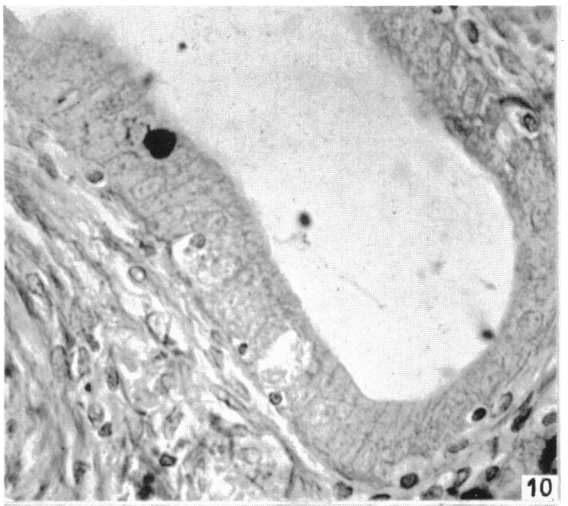
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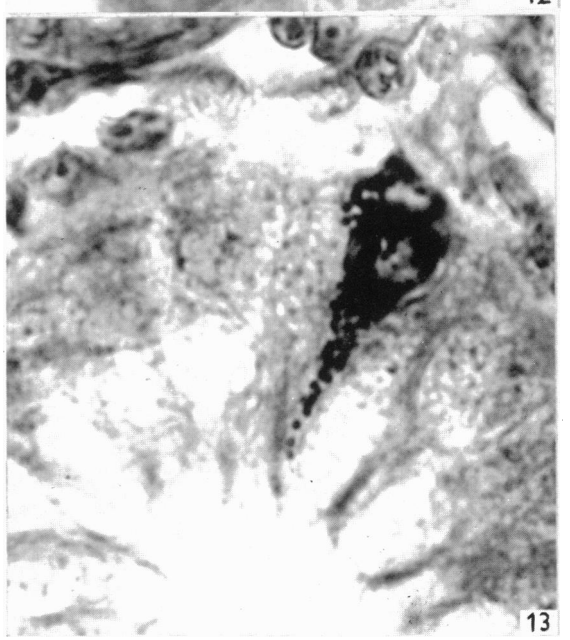
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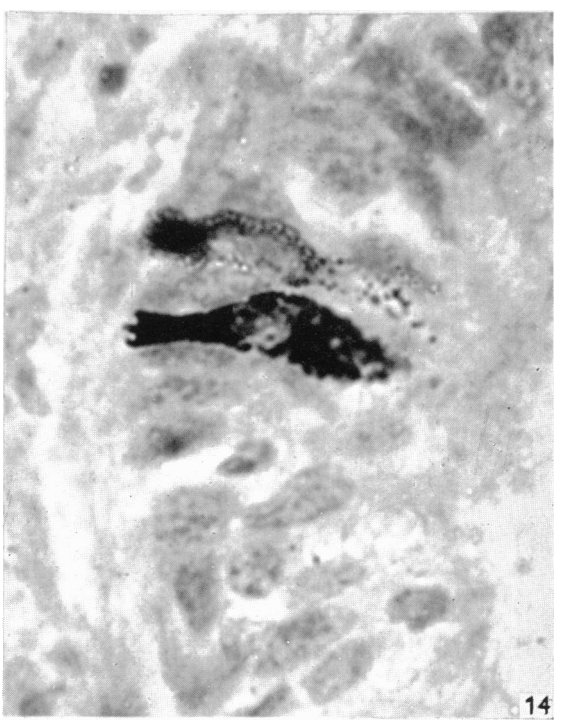
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