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The distribution and ontogeny of gastrin/CCK-, somatostatin- and neurotensin-immunoreactive cells in the gastrointestinal tract of the chicken

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Summary. The distribution and time of appearance of cells with gastrin/CCK-, neurotensin- and somatostatin-like immunoreactivity were studied in samples from eight regions of the gastrointestinal tract of chick embryos from 11 days of incubation to hatching. No immunoreactive cells were found in any region at 11 days of incubation. Somatostatin- and neurotensin-immunoreactive cells appeared for the first time in the proventriculus, pyloric region and duodenum at 12 days of incubation with cells immunoreactive for neurotensin occurring in the rectum at the same stage. Gastrin/CCK-immunoreactive cells were detected in the small intestine first at 14 days and in the pyloric region two days later. Cells immunoreactive for somatostatin and neurotensin appeared in the upper and lower ileum at 14 days of incubation for the first time; neurotensin-immunoreactive cells, present in the caecum at 14 and 16 days, were rare. Cells of all three types were plentiful in the pyloric region by 17½ days of incubation. No immunoreactive cells were detected in the gizzard at any stage studied.

Endocrine cells were present in the relatively undifferentiated surface epithelium which occurs throughout the gastrointestinal tract of chick embryos at 12 days of incubation. Thereafter cells of all three types were detected in the glandular epithelium at or very soon after morphogenesis and differentiation of the latter had occurred.

Key words: Ontogeny, Gut endocrine cells, Gastrointestinal tract

Introduction

Extensive immunocytochemical studies have been carried out on the distribution of gut endocrine cells in a wide variety of invertebrates and of vertebrates, especially mammals, (Rufener et al., 1975; Sundler et al., 1977; Alumets et al., 1977; Helmstaedter et al., 1977; Seino et al., 1979). Studies on avian gut have been conducted at hatching (Rawdon and Andrew, 1981) and thereafter in young birds, (Larsson et al., 1974) and adults (Aitken, 1958; Yamada et al., 1979).

Immunocytochemical studies have also demonstrated immunoreactive cells of various types in early rat and human foetal gut (Larsson et al., 1975; Dubois et al., 1976; Dubois et al., 1976; Larsson et al., 1977; Dupouy et al., 1983; Kataoka et al., 1985). There is, however, much less published work on embryonic avian material; that of Sundler et al. (1977), Alumets et al. (1977) and Salvi and Renda (1986) reports on the distribution and ontogeny of certain gut endocrine cell types in chick embryos at different stages of development, but with somewhat differing results.

The present study was therefore undertaken to determine the distribution and time of first appearance of gut endocrine cells in chick embryos in all regions of the gastrointestinal tract. The cell types reported on here show immunoreactivity for gastrin/CCK, somatostatin and neurotensin; others will follow later.

Materials and methods

Chick embryos (Black Australorp strain), on removal from incubated eggs were staged according to the normal table of Hamburger and Hamilton (1951). Tissues from eight regions of the gut (Fig. 1) at 11-, 12-, 14-, 16-, 17½- and 19 days of incubation (stages 37-45) and at hatching (stage 46) were rapidly dissected out. One mm-wide strips of gut were quenched in melting methyl butane and freeze dried overnight at -40°C and 10^{-3} Torr. All tissues were subsequently fixed in parabenzoquinone vapour (Pearse and Polak, 1975) prepared from recrystallized PBQ (Merck), for 3h at 60°C, infiltrated with Epon-Araldite under vacuum for 3h and embedded in resin which was the cured for 48h at 60° C.

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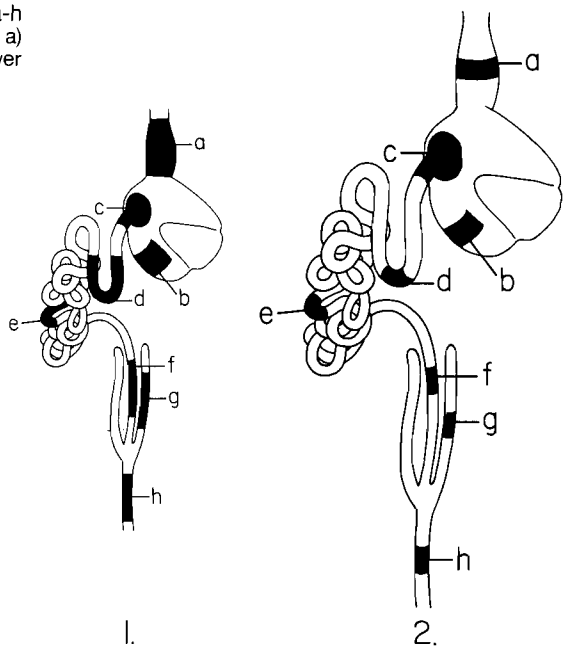
Fig. 1. Gastrointestinal tract of the chick embryo indicating the regions a-h studied 1) 11-14 days of incubation 2) 16 days of incubation-hatching a) Proventriculus b) Gizzard c) Pyloric region d) Duodenum e) Upper ileum f) Lower ileum g) Caecum h) Rectum.



Sets of four consecutive one micron sections were mounted in wells on PTFE-coated slides (Rawdon, 1978). They were deplasticized according to the method of Mayor et al. (1961). The first and third sections of each set were stained with primary antiserum, the second and fourth sections being used for immunocytochemical controls. An indirect immunoperoxidase method (Rawdon and Andrew, 1979) was used to demonstrate sites of antibody-antigen interaction. Some sections were mounted in veronal buffered glycerol and studied by interference microscopy; others were weakly counterstained with Heidenhain's haematoxylin mounted in DPX, and studied by phase contrast microscopy.

Details of the primary antisera used are listed in Table 1. All had been raised in rabbits. Immunocytochemical controls included replacement of the primary antiserum with non-immune rabbit serum, with diluent alone and with primary antiserum pre-incubated with its corresponding antigen for 22h at 4°C at the concentration shown in Table 2.

Table 1. Antisera used.

Antiserum raised to	Code	Specificity	Dilution	Source
cholecystokinin (CCK 8-11)	L48	COOH-terminal	1:8000	*G.J. Dockray, Liverpool
neurotensin (synthetic bovine)	R-94	COOH-terminal	1:100	*P. Emson, Cambridge
somatostatin (synthetic cyclic bovine)	195-8-11-8-76	–	1:8000	*M.P. Dubois, Nouzilly

* I gratefully acknowledge these gifts.

Table 2. Antigens used for absorption purposes

Antigen	Concentration	Source
CCK 39	20μg/ml	* V. Mutt, Stockholm
neurotensin (synthetic bovine)	20μg/ml	Sigma
somatostatin (synthetic toad)	20μg/ml	Sigma

* I gratefully acknowledge this gift.

The specificity of the primary antisera used in this study had previously been demonstrated by Rawdon and Andrew (1981) by absorbing each with a range of structurally related and unrelated gut peptides including those mentioned in Table 2. Antiserum L48 is directed against the COOH-terminal pentapeptide of cholecystokinin (CCK), a sequence common to both gastrin and CCK. Thus this antiserum would not be expected to distinguish between these two peptides. Cells reacting with antiserum L48 are hence collectively referred to as gastrin/CCK cells.

The «high salt» procedure advocated by Grube (1980) to reduce non-specific staining was used with the neurotensin antiserum.

The distribution of a given endocrine cell type in each region was studied in specimens from two to five embryos at each stage.

Results

All immunocytochemical controls gave satisfactory

results (Fig. 2a-c). Although the «high salt» procedure of Grube (1980) did not always reduce background staining by the neurotensin antiserum, neurotensin immunoreactive cells were nevertheless distinguishable.

The relative frequency of the different types of immunoreactive cells in the various regions of the gastrointestinal tract was subjectively graded into different categories (Fig. 3).

No cells immunoreactive to any of the three antisera used were detected at 11 days of incubation, the earliest stage at which cells were sought. The first endocrine cells appeared at 12 days of incubation. They were very sparsely distributed and some contained only a few granules.

Immunoreactive somatostatin and neurotensin cells

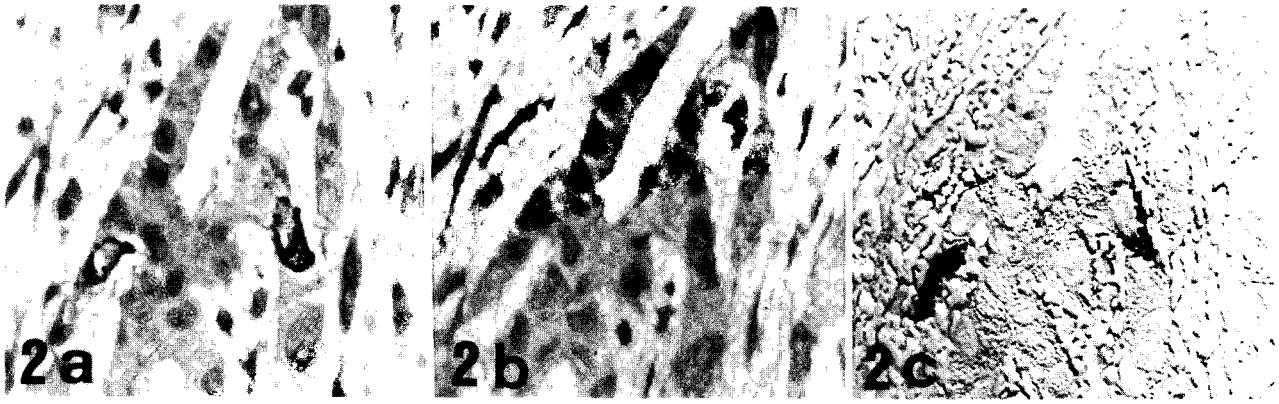


Fig. 2. Consecutive sections of the pyloric region at 17½ days of incubation demonstrating a) immunoreactive somatostatin cells; counterstained c) immunoreactive somatostatin cells; unstained b) absorption control of a and c; counterstained. Benzoquinone vapour fixation. × 900

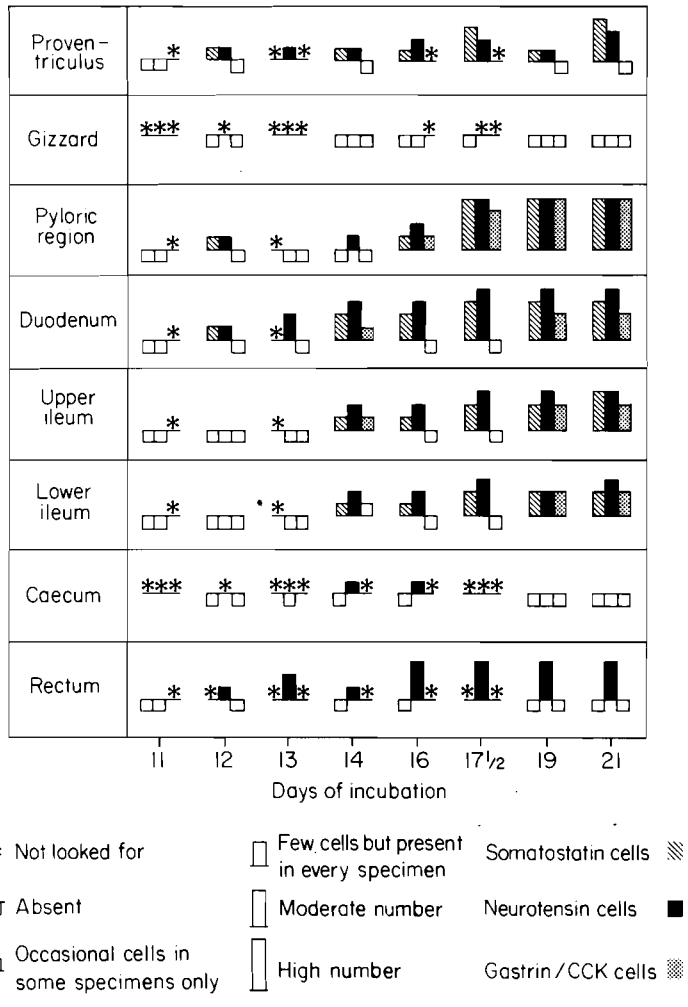


Fig. 3. Block diagram to demonstrate the distribution and frequency of cells showing immunoreactivities for gut peptides in the gastrointestinal tract of chick embryos.

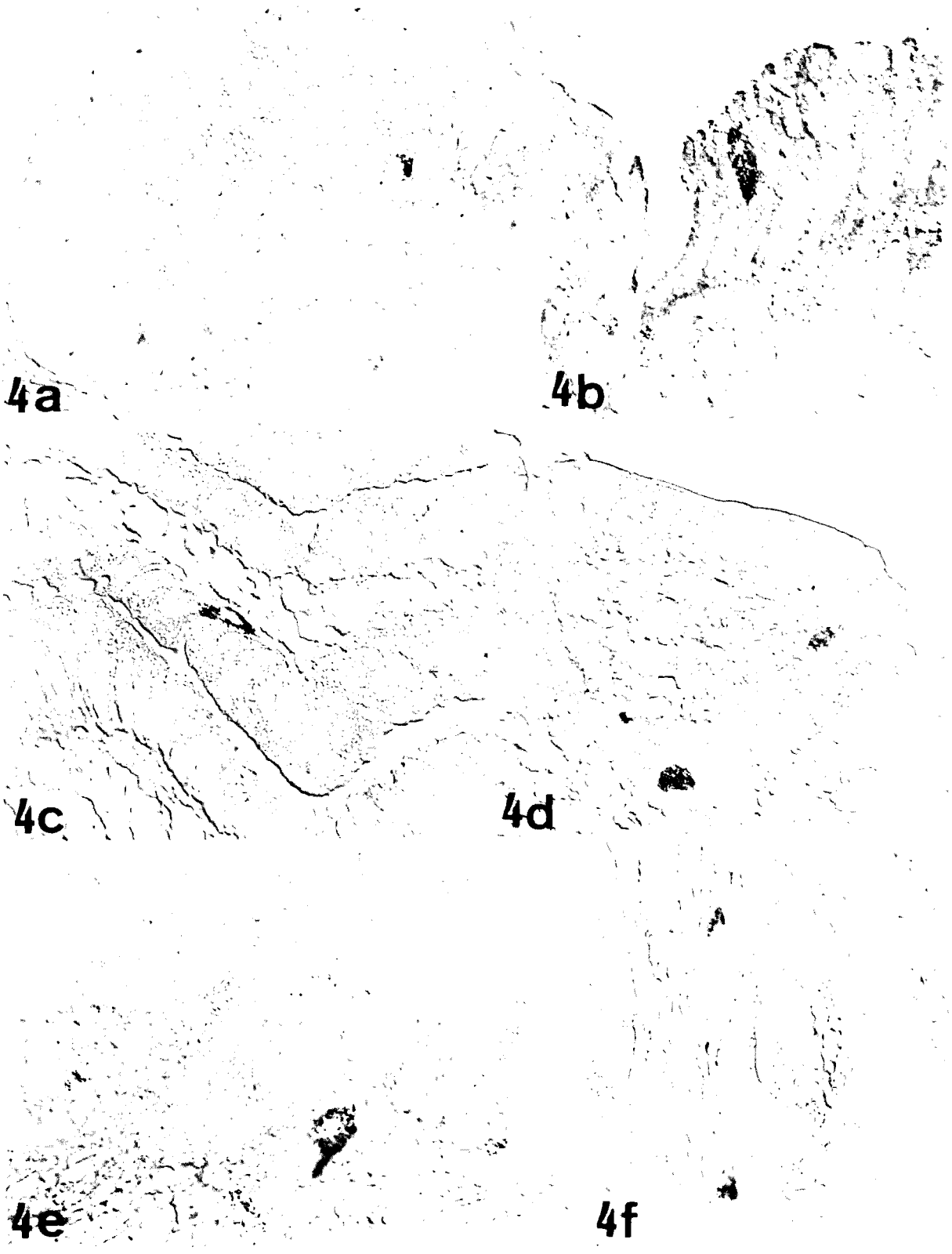


Fig. 4. Regions of chick embryonic gut showing the early appearance of different types of endocrine cells. Immunoreactive neurotensin cells at 12 days of incubation in a) the proventriculus b) the pyloric region c) the duodenum d) the rectum at 14 days of incubation e) an immunoreactive somatostatin cell in the pyloric region at 12 days and f) immunoreactive gastrin/CCK cells in the pyloric region at 16 days of incubation. Benzoquinone vapour fixation; unstained. × 900

first appeared at the same stage of incubation and in almost all the same regions of the tract, cells of both types occurring by the 12th day of incubation in the proventriculus, pyloric region and duodenum, neurotensin

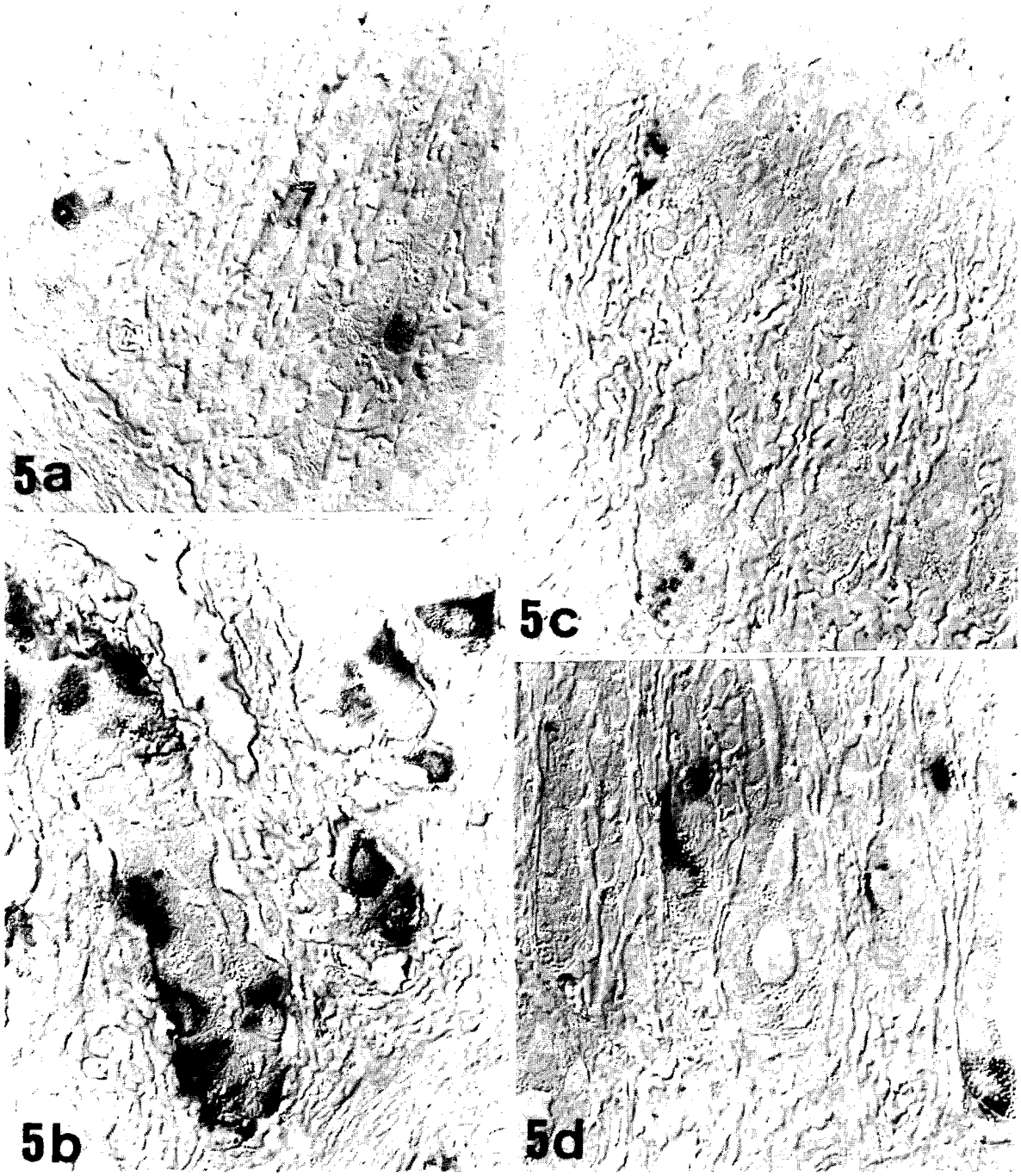


Fig. 5. Immunoreactive gastrin/CCK cells in the pyloric region at a) 17½ days of incubation b) 19 days of incubation. Immunoreactive somatostatin cells in the pyloric region at c) 16 days and d) 17½ days of incubation. Benzoquinone vapour fixation; unstained. × 900

immunoreactive cells being detected in the rectum, too, at this stage (Fig. 4a-e). Cells immunoreactive for somatostatin were not looked for at 13 days of incubation as cells of this type had already been found in the proventriculus, pyloric region and duodenum at 12 days.

Cells containing a gastrin/CCK-like peptide first appeared on the 14th day in all parts of the small intestine. Such cells were first detected in the pyloric region at 16 days of incubation (Fig. 4f), the latest stage at which immunoreactive cells first occurred in any region.

The only endocrine cells found in the rectum and caecum were cells immunoreactive for neurotensin. These became fairly abundant in the rectum at 16 days of incubation; in the caecum only a single cell was found on the 14th day and another on the 16th day of incubation. No cell types other than perhaps the cells immunoreactive for neurotensin in the caecum, appeared and then disappeared before hatching. As only two neurotensin immunoreactive cells were seen there, this may be attributable to sampling.

No immunoreactive cells were found in the gizzard at any of the stages examined.

Subjective assessment of the immunoreactive cells of all three types showed that, as a rule, the cells increased fairly steadily in number in all regions in which they were present. Hence by 16- or 17½-days of incubation numbers had mounted, especially in the regions where cells were numerous at hatching, i.e. the distribution and frequency of cells at hatching generally appeared to be attained by 17½ days of incubation for cells with neurotensin- and somatostatin-like immunoreactivity but only at 19 days for cells immunoreactive for gastrin/CCK (Figs. 5a-d).

From 17½ days of incubation until hatching all three types of immunoreactive cells were generally much more numerous in the pyloric region than in other parts of the tract.

Where cells of a given type were sparsely distributed they were not necessarily detected at every successive stage or in every specimen at the same stage e.g. cells with gastrin/CCK-like immunoreactivity in the small intestine on days 16 and 17½; cells immunoreactive for neurotensin on day 13 in the pyloric region; and cells immunoreactive for somatostatin in the pyloric region on days 13 and 14, and in the proventriculus and duodenum on day 13.

The morphology and differentiation of the gut in relation to the time at which endocrine cells first appear is worthy of note. At 12 days of incubation the epithelium lining the gastrointestinal tract is relatively undifferentiated in all regions. Somatostatin and neurotensin immunoreactive cells first appeared in the proventriculus in the pseudostratified surface epithelium only, although a few short simple tubular glands were present. At 14 days a few immunoreactive cells of both types were found in the newly developed proventricular compound glands, none being detected yet in the short simple tubular glands. By 17½ days these two cell types were present in both the simple and compound glands which were by then well differentiated. In the pyloric region when the first somatostatin- and neurotensin-immunoreactive cells appeared, also at 12 days of incubation, the surface epithelium here too, was low and pseudostratified with no sign of gastric pits or glands. At 14 days immunoreactive cells of both types were very sparse in the few short simple tubular glands formed. At 17½ days, in the now well developed simple tubular glands, cells immunoreactive for gastrin/CCK, somatostatin and neurotensin were plentiful. In the small intestine, too, a few neurotensin- and somatostatin-immunoreactive cells were found in the low surface epithelium at 12 days of incubation.

At 14 days of incubation when cells showing gastrin/CCK-like immunoreactivity first appeared, all three cell types occurred in newly-formed zigzag villous folds which were lined by a simple columnar epithelium. As the mucosa differentiated to form crypts and villi, by 17½ days of incubation immunoreactive cells had become more numerous there.

Generally in some of the first immunoreactive cells to appear, the granules were few, tending to be located towards the bases of the immunoreactive cells. Later in development when the epithelium had undergone differentiation and morphogenesis and resembled that of chicks at hatching, as a rule, the immunoreactive granules present filled the cells.

Discussion

Since Polak et al. undertook a survey of endocrine cells in the gastrointestinal tract of adult chickens in 1974, further information has accumulated on these cells in birds. As regards the distribution of avian endocrine cells at hatching, the findings of the present study correspond reasonably well with those recorded in a survey carried out by Rawdon and Andrew (1981) and with those of other workers on avian material (Larsson et al., 1974; Polak et al., 1974; Alumets et al., 1977; Sundler et al., 1977).

Less attention has, however, been paid to embryonic avian tissue. In the present study on embryonic material it was of interest to note that endocrine cells (albeit sparsely granulated) were already developing in the relatively undifferentiated surface epithelium of the gut; thereafter they were present in glands at or very soon after morphogenesis of the latter had begun.

The paucity of the endocrine cells at the time of first detection is to be expected. It is, of course, possible that some cells may have differentiated earlier but were missed due to sampling. It is probable that sampling also accounts for the interrruptions in the occurrence of cells at certain times, particularly since this was observed in regions in which the cells were sparse.

Immunoreactive cells of all three types studied showed a general increase in density during development until the pattern at hatching was established. It may be that this trend changes shortly after hatching as has been shown for pancreatic polypeptide cells by Alumets et al., (1978). Further changes may well occur in young birds to attain the adult pattern.

A previous study has shown the gizzard in embryos to be an area lacking in endocrine cells (Sundler et al., 1977). At hatching, none of the types sought in the present study was found; Rawdon and Andrew (1981) reported very rare cells of two other types. On the other hand, Yamada et al. (1986) have demonstrated various endocrine cells, including somatostatin-immunoreactive cells, in adult gizzard, particularly in the cranial and caudal regions which were not sampled in the present study. A comprehensive survey of the embryonic gizzard might perhaps reveal more endocrine cells.

The earliest stage at which endocrine cells are here reported to appear in chick embryos is at 12 days of incubation. Cells immunoreactive for neurotensin and somatostatin were found at this time in the proventriculus and pyloric region as well as in the duodenum. The fact that no immunoreactive cells were detected at 11 days of incubation may, as already mentioned, be attributable to limited sampling.

In this study neurotensin-immunoreactive cells were one of the earliest endocrine cell types found in the gut of chick embryos. They were detected very much earlier than by previous workers. A few immunoreactive cells were distinguished in the proventriculus already at 12 days of incubation, the numbers increasing thereafter; Sundler et al. (1977), however, found no cells with neurotensin-like immunoreactivity in this region from 10 days of incubation on, or even at hatching. Whereas these authors reported such cells in the colon first at 18 days of incubation, in the small intestine at 19 days and in the pyloric region at 20 days, in the present study they have been identified in all these regions at 12 days.

The occurrence of neurotensin-immunoreactive cells in the caecum was rare as noted by Sundler et al. (1977); Rawdon and Andrew (1981) detected none in chicks at hatching. Cells with neurotensin-like immunoreactivity occurred from the proventriculus to the rectum, showing a wide distribution in the embryo as they do in chicks at hatching (Rawdon and Andrew, 1981). A variable staining intensity of the neurotensin-immunoreactive cells was observed in the pyloric region from 17½ days of incubation, as reported at hatching by Sundler et al. (1977) and Rawdon and Andrew (1981).

Somatostatin-immunoreactive cells, like neurotensin-immunoreactive cells, appeared at 12 days of incubation, and in the same regions (proventriculus, pylorus, duodenum), except for the rectum, whereas Alumets et al. (1977) reported them to be confined to the proventriculus at 12 days. Wium too, (pers. commun.) has detected somatostatin-immunoreactive cells here at this stage. At 14 days they were found in addition in the upper and lower ileum in the present study. In the pyloric region, Salvi and Renda (1986) found cells with somatostatin-like immunoreactivity at 12 days but in the duodenum approximately one day earlier. In the present study somatostatin-immunoreactive cells were already numerous in the duodenum at 16 days and in the pylorus at 17½ days of incubation, contrary to the findings of Alumets et al. (1977) who reported the first appearance of these cells in the duodenum at 16 days of incubation and in the pyloric region three days later.

There is some evidence that antiserum L48 used here, can distinguish a CCK-like peptide from gastrin in chick gut, cells containing the former being found in the upper and lower ileum and the latter in the pyloric region and duodenum (Rawdon and Andrew, 1981). This seems reasonable since in mammals gastrin cells are concentrated in the pyloric antrum and in some species there are a few in the duodenum, whereas CCK cells are confined to the intestine. Hence the gastrin/CCK-immunoreactive cells found in the pyloric region and duodenum in the present study in embryos at hatching are likely to be gastrin cells and those in the ileum, CCK cells. In young chickens, Larsson et al. (1974) also found cells immunoreactive for gastrin/CCK in these regions. In the present study, despite examination of at least two specimens and sometimes five of each of these regions at 12, 13 and 14 days of incubation, gastrin/CCK-immunoreactive cells were first detected only on the 14th day in the small intestine and at 16 days in the pyloric region. Even then the cells were rare. Salvi and Renda (1986), however, report such cells on or about 11 days of incubation in the pyloric region and the duodenum.

Although it is possible that the very first cells of the types studied here may appear a little earlier than reported, the results give a clear indication of the time and the specific regions in which they may first be expected. They also show when any are certain to be present in reasonable numbers in a given region of the gastrointenstinal tract. This information is of particular value in, for instance, the planning of experiments on the differentiation of gut endocrine cells or studies of the co-existence of gut peptides in the same cells in embryonic tissue.

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