Enterochromaffin cells are known to be present from the lower end of the oesophagus to the rectum in man and other mammals studied (Kull, 1925; Friedmann, 1934; Hamperl, 1925; Jacobson, 1939; Gillman, 1942). Their presence in the human? oesophagus is mentioned by Ham and Leeson (1961), and in the guinea pig oesophagus by Kull (1925) but Dawson (1945) fails to find them in the oesophagus of the guinea pig, rabbit, rat, mouse and hamster.

There is considerable controversy regarding the presence and distribution of enterochromaffin cells in the human stomach. Kull (1925) and Gillman (1942) state that they are absent in the normal mucosa of the human stomach; Hamperl (1927) and Friedmann (1934) find only a few cells; but most investigators agree that they are present (Bloom and Fawcett, 1962; Ham and Leeson, 1961; Macklin and Macklin, 1928; Jacobson, 1939). Bloom and Fawcett (1962) and Sharples (1945b) observe that these cells are more numerous in the fundus of the stomach than in the pylorus, but Jacobson (1939) states that the cells occur in the cardiac region, are practically absent in the fundus and are always present in fair numbers in the pylorus.

The presence of enterochromaffin cells has been reported in the stomach of several animals including the guinea pig (Kull,
The enterochromaffin cells are found in a variety of mammals but not all species have them. For example, they are present in the stomach of the mouse and rat (Kahlau, 1925; Dawson, 1945); the horse, pig, and cat (Tehver, 1930; Jacobson, 1939); sheep (Tehver, 1930) and rabbit (Dawson, 1945). However, their presence in the stomach of the guinea pig and rat is denied by Jacobson (1939). They are also reported to be absent in the antestomach or crop of ruminants and in the cutaneous mucous membrane covering part of the horse and pig stomach (Tehver, 1930).

In the pig, the enterochromaffin cells occur in the cardiac region, are practically absent in the fundus and are found in fair numbers in the pylorus; in the horse they are present in the pyloric region, very rare in fundus, and absent in cardia (Jacobson, 1939). In the cat and sheep they are virtually absent in the pylorus (Tehver, 1930; Jacobson, 1939). In the rabbit they are most numerous in the cardiac region, less in the fundic region and sparse in the pylorus (Dawson, 1945). In the guinea pig they are most numerous in the fundus, fewer in the cardiac region and scattered in the pylorus (Dawson, 1945; Won, 1964). In the mouse they are numerous in the fundus, and few in cardiac and pyloric regions (Dawson, 1945).

A cranio-caudal gradient in the distribution of enterochromaffin cells of the human small intestine has been reported by several workers (Bloom and Fawcett, 1962; Schofield, 1953; Hamperl, 1925; Jacobson, 1939; Gillman, 1942). A similar gradient has been reported in the guinea pig (Hoeschen, 1937; Schofield, 1951), rat (Münch, 1939), and chick (Monesi, 1960).
Hamperl (1925) and Jacobson (1939) describe a progressive diminution of enterochromaffin cells from caecum to rectum in man. Friedmann (1934) describes a gradient in the opposite direction; but Schofield (1953) fails to confirm this finding. The number of enterochromaffin cells shows a progressive decrease from caecum to anus in the guinea pig (Hoeschen, 1937) and in the rat (Münch, 1939). In cattle their number increases in the terminal part of the large intestine (Tehver, 1930).

Enterochromaffin cells are generally considered to be abundant in the human appendix (Bloom and Fawcett, 1962) but Gillman (1942) finds only a few cells. They are also reported in the gall bladder, bile and pancreatic ducts, prostatic glands and prostatic urethra (Ackerman and Del Regato, 1962; Clara, 1957; Hamperl, 1952). Their presence in Meckel's diverticulum is indicated by the frequent occurrence of carcinoids in this situation (Willis, 1960).

Previous studies on distribution of the enterochromaffin cells in the human gastro-intestinal tract are incomplete in that they are not based on quantitative estimations. The only systematic studies on distribution of these cells are those of Hoeschen (1937) in the guinea pig and of Münch (1939) in the rat. The distribution of the enterochromaffin cells must undoubtedly bear a significant relationship to their function and it is therefore important to make a systematic study of their distribution in man.
MATERIAL AND METHODS:

The distribution of enterochromaffin cells has been studied in 11 human foetuses (marked * in Table 1) having a C.R. length of 65 mm or more. Foetuses of this age group are chosen as enterochromaffin cells have appeared throughout the human gastrointestinal tract by this stage of development (Page 45). Three of these foetuses (H 968, H 699, H 744) are serially sectioned and stained at appropriate levels by the Bodian method. In the remaining foetuses the entire gastrointestinal tract from the lower end of the oesophagus to the rectum has been removed, cut up into appropriate pieces and embedded in paraffin as usual.

Block of the stomach of three foetuses (H 986, H 828, H 818) have been prepared and sectioned in such a way as to provide sections showing the entire stomach including the lower end of the oesophagus and the pyloro-duodenal junction in one section. This has been achieved by cutting the stomach along the greater and lesser curvatures and placing the cut surface on a perfectly flat object at the time of blocking. Sectioning in this manner greatly facilitates the comparison of enterochromaffin cell density in various parts of the stomach.

The small intestine of six foetuses (H 986, H 828, H 818, H 839) has been cut up into pieces approximately one centimeter in length. All pieces from one foetus (varying from 15 to 42 in number) are arranged in serial order within one or two blocks to facilitate sectioning and staining, and to eliminate possible errors in cell counts.

* Only alternate pieces were processed in the 220mm and full term foetuses.
counts due to variation in section thickness or in staining. The arrangement of a large number of pieces of intestine in one block without possibility of confusion of their serial order is accomplished as follows:

(i) Two sewing needles are passed through the two ends of the most cranial piece of intestine, which thus comes to lie nearest the 'eyes' of the needles (Fig. 25a).

(ii) Five to six pieces of intestine are, in this way, threaded on to this set of needles, their serial order being indicated by their position relative to the eyes (Fig. 25b). These pieces can now be treated as one unit through dehydration, clearing and impregnation.

(iii) In making the blocks these units are placed one on top of another as shown in Fig. 25c. After the paraffin has set the block is trimmed to expose the ends of the needles which are then pulled out. Appropriate marks are made on the block indicating the proximal side of each unit and also the most proximal unit. Sections are cut in the plane of the arrow in Fig. 25b.

As a rule the large intestine has been similarly treated, the appendix being incorporated in the block at the appropriate position. In H 812 the entire large gut is longitudinally sectioned. Again it is found possible to make the block and section it in such a way as to get portions of almost the whole large gut in a single section.

The methods used for quantitative estimations are considered at length in Chapter 6.
Method of arranging several pieces of gut in serial order within one paraffin block.
OBSERVATIONS:

A. Mouth, pharynx, oesophagus:

Observations on serially sectioned human foetuses stained by the Bodian method fail to reveal any cells with silver reducing granules in the epithelial lining of the mouth, pharynx and the thoracic part of the oesophagus. However, argentaffine as well as argentaffin cells can be consistently found in the stratified squamous lining of the lowest part of the oesophagus. The cells are few in this situation and are as a rule confined to the deepest layer of the epithelium. They assume irregular shapes that are presumably dependent on pressure of neighbouring cells. Occasionally these cells can be seen extending right upto the lumen of the oesophagus.

B. Stomach:

Cells giving positive argentaffin, Schmorl and diazonium reactions can be identified in the mucous membrane all over the stomach in all the foetuses studied. In addition, there is a much larger number of cells that are argentophile and do not give positive results with the other reactions. Both types of cells are most numerous near the cardiac end and progressively decrease in number towards the pylorus (Fig. 26). The cells are present only in relation to the epithelial lining of the glands of the gastric mucosa. No argentophile cells are seen either on the surface epithelium or in the foveolae. Numerous argentaffin cells can always be seen in islets of
intestinal mucosa that are frequently present in the pyloric part of the adult stomach.

C. Small intestine:

Argyrophile and argentaffin cells have appeared throughout the small intestine by the 35 mm and 55 mm stages respectively. At these stages of development, the cells are seen in greatest number in the duodenum and progressively diminish towards the terminal ileum.

At the 65 mm stage, a definite cranio-caudal gradient of distribution is demonstrable. By the 75 mm stage, however, the pattern has changed to that illustrated in figure 28 a and b. It is seen that the cell density decreases from the duodenum to about the middle of the small intestine and thereafter again progressively increases towards the ileo-caecal junction. The density of cells in the terminal ileum exceeds that in the duodenum.

Both argyrophile and argentaffin cells show the same pattern of distribution.

Estimations at the 97 mm, 140 mm and 220 mm stages reveal a pattern of distribution similar to that at the 75 mm stage. The increase in cell density in the distal half or so of the small intestine is seen to be more pronounced for argentaffin cells than for argyrophile cells.

D. Large intestine:

Both argyrophile and argentaffin cells have appeared throughout the large intestine by the 65 mm stage. At this stage argyrophile
EXPLANATION OF FIGURES 26 - 28

Fig. 26 : Graph showing the distribution of argentaffin cells in the stomach of a 140 mm foetus. Cells were counted in successive microscopic fields beginning at the cardiac end and proceeding to the pylorus along the greater curvature.

Fig. 27 : Graph showing the distribution of argyrophile cells in the small intestine of a 65 mm human foetus.

Fig. 28 : Graphs showing the distribution of (a) argyrophile and of (b) argentaffin cells in the small intestine of a 75 mm human foetus.
FIG. 29

Graphs showing the distribution of (a) argyrophile cells, and (b) argentaffin cells in the small intestine of a 220 mm human foetus.
cells are least abundant in the caecum and sharply increase in number as one proceeds towards the rectum (Fig. 30). Argentaffin cells are easily identified in the rectum, but can be located in the colon only on prolonged searching.

By the 75 mm stage the pattern of distribution has changed to that shown in figure 31. The proximal half of the large intestine shows a fall in density of both the argyrophile and argentaffin cells, whereas the distal half again shows a rise.

The pattern of argyrophile cell distribution at the 97 mm stage is the same as at the 75 mm stage. Argentaffin cell density, however, shows no distinct cranio-caudal gradient.

By the 140 mm stage the cranio-caudal gradient seen at the 65 mm stage has been reversed; there is a definite decrease in argyrophile and argentaffin cell density in proceeding from caecum to rectum (Fig. 32).

In the 220 mm foetus studied there is no obvious cranio-caudal gradient (Figs. 33 a, b). However, the findings are suggestive of the possible existence of a pattern similar to that seen at the 75 mm stage. Both in figure 33a and 33b the first five points on the graph suggest a progressive cranio-caudal decrease in enterochromaffin cell population. On the other hand if the first point on the graph is ignored the rest of the graph indicates a gradient in the opposite direction. This interpretation of the findings in this foetus is supported by the fact that at full term the pattern observed (Figs. 33 c, d) is the same as at the 75 mm and 97 mm stages.

The distribution of enterochromaffin cells has also been studied in the large intestine of a 13 year old girl. In this specimen (removed surgically) the distal part of the pelvic colon and the rectum were not available for study. The pattern of distribution of the argyrophile and argentaffin cells (Figs. 33 e, f) is the same as in the 65 mm foetus.

(See discussion on page 70)

E. Appendix:

It has been shown earlier (Page 51) that after a relatively late onset of differentiation cells of the enterochromaffin system reach
FIG. 30

Distribution of argyrophile cells in the large intestine of a 65 mm human foetus.

FIG. 31

Distribution of (a) argyrophile and (b) argentaffin cells in the large intestine of a 75 mm human foetus.
3000 -i
<D
OCO
<MUP to
2000 - 4^bOI G
G
CO
H 1000 - rH 
CD 
O
65 mm C.R 0
LARGE INTESTINE ...  1 ........... "I    I
2 4 6 8
Cranio-caudal level
FIG. 30

75 mm C.R.
LARGE INTESTINE
ARGYROPHILE

75 mm C.R.
LARGE INTESTINE
ARGENTAFFIN

Cells/\text{mm}^2 \text{ gut surface}

Cranio-caudal level
FIG. 31a

Cranio-caudal level
FIG. 31b
FIG. 32

Distribution of (a) argyrophile and (b) argentaffin cells in the large intestine of a 140 mm human foetus.

FIG. 33a,b.

Distribution of (a) argyrophile and (b) argentaffin cells in the large intestine of a 220 mm human foetus.
140 mm C.R
LARGE INTESTINE
ARGYROPHILE

220 mm C.R
LARGE INTESTINE
ARGYROPHILE

140 mm C.R
LARGE INTESTINE
ARGENTAFFIN

220 mm C.R
LARGE INTESTINE
ARGENTAFFIN
a peak level of density by about the 120 mm stage. Thus it is valid to compare the cell density in the appendix with that of other regions of gut only after this stage. From Table 4 it is seen that when estimations are made in terms of basement membrane the cell density in the appendix is significantly greater than in the duodenum between the 97 mm and 220 mm stages. However, as the amount of mucosa related to a given surface area of duodenum or caecum is much greater than that in the appendix, the density of cells per mm² gut surface of appendix is found to be more or less the same as in the duodenum and caecum. Further, because of the smaller diameter of the appendix the number of cells per unit length of appendix is less than in the duodenum, terminal ileum or caecum.

By full term the density in the appendix (estimated per mm² of basement membrane - Table 4) has fallen to the adult level and is now less than in most of the rest of the gastro-intestinal tract.

THE RELATIVE NUMBER OF ARGYROPHILE AND ARGENTAFFIN CELLS IN THE GASTRO-INTESTINAL TRACT OF HUMAN FOETUSES:

A comparison of the numbers of argyrophile and argentaffin cells at various levels of the gastro-intestinal tract reveals a remarkable degree of variation in the relative proportion of the two types of cells. As stated earlier, in the stomach the number of argentaffin cells is small, but argyrophile cells are numerous. In the small intestine a plot of the numbers of argentaffin cells at various cranio-caudal levels against the numbers of argyrophile cells fails to reveal any correlation between the two types (Figs. 34, 35). However, when the argentaffin cells present are expressed as a percentage of the argyrophile cell population and this percentage is plotted against the cranio-caudal level, it is found that the relative proportion of argentaffin cells undergoes a decrease in the proximal part of the small intestine and thereafter shows a marked increase so that near the termination of the ileum nearly all
FIGS. 34 & 35

Graphs showing plots of the numbers of argentaffin cells against the number of argyrophile cells seen at various cranio-caudal levels of the small intestine of a 140 mm foetus (Fig. 34) and a 220 mm foetus (Fig. 35).

FIGS. 36 & 37

Graphs showing the variation in the percentage of argyrophile cells that are argentaffin at various cranio-caudal levels of the small intestine of a 140 mm foetus (Fig. 36) and a 220 mm foetus (Fig. 37).
Argyrophile cells per section

![Graph 1: 140 mm C.R SMALL INTESTINE](#)

![Graph 2: 220 mm C.R SMALL INTESTINE](#)

% argyrophile cells that are argentaffin

![Graph 3: Cranio-caudal level](#)

![Graph 4: Cranio-caudal level](#)
FIGS. 38 & 39

Graphs showing plots of the numbers of argentaffin cells against the number of argyrophile cells seen at various cranio-caudal levels of the large intestine of a 140 mm foetus (Fig. 38) and a 220 mm foetus (Fig. 39).

FIGS. 40 & 41

Graphs showing the variation in the percentage of argyrophile cells that are non-argentaffin (pre-enterochromaffin) at various cranio-caudal levels of the small intestine of a 140 mm foetus (Fig. 40) and a 220 mm foetus (Fig. 41).
Non-argentaffin cells
Argyrophile cells

Argentaffin cells

FIG. 38

Argentaffin cells

FIG. 39

% non-argent. argyro. cells

% non-argent. argyro. cells

FIG. 40

FIG. 41
argyrophile cells are argentaffin (Figs. 36, 37). In the appendix also almost all argyrophile cells are argentaffin.

In the large intestine, the relationship between the proportion of the two cell types is more consistent and a positive correlation is demonstrable between counts of argyrophile and argentaffin cells (Figs. 38, 39). This is due to the fact that the percentage of argyrophile cells that are argentaffin has no correlation with cranio-caudal level in the large intestine.

As a corollary of what has been stated above it follows that in proceeding from the cranial to the caudal end of the small intestine the proportion of non-argentaffin argyrophile (or pre-enterochromaffin cells) first undergoes a moderate increase and then sharply decreases towards the terminal ileum (Fig. 40, 41). However, in the large intestine the proportion of these cells is not correlated with the cranio-caudal level.

DISCUSSION:

1. Stomach:

The pattern of distribution of enterochromaffin cells observed in the stomach fits in with the findings of Bloom and Fawcett (1962) and Sharples (1945b), but disagrees completely with those of Jacobson (1939). Failure of some workers (Kull, 1925; Gillman, 1942) to observe enterochromaffin cells in the stomach may be attributed
to imperfect fixation (See Chapter 10); to the fact that the morphology of these cells in the stomach is strikingly different from that in the intestine and that unless this is kept in mind they elude observation; and to the fact that though these cells are well demonstrated by the Gomori-hexamine silver method (and in much greater numbers by argyrophile methods), the Masson-Hamperl, Schmorl and diazonium methods give unsatisfactory staining.

The enterochromaffin cells of the stomach are of special interest in that they show a number of unusual features. As stated above their morphology is atypical. There is a marked preponderance of argyrophile cells over argentaffin ones suggesting that the non-argentaffin argyrophile cells may not only be precursors of the enterochromaffin cells proper, but may also have a significance of their own. This is to be correlated with the fact that (a) in human embryos there is a much greater time lag between the appearance of argyrophile cells and of argentaffin ones in the stomach than in other parts of the gastro-intestinal tract (Page 47); and (b) that in the proventriculus and gizzard of the chick (Dawson and Moyr, 1948) and in the fundus of the stomach of the rat (Dawson, 1948) the argyrophile cells never develop into argentaffin cells.

2. Small intestine:

The findings of the present investigation show that, at least in foetuses, the pattern of distribution is not as simple as has been presumed. Over a given region of small intestine the density
of the enterochromaffin cells is not uniform and the number of cells to be found in adjoining serial sections varies considerably. Quantitative estimations at a large number of cranio-caudal levels are, therefore, necessary to reveal the real pattern of distribution. This has not been done in the human adult so far and further investigation is necessary to determine whether the findings in foetuses are applicable to adults.

Münch (1939) has described the presence of a cranio-caudal gradient in the distribution of enterochromaffin cells in the albino rat. However, a critical examination of the actual cell counts published by him shows that there is a small but definite increase in enterochromaffin cell population in the distal part of the small intestine thus demonstrating a parallelism with the pattern seen in human foetuses. The possible functional significance of variations in enterochromaffin cell density in various parts of the small intestine is discussed in Chapter twelve.

3. Large intestine:

Enterochromaffin cells of the large intestine have attracted far less attention than those of the small intestine in spite of the fact that, notwithstanding statements to the contrary, they are abundant in this situation. Hampel (1925) and Jacobson (1939) describe a progressive diminution of cells from cecum to rectum, but Friedmann (1934) describes a gradient in the opposite direction. Friedmann's view is supported by Tehver's observation that in
cattle the number of enterochromaffin cells increases in the terminal part of the large intestine, and by Faustini's\(^{(1955)}\) finding that in the colon of the adult bull, calf, sheep, pig, adult horse and foal the maximum concentration of 5-hydroxytryptamine is to be found in the distal part.

In this regard the findings of the present investigation show that there is some element of truth in both these conflicting views. A consideration of the findings in the 65 mm, 75 mm, and 140 mm foetuses strongly suggests that the patterns seen in these foetuses represent progressive stages in the evolution of a definitive pattern. However, the findings in the 220 mm and full term foetuses and in the specimen from a 13 year old girl show that the position is not so simple. Each of the patterns seen at the 65 mm, 75 mm and 140 mm stages appears to be capable of persisting into post-natal life. This is shown by the following:

(i) Persistence of the pattern seen at the 65 mm stage is indicated by the findings in the large intestine of the 13 year old girl. This also fits in with the observations of Friedmann\(^{(1934)}\).

(ii) Persistence of the pattern seen at the 140 mm stage would account for the observations recorded by Hamperl\(^{(1925)}\) and Jacobson\(^{(1939)}\).

(iii) On the basis of the material studied in the present investigation, however, it appears that in the majority of specimens there is a persistence of the pattern seen at the 75 mm stage. The presence of this pattern at the 75 mm, 97 mm, 220(?), and full term...