Further Observations on the Development of the Peptic Cells of the Rabbit's Stomach

By GORDON MENZIES

(*From the Department of Anatomy, St. Mary's Hospital Medical School, London*)

Summary

1. The loss of the mucinogen component of the peptic granules begins during the fourth week of post-natal life (400 g) at a site on or near the greater curvature of the stomach adjacent to the pyloric antrum. It reaches the lesser curvature at about the sixth week (750 g).

2. The loss progresses slowly across the ventral and dorsal walls of the stomach and more rapidly along the greater curvature.

3. At 11–12 weeks (1,800 g) only a few peptic cells on the greater curvature and adjacent to the oesophageal opening contain PAS-positive granules.

4. Except for a few granules in an occasional peptic cell, no mucinogen can be demonstrated in these cells after the fourteenth week (1,900 g).

5. The stage seems to be correlated more closely with the weight than with the age of the animals.

Introduction

IT has recently been shown (Menzies, 1958) that during the early weeks of post-natal life all the granules in the peptic cells of the rabbit's stomach contain a mucinogen component. This component, as shown by the PAS reaction, is lost, first in the cells at the base of the glands and then up the tubules towards the crypts. As all the samples of the gastric mucosa were taken from a similar situation on the greater curvature of the stomach, where it was shown that the loss occurs between the fourth and sixth weeks of post-natal life, it seemed desirable to ascertain if this loss occurs in all areas of the stomach at the same time, and that is the purpose of the present investigation.

Materials and Methods

The stomachs from three adult rabbits were used to find the exact areas that contained peptic cells. Fifty-six young animals were used, from 10 litters. These were killed and weighed at intervals from 2 to 16 weeks after birth. Not less than 4 animals were examined at each interval from the third to the eleventh week inclusive. In order to try to prevent an excessive loss of peptic granules through feeding, all animals over 4 weeks old were starved for a period of 12 h before being killed. During this period of starvation they were kept in separate cages and allowed fluid to drink as much as they wished.

As it has been suggested that pyronin increases protein synthesis (Hoffman, 1952), two other litters of rabbits were examined that had been fed with 1% pyronin (Gurr's G). This was placed in the drinking fluid of the mothers from the time of conception, and in the drinking fluid of the young rabbits from these litters after the time of weaning (5 weeks).

[Quarterly Journal of Microscopical Science, Vol. 101, part 1, pp. 19–24, March 1960.]

Histochemical and cytological techniques

In order to construct a map of the sites of the peptic cells that contained either (*a*) PAS-positive granules, or (*b*) granules that had lost their PAS-positive reaction (Menzies, 1958), from 12 to 26 representative pieces of the stomach wall were removed and examined histologically. The reader is referred to fig. 1, which shows the sites of the pieces of the mucosa examined.

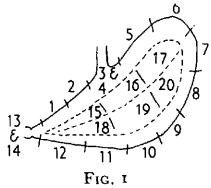

FIG. 1

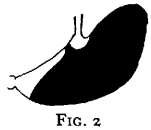

FIG. 2

FIG. 1. Diagram of the ventral aspect of the stomach to show the site and extent of the pieces of mucosa taken.

FIG. 2. Diagram showing the distribution of the peptic cells (black), in relation to the greater and lesser curvatures and the ventral or dorsal walls of the adult rabbit's stomach. This diagram could equally well represent any typical rabbit weighing more than 200 g (i.e. at least 3 weeks old). Note the relatively small area occupied by peptic cells on the lesser curvature.

Nos. 1 and 2 were taken from the ventral and dorsal walls of the stomach to include the lesser curvature from the pylorus to the oesophagus. Nos. 5 to 12 include the whole of the greater curvature and adjacent stomach wall. Nos. 3 and 4 include both sides of the oesophageal inlet and the adjacent portions of greater and lesser curvature of the stomach. Nos. 13 and 14 include both sides of the stomach adjacent to the pyloric sphincter. The remaining two pieces left were the ventral and dorsal walls; these were each cut into about 6 pieces and numbered. Nos. 15 to 20 represent the ventral wall of the stomach. Nos. 21 to 26, not shown in the diagram, represent the dorsal wall. It was found that the number of pieces necessary to cover the above areas could be reduced to 12 in animals under 5 weeks old. The pieces of mucous membrane were pinned out on cork, orientated, numbered, fixed for Bowie's method (Bowie, 1936), and subjected to the following procedures.

A. For the mucinogen component: the periodic acid / Schiff technique.

B. For the pepsinogen component: Bowie's method.

After appropriate fixation all pepsinogen granules at all stages colour blue with Bowie's method, whether they contain a mucinogen component or not, whereas only those containing a mucinogen component colour with the periodic acid / Schiff technique.

RESULTS

Peptic cells in adult rabbits

As has been reported in other species, the peptic cells in the rabbit are found (fig. 2) only in the body (or fundus) of the stomach, being absent from the pyloric area and a small region around the oesophagus (both these areas

being white in fig. 2). Peptic cells occupy only a very small area on the lesser curvature (figs. 2 and 3), an area much smaller than any I have seen described in the literature for other mammals; but they approach much nearer to the pyloric sphincter on the greater curvature (see fig. 2). On the ventral (fig. 2) and dorsal walls of the stomach the limit of the area occupied by peptic cells is represented by a line gently curved towards the pylorus from greater to

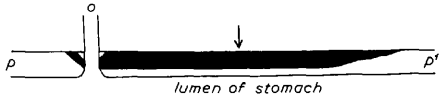

FIG. 3. Diagram of the distribution of peptic cells (black) along the greater and lesser curvatures of the adult rabbit's stomach. *o*, oesophagus; *p*, the pyloric part of the stomach on the lesser curvature; *p'*, the pyloric part of the stomach on the greater curvature. The arrow is at the site on the greater curvature opposite the opening of the oesophagus.

lesser curvature. Except in a small transitional area adjoining this line, the peptic cells are found in the bases and bodies of the gastric tubules. As this line is approached they are seen to be absent first from the upper regions of the bodies and later from the lower portions of the bodies, being thus present only in the bases of the tubules, until finally they completely disappear rather abruptly.

Peptic cells in young rabbits

Animals 3 weeks old. The distribution of peptic cells at this age is similar to that of the adult (see fig. 2), but whereas in the latter they are found in the lower two-thirds of the gastric tubules, at 3 weeks old they show an uneven distribution in all cases, being in many areas absent from the bases of the tubules but in others extending up to the crypts. The majority, however, are found in the bases and bodies of the tubules. This irregular distribution of peptic cells within the gastric tubules seems to bear no relation to the position of the tubules in the stomach wall. One animal (examined at 2 weeks old) showed peptic cells full of Bowie-positive granules, occupying three-quarters of the length of the lesser curvature. This occurrence, which according to Hirschowitz (1958) may be a seasonal phenomenon, was not seen in any of the other animals.

All the peptic granules examined in animals 3 weeks old are PAS-positive (fig. 4, A).

Animals 4 weeks old. The results with one animal (of 260 g) could not be distinguished from those of the preceding group; but the others (8 animals, each weighing between 350 and 480 g) show a marked increase in the number of peptic cells present, and a distribution in the tubules very similar to that in the adult.

Except for a small area on the lesser curvature of the stomach and the adjacent portions of the ventral and dorsal walls, containing peptic cells whose granules are still PAS-positive, the granules in all the peptic cells

lying next to the pyloric area of the stomach have lost their PAS-positive component (fig. 5, A). In sections from areas further away from the pylorus, islands of peptic cells containing PAS-positive granules appear situated near the crypts of the tubules (fig. 4, B), whilst the peptic cells in the bases and bodies of the glands contain only PAS-negative granules. In sections still further from the pylorus only the peptic cells in the bases of the glands (fig. 4, B) contain PAS-negative granules, until finally, on the greater curvature of

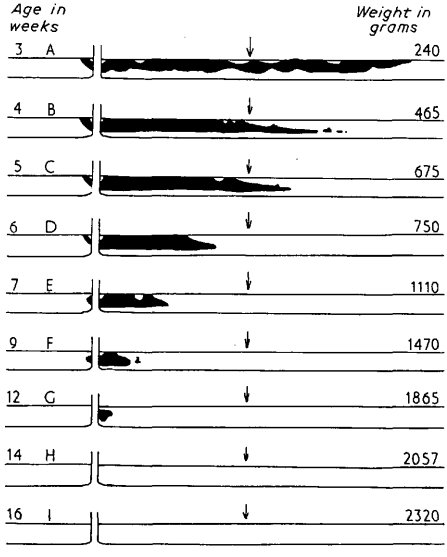

FIG. 4. Diagram of a series of young rabbits from 3 to 16 weeks old, showing (black) the distribution of peptic cells containing granules which are PAS-positive. The diagram represents the oesophageal opening with the lesser curvature to the left and the greater curvature to the right, each end being the pylorus. The arrow is at the site on the greater curvature opposite the oesophageal opening. (Compare fig. 1.)

the stomach (and the adjacent walls) at a site opposite the oesophageal opening, only small islands of peptic cells containing PAS-negative granules occur and these are deep in the bases of the glands (fig. 4, B). These cells are amongst other peptic cells whose granules are PAS-positive (as already reported, Menzies, 1958).

The 'area' of peptic cells whose granules have lost their PAS-positive component is widest on the greater curvature of the stomach and becomes progressively narrower as it proceeds up the ventral and dorsal walls of the stomach, as is shown in fig. 5, A.

It might be appropriate to stress here that fig. 4 represents peptic cells

containing granules in a PAS-positive phase. All peptic cell granules from 4 weeks up to and including the adult stage react positively with Bowie's method and show a pattern of distribution not essentially different from that shown in fig. 3.

Animals from 5 to 12 weeks old. There is a progressive loss of the PAS component of the peptic granules starting in the cells in the bases of the glands and proceeding up the tubules (fig. 4, C–G), and starting in the cells near the pylorus and on the greater curvature and proceeding slowly across

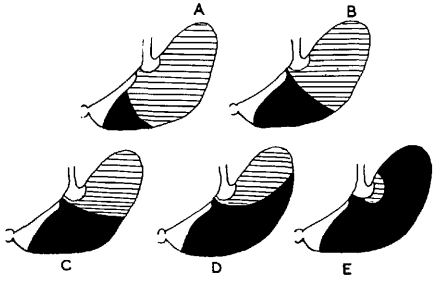

FIG. 5, A–E. Diagrams from animals aged (A) 4, (B) 6, (C) 7, (D) 9, (E) 11–12 weeks. Black represents peptic cells containing PAS-negative granules. Parallel lines represent peptic cells containing PAS-positive granules. Note that in both sets of peptic cells the granules are Bowie-positive.

the ventral and dorsal walls of the stomach to the lesser curvature and more quickly along the greater curvature towards the oesophagus (fig. 5, B–E). The first peptic cells on the lesser curvature to lose their PAS-positive granules are seen at the sixth week (fig. 5, B). Finally, at the eleventh or twelfth week only a few peptic cells remain whose granules are PAS-positive. These cells are situated on the greater curvature near the oesophagus and in some animals they are seen (fig. 5, E) on the ventral and dorsal walls of the stomach around the oesophagus.

The changes described above are correlated more closely with the weight of the animal than with its age: animals of the same weight, whatever their age, show similar changes. Furthermore, although the loss of the PAS-positive component of the peptic granules is progressive as already described, it is an irregular progression. There are some sections (from all the animals described above) that show no PAS-positive material in the peptic cells, whereas other sections from the same *blocks* treated at the same time show a few 'islands' of peptic cells whose granules are still PAS positive.

Animals from 14 to 16 weeks old, and those fed on pyronin. Apart from a very few granules in an occasional peptic cell, no PAS-positive material is seen in peptic cells in any of the sections examined from animals over 12 weeks old (or weighing more than 1,900 g—see fig. 4, H–I).

With animals of the same weight, the results observed on those that had been fed on pyronin are similar to those of the normal animals.

Discussion

As already stated, it has been reported from the examination of sections of the gastric mucosa which had been taken from the greater curvature of the stomach opposite the oesophageal entrance, that the loss of the mucinogen phase of the peptic granules was completed by the end of the sixth week after birth. The present investigation confirms that statement in so far as that particular area of the stomach is concerned, but shows that in other areas the change is not completed until the end of the twelfth week. The importance must therefore be stressed of examining sections from all areas of an organ before concluding that serial changes take place within its cells at any specific time. But if this is impracticable, as is usually the case, it would seem essential that all sections to be compared should be taken from similar anatomical sites on the organ concerned.

It is fortunate that the rabbit was chosen for the present series of studies, for in this animal the loss of the mucinogen component of the peptic granules occurs slowly and at a relatively late and well-developed stage of the gastric mucosa, and can therefore be easily observed. It does, however, occur in other mammals, as, for example, in man and the rhesus monkey (Menzies, 1958), and in the rat, mouse, hamster, cat, and dog (personal observations). But with some it is difficult to obtain the material necessary to carry out a complete investigation; with others (rat and mouse) it occurs very early in development, before the gastric tubule has attained any great length, and insufficient cells are present to show any order of loss; whilst with yet others the histological picture is complicated either by a deep-seated layer (mouse) or an irregular distribution (cat and dog) of mucous neck cells, which themselves contain PAS-positive mucinogen granules at all stages. It is not immediately obvious why in the rabbit the peptic granules should lose their mucinogen component in the order in which they do. It is unlikely to be related to the onset of peptic activity, for pepsin has been demonstrated in the gastric juice of the rabbit at 1 to 2 weeks old (Hammarsten, 1874), and furthermore in some mammals, for example the guinea pig and porcupine, most, if not all, of the pepsinogen granules retain their mucinogen component even at the adult stage.

REFERENCES

Bowie, D. J., 1936. 'A method for staining the pepsinogen granules in gastric glands.' Anat. Rec., **64**, 537.

Hammarsten, O., 1874. 'Beobachtungen über die Eiweissverdauung bei Neugeborenen wie bei saugenden Thieren und Menschen.' *Beitr. Anat. Physiol.*, Festgabe C. Ludwig, 116.

Hirschowitz, B. I., 1958. (Personal communication.)

Hoffman, H., 1952. 'Acceleration and retardation of the process of axon-sprouting in partially denervated muscles.' Aust. J. exp. Biol. med. Sci., **30**, 541.

Menzies, G., 1958. 'Observations on the developmental cytology of the fundic region of the rabbit's stomach, with particular reference to the peptic cells.' Quart. J. micr. Sci., **99**, 485.