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THE PRESENCE OF PANETH CELLS CONFIRMED IN THE PIG*

M. S. MYER, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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The presence of Paneth cells, traditionally believed to be absent in the crypts of the domestic pig, *Sus scrofa* (Linnaeus, 1758), has been confirmed in this report. The cells were found approximately 104 cm from the pyloric valve and occurred mainly along the sides and in the lower half of the crypts of Lieberkühn.

INTRODUCTION

Paneth cells, normally occurring as a small group or as isolated cells in the deepest part of the duodenal crypts of Lieberkühn, have traditionally been described as absent in the crypts of the domestic pig, *Sus scrofa* (Linnaeus, 1758), by such authors as Trautmann & Fiebiger (1952), Krölling & Grau (1960) and Creamer (1967). This erroneous concept has even been perpetuated in recent textbooks such as those by Ham (1974) and by Moon (1976). This widely-held belief, however, has recently been challenged by the description of such cells in the pig by Louwers & De Vos (1971).

The morphology and incidence of the Paneth cells received special attention in my recent systematic survey of the histology of the pig intestine. The findings reported here in an attempt to amend the traditional but erroneous concept is in support of the work by Louwers & De Vos (1971).

MATERIALS AND METHODS

Specimens, each approximately 1 cm long, were taken at 25 cm intervals from the small intestine of a 5-month-old pig and fixed for 8 h in the standard Zenker's solution. After overnight washing in running tap water, the tissue blocks were dehydrated, embedded in paraffin wax and sectioned at 3 μm and 5 μm. The thicker sections were stained with haematoxylin and eosin, while the others were stained by the following methods: Periodic Acid-Schiff (PAS) for mucopolysaccharides (Pearse, 1961); Mallory's phosphotungstic acid haematoxylin (MPAH) for mucoproteins (Mullen & McCarter, 1941; Luna, 1968); phloxine-tartrazine (PT) for cell granules (Lendrum, 1947); Alcian blue for acid-mucopolysaccharides (Luna, 1968).

RESULTS

In one of the specimens examined Paneth cells were located only in the crypts of Lieberkühn about 104 cm from the pyloric valve, and they were grouped mainly along the sides in the lower half of the crypts (Fig. 1).

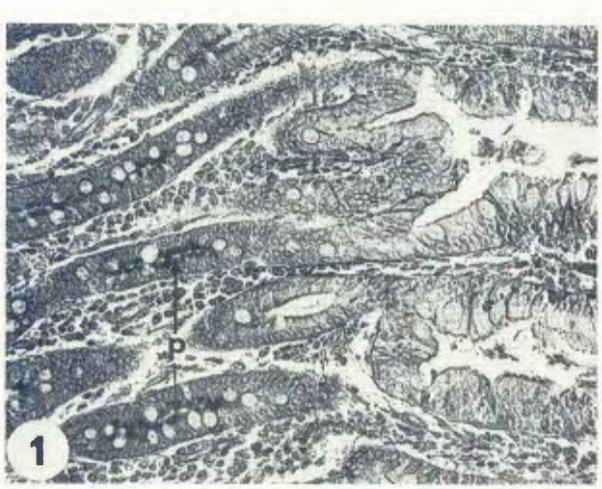

FIG. 1 Longitudinal section through the crypts of Lieberkühn. Paneth cell granules (p) are mainly grouped along the sides in the lower half of the crypts: MPAH × 200

They had the appearance of truncated pyramids, with a proximally situated nucleus and distally placed secretory granules (Fig. 2). Proximally, the Paneth cells were lightly basophilic, whole the Golgi apparatus was seen to be situated just distal to the nucleus, where, because of lack of staining ability, it appeared as a negative image (Fig. 2).

Both MPAH and PT tended to stain the cores of the granules, with MPAH staining less intensely (Fig. 3). Cores showed up only faintly with HE and PAS, and not at all with Alcian blue. The number of Paneth cells varied in different crypts, and some crypts had no Paneth cells at all (Fig. 4). Some cells contained fine-cored granules (Fig. 5), others had coarser cored granules (Fig. 6), and still others both fine- and coarser cored granules (Fig. 7).

DISCUSSION

Finding Paneth cells in the crypts of the jejenum of the pig, *Sus scrofa* (Linnaeus, 1758), in the present study, will finally correct an erroneous concept that has been perpetuated in textbooks for many decades, namely, that Paneth cells are absent from the porcine crypt epithelium. Attention was first drawn to this erroneous concept by Sloss (1954), who described "granular cells of Paneth" in the basal portion of the pig's crypts of Lieberkühn, but her findings were ignored by later authors. Louwers & De Vos (1971) also referred to the existence of Paneth cells in the pig, and classified them according to the staining reactions of their granules.

There is evidence from the literature that the staining reaction of the granules depends largely on the fixative used. In the present study, phloxine-tartrazine was found to be the ideal stain for the cores of the granules. This may have been because the sections were initially fixed in Zenker's solution in which the mercuric chloride in conjunction with potassium bichromate could have served as an excellent mordant. Acid fixatives appear to dissolve the granules (Lillie, 1958). Thus Selzman & Liebelt (1962) showed that, after fixation in trichloracetic acid, Paneth cells appeared to be devoid of granules. When Carnoy's fluid is used, the situation is less clear. Some workers (Selzman & Liebelt, 1962) found no granules, while others (Hampton, 1965) found granules, but only when they used contrast microscopy.

The separation of the granules into core and capsule might also depend on the method of fixation used, since Hampton (1965) reported that considerable shrinkage of the granules occurred in glutaraldehyde fixation, unless 1% sucrose solution was added. Dalton (1951) had previously suggested that halos seen around Paneth cell granules were due to shrinkage. The failure of Hampton (1965) to stain the halos of mouse Paneth cell granules with Alcian blue supported the idea that, in some cases at least, the halo/capsule was a fixation artefact. In the present study of pig Paneth cell granules, it was impossible, with the fixative used, to demonstrate the capsule of the granules with Alcian blue.

Spicer, Staley, Wetzel & Wetzel (1967) stated that, in the differentiation of Paneth cell granules, the proportion of acid mucopolysaccharides and protein in the capsule and core could vary with increasing maturity and finally

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end up as a PAS-positive capsule of acid mucopolysaccharides and a predominantly protein core. In the present study, the pig Paneth cell granules did not stain very distinctly with PAS. According to Glerean, Nylceo & De Castro (1965), the granules of Brazilian ant-bear Paneth cells, for instance, gave different staining reactions: some appeared unstainable, others had a PAS-positive core and an unstainable halo, still others had halos which gave a positive Alcian blue reaction and cores which were PAS-positive. The author suggests that the different types of granule described above might all be part of a cycle of differentiation, starting as unstainable granules, and finally ending up as granules with a PAS-positive core and an Alcian blue stained halo. On elimination into the glandular lumen, almost the entire granule would react for mucopolysaccharide, giving a positive Alcian blue reaction.

Various hypotheses have been put forward regarding the differentiation of Paneth cells. Bjerknes & Cheng (1981) considered Paneth stem cells to be present more or less halfway down the crypt. In this stem cell zone, small granules would be present. Deeper down the crypt, the granules would be larger. Troughton & Trier (1969), on the other hand, found undifferentiated cells between the Paneth cells at the bases of the crypts in the mouse duodenum. They also reported on the existence of laterally located stem cells. In essence, therefore, 2 stem cell zones might be operating: one at the bases of the crypts and the other on the sides higher up. The latter, in addition, must also be the source of the columnar absorptive and goblet cells. In this investigation, granules of varying sizes were found in all Paneth cells, and no pattern of increasing granule size as the Paneth cells migrated was seen. No proof could be found that maturing Paneth cells selectively migrate towards the bases of the crypts, as reported by Bjerknes & Cheng (1981).

That Paneth cells were found in the jejunum, as opposed to the duodenum of the pig in this particular investigation, is not unusual. Taylor & Flaa (1964) reported that in their study of Paneth cell granules in rats the cells increased in number from the duodenum to ileum, the greatest number occurring in the ileum. However, the small size of the Paneth cell granules, together with their infrequent occurrence throughout the crypts of the small intestine of the pig, might have some bearing on the controversy surrounding the existence of these granules in the pig.

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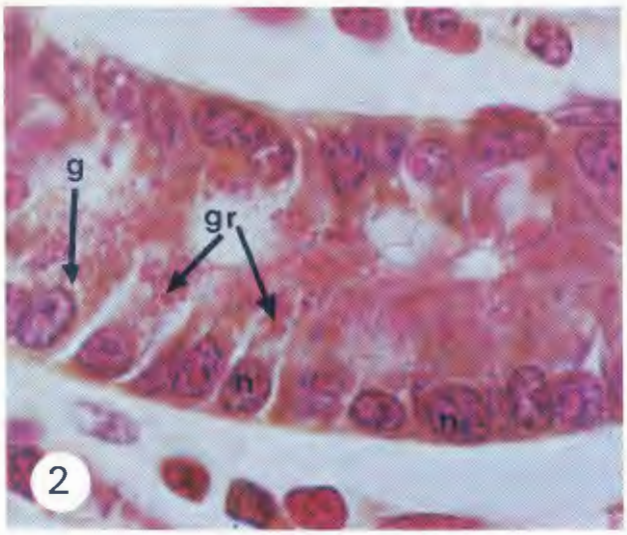

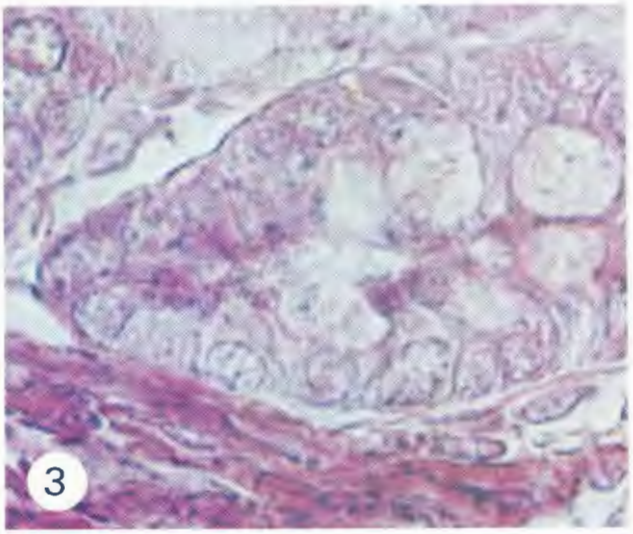

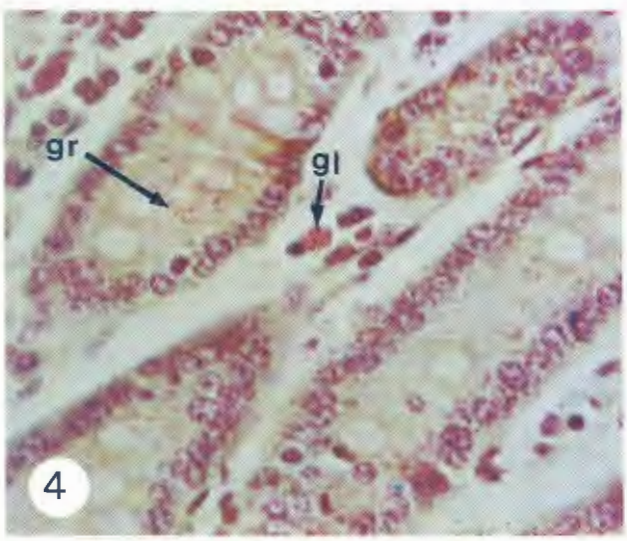

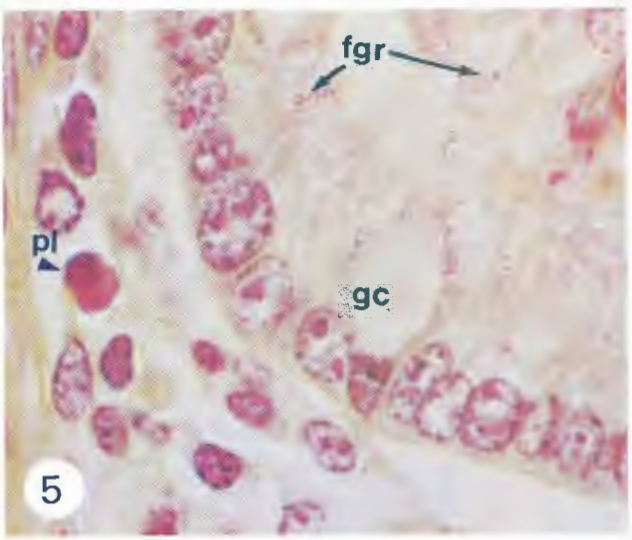

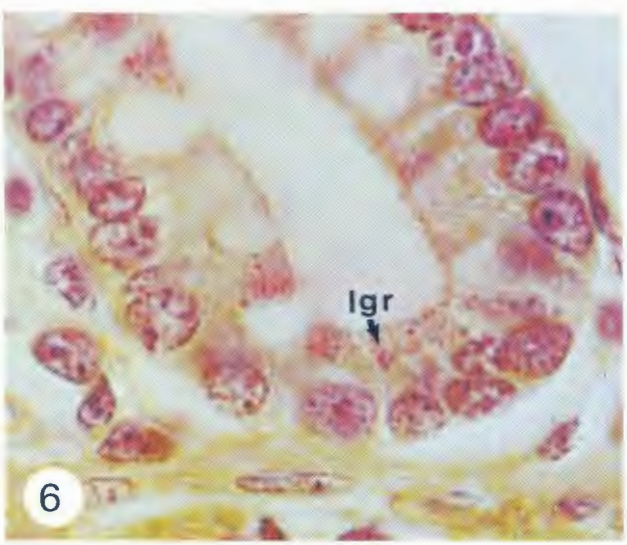

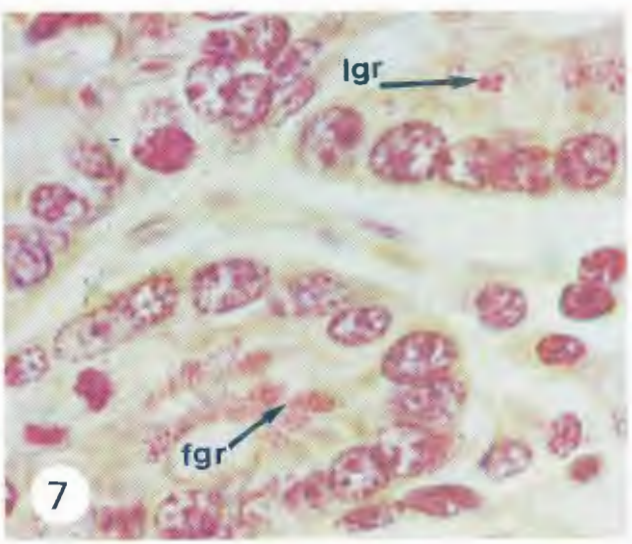

FIG. 2 Basal portion of crypt, showing Paneth cells with proximally situated nuclei (n) surrounded by basophilic cytoplasm and secretory granules (gr) placed distally. The Golgi apparatus (g) appears as a negatively stained area distal to the nucleus: PT × 1200

FIG. 3 Basal portion of crypt, showing Paneth cell granules with their cores stained up with MPAH: MPAH × 1200

FIG. 4 Paneth cell granules (gr) sometimes occur very infrequently in the crypts of Lieberkühn. PT also stains the granules of a globule leucocyte (gl): PT × 500

FIG. 5 This crypt shows exclusively the formation of fine cored granules (fgr) in the Paneth cells. A goblet cell (gc) as well as a plasma cell (pl) are also visible: PT × 1200

FIG. 6 Paneth cells, situated at the extreme basal portion of this crypt, show almost exclusively the formation of large cored granules (lgr): PT × 1200

FIG. 7 Both fine (fgr) and coarser cored granules (lgr) can occur in cells of the same crypt, or separately in cells of adjacent crypts: PT × 1200