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Histological changes in the small intestine of the young pig and their relation to macromolecular uptake

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INTRODUCTION

There is general agreement that in the suckling pig the transfer of immune globulins from the colostrum to the circulation takes place only during the first 24–48 h after birth and that during this period many other proteins can pass from the gut into the circulation (Norbring & Olsson, 1957, 1958; Olsson, 1959*a*, *b*; Lecce, Matrone & Morgan, 1961*a*, *b*; Pierce & Smith, 1967*a*; Hardy, 1969*b*). It has also been demonstrated that polyvinyl pyrrolidone (PVP) of mean mol.wt. 40000 (K. 30) can be absorbed into the circulation during this period (Lecce *et al.* 1961*a*, *b*). In addition, a quantitative study of factors which influence the absorption of the larger polymer PVP K. 60 (mean mol.wt. 160000) has been reported (Hardy, 1969*a*).

The investigations referred to above have been mainly concerned with the passage of macromolecular solutes from the gut into the blood, so that, with the exception of qualitative observations of the presence of fluorescein-labelled proteins in the intestinal epithelial cells, there has been no systematic exploration of the initial uptake of macromolecules by the intestinal epithelial cells.

A technique has been developed in the young rat which allows the quantitative determination of the uptake of [^{125}I]PVP K. 60 by the small intestine as a whole, and also the identification of those cells which actually take up the polymer (Clarke & Hardy, 1969*a*). The first section of the results to be reported in this paper concerns the application of this technique to the young pig, and from this it will be shown that the cells of the terminal small intestine retain the ability to take up PVP for up to 12 days after the time at which its transfer into the blood ceases. The second part of the paper is devoted to an examination of the histological changes at different levels of the small intestines of pigs during the first 3 weeks after birth and to the relation between these changes and PVP uptake.

METHODS

Pigs. Suckled piglets were obtained from local farms, transported to the laboratory in heated boxes and fed approximately 1 h from the time they were removed from the sow.

Method of feeding. Animals were fed 20 ml of a 2 % aqueous solution of polyvinyl

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pyrrolidone K. 60 (Fluka A. G.) containing 2–4 μc [^{125}I]PVP K. 60 (Radiochemical Centre, Amersham) by stomach tube.

Measurement of PVP uptake. Four hours after feeding, the pigs were killed by an intraperitoneal injection of sodium pentobarbitone, weighed and sexed. The age of an animal specified in the text refers to the age when killed. The following samples were obtained from each animal and were placed in glass tubes to await the estimation of radioactivity: intracardiac blood sample, lungs, stomach and contents, and large intestine and contents. In larger animals the stomach and large intestine were homogenized (Ato-mix: M.S.E.) and the [^{125}I] content estimated from representative aliquots. The small intestine was dissected free of mesentery and placed, without stretching, on a centimetre scale. The following samples were taken for histology (H) and scintillation counting (C) (pylorus = 0): 0–2 cm (H), 3–12 (C), 40–49 (C), 50–52 (H), 90–99 (C), 100–102 (H), etc. This sequence was repeated until the ileocaecal valve was reached, when the final 2 cm were taken for histology and the immediately preceding 10 cm taken for counting. The intestine was divided with a sharp razor blade and samples for histology were placed immediately in Bouin's fluid. The 10 cm segments for scintillation counting were flushed through with 0·9 % NaCl while being gently massaged with forceps to ensure the expulsion of [^{125}I]PVP remaining in the lumen (Clarke & Hardy, 1969*a*). After washing, gut segments were placed in glass tubes for scintillation counting.

This technique allowed the estimation of [^{125}I]PVP uptake and an histological examination of the immediately adjacent tissue from the first and the last 10 cm of the small intestine and from the intervening gut at 50 cm intervals.

Calculation of total PVP uptake. [^{125}I]PVP uptake by the small intestine in rats was expressed as a percentage of the [^{125}I]PVP entering the small intestine ([^{125}I]PVP fed − [^{125}I]PVP in stomach) (Clarke & Hardy, 1969*a*). The same index of uptake (percentage PVP uptake) is used in this paper, but since [^{125}I]PVP uptake was measured in only 10 cm of each 50 cm length, it has been assumed that the total uptake for each 50 cm length can be represented by 5 times the uptake of the 10 cm sample.

Site of PVP uptake in the small intestine. To facilitate comparison with other species this has been represented as described for rats by Clarke & Hardy (1969*a*). Thus in Figs. 2 and 25 the width of each bar represents the length of the gut fraction relative to that of the whole small intestine. The area of each bar represents the net radioactivity in that gut fraction as a percentage of the total radioactivity which had passed the pylorus:

$$\text{relative PVP uptake} = \frac{\text{net counts/min of fraction}}{\text{total net counts/min passed pylorus}} \times \frac{\text{length of small intestine}}{\text{length of fraction}}$$

Measurement of radioactivity. Samples were analysed in a Nuclear Chicago model 4222 automatic gamma counting system, to an accuracy of 1 % or better.

Histology. Fixation for 24 h in Bouin's fluid was followed by dehydration, paraffin-wax embedding and sectioning at 5μm. Sections were stained with Mayer's haemalum, Van Gieson and alcian blue, or Heidenhain's iron haematoxylin and Van Gieson or the periodic acid–Schiff (PAS) technique and alcian blue.

RESULTS

The effect of age on [^{125}I]*PVP uptake*

In unsuckled pigs [^{131}I]PVP K. 60 is readily transferred from the intestinal lumen into the circulation if it is dissolved in colostrum. However, if pigs are allowed to suckle for 2 h immediately after birth and are then fed [^{131}I]PVP K. 60 at 5 h of age it is found that virtually none of the [^{131}I]PVP fed passes into the blood during the subsequent 6 h (Hardy, 1969*a*).

The present experiments were undertaken to estimate PVP uptake by the intestine and would have been prejudiced by any loss of PVP from the epithelial cells into the blood. For this reason suckled animals were used and the absence of [^{125}I]PVP transfer into the blood has been repeatedly confirmed by the analysis of blood samples at the end of the 4 h absorption period.

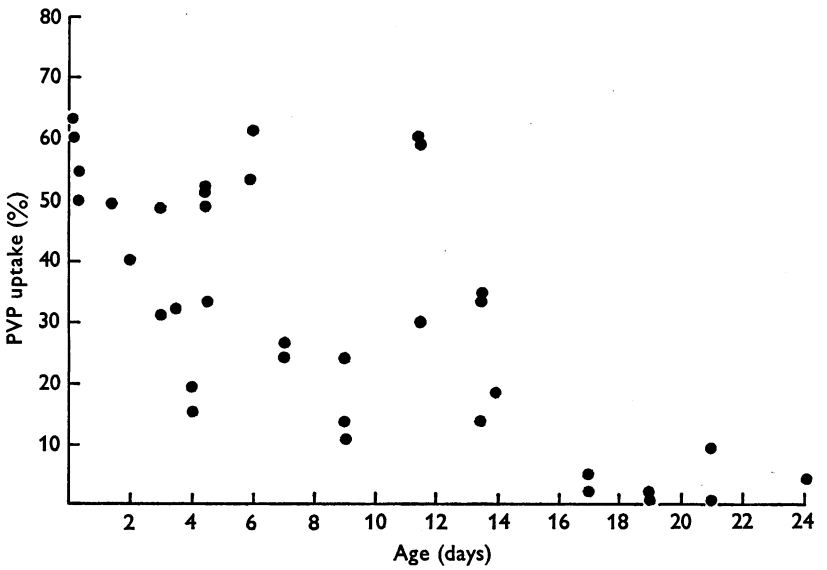


Fig. 1. Changes with increasing age in the uptake of [^{125}I]PVP by the small intestine of the young pig.

The results of measurements of PVP uptake in 36 pigs aged between 9 h and 24 d are shown in Fig. 1. There was considerable and as yet unexplained variation between individuals at certain ages, but it is clear that in some animals the ability to take up PVP was retained for up to 12 d after birth and that there was a decline in uptake in animals tested at 12–15 d of age. However, in certain individuals, PVP uptake was reduced at a much earlier age. These differences in PVP uptake with age and between individuals can be consistently related to the histological appearance of the small intestine (see below).

The site of the [^{125}I]*PVP uptake*

The way in which the longitudinal pattern of [^{125}I]PVP uptake by the small intestine varied with age is represented in Fig. 2. In the 9 h old animal, the polymer

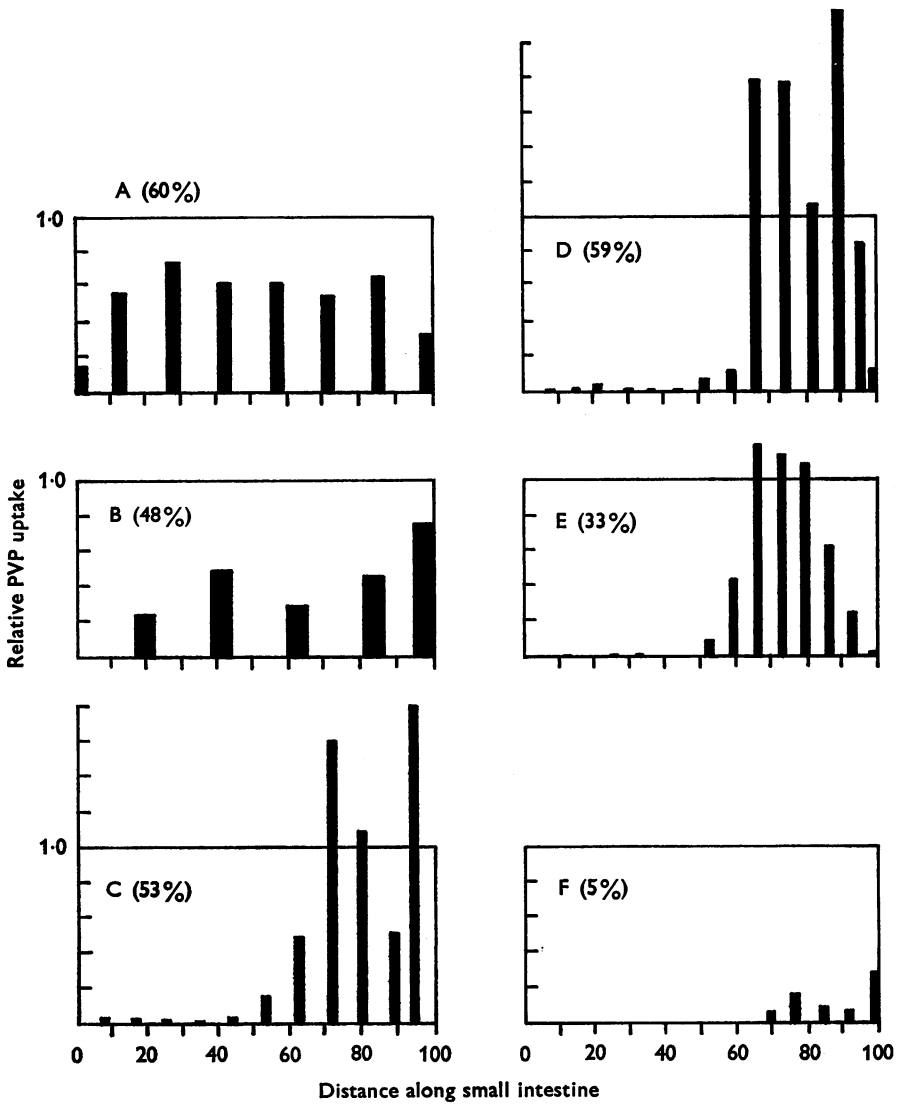


Fig. 2. Changes with age in the longitudinal distribution of [^{125}I]PVP uptake in the small intestine of the young pig 4 h after feeding. A, 9 h; B, 37 h; C, 6 d; D, 11½ d; E, 13½ d; F, 17 d. Abscissa: distance along small intestine; 0, pylorus; 100, ileocaecal valve. Figures in parentheses indicate total uptake of [^{125}I]PVP as a percentage of [^{125}I]PVP which had passed the pylorus. Uniform and total uptake of all PVP passing the pylorus would be represented by the area enclosed by the rectangle.

was taken up relatively uniformly throughout the intestine, with the exception of the two extremities. Between this age and 6 d after birth PVP uptake gradually became restricted to the terminal ½–⅓ of the intestine, although there was little decline in the total uptake. In the 11½ d old animal PVP uptake remained high in the terminal third of the intestine while in the two final animals aged 13½ and 17 d there was a decline in total PVP uptake.

In those animals in which PVP uptake fell to low levels soon after birth, the sequence of changes in the longitudinal distribution of PVP uptake was similar to that described above.

Histological Observations

Two major morphological features distinguish the epithelium of the villi of the newborn suckled piglet from that of a piglet 1 month old. These are (i) the presence in the cytoplasm of watery vacuoles and (ii) the position of the nucleus at the apical pole of the cell.

To facilitate understanding of the relation of these features to macromolecular uptake by the epithelial cells, and macromolecular absorption into the blood-stream, the small intestine has been divided, for descriptive purposes, into four zones. The changes with age in the structural features of each zone will be described.

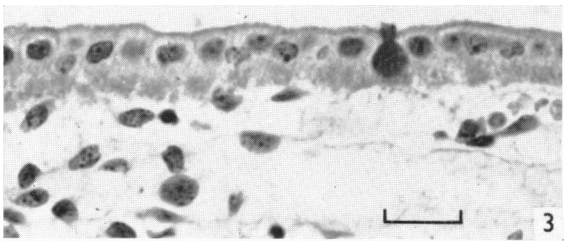
Zone 1

The first zone ('upper duodenum') comprises the first few cm of the small intestine. The histological appearances vary with the precise site from which the specimen is taken, but in general, close to the pylorus, the nuclei are basal, vacuolation is absent, and little morphological change, apart from enlargement, occurs with age.

Zone 2

The next zone ('duodenum/jejunum') stretches to a point about half-way down the small intestine. In unsuckled piglets 5 and 23 h old, the villus epithelium is cuboidal or low columnar, non-vacuolated, with the nucleus nearer the apical than the basal pole of the cell (Fig. 3). The subnuclear cytoplasm is dense and acidophilic, but has no structure discernible at this resolution. The appearance in the 77 h unsuckled piglet is similar, except that moderate subnuclear vacuolation is now present. In the suckled piglet 12 h of age, however, dramatic changes have occurred. All the villus cells, with the exception of a few at the base of each villus, have nuclei displaced to the apical pole of the cell by a large vacuole (Figs. 4, 5). Over the space of a few cell positions (Fig. 5), the vacuole increases rapidly in size, but is always basal to the nucleus. On the upper part of the villus (Fig. 4) the vacuoles are full of acidophilic protein, but the vacuoles on the lower part of the villus appear to be empty (Fig. 5), although protein is present in the lumen of the intestine. This protein has a scalloped, eroded appearance where it is in contact with the epithelial cells (Fig. 4). In addition, the sub-epithelial tissue contains protein, as do the lacteals (Fig. 4).

At 36 h the vacuoles are smaller, and only the upper 80–90 % of the villus is clothed with these cells, which still have apical nuclei. The basal cytoplasm is still dense, and mildly acidophilic. The sub-epithelial tissue and lacteals no longer contain obviously stainable protein. From 74 h the proportion of villus clothed by vacuolated cells is progressively reduced (Fig. 6), the reduction occurring more rapidly distally than proximally. Thus proximally the upper 60 % of the villus epithelium is vacuolated at 74 h, while half-way down the small intestine the proportion has fallen to 30 %. In addition, the lowermost vacuolated cells on the villus now have nuclei basal to the vacuole; a few cell positions higher the nucleus may be sandwiched between two vacuoles, while higher still, most of the nuclei are still apical (Fig. 6).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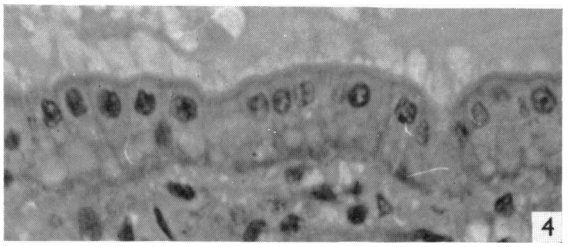
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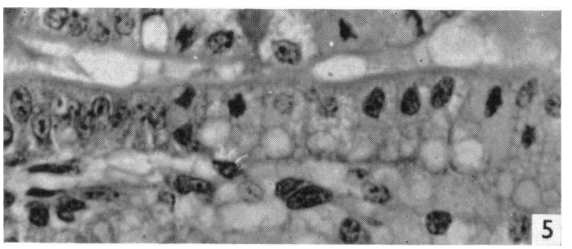
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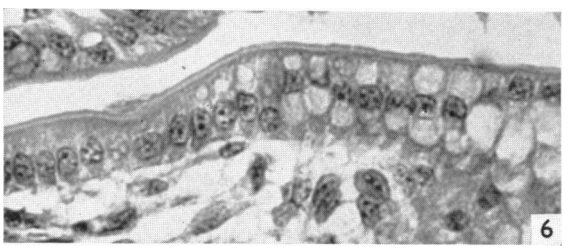
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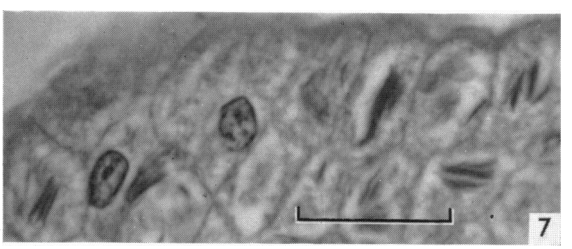
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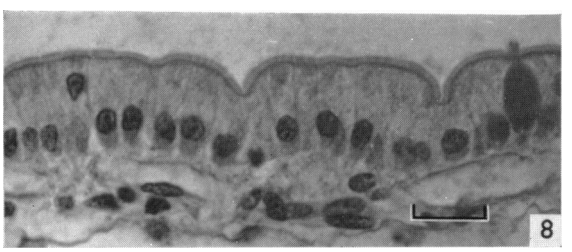
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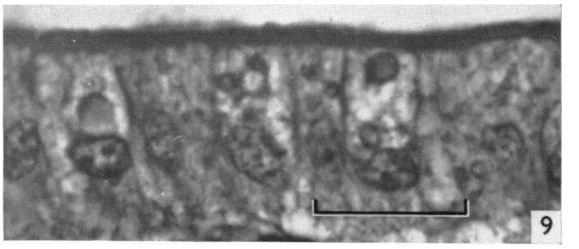
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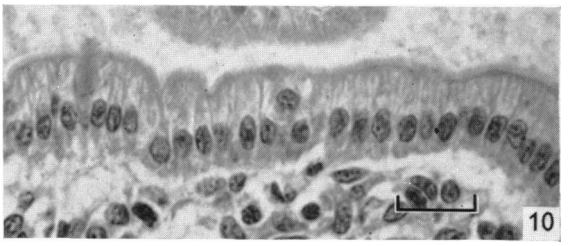
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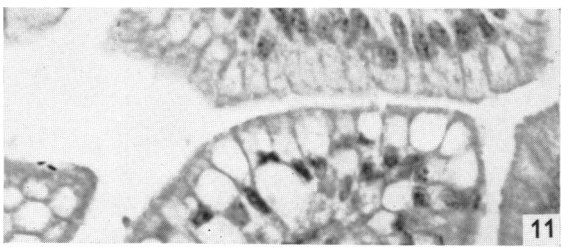
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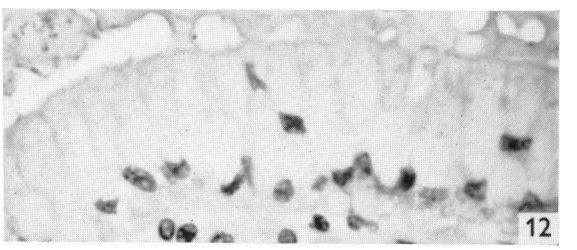
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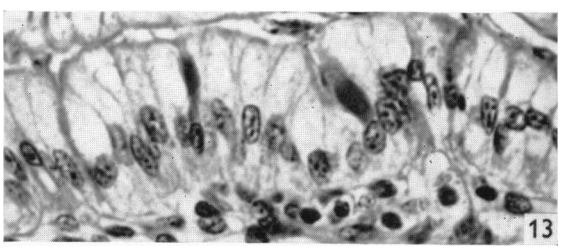
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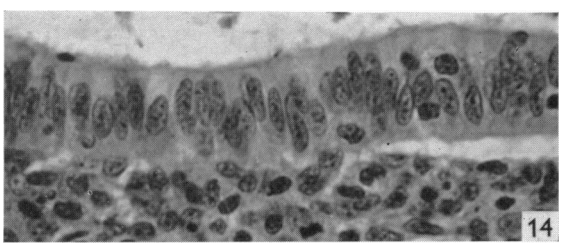
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At 86 h the vacuolated epithelium is still further restricted to the upper part of the villus, and the nuclei are less consistently apical, but, in both animals examined at this age, there are visible in some of the vacuoles lamellar bodies (Fig. 7) staining with haemalum and with iron haematoxylin whose appearance is suggestive of the shell of a vacuolar structure from which the fluid contents have been evacuated.

By 108 h virtually all traces of vacuolation have disappeared, and the apical cytoplasm of the uppermost cells on the villus appears to contain an area of granular or reticular material, not resolvable as vesicular structures (Fig. 8). Apical nuclei are relatively uncommon at this age, and the acidophilia of the basal cytoplasm is less pronounced. Cells lower on the villus at this age have an appearance similar to that of the cells seen covering the villi of older piglets. At 176 h occasional vesicles and vacuoles are seen in the apical cytoplasm (Fig. 9), but they are not present at 236 h (Fig. 10).

The only other noteworthy feature of this zone is that one of the older piglets (14 d), which was small for its age, had a short small intestine, with stunted villi and mitoses in the crypts so numerous as to be obvious to casual inspection (Fig. 15). This appearance is reminiscent of the coeliac syndrome in man.

The third zone ('jejunum/ileum') combines features of the second and fourth zones, and its description will, for clarity, follow that of the latter.

Zone 4

The fourth zone ('ileum') stretches from about three-quarters down the small intestine to the ileocaecal valve. The terminal ileum of the unsuckled piglets (5 and 23 h old) contains villi bearing variable numbers of cells with empty vacuoles of

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Figs. 3–14. Stained with haemalum, Van Gieson and alcian blue. Scale line is 20μm; it applies to the figure marked, and to all subsequent figures until a change in magnification occurs. For each figure, the age of the specimen at death is given, together with the site in the intestine from which it was taken (pylorus = 0, ileocaecal valve = 100), and whether it is from the upper (U), middle (M) or basal (B) third of the villus. Where relevant, the bottom of the villus is to the left, the top to the right. All animals suckled unless specified.

Fig. 3. 9 h, unsuckled. 15. U. Epithelial nuclei apical; basal cytoplasm dense, with no vacuoles.

Fig. 4. 12 h. 15. U. Nuclei apical in epithelial cells; protein in lumen, vacuoles, connective tissue and lacteals.

Fig. 5. 12 h. 15. B. Nuclei apical in vacuolated cells; vacuoles empty, although protein in lumen of intestine.

Fig. 6. 74 h. 25. M. Onset of vacuolation now in mid-villus; vacuole initially apical to nucleus.

Fig. 7. 86 h. 14. U. Lamellar structures in cytoplasm.

Fig. 8. 108 h. 29. U. No vacuolation of epithelium resolvable; nuclei basal.

Fig. 9. 176 h. 44. U. Vacuoles of varying size in epithelium.

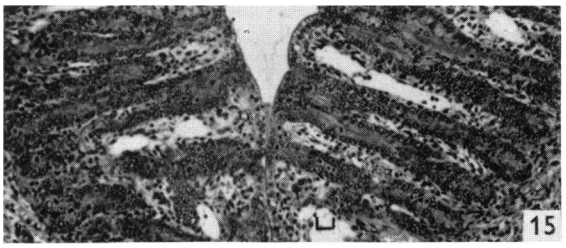
Fig. 10. 233 h. 33. U. Vacuoles absent once more.

Fig. 11. 23 h, unsuckled. 86. U. Variable vacuolation and nuclear position from one villus to another.

Fig. 12. 12 h. 100. U. Compare with Fig. 4. Nuclei basal; protein in lumen, vacuoles, connective tissue and lacteals.

Fig. 13. 320 h. 94. U. Vacuolated cells in region free of lymphoid tissue.

Fig. 14. 320 h. 94. U. Same section as Fig. 13. Absence of vacuolation on villi overlying lymphoid tissue.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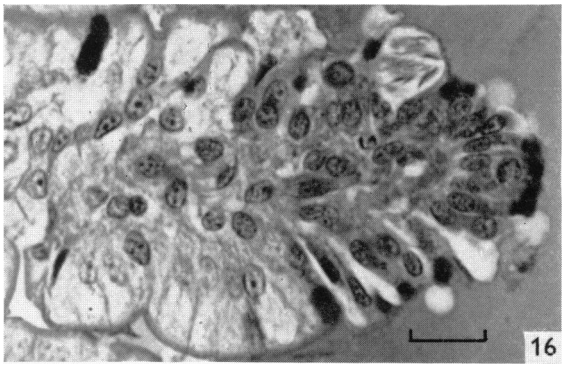
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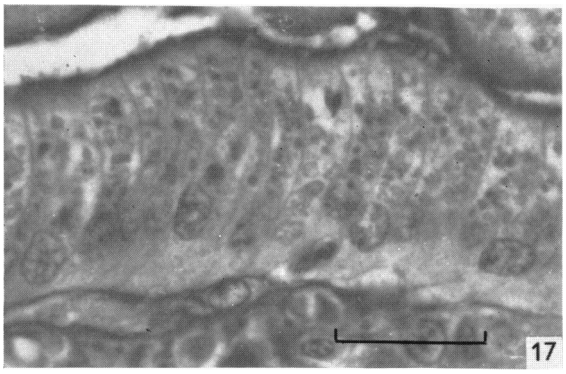
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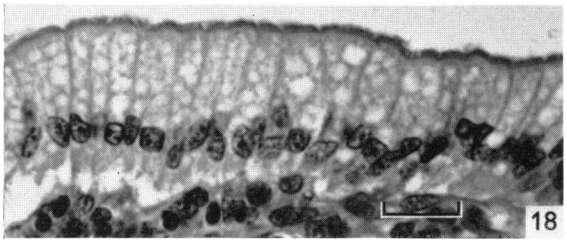
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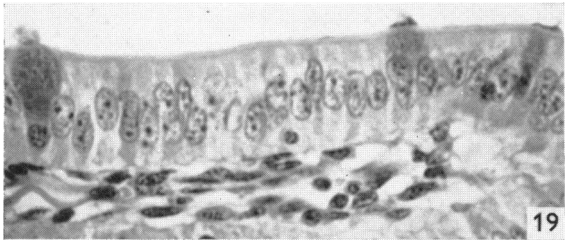
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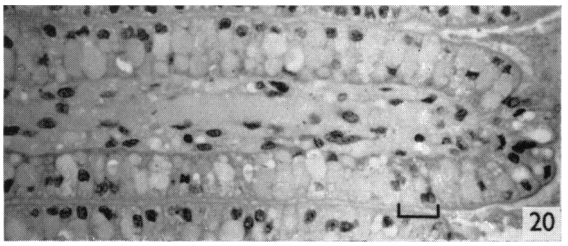
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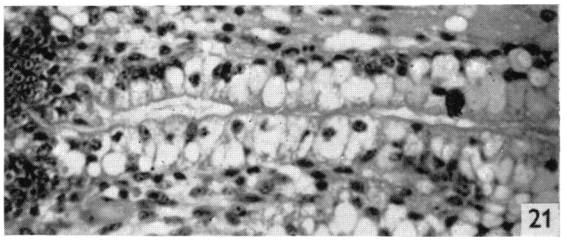
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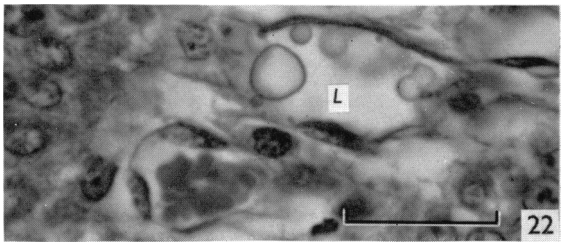

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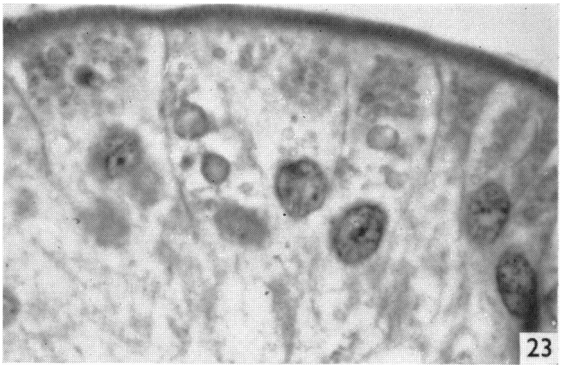
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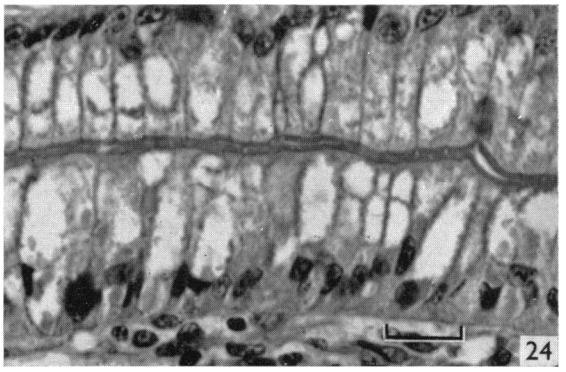
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moderate size and with variable nuclear position (Fig. 11). In the 77 h unsuckled piglet the nuclei are still apical, but the cytoplasm shows a reticular rather than vacuolated appearance. The 12 h suckled piglet, however, shows a more heavily vacuolated, protein-containing epithelium, similar to that of the second zone at this age, except that the nuclei are basal in the cell (Fig. 12). Acidophilic protein, both intra-epithelial and subepithelial, is present in young animals, but is not visible in the sections from older animals (70 h). The nuclei in the period 76–98 h may be apical in the cells at the top of the villus, and for a period after this (up to 151 h) there may be a zone half-way up the villus where some nuclei occupy an apical position in the cell.

The vacuolated cells are replaced from below by cells which show little or no sign of vacuolation. Thus at 86 h the vacuolated cells are restricted to the top of the villus. They may contain the lamellar bodies described above, and in animals of this age clumps of cells are seen at the tops of some villi, with a structure similar to that of the surface mucous cells of the gastric mucosa (Fig. 16). At 103 h, however, the cells on the villus begin to show not the single large vacuole characteristic of the earlier period, but a more variable, diffuse, multiple vacuolation (Fig. 17). From this age up to 14 d there is a tendency for the degree of vacuolation of these cells to increase while most of the villus is covered by these cells (Fig. 18). From 14 d the vacuolated cells, as in the proximal small intestine, tend to be restricted to the upper part of the villus, and they disappear by 21 d (Fig. 19).

In the terminal ileum variable amounts of lymphoid tissue are seen which tend to increase in quantity with age. In animals of up to 86 h of age there is relatively little lymphoid tissue underlying the mucosa and there is little difference between the degree of vacuolation of villi overlying and those not overlying lymphoid tissue. Beyond this age, as lymphoid tissue increases both in its depth and in the proportion of the intestinal circumference involved, vacuolation becomes less extensive on villi

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Figs. 15–24. Stained with haemalum, Van Gieson and alcian blue. Scale line is 20μm; it applies to the figure marked, and to all subsequent figures until a change in magnification occurs. For each figure the age of the specimen at death is given, together with the site in the intestine from which it was taken (pylorus = 0, ileocaecal valve = 100), and whether it is from the upper (U), middle (M) or basal (B) third of the villus. Where relevant, the bottom of the villus is to the left, the top to the right. All animals suckled unless specified.

Fig. 15. 336 h. 44.—. Long crypts and short villi as in coeliac syndrome. Malabsorption may account for the small size of this piglet.

Fig. 16. 86 h. 86. U. Vacuolated cells (some containing lamellar bodies), and cells like the surface mucous cells of the gastric mucosa.

Fig. 17. 108 h. 88. U. Multiple, diffuse vacuolation of epithelial cells.

Fig. 18. 320 h. 80. U. Vacuoles larger.

Fig. 19. 504 h. 85. U. Epithelium no longer vacuolated.

Fig. 20. 12 h. 74. U. Protein in lumen, vacuoles, connective tissue and lacteals. Some epithelial nuclei are apical, like those higher up the intestine; others are basal, like those between this point and the ileocaecal valve.

Fig. 21. 12 h. 74. B. Same section as Fig. 20. Vacuoles are empty, and some nuclei are apical.

Fig. 22. 82 h. 72. U. Vacuolar structures of various sizes in the lumen of the lacteal. (L).

Fig. 23. 151 h. 63. U. Small vacuoles in the epithelial cells.

Fig. 24. 270 h. 75. U. Large vacuoles in epithelial cells; nuclei basal.

overlying lymphoid tissue. Indeed it may be absent altogether (Fig. 14), although neighbouring villi, not overlying lymphoid tissue, may still be relatively heavily vacuolated (Fig. 13).

Zone 3

The changing histological appearances of the third zone ('jejunum/ileum') comprising approximately the third quarter of the small intestine, are complex, but may be summarized by saying that in the young piglet this zone is similar to the second zone, but becomes more like the fourth as the piglet develops. Thus, in the unsuckled 5 h piglet, non-vacuolated cells with apical nuclei (compare with Fig. 3) extend well into the fourth quarter of the small intestine. By 77 h in the unsuckled animal the nuclei are all basal, with empty apical cytoplasmic vacuolation on the upper part of the villus. However, in the 12 h suckled piglet the nuclei are apical to protein-filled vacuoles in the entire third quarter, and similar cells on the upper villus are seen (Fig. 20) at the junction with the fourth quarter while the lower villus is clothed by vacuolated cells, usually with basal nuclei (Fig. 21). As development proceeds, the cells with apical nuclei are progressively restricted to the top of the villus. They are succeeded by vacuolated cells with basal nuclei, and at 86 h vacuolar structures are seen in the lacteals (Fig. 22). The villus then becomes clothed with cells similar to those in the fourth zone. These are not obviously vacuolated at first, but subsequently contain small vacuoles (Fig. 23). These cells in turn appear to be replaced at 14 d, for a brief period, by more obviously vacuolated cells resembling those in the distal quarter (Fig. 24), and, likewise, these become restricted to the top of the villus, ultimately giving way to 'mature' cells by about 17 d of age (cf. Fig. 19).

DISCUSSION

Most previous histological investigations of the intestinal epithelium of young pigs have neglected two facts: first, that the epithelium is still active in macromolecular uptake for many days after transfer of macromolecules into the blood has ceased; and secondly, that there is a longitudinal zonation of structure.

There is general agreement that, except in the terminal ileum, the epithelium in the unsuckled pig is not vacuolated (Sibalin & Björkman, 1966; Pierce & Smith, 1967*b*; Kraehenbuhl & Campiche, 1969), but vacuoles appear after suckling (Sibalin & Björkman, 1966) and contain macromolecular material (Payne & Marsh, 1962*a*, *b*; Kaeberle & Segre, 1964). Matisson & Karlsson (1966) did not see the large vacuoles described by all other investigators. Reports of the relative positions of nuclei and vacuoles are conflicting; our findings confirm those of Pierce & Smith (1967*b*), who show nuclei apical in the cells of our zones 2 and 3 and basal in our zone 4. Matisson & Karlsson (1966), however, describe basal nuclei in the 'mid-jejunum' of the fasting new-born pig, although their Fig. 1 shows clearly that the nuclei are in the apical half of the cell. Comline, Pomeroy & Titchen (1953) did not state the precise site from which their specimens were taken, but describe mainly apical vacuoles in piglets 6–8 h after suckling. This finding is compatible with our observations on zone 4, but their observation of basal vacuoles in piglets 15–18 h after suckling is consistent with specimens taken from our zones 2 and 3.

Those who have examined older animals describe the disappearance of vacuoles from the cells after about 36 h of age, but their specimens have been taken from our zone 2 ('proximal jejunum', Sibalin & Björkman, 1966; 'mid-jejunum', Kenworthy, Stubbs & Syme, 1967).

The present results are in agreement with the electron microscope studies of Hardy, Hockaday & Tapp (1970). We have found no other descriptions of the structure of the distal small intestine of piglets during the period of macromolecular uptake we have described.

The association between vacuolation and the ability to take up PVP in the pig is similar to that found in the rat (Clarke & Hardy, 1969*a*, *b*), the goat (Clarke & Hardy, 1971) and the rabbit, ferret, guinea-pig and kitten (Clarke & Hardy, 1970).

In some species the age at which PVP uptake ceases coincides with the onset of ingestion of solid food. Indeed, in the pig, PVP uptake falls at about 2 weeks of age, which is the time that the young first show interest in creep feeds. However, it has been demonstrated that, in the rat at least, closure is not caused by the ingestion of solid food (Halliday, 1956).

The demonstration during the present investigation that the terminal small intestine can take up [^{125}I]PVP K. 60 up to 12–14 d after birth in some animals necessitates the reappraisal of previous work relating to the cessation of macromolecular absorption ('closure') in the young pig.

There is general agreement in the literature that the transfer into the blood of specific antibodies (Young & Underdahl, 1950; Speer, Brown, Quinn & Catron, 1959; Miller *et al.* 1962), PVP K. 30 (Lecce & Morgan, 1962; Lecce, Morgan & Matrone, 1964), egg proteins (Lecce & Morgan, 1962) and [^{131}I]PVP K. 60 (Hardy, 1969*a*) ceases after suckling, although the delay reported between suckling and the cessation of transfer varies between 5 h for PVP K. 60 and 30 h for egg protein; this discrepancy has been discussed by Hardy (1969*a*). In the unsuckled animal the transfer into the blood of PVP K. 30 and egg protein persists for at least 86 h after birth (Lecce & Morgan, 1962) and that of [^{131}I]PVP K. 60 for at least 65 h after birth, although in the latter case the amount transferred is much reduced in comparison with transfer immediately after birth (Hardy, 1969*a*).

In the unsuckled animal absorption, as measured by the transfer of egg proteins and PVP K. 30 from the gut lumen into the circulation, can be curtailed by the oral administration of dialysates of colostrum or milk (Lecce *et al.* 1964), or amounts of glucose in excess of 300 m-equiv (Lecce, 1966). These authors consider that the termination of macromolecular transfer by these agents reflects the process normally initiated by suckling and have assumed that both these phenomena may be attributed to the cessation of pinocytosis. Furthermore, Lecce (1966) has proposed that pinocytosis is curtailed by the exhaustion of supplies of apical cell membrane due to the pinocytotic stimulant action of glucose or of low mol. wt. agents in colostrum. Such an explanation now seems unlikely, since pinocytosis is probably not the limiting process in the transfer of macromolecules to the blood and persists after such transfer has ceased.

The present demonstration of the protracted ability of the suckled pig to take up PVP conflicts with the reports of Payne & Marsh (1962*a*, *b*) that suckling terminates the uptake of fluorescein-isothiocyanate (F.I.T.C.) tagged γ globulins.

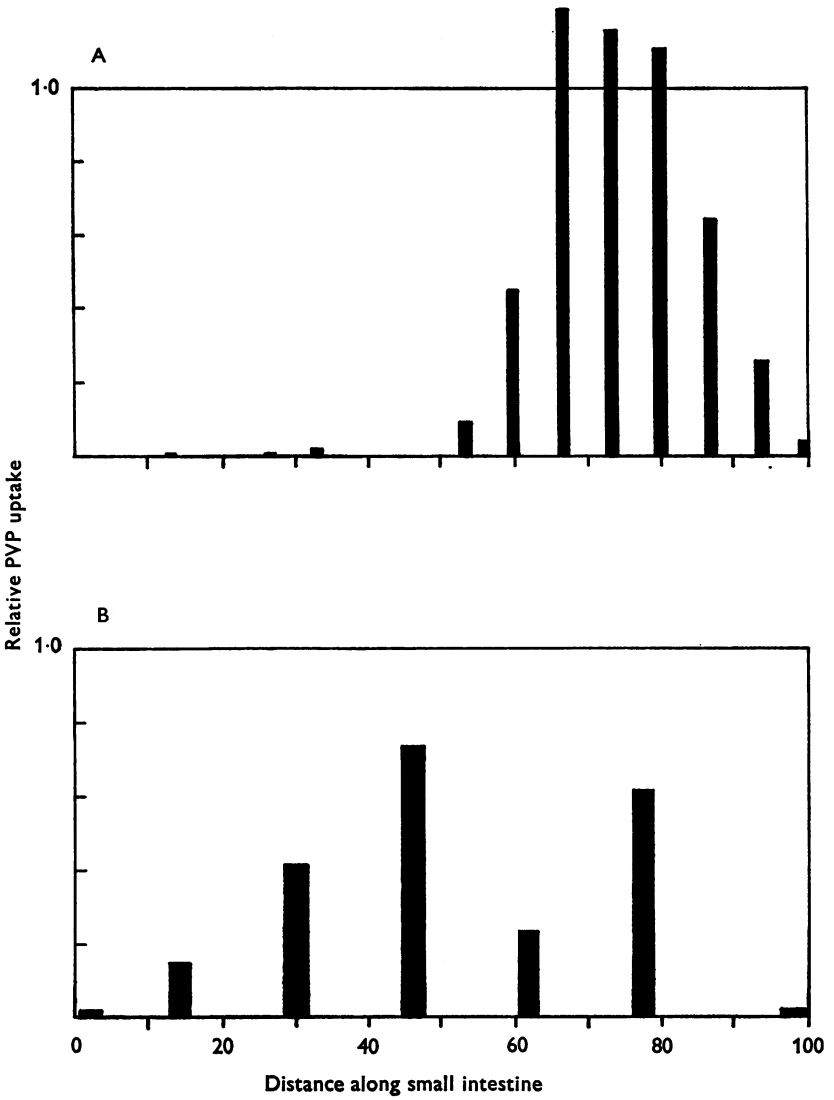

Fig. 25. Effect of starvation on the longitudinal distribution of [^{125}I]PVP uptake by the small intestine of the young pig. Both pigs 77 h old, killed 4 h after feeding PVP. A, Pig left with mother from birth to 72 h of age. B, Pig removed from mother immediately after birth, before suckling, and maintained in an incubator until killed: intramuscular injections of 0·25 ml distavone (75000 i.u. procaine penicillin; 62·5 mg dihydrostreptomycin sulphate) and 20 ml water fed by stomach-tube at 1, 25, and 49 h after birth. Abscissa as Fig. 2.

These workers examined sections from an unspecified region of the small intestine after oral administration of F.I.T.C. γ globulin or injection of this protein directly into the intestine in animals anaesthetized with pentobarbital sodium. However, if in their experiments the segments examined for F.I.T.C. γ globulin in both suckled and unsuckled animals were taken from the proximal third of the intestine, the

apparent cessation of uptake after suckling can be explained. Fig. 25 in the present paper illustrates the difference in the distribution of [^{125}I]PVP uptake in suckled and in unsuckled animals killed 77 h after birth. In the suckled animal PVP uptake is essentially restricted to the distal two-thirds of the intestine, whereas there is still considerable uptake in the more proximal portion of the intestine of the unsuckled animal. This result also affords an explanation for the effect on F.I.T.C. γ globulin uptake of ligating the intestine before suckling, reported by Payne & Marsh (1962*a*).

Previous observations on the transfer of macromolecular material from the lumen of the small intestine into the circulation are compatible with the present results on uptake into the villous epithelial cells, if it is assumed that closure takes place in two stages. We propose that in the unsuckled animal there are cells which can take up macromolecules from the intestinal lumen and then expel them through the lateral or basal surfaces of the cell into the circulation. Subsequently a change occurs in the characteristics of these cells so that they lose the ability to release intracellular macromolecules from the lacteal border but retain the power to take them up from the intestinal lumen. The second stage of closure comprises the progressive loss of the ability to take up macromolecules into the cells of the terminal ileum; this process shows considerable individual variation but is delayed in some animals until 12–14 d after birth.

The second stage of closure—the termination of PVP uptake by the terminal ileum—can be related histologically to the progressive replacement of vacuolated cells by non-vacuolated cells from below. This second stage thus resembles the mechanism of decline in PVP uptake between 18–21 d after birth seen in the rat, in which species there is good agreement between the time course of closure and the turnover time of the villous epithelial cells as determined by tritiated-thymidine autoradiography (Clarke & Hardy 1969*b*).

The first stage of closure in the pig—that is, the cessation of expulsion of intracellular PVP—may require a change in the properties of the villous cells *in situ* since it is probably too rapid to be explained by cellular replacement, although the rate of this replacement has not been determined in pigs.

SUMMARY

1. The uptake of [^{125}I] polyvinyl pyrrolidone (PVP) of mean mol.wt. 160,000 (K. 60) by the epithelium of the small intestine has been measured after oral administration to suckled pigs up to 24 d after birth.

2. There was considerable individual variation in uptake, but in many animals less than 12 d old at least 50 % of the [^{125}I]PVP passing into the small intestine entered the intestinal wall. A decline in uptake occurred between 12 and 15 d so that the intestine in animals more than 15 d old took up little PVP.

3. The pattern of PVP uptake along the intestine changed with age. In animals 5 h old when fed, PVP was uniformly taken up at all levels, but in piglets 6 d of age and older uptake was restricted to the distal third of the intestine.

4. In the proximal half of the small intestine vacuolated epithelial cells were progressively restricted to the top of the villus during the first 4 d of life; distally,

however, vacuolated cells persisted into the third week of life.

5. There was a positive correlation between the ability of a segment of intestine to take up PVP and the presence of vacuolated cells in the epithelium of that segment.

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