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Maturation in the ferret ileal epithelium and the effect of cortisone acetate*

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INTRODUCTION

The epithelial cells covering the villi in the neonate rat ileum are specially adapted for intracellular digestion during the limited period of immunoglobulin uptake by the proximal intestine (Clark, 1959; Graney, 1968). They undertake non-selective pinocytosis of macromolecules from the intestinal lumen by way of an extensive vacuolar system (Clark, 1959). From 18 days of age, immunoglobulin uptake declines (Halliday, 1955) and the vacuolated cells covering the villi in the ileum are replaced by a mature, non-pinocytosing epithelium emerging from the crypts (Clark & Hardy, 1969). This is known as ileal maturation or 'closure'. In light microscopic studies of the neonate ferret, a similar ileal maturation has been indicated between about 34 and 40 days of age (Williams & Beck, 1969; Clark & Hardy, 1970).

The ability of various corticosteroids, such as cortisone acetate, to promote the onset of maturation in either the proximal or the ileal epithelia has been studied in detail in the rat (Halliday, 1959; Daniels, Hardy, Malinowska & Nathanielsz, 1973). Attempts to influence intestinal epithelial maturation in the dog, hedgehog, calf and pig have so far proved conflicting or negative (Gillette & Filkins, 1966; Morris & Steel, 1964; Husband, Brandon & Lascelles, 1973; Payne & Marsh, 1962; Patt, & Eberhart, 1976). These investigators, however, studied antibody uptake only in the proximal intestine. Ileal maturation was not looked for in these animals.

In this paper the effect of large doses of cortisone acetate on the ultrastructural features of the ileal epithelium in the ferret is assessed.

MATERIALS AND METHODS

Animals

Litters from three varieties of ferret (polecat, albino and polecat/albino crosses) were used. Pregnant animals were separated and placed in a quiet room a week prior to littering. The day of littering was designated as day 1. Animals were obtained from A. S. Rowe, Thetford.

Injections

Ferrets, 21 days old, received either a single intraperitoneal injection of cortisone acetate ('Cortistab', Boots) (2·5 mg/ml) or an equivalent volume of 0·9 % saline solution. Seven doses of cortisone acetate were used: 0·25, 0·875, 1·0, 1·1, 1·2, 1·5 and 1·87 mg/g body weight. A 21 days old ferret weighed on average 62 g.

* Reprint requests to Professor F. Beck.

Table 1. *The effect of cortisone acetate on ferret and rat ileum*

Dose (mg/g body weight)	Animal	No. of animals treated	Ileum	No. of control animals
0·25	Rat	96	Closed	64*
0·25	Ferret	11	Open	11
0·875	Ferret	2	Open	1
1·0	Ferret	3	Open	2
1·1	Ferret	4	Open	2
1·2	Ferret	5	Open	4
1·5	Ferret	4	Animals died	3
1·87	Ferret	5	Animals died	5

* Carlile (1980).

Preparation of tissue for electron microscope study

Animals used for study of normal maturation were killed at weekly intervals between 1 and 7 weeks of age in a preliminary study to isolate the 'closure' period. A further one or two animals were killed at 32, 35, 37, 39 and 42 days of age in a detailed study of this period.

For the study of the effects of cortisone acetate, other litters were divided into a majority treated with cortisone acetate and a smaller number of saline-treated controls. The animals were killed at either 5 days (0·25 and 0·875 mg/g body weight doses of cortisone acetate) or 3 and 5 days (1·0, 1·1 and 1·2 mg/g body weight doses of cortisone acetate) after treatment.

The gut was fixed by cardiac perfusion with a 3 % glutaraldehyde/1 % paraformaldehyde fixative in phosphate buffer (pH 7·4). Samples of ileum were taken from a point 75 % along the length of the intestine as measured from the pylorus. Samples were fixed for a further 3 hours and rinsed in a sucrose/phosphate buffer wash before post-fixation in 1 % osmium tetroxide in sucrose/phosphate buffer for one hour. Following dehydration and clearing, samples were embedded in Araldite. Thick sections stained with toluidine blue were used to orientate the blocks so that the villi were cut longitudinally. Silver sections were mounted on single slot grids and stained with 10 % uranyl acetate and lead citrate before viewing under a Jeol 100S transmission electron microscope at an accelerating voltage of 80 kV.

RESULTS

Normal maturation

Maturation of the ileal epithelium occurred, between 35 and 39 days of age, by a process of cellular replacement. The morphological features of the 35 day epithelium were similar to those seen on the villus in the rat ileum at 18 days or earlier (Clark, 1959). There were numerous invaginations in the apical plasma membrane indicating pinocytosis. The apical cytoplasm contained several small vesicles and the rest of the cell was occupied by a single giant supranuclear vacuole. By 37 days the first few cells of a new, mature non-pinocytosing cell population could be seen emerging from the crypts. The vacuolar system in the cells already on the villus was unaffected by the onset of the maturation process. Thirty nine days after birth the new mature cell population had reached the tip of the villus.

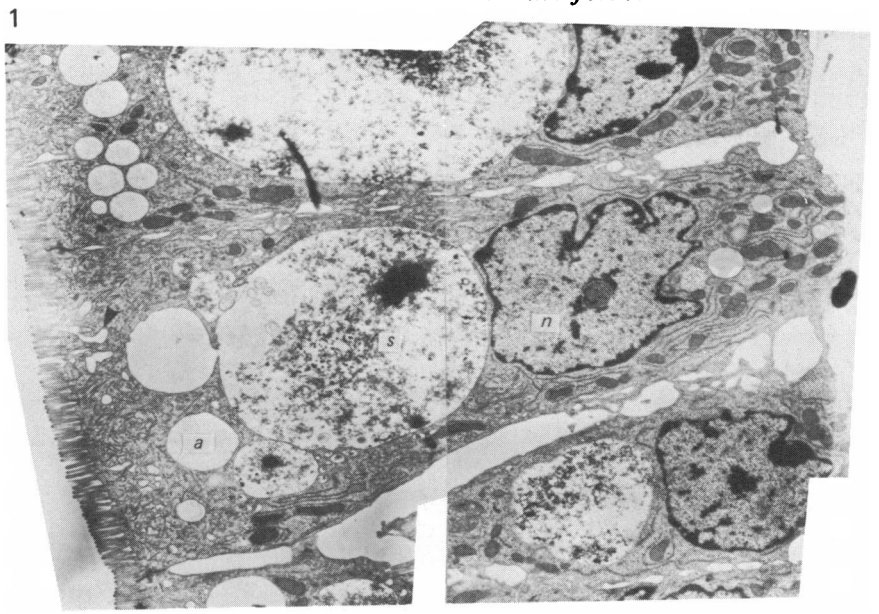

Fig. 1. A typical fully vacuolated epithelial cell seen on a villus in the ferret ileum 5 days after treatment with cortisone acetate (1·2 mg/g body weight) at 21 days of age. Note the presence of a complete vacuolar system. Arrowhead, pinocytosis; *a*, apical vesicles; *s*, supranuclear vacuole; *n*, nucleus. × 4200.

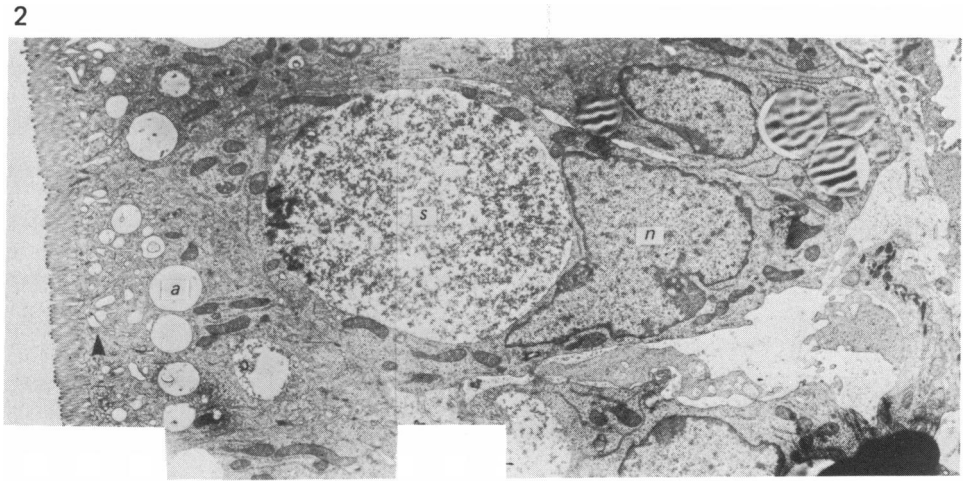

Fig. 2. A typical fully vacuolated epithelial cell seen on a villus in the ferret ileum of a 26 days old litter mate control to Fig. 1. Key as for Fig. 1. × 4200.

The effect of cortisone acetate

The ability of the various doses of cortisone acetate tested to induce maturation in the ferret ileal epithelium are summarized in Table 1. The two highest doses, 1·5 and 1·87 mg/g body weight, were fatal soon after administration. At all the other doses used detailed comparison at the electron microscope level with litter mate controls showed no morphological changes up to 5 days after treatment. Examples of a ferret ileum treated at 1·2 mg/g body weight, and its litter mate control, are

illustrated in Figures 1 and 2. That the villous epithelial cells continued to undertake pinocytosis was evident from the presence of invaginations in the apical plasma membrane. The rest of the cell was filled with the typical apical vesicles and the giant supranuclear vacuole.

DISCUSSION

Normal maturation ('closure') in the ferret ileal epithelium was proven to be between 35 and 39 days after birth. This agrees with estimates made by other investigators (Williams & Beck, 1969; Clark & Hardy, 1970). It occurred by a process of cellular replacement similar to that seen in the rat ileum (Clark & Hardy, 1969).

Administration of several dose levels of cortisone acetate to young ferrets failed to induce any morphological changes in the ileal epithelium indicative of maturation. The doses used here, excluding the two fatal ones, were one to five times the dose of cortisone acetate used to promote complete ileal maturation in the rat (Daniels *et al.* 1973; Morris & Morris, 1976) (see Table 1).

Our results should be seen against the background of reports by other investigators who have demonstrated the failure of various corticosteroids to promote maturation of the epithelium of the proximal intestine in the dog, hedgehog, calf and pig (Gillette & Filkins, 1966; Morris & Steel, 1964; Husband, Brandon & Lascelles, 1973; Payne & Marsh, 1962; Patt & Eberhart, 1976).

This is the first time that the effect of corticosteroids on the ileal epithelium has been investigated in a non-rodent species. The failure of cortisone acetate to induce maturation suggests that its maturing effect on the ileal epithelium in the young rat might be more specific than is generally realised.

SUMMARY

Normal maturation ('closure') in the ferret ileal epithelium occurs, between 35 and 39 days after birth, by a process of cellular replacement. The administration of cortisone acetate at doses up to 1·2 mg/g body weight for 5 days failed to promote any morphological changes in the ileal epithelium in this non-rodent species, and the results suggest that the maturing effect of cortisone acetate in the rat might be peculiar to the rodents.

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