THE DISTRIBUTION OF IMMUNOGLOBULINS IN THE INTESTINE OF THE NEONATAL CALF

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Résumé

LA DISTRIBUTION DES IMMUNOGLOBULINES DANS L'INTESTIN DU VEAU NOUVEAU-NE. — La distribution des immunoglobulines G, M et A dans l'intestin grêle de veaux ayant reçu du colostrum a été suivie au moyen d'une technique d'immunofluorescence. Les immunoglobulines colostrales ont été localisées dans la région de l'intestin grêle, où elles ont été trouvées soit dans la lamina propria, dans les cellules épithéliales ou sur la surface muqueuse des cellules épithéliales. La distribution varia avec l'âge du veau examiné. Chez les très jeunes animaux, les cellules épithéliales villeuses absorbent les trois classes d'immunoglobulines, tandis que, chez des veaux de 4 jours, les cellules épithéliales ne contenaient généralement pas d'immunoglobulines. Chez des veaux n'ayant pas reçu de colostrum, les immunoglobulines intestinales étaient synthétisées dans les plaques de Peyer et la région des cryptes de la muqueuse de l'intestin grêle, et des immunoglobulines étaient présentes dans les cellules épithéliales des cryptes et dans les cellules lymphoïdes entourant la lamina propria.

Introduction

In a study of the immunity of the calf to colibacillosis, Logan and Penhale (1971 a and b) noted that colostral immunoglobulins have a dual protective role. Those immunoglobulins which are absorbed from the small intestine into the circulation protect against septicaemia, whilst the immunoglobulins which remain in the small intestine have local protective effect against diarrhoea. These different protective functions appear to be independant of each other. High serum immunoglobulin levels do not prevent diarrhoea nor do high intestinal levels prevent death from colisepticaemia. Furthermore, the protection in the intestine is mainly prophylactic and it would appear essential that some form of immunological barrier is established prior to infection (Logan, Pearson and McNulty, 1977). For example, if adherence of enteropathogenic *E. coli* to the intestinal epithelium occurs then the protective action of the colostral immunoglobulins is very limited. In view of the importance of this finding in relation to neonatal diarrhoea it was considered pertinent to examine the local distribution of the individual classes of colostral immunoglobulin in the small intestine of calves and to compare it with that of actively produced immunoglobulins.

Materials and methods

CALVES

Newborn Friesian bull calves were purchased from 2 farms. On arrival at the laboratory the calves were kept in isolation and subjected to the following experimental protocols.

Calves 1 and 2. These calves were unsuckled and post-mortem examination of the intestine was carried out at 12 hours old.

Calves 3 and 4. These calves were fed 1 500 ml of colostrum at birth and 4 hours after the intestine was examined.

Calves 5 and 6. These calves received 1 500 ml of colostrum at birth and then 400 ml daily for 3 days prior to post-mortem examination which was carried out on the fourth day.

Calves 7 and 8. These calves were unsuckled and were fed milk substitute twice daily. Each was given intravenously 4 g of a serum IgM-rich fraction to prevent coli-septicaemia (Logan and Penhale 1971 c). Calf 7 was killed at 3 days old. Calf 8 was experimentally infected with rotavirus at 48 hours old and was killed 11 days later, when it was clinically normal.

COLLECTION OF INTESTINAL SAMPLES

Calves were anaesthetised using pentobarbitone and samples were taken from 10 sites throughout the length of the small intestine for immunofluorescent examination.

Fig. 1.—4-hour old colostrum-fed calf. Two villi stained with anti bovine IgG FITC-conjugated serum. Immunofluorescent staining on luminal surface of epithelial cells (arrow), within the cells with nuclei outlined, and in lamina propria (L).

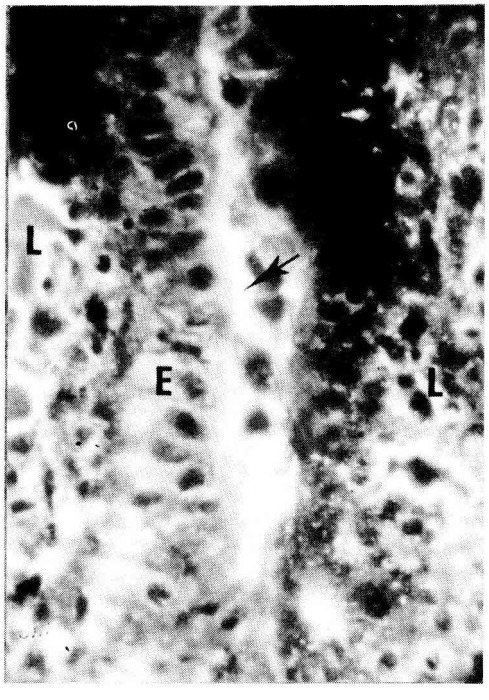

Fig. 2.—4-hour old colostrum-fed calf. Villi stained with anti bovine IgM FITC-conjugated serum. Immunofluorescence on luminal surface of epithelial cells (arrow), throughout epithelial cell cytoplasm (E) and in lamina propria (L).

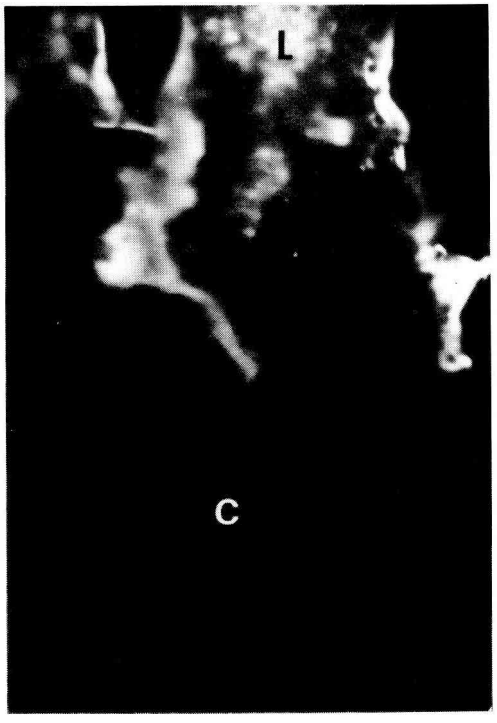

Fig. 3.—4-hour old colostrum-fed calf. Base of villus showing immunofluorescence against anti bovine IgA FITC-conjugated serum on surface, within epithelium and in the lamina propria (L). Crypt area (C) shows no immunofluorescence.

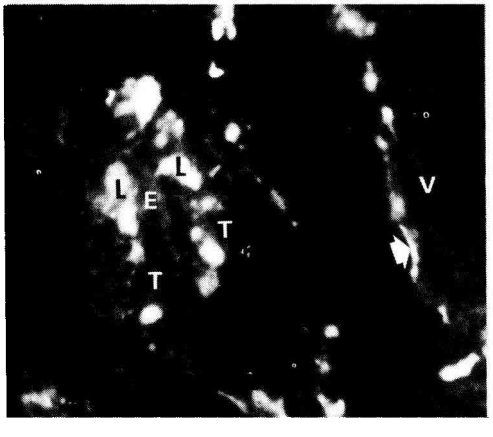

Fig. 4.—4-day old colostrum-fed calf. Villi cut longitudinally (V) and tangentially (T) stained with anti bovine IgA FITC-conjugated serum. Immunofluorescence on surface of villus (arrow), in a few epithelial cells (E) and in lamina propria (L).

Each sample was placed on a small piece of cardboard and immediately deep frozen in 2-methyl-butane using liquid nitrogen.

IMMUNOFLUORESCENT STUDIES

The preparation of specific antisera to bovine IgG, IgM and IgA has been described previously (Logan and Penhale 1972). Each antiserum was conjugated with fluorescein isothiocyanate (F.I.T.C.) according to the method of Allen and Porter (1970). Antisera were finally absorbed with lyophilised homogenated foetal intestine at the rate of 100 g per ml of conjugated antisera.

6μ sections of frozen tissue were cut and fixed as described by Allen & Porter (1970). Samples for IgG and IgA examination were fixed in 70 % ethanol for 30 minutes, those for IgM examination in methanol for 20 minutes.

Sections were then incubated with the

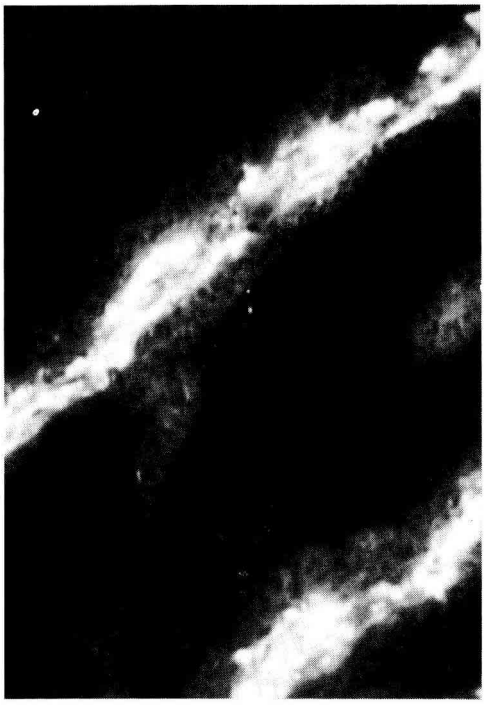

Fig. 5.—4-day old colostrum-fed calf. Villi stained with anti bovine IgG FITC-conjugated serum. Immunofluorescence in villous lamina propria. Epithelial cells show no fluorescence.

conjugated antiserum in a moist chamber for one hour at 37 °C. They were rinsed in 0.01 M phosphate buffered saline and then washed twice (10 minutes each wash) in PBS with gentle shaking. Following washing, sections were mounted in glycerol and examined using UV light.

The specificity of the reactions was checked by blocking with (a) non-immune serum, and (b) unconjugated antiserum, before incubating with the respective conjugated antiserum. The tissues from the 12 hour old unsuckled calves which were stained with the specific conjugated antisera, also acted as controls.

Results

The villi and crypts of the 12 hours old unsuckled calves did not show any fluorescence when stained with IgG and IgM antisera. Using the anti-IgA serum, villi were

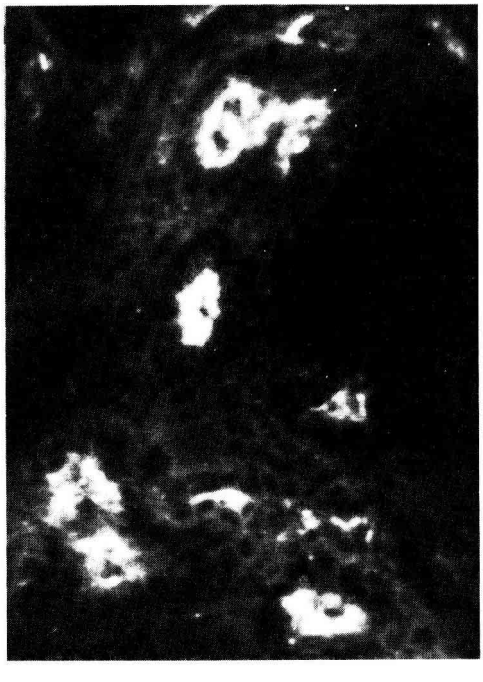

Fig. 6.—4-day old colostrum-fed calf. Cross section of crypt area showing immunofluorescence with anti bovine IgM FITC-conjugated serum in apical cell cytoplasm.

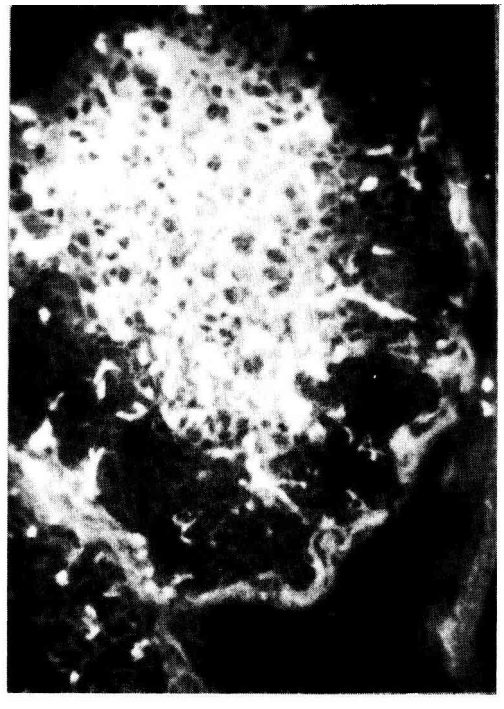

Fig. 7.—3-day old unsuckled calf. Lymphoid follicle of Peyers' patch stained with anti bovine IgG FITC-conjugated serum.

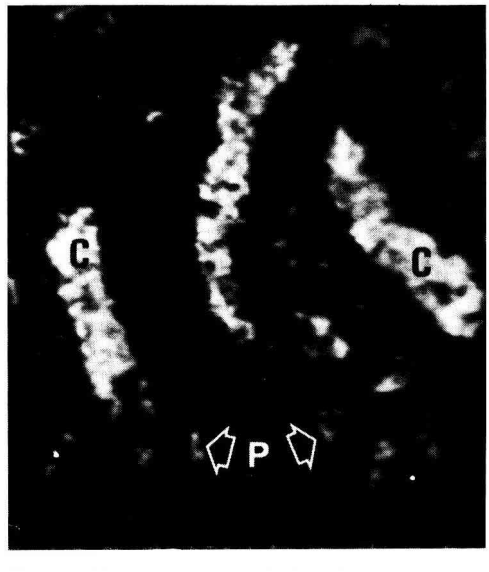

Fig. 8.—14-day old unsuckled calf. Longitudinal section of crypt epithelium (C) stained with anti bovine IgG FITC-conjugated serum. Plasma cells (P) are present in lamina propria.

also negative but a few cells in the crypt area of some sections fluoresced. However, fluorescence was very weak.

In contrast, in the 4 hour old colostrum-fed calves there was very strong fluorescence throughout the length of the intestine using all three antisera. Immunoglobulin G, which had a fine granular appearance (fig. 1), could be identified on the surface of villous epithelial cells, within epithelial cells where the nuclei were outlined, and in the lamina propria. In many sections the lacteals were full of immunoglobulin. Only in the distal ileum was it seen free in the lumen of the intestine. In general, the distribution of immunoglobulins M and A appeared to be similar to that of IgG (figs. 2 and 3). These immunoglobulins were also present in the lumen of the distal ileum. The crypts showed no fluorescence using any of the antisera (fig. 3).

In the colostrum-fed 4 day-old calves, the most prominent feature was the almost complete absence of immunoglobulin from the cytoplasm of the villous epithelial cells. Immunoglobulin of all 3 classes could be seen fluorescing on the surface of the villous epithelial cells (fig. 4) or in the lamina propria of the villi (fig. 5). Only very occa-

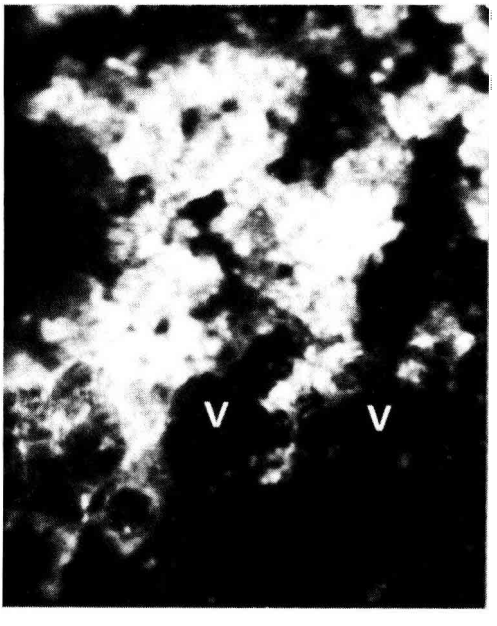

Fig. 9.—14-day old unsuckled calf. Section stained with anti bovine IgG FITC-conjugated serum. Immunoglobulin G fluorescing in lumen of distal small intestine. Unstained villi (V).

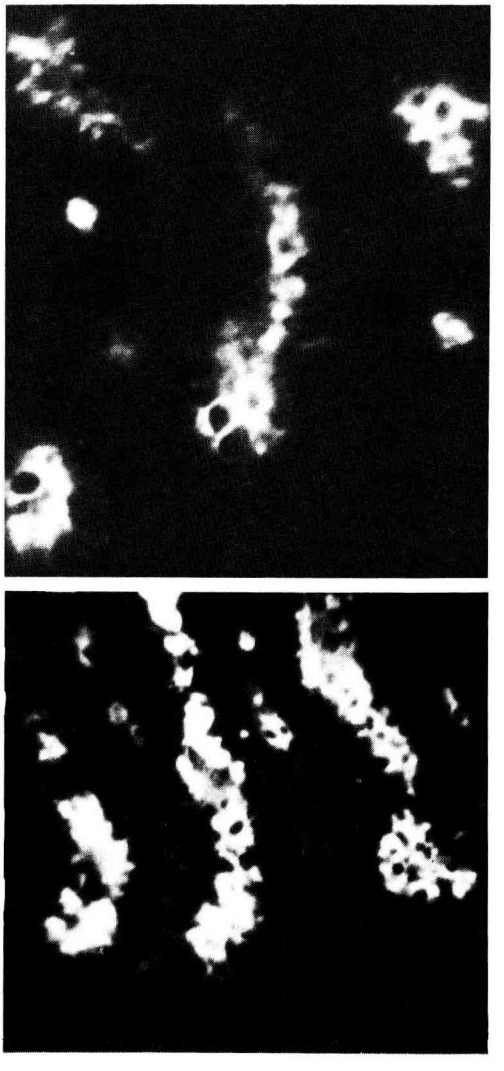

Fig. 10.—14-day old unsuckled calf. Longitudinal section of crypts stained with anti bovine IgA FITC-conjugated serum (upper) and anti bovine IgM FITC-conjugated serum (lower). Immunofluorescence in apical cell cytoplasm.

sionally was immunoglobulin seen fluorescing in isolated epithelial cells. The crypt areas did not fluoresce when examined with anti-IgG serum. However, some fluorescence was observed using the anti-IgM and anti-IgA sera. The fluorescence was quite strong and appeared to be confined to the apical cytoplasm of the crypt epithelial cells (fig. 6). Only a few plasma cells were seen.

In the 3 day old and 14 day old unsuckled calves the distribution of immunoglobulin was markedly different to that of the colostrum-fed calves. In the 3-day old calf, there was no evidence of IgG in either the villi or crypts. In the Peyers patches, there was strong fluorescence to IgG within the sinuses and numerous stained cells were detected (fig. 7). In the 14 day old calf, IgG was present in the crypt epithelium. It again had a granular appearance and was present in the lumen of the crypts and often throughout the depth of epithelial cells. Stained lymphoid cells were plentiful in the lamina propria (fig. 8). Fluorescence was not observed in the villi at all sites examined in the small intestine but in the distal ileum some IgG was detected in the lumen (fig. 9). It did not appear to be adherent to epithelial cells.

The distribution of IgA and M appeared to be similar in both calves. In the crypts, the immunoglobulins were found both on the surface of and in the cytoplasm of the epithelial cells where they tended to form distinct bands in the apical regions (fig. 10). There were numerous fluorescing cells in the lamina propria, and in Peyers patches. Villous epithelial cells did not stain with either anti-IgA or anti-IgM sera but fluorescing cells could be observed in the lamina propria and IgM and IgA were present on the surface of epithelial cells (fig. 11).

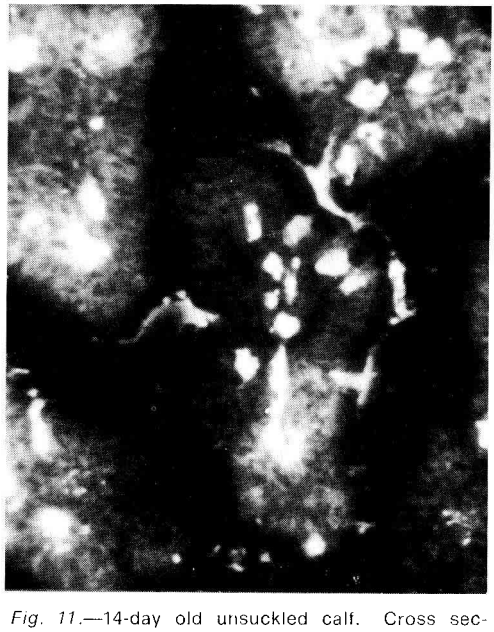

Fig. 11.—14-day old unsuckled calf. Cross section of villi stained with anti bovine IgA FITC-conjugated serum. Immunofluorescence on luminal surface of epithelium and in lamina propria.

Discussion

The present study demonstrated that in colostrum-fed calves immunoglobulins G, M and A can be detected at all levels of the small intestine. The distribution of these immunoglobulins not only varied with the age of the calf but was substantially different to the distribution of those immunoglobulins synthesised by the calf. The colostral immunoglobulins were present only in the villous region whereas the actively produced immunoglobulins were found predominantly in the Peyers patches, crypt epithelium and lumen.

In the 4-hour old calves the picture was that of active absorption of immunoglobulin (Fey, 1971). Immunoglobulins could be seen either on the villous epithelial surface, undergoing transport across the epithelial cells or within the lacteals of the lamina propria. Earlier workers have suggested that absorption occurs primarily in the anterior small intestine (El-Nageh, 1967 ; Fey, 1972) but in the present study no regional differences were observed ; villous epithelial cells throughout the small intestine being capable of absorbing immunoglobulin.

By contrast, in the 3 day old colostrum-fed calves very little immunoglobulin was detected in the villous epithelial cells. This was an expected finding as the epithelial cells are thought to cease absorbing immunoglobulin by 24 hours of age (reviewed by Fey, 1972). Nevertheless, some isolated epithelial cells did contain immunoglobulin

which suggests that they were still capable of limited absorption even in the 4 day old calves. However, their contribution to the serum pool of immunoglobulin is probably negligible. Immunoglobulin of all 3 classes was present in quantity on the surfaces of the villi and it is probable they form an immunological barrier which prevents the adhesion of enteropathogenic microorganisms.

The observation of immunoglobulin in the crypt area of the small intestine of these calves would suggest that they were actually synthesising immunoglobulin even in the presence of maternal immunoglobulin.

The findings in the colostrum deprived calves are similar to those of Porter, Noakes and Allen (1972) and Newby and Bourne (1976). However, in the 14 day old calf probably as a consequence of the experimental rotavirus infection there appeared to be more immunoglobulin in the crypt area than reported by the earlier workers. Furthermore, immunoglobulins A and M were adherent to the luminal surface of the villous epithelium.

The presence of immunoglobulin in the intestine of the 3 day old calf again emphasises the immunocompetence of the neonatal calf (reviewed by Schultz, 1973).

It is clear from the present study that in both unsuckled and colostrum fed calves the distribution and secretion of intestinal immunoglobulins are of a very complex nature and much research will be needed to identify which immunoglobulins are most important in the defence of the intestine against infection with enteropathogens. To date the only direct evidence available suggests that in enteric colibacillosis the three classes of immunoglobulin have complementary roles (Logan *et al.*, 1974).

Acknowledgements

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Summary

The distribution of immunoglobulins G, M and A in the small intestine of colostrum fed calves was monitored using immunofluorescent techniques. Colostral immunoglobulins were located in villous region of the small intestine where they were found either in the lamina propria, in the epithelial cells or on the mucosal surface of the epithelial cells. The distribution varied according to the age of calf examined. In very young calves, the villous epithelial cells were seen to be absorbing immunoglobulin of all 3 classes, whereas in 4 day old calves, the epithelial cells were generally devoid of immunoglobulin. In unsuckled calves, the intestinal immunoglobulins were synthesised in the Peyers patches and crypt region of the mucosa of the small intestine and immunoglobulin was found n the crypt epithelial cells and in plasma cells in the surrounding lamina propria.

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