JEJUNAL MALABSORPTION IN THE RAT INFECTED BY THE NEMATODE *NIPPOSTRONGYLUS BRASILIENSIS*

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Abstract

Symons L. E. A., Gibbins J. R. and Jones W. O., 1970. Jejunal malabsorption in the rat infected by the nematode *Nippostrongylus brasiliensis*. International Journal for Parasitology, 1: 179–187. The rate of jejunal absorption of a range of substances absorbed actively or by diffusion was depressed in the rat infected by the nematode *Nippostrongylus brasiliensis*. The degree of malabsorption of actively absorbed substances was directly related to the severity of the infection.

Malabsorption could not be explained by any failure of the respiratory activity of isolated epithelial cells or mucosal mitochondria. It was concluded that it could be explained by the numbers of immature crypt-like cells that occurred on the jejunal villi of the infected rat and by the enzyme deficiencies shown earlier to be associated with the brush borders.

INDEX KEY WORDS: Rat; *Nippostrongylus brasiliensis*; nematode; jejenum malabsorption; intracellular; respiration; electron microscopy; epithelium; mucosa; villi; crypts; brush borders; glycocalyx.

INTRODUCTION

Earlier work had shown that in the rat parasitized by the nematode *Nippostrongylus brasiliensis* the net jejunal fluxes of sodium, chloride and water (Symons, 1960b) and the rate of glucose absorption (Symons, 1960c) were reduced. These changes were associated with an increase in the total weight of mucosa which was accompanied by various morphological changes to the villi, including atrophy. There was an apparent abnormality of the brush borders of the epithelial cells, a marked loss of enzyme activity and increased levels of RNA and phospholipid of the mucosa (Symons & Fairbairn, 1963). The ribonucleic protein (RNP) of the epithelial cells was also increased (Symons, 1965). These abnormalities are similar to those reported to occur in other unrelated diseases of malabsorption (Padykula et al., 1961; Townley et al., 1964).

Because it was not known whether there was jejunal malabsorption of substances other than glucose, electrolytes and water, the absorption of amino acids, fatty acid and bromsulphalein (BSP) was measured.

Examinations were conducted to explain the jejunal malabsorption of this range of substances. The respiratory activity of jejunal tissue was measured because it had been shown earlier by histo-chemistry that there was a depression of the activity of succinic dehydrogenase in the cytoplasm of jejunal epithelial cells (Symons & Fairbairn, 1963). The jejunal epithelium was then examined by electron microscopy. It was shown that a significant proportion of the villous cells were similar to the undifferentiated cells of the crypts of Lieberkühn. Malabsorption is discussed in relation to the findings of these examinations.

MATERIALS AND METHODS

Male albino rats weighing 100–200 g were infected by the subcutaneous injection of 3500–4000 larvae of *N. brasiliensis*. All experiments described in the text were made with

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tissue collected on the 9th–11th days after the injection (10-day rats) when the response to infection was at its height (Symons & Fairbairn, 1963). All rats were fed stock laboratory cubes containing about 21 per cent protein.

The infected animals selected for use in any experiment were those that showed clinical evidence of disease. In general they were depressed, obviously in poor condition and might have diarrhoea, although this was not invariable. It was common experience that these animals would be inappetant with a poor growth rate, although these parameters were not measured in the present experiments (Symons, 1969). The severity of the infection in each animal was graded subjectively by L.E.A.S. before any experiment was performed (Symons, 1960b).

Absorption of glucose, amino acids, palmitic acid and BSP from jejunal segments

Before the preparation of jejunal loops for the measurement of absorption in vivo, the rats were fasted overnight and then anaesthetised by the intraperitoneal injection of urethane at about 200 mg/150 g body wt. The abdomen was opened by laparotomy and, except for measurement of palmitic acid absorption, from 10 to 20 cm of the jejunum was isolated between ligatures with minimum interference with the blood supply. The proximal ligature was always applied to the point at which the small intestine appeared on the left side of the abdominal cavity. The loop was not washed out. After the injection of the test solution through a fine hypodermic needle, the loop was returned to the abdominal cavity without kinking and the wound closed with metal clips. In the palmitic acid experiments a cannula was inserted into the duodenum a short distance distal to the pylorus so as not to interfere with the entry of the bile duct. The jejunum was then ligated about 20 cm distal to the cannula, which finally projected through the laparotomy incision and through which the test solution was injected. Throughout the experiments the rats were kept warm beneath a blanket on a hotplate.

Two ml of one of the following test solutions were administered to each rat:—
(a) 15 mM NaCl containing 200 mM D-glucose and 100 mM L-methionine or L-histidine.
(b) 10 % bovine serum albumin (BSA) to which was bound 20 moles of unlabelled palmitic acid and 1 μCi 14C-palmitic acid.
(c) 15 mM NaCl containing 10 mg BSP.

Absorption was measured with pairs of rats, one infected and one control, at a time. Twenty minutes were allowed for the concurrent absorption of glucose and one or other of the amino acids, and 15 min for palmitic acid and BSP, each in separate experiments. The segments were then removed rapidly, measured, emptied into graduated vessels and made up to a suitable volume. Protein was precipitated from the washings with ZnSO₄ and Ba(OH)₂ prior to the estimation of the residual glucose, amino acids or BSP.

In each experiment the dry weights of the mucosae were estimated from the lengths of the segments according to formulae calculated in earlier experiments (Symons, 1960a):

Control rats 15 ± 3 mg/cm
Infected rats 23 ± 2 mg/cm

Absorption was then calculated as mM or mg/g dry mucosa/h, but as the rats were examined in pairs, the results were expressed as the ratio of the rates of absorption of infected rats compared with their controls.

Respiration of epithelial cells and isolated mucosal mitochondria

Epithelial cells. These cells were isolated by incubating everted jejunum at 35°C in the trypsin–pancreatin medium of Stern & Reilly (1965). Eversion of the intestine allowed the
removal of all parasites. After incubation, the loops of intestine were pulled several times beneath a glass roller in a petri dish containing Stern and Reilly's sucrose-phosphate medium, and the released cells and medium transferred to an ice-bath. They were then taken to the cold room for subsequent handling. After washing in the same medium, the cells from two rats were pooled so that after centrifugation about 1 ml lightly packed cells was isolated. These were diluted to about 1:7 in the same medium, to which was added 9-5 mM succinate (final concentration). Three ml of this suspension (in duplicate) were transferred to a Warburg flask and incubated at 37°C in O₂ for 20 min after an equilibration period of 10 min. The QO₂ was expressed as µl O₂/mg of protein/h.

Isolated mucosal mitochondria. Mitochondria were isolated as described previously from the mucosa scraped from everted jejuna, and homogenized in the presence of 1·5% BSA to prevent inhibition of respiration by unsaturated fatty acids (Symons, 1966a). Pooled mucosa from two rats was used for each measurement. The activities of the enzymes succinic, malic, α-oxoglutaric and glutamic dehydrogenases were measured.

The activities were expressed as µl O₂/mg N/h and estimated from the first 30 min after the 10 min equilibrium period. The chemical and isotopic analyses are given below:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>by the glucose oxidase method of Dahlqvist (1961).</td>
</tr>
<tr>
<td>L-methionine</td>
<td>by a slight modification of the method of McCarthy &amp; Sullivan (1941).</td>
</tr>
<tr>
<td>L-histidine</td>
<td>by the method of Macpherson (1946), as modified by Birt &amp; Hird (1956).</td>
</tr>
<tr>
<td>BSP</td>
<td>1 ml diluted washings plus 4 ml 0·25N NaOH. The standard was 50 µg/ml of BSP and suitable dilutions thereof. The optical density was measured at 580 nm. Care was taken to ensure that all the dye had been removed from the mucosa.</td>
</tr>
<tr>
<td>Protein</td>
<td>by the method of Lowry et al. (1951).</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>by micro-Kjeldahl.</td>
</tr>
<tr>
<td>¹⁴C-palmitic acid</td>
<td>Duplicate aliquots of washings added to PPO and dimethyl POPOP in toluene plus Triton X-100 (Patterson &amp; Greene, 1965).</td>
</tr>
</tbody>
</table>

The isotope was purchased from the Radiochemical Centre, Amersham, England, and all counting was done with a Packard liquid scintillation spectrometer, Model 3375. External standards were used to estimate quenching.

Electron microscopy

The abdomen was opened after the rats had been anaesthetized, and cold 3% gluteraldehyde in sodium cacodylate buffer was injected into the lumen of a loop of jejunum showing maximum response to the infection. Fixative was also dropped onto the serosal surface of the loop, which was then removed into a dish containing more cold fixative. Rings about 1-2 mm long were sliced off with stainless steel blades and transferred to cold fixative for 1·5 h. After washing they were post-fixed in 2% osmium tetroxide in phosphate buffer at room temperature for 1 h. The dehydrated blocks were embedded in Araldite. Tissue was also prepared from the same region of the jejunum of normal rats.

Care was taken to ensure that cells examined were from the upper third of the villi. This was done by prior examination by phase contrast microscopy of thick (1 µm) sections and subsequently trimming the block under a dissecting microscope, using a suitable landmark such as part of a parasite. After cutting on an ultra-microtome, the sections were stained with uranyl acetate and lead citrate before examination in a Siemens Elmiskop I microscope.

Other sections from tissue fixed in 2% osmium tetroxide and embedded in methacrylate
were stained by the method described by Ito (1965) to demonstrate the apical mucopoly-
saccharide surface coat or glycocalyx of the microvilli. Some of these sections were stained
with Alcian blue as described by the same author for examination of the brush borders by
light microscopy.

Other jejunal segments were cut in a cryostat and stained with Oil Red O after the
animals had been fasted for 24 h.

Statistical analyses

The results of the respiratory experiments were analysed by Student's 't' test.

RESULTS

Absorption in vivo by jejunal segments

The results of these experiments are set out in Table 1, in which it can be seen that the
rate of absorption/g dry mucosal tissue was reduced in the infected rat. It was also apparent
that the depression of absorption of glucose and amino acids was related directly to the
severity of the infection, which was judged subjectively from the appearance of the rat and
its jejunum. The infections of the rats in which the absorption of both glucose and methion-
ine was measured were judged to be heavy, but those in which the absorption of both glucose
and histidine were measured were only lightly infected, so that the ratio of the rates of the
infected and their controls was 0.76 and 0.78, respectively. The experiment with histidine,
without glucose, was repeated in a second group of rats judged to be heavily to very heavily
infected. The ratio then fell to a mean of 0.51. The effect of the severity of the infection was
not as clear in the palmitic acid and BSP experiments because the range of infections was
limited, although it is interesting that the depression of absorption of the former was marked
in spite of the fact that the infections were generally only moderately severe.

Respiration in vitro

The microscopic appearance of the jejunum after the removal of the epithelial cells
appeared identical with that illustrated by Harrer et al. (1964), whose method as modified
by Stern & Reilly (1965) was used in the present experiment. The epithelial cells were
removed from the villi but were left intact in the crypts of Lieberkühn.

The results of the experiments which measured the $QO_2$ of epithelial cells and of their
isolated mitochondria in the presence of Krebs' Cycle substrates are shown in Table 2. In
both instances it can be seen that there was no statistically significant difference between
the infected rats and their controls. The rats were judged subjectively to be affected lightly

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### Table 1.—The Absorption in vivo of L-Methionine and L-Histidine (Compared Concurrently with D-Glucose), Palmitic Acid and BSP from Jejunal Loops of Rats Infected with *N. brasiliensis*. Results, Originally Calculated as Absorption/g Dry Mucosa/h, Expressed as the Ratio of Pairs of Infected Rats and Their Controls

<table>
<thead>
<tr>
<th></th>
<th>Methionine</th>
<th>Glucose</th>
<th>Histidine</th>
<th>Glucose</th>
<th>Palmitic</th>
<th>BSP</th>
</tr>
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<tr>
<td></td>
<td>(5) 0.65 ± 0.19</td>
<td>0.53 ± 0.15</td>
<td>(4) 0.78 ± 0.18</td>
<td>0.76 ± 0.22</td>
<td>(6) 0.60 ± 0.11</td>
<td>(6) 0.76 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>(5) 0.51 ± 0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

The figures in parenthesis = number of pairs of rats.

$±$ = standard deviation.
TABLE 2.—Respiratory activity of epithelial cells and mucosal mitochondria from the rat infected with *N. brasiliensis*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Controls</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial cells</td>
<td>20.6 ± 1.45</td>
<td>18.4 ± 1.8</td>
</tr>
<tr>
<td>μl O_2/mg protein/h</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Mucosal mitochondria</td>
<td>564 ± 131</td>
<td>615 ± 123</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Malic dehydrogenase</td>
<td>418 ± 105</td>
<td>431 ± 112</td>
</tr>
<tr>
<td>α-Oxoglutaric dehydrogenase</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Glutamic dehydrogenase</td>
<td>288 ± 89</td>
<td>284 ± 77</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parenthesis = number of experiments.

The difference between the infected rats and their controls for both epithelial cells and mucosal mitochondria was non-significant.

to very severely in each experiment, but there was no evidence that the respiration of tissue from the most severely affected was any lower than from the lightly affected.

**Electron microscopy**

Because the electron microscopic appearance of the normal intestinal epithelial cells, including that of the rat, has been described many times, it is not necessary to discuss it in detail, but there are one or two points to be made. First, the brush borders of the epithelial cells in the normal rat form a dense and evenly distributed pallisade round the entire edge of the apex of the villus (Fig. 1). On the other hand, the microvilli on the cells of the villi from the infected rat are abnormal and their density is highly variable (Figs. 3 and 4). This abnormality of the microvilli is not confined to individual isolated cells. Secondly, it can be seen from the state of the intercellular spaces that the mucosa of the normal rat is in an active absorptive state, while there is much less evidence of this in the infected mucosa (Fig. 3). Possibly this difference is a reflection of a difference of absorptive efficiency, but it may be due merely to anorexia in this heavily infected animal. Both rats were killed at the same time.

In epithelial cells from villi of normal rats the mitochondria tend to be elongated in shape and are densely stained (Fig. 2). In infected rats, on the other hand, the mitochondria are rounded, poorly stained and vacuolated (Fig. 4). In electron micrographs of higher magnification than are illustrated in the present paper, there appeared to be many more free ribosomes in the cells from the infected rat. The Golgi apparatus, which is prominent in the cells from the normal rat, is sometimes displaced and dispersed in those from the infected animals whereas in others it may be very active.

Figures 6 and 7 are micrographs of crypt epithelial cells from normal and infected rats, respectively. Similar rudimentary microvilli were found on cells from each animal. In fact, the brush border of the villous cells in the infected rat (Figs. 3 and 4) are almost identical to the brush borders of crypt cells in normal animals. It must also be noted that the mitochondria in the crypt cells from both infected and normal animals are more rounded and lightly stained than those found in the mature villous cells of the normal animal. There appeared to be a high concentration of free ribosomes in crypt cells from both animals. This too is similar to the presence of these particles in the villous cells of the infected rat. Morphologically it would appear that in the infected animals, crypt cells have moved from the crypt up on to the villus, but that the cytoplasmic differentiation that would normally
accompany this movement has not occurred. The elongated bodies in the crypt of the infected rat are believed to be *Giardia muris*, a common protozoal inhabitant of this animal (Fig. 7). The number of these parasites often, but not invariably, increases considerably in infections by *N. brasiliensis*.

In contrast to the relatively thin basement membrane found beneath the villous epithelial cells of the normal jejunal mucosa (Fig. 2) the basement membrane of the villous cells of the infected rat was often markedly thickened (Fig. 5). Cytoplasmic processes extend from the epithelial cells into this thickened basement membrane and through the membrane to contact a connective tissue cell in the core of the villus (Fig. 5).

It is interesting to note that in the infected animal the basement membrane lining blood vessels in the core of the villus is also grossly thickened (Fig. 5). The basement membrane lining the crypts in the infected rat was unchanged and identical with that in crypts of the normal animal.

Large homogeneous and electron opaque inclusions were often found in the villous epithelial cells of infected animals (Figs. 3 and 9) but never in those cells of normal animals. It was found that if conventionally prepared sections were stained with Oil Red O, positively stained inclusions were found in infected animals but not in their controls. It was concluded that these inclusions were lipid droplets.

The abnormalities described above are not distributed uniformly over all the villi of the infected mucosa. The villi illustrated in this paper were deliberately selected from a region of the jejunum which macroscopically showed maximum response to infection. On some villi at least, a large proportion of the epithelial cells were abnormal, whereas elsewhere it was not possible to detect much variation. When sections from rats used in the present work were stained with Alcian blue, it was seen that derangement was not confined to isolated cells but occurred in groups of cells, and occasionally, over much of one villus.

Figures 8 and 9 compare the glycocalyces of the brush borders of the epithelial cells of villi of normal and infected rats, respectively. It was commonly found that the glycocalyx while rudimentary or absent in the control rat, as reported by Ito (1965), was prominent in the infected animal. Similarly, Alcian blue did not reveal a glycocalyx in control rats, whereas there was a line of variable thickness denoting this structure in sections from the infected rat.

Figure 9 clearly illustrates the droplets of lipid which are described above and found solely in the infected jejenum.

**DISCUSSION**

The present experiments showed that jejunal malabsorption per unit dry weight of mucosa was not confined to water and electrolytes (Symons, 1960b) and glucose (Symons, 1960c) as was reported earlier. It has now been shown that there was also malabsorption of the amino acids methionine and histidine, palmitic acid and BSP. Glucose was used in both the earlier and present experiments to enable comparison between them to be made. The degree of malabsorption in the present experiments was not as great as in the earlier, in which a segment of the jejunum was infused and collections made from its distal end. In the present experiments ligated segments were used. The former was a more physiologically satisfactory technique.

The severity of the infections was directly related to the degree of malabsorption of the actively absorbed glucose and amino acids. The absorption of BSP, which is by diffusion, was not as markedly affected as was the actively absorbed substances. On the other hand the absorption of palmitic acid, believed also to be by diffusion, was markedly affected.
Fig. 1. Electron micrograph of jejunal epithelial cells of the normal rat. mv = microvilli; ics = intercellular space. ∗ 4200.

Fig. 2. Basal portion of the jejunal epithelial cell of the normal rat illustrating typical mitochondria (m); bm = basement membrane; bv = blood vessel. ∗ 15,200.
Fig. 3. Jejunal epithelial cells of the infected rat illustrating lipid inclusions (l). × 5800.
Fig. 4. Jejunal epithelial cells of the infected rat illustrating typical mitochondria. \( \times 11,200 \).

Fig. 5. Basal portion of a jejunal epithelial cell of the infected rat illustrating the basement membrane. \( \mathrm{cp} = \) cytoplasmic process. \( \times 12,800 \).
Fig. 6. Jejunal epithelial cells of the crypts of Lieberkühn of the normal rat. × 4000.

Fig. 7. Jejunal epithelial cells of the crypts of Lieberkühn of the infected rat. eb = elongated bodies (Giardia muris). × 4000.
FIG. 8. Apical portion of a jejunal epithelial cell of a normal rat illustrating the microvilli and almost total absence of a glycocalyx. x 30,000.

FIG. 9. Apical portion of a jejunal epithelial cell illustrating the microvilli, presence of a glycocalyx (g) and lipid inclusions in the cytoplasm. x 30,000.
The rate of absorption was expressed in terms of dry weight of mucosa and not in the more familiar terms of cm length of intestine, because earlier work had shown that it would have been meaningless to have compared absorption between infected rats and their controls in the latter terms when it was intended to measure absorption in terms of a suitable unit of mucosa, the tissue concerned. In the infected rat the size and weight of the small intestine (Symons, 1957) and of the weight of mucosa/cm length of jejunum is greatly increased (Symons, 1960a). At that time it was decided that absorption/g dry wt. of mucosa was the best available compromise. Later measurements showed that there were 23–25 per cent more epithelial cells/cm length of jejunum in the infected rat (Symons & Fairbairn, 1963). The expression of absorption/g dry wt. of mucosa, therefore, actually over-estimated the rate of absorption per cell by the infected rat relative to its control. As there was an unequivocal slower rate of absorption/g dry wt. of mucosa in the infected rat, the relatively small error introduced would not affect the physiological significance of the present measurements.

It must also be pointed out that if the rate of glucose absorption was expressed per cm length of jejunum, the difference between the infected rat and its control was reduced, although it was not eliminated, because of the greater weight of mucosa in the diseased animals. Furthermore, there was no evidence of a failure of digestion or absorption by the infected rat if measurements are made over the entire length of the small intestine, rather than the jejunum alone (Symons, 1960c, 1966b; Symons & Jones, 1970). The depression of jejunal absorption was compensated by increased activity in the ileum (Symons, 1961).

It is now necessary to explain the depression of jejunal absorption of this range of substances. Limited evidence did not indicate any biochemical derangement which could account for this failure. The infection did not impair the respiration of the epithelial cells when judged by the $QO_2$ of isolated cells or of mucosal mitochondria. It was noteworthy that these direct measurements of respiratory activity did not support the histochemical evidence of depression of succinic dehydrogenase reported earlier for this infection (Symons & Fairbairn, 1963). Histochemical evidence of a reduction of this enzyme in the mucosa of protein-depleted rats was also found by Tandon et al. (1969), who discussed briefly its possible connection with malabsorption in clinical protein-calorie malnutrition. The results of the present experiments suggested that a histochemical examination of the activity of this enzyme may be misleading, as direct measurement failed to show any such deficiency.

On the other hand, some evidence of a biochemical abnormality was reported briefly by Playoust & Gallagher (1968), who found a marked depression of stearic acid absorption by rats with nippostrongylosis. They concluded that there was a correlation between fatty acid malabsorption and the increased level of unesterified fatty acid in the mucosa. Subsequently, this was shown to be due to derangement of the phosphatidic acid step in the esterification process (Playoust, Gallagher & Symons, unpublished). It was then concluded that this was due to the presence of undifferentiated cells on the villi. However, other enzymological steps associated with absorption may be affected.

Although jejunal malabsorption cannot be explained by the results of the limited number of biochemical examinations that have been made, it was clear that electron microscopic changes were significant. A comparison of the characteristics of the crypt cells of the infected rat and its control do not reveal any differences, but the epithelial cells from the villus of the infected rat appear to be identical with crypt cells (Figs. 1 and 2, cf. Figs. 3 and 4). It was concluded, therefore, that these villi contained a number of immature or undifferentiated cells. In one electron micrograph of low magnification (not illustrated in the present paper) it was obvious that all the cells visible on three villi were of this type. How-
ever, on other villi from infected rats, not all the epithelial cells showed the characteristics of crypt cells. In other sections stained with Alcian blue and examined by light microscopy it was shown that there were patches of cells with poorly stained microvilli. This was consistent with the patchy distribution of poor PAS staining of brush borders reported earlier and taken to imply a similar distribution of abnormal brush borders, and hence a patchy distribution of crypt-like cells (Symons & Fairbairn, 1963). It was concluded that, although the proportion of these cells on the villi of the jejunal mucosa of the infected rat could not be estimated accurately, malabsorption could be explained on this basis. It is well known that undifferentiated crypt cells are not absorbing cells.

If there is a relatively large number of crypt cells on these villi, the low levels of the enzymes maltase, leucine-aminopeptidase and alkaline $\beta$-glycerophosphatase previously reported (Symons & Fairbairn, 1963) can be readily explained. It has been reported by others that the ratio of the activities in the intestinal crypts of the rat compared with those on the villi, of maltase, dipeptidase and alkaline phosphatase was 0.17, 0.43 and 0.49 respectively (Fortin-Magana et al., 1970). It would be interesting to determine whether the lack of the phosphatidic acid step in the esterification process mentioned above could be explained on the same basis.

The occurrence of crypt-like cells on the villi is presumably due to the increased rate of turnover and proliferation of jejunal epithelial cells previously reported for the rat with nippostrongylosis (Symons, 1965).

The apparent increase in the glycocalyx is interesting, although its significance in relation to malabsorption can only be conjectured. This layer appears denser in celiac disease than in normal man, but this may be subject to technical variation and therefore not a true finding (Dr. Margot Shiner, personal communication).

The accumulation of lipid droplets in the epithelial cells of the infected jejunum cannot be explained with certainty. It cannot be accounted for in terms of the accumulation of fatty acid occurring during malabsorption of lipid, as this was unesterified (Playoust & Gallagher, 1968). The induction of a fatty liver as occurs with carbon tetrachloride hepatotoxicity for instance, may be due to the failure of the biosynthesis of lipoprotein molecules necessary for the secretion of lipid from that organ or from the failure of hepatitic protein synthesis (Recknagel, 1967). A similar failure of secretion from intestinal epithelial cells could be postulated, but, as earlier experiments had demonstrated, there is evidence of an increase, not a depression of protein synthesis in this infection (Symons & Fairbairn, 1963; Symons, 1965). Direct measurements of protein synthesis have confirmed this (Symons, unpublished). For the same reason it appeared unlikely that failure of transport was due to a failure to synthesise the protein moiety of chylomicrons.

Finally, because the morphological and biochemical abnormalities of the rat infected with the nematode *N. brasiliensis* as described in this and earlier papers are similar to those of unrelated malabsorptive diseases of man, it is suggested that this disease might be a useful model for further studies of the explanation of malabsorption.

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MALABSORPTION IN RAT INFECTED WITH *N. brasiliensis*


