Changes in Rat Intestinal Villi After Challenge with Wheat Protein

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Summary

Rats reared on normal diet and on a non-wheat protein diet were subjected to an oral dose of a wheat protein mixture. Those reared on rat diet showed no change in the appearance of the intestinal villi, whereas the rats reared on a non-wheat protein diet showed much broadening of the villi, this being more pronounced in the distal intestine. It is suggested that this is due to a "first encounter" phenomenon, and might have immunological as well as morphological aspects.

Introduction

Uptake of protein by adult rat intestine is believed to be a non-selective process, and whatever is presented to the intestine, in physiological doses is taken up by these cells, not necessarily as native protein (Walker et al., 1975; Williams, 1978a), but as breakdown products of molecular weight 20,000 to 50,000 daltons (Hemmings and Williams, 1978).

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Villous atrophy and other intestinal conditions have been demonstrated in human intestinal villi especially when a coeliac condition is present, and it has been suggested by many workers that the intake of wheat protein can make the condition worse. Normal villi of the intestine show up under the microscope as finger like protrusions extending from the terminal web area of the cells: they consist of layers of epithelial cells and have a slightly blunted end. Atrophy of the villi show the protrusions to be more blunt, broader, and showing a reduced surface area.

The effect of wheat proteins on the morphology of intestinal cells has long been recognized (Strober, 1978), and it has been suggested that these proteins affect the gut mucosa causing more protein to traverse the gut barrier and be released into the general circulation. Previous work by Williams (1979) using the antigen/antibody technique showed that when animals were kept on a non-wheat diet and later subjected to an oral dose of wheat proteins, more protein in the antigenic form was detected in body tissues compared with animals that had been reared on a wheat diet. This suggests that there is either an immuno-

logical barrier due to previous experience of the protein in the wheat fed animals, or that there is an incomplete immunological barrier, or a more permeable intestine in the non-wheat rats.

In the present study, histological observations were carried out on rat intestines where rats had received normal rat diet, containing wheat protein, comparing them with the intestines of rats that had been reared on a non-wheat protein diet of commercial dog food, carrots, apples and greens. The object was to see if a morphological difference of the intestinal villi occurred, and also to see if a wheat protein challenge changed the appearance of the villi of the non-wheat protein diet animal.

Materials and Methods

Albino rats of the Wistar strain were used throughout, with the exception of the 18 day old rats, all were allowed to reach maturity before experimentation. Water was available ad lib to all, in the first group of 12 rats, rat food diet was rationed so as to have comparable weight rats, this being 260 to 280 grams.

The second group of eight rats was reared from pre-gestation period on a diet not containing any wheat proteins. This entailed putting mothers on a non-wheat protein diet of commercial dog food not containing wheat, carrots, apples and greens before mating, so as to have siblings with no previous experience of wheat protein. All rats were killed by a blow on the head prior to dissecting out the intestine and washing it through with 25 mls phosphate buffered saline (PBS), pH7.0.).

Wheat proteins were prepared from milled bouquet wheat (Rank Hovis McDougall). After milling the wheat grain in a Christie hammermill using a 0.5mm filter attachment, stirring at 4°C in a volume of PBS, the supernatent containing the wheat proteins was concentrated in a Rotavapor to give a final concentration of 2 percent protein as determined by the Nessler method (Thompson and Morrison, 1951).

Feeding of protein was by gavage directly into the stomach by means of a polyethylene cannula attached to a syringe. Animals receiving protein in this manner were slightly anaesthetised with Ether. The dose was 4 mls of the 2 percent solution. Diet reared animals were given a similar volume of homologous rat serum at a concentration of 2 percent. This maintained similar concentrations and volumes in the intestines. The animals were allowed to recover and a period of three days passed before they were killed. Feeding of proteins was repeated once daily over that period.

Histological studies were carried out on both proximal and distal segments of the intestine, tissue was taken from standard sites in the gut to ensure comparability; this was prepared as soon as possible after death, fixed in Bouin's fixative for 24 hours before it was dehydrated and subjected to normal histological methods. Embedding was in paraffin wax (mp 56°C) and sections were cut at 7u thickness on a Beck microtome. Staining was Haematoxy-lin/Eosin. Prints were prepared from slides on Kodak PanX film after exposure of 4 seconds or more in a Zeiss Ultraphot 2 microscope.

For determining the cross sectional areas, a Hyashi Denco Auto Area Meter AAM 7 was used. Negatives of the sections were placed in a photographic enlarger, and the images projected onto white paper, villi were carefully copied, and the image cut out prior to putting through the area meter. Many sections were given this treatment and the results were given in the table. Also determined were villous height, and it can be seen by looking at the photographs that a difference in villi height occurred.

Results

The following micrographs depict the appearance of the intestinal villi from 18 day old and mature rats. At the age of 18 days there was no difference in intestinal villi of intestines from pups of mothers that had been reared on rat food diet and those of special diet rats. The micrographs are presented so as to ascertain that at this stage of villous development no difference in morphology is observed (Figs 1-4). Villous height and cross sectional areas were also

noted to be very similar (Data not included). In mature animals however, a marked difference in appearance was noted (Figs. 5-12). In the proximal segments (Figs. 5-8) it can be seen that in the rat diet animals, the villi are of moderate length, whereas in the non-wheat diet animals, there is a difference, the villi are longer, not as broad and appear quite healthy. After feeding wheat proteins to these rats it was observed that in the rat food reared rats there was no difference in the morphology of the villi, and it was also noticed that there was little difference in proximal intestine after feeding wheat protein to special diet rats, the villi were observed to be very similar, perhaps showing a slight blunting effect with some broadening of the villi. When comparison was made of villous height, it was noticed that the normal diet rat was similar to the normal diet rat after a feed of wheat protein. The special diet rat proximal villi appeared longer than the normal diet and the length of the special diet villi was reduced after feeding wheat protein. Similarly, the Table shows a small difference in the cross-sectional area.

When sections from the distal intestine of mature rats were examined a totally different picture was seen (Figs. 9-12). The villi of the rat diet fed animal had a blunt, broad appearance; this was also observed after feeding wheat protein (Figs 9 & 11), villous height was observed to be near equal with areas not being very different, whereas the villi of the special diet animals were seen to be long and slender, (Fig. 10) a larger cross-sectional area and a villous height of 1.5x was recorded. Both types appeared quite healthy. However, after a feed of wheat protein a change was observed in the villi of the special diet animals; they took on the appearance of the rat diet villi and became blunt and broader (Fig. 12); the height of the villi was reduced, together with the cross sectional area.

Another observation was made in that after feeding non-wheat rats on wheat protein for a short period of time, and then returning them onto non-wheat diet, the intestinal villi did not revert to being long and slender, but gave the appearance of the rat diet fed animals' (Figs. not included). The figures presented are typical findings from many sections examined, and care was taken to study similar segments of intestine.

It was observed that when non-wheat diet rats were given their first dose of wheat protein, a shock condition developed, they became lethargic, and seemed to be gasping for air; this could not be an anaphylactic type shock as it was the animals' first encounter with the protein, therefore it must have been some form of systemic reaction to wheat protein. Apart from this initial period lasting around 20 minutes, this condition was not reproduced on subsequent feeding, and the animals showed no further discomfort.

Analysis carried out by scanning the cross sectional area of the villi using a Hyashi Denco Auto Area Meter AAM 7 was also made (Table 1). The difference in area of the proximal sections was not great, and it was found that only the shape differed when comparisons were made of rat diet and non-wheat diet rat intestines. When the distal intestines were compared however, the cross sectional area of the non-wheat diet was found to be nearly 1.5x the cross sectional area of the rat diet animal. After feeding wheat protein, this was reduced; this holds true also for the villous height. It was also observed that after special diet rats were given wheat protein, and later put back onto non-wheat diet, it was found that the intestinal villi were as rat diet, a process of reverting to being long slender villi was not seen.

TABLE 1

AVERAGE CROSS SECTIONAL AREAS OF VILLI OF ADULT RATS FED NORMAL DIET (A). AND NON WHEAT PROTEIN FED ADULT RATS (B). (Average of many sections examined). Number of rats 12.

	A Area	B Area
Proximal Intestine	**26.2mm^2 ±1.1 mm**	**25.5mm^2 ± 1.4mm**
Distal Intestine	**21.1 mm^2 ± 0.9mm**	**29.2mm^2 ± 2.3mm**

AVERAGE CROSS SECTIONAL AREAS OF VILLI OF NON-WHEAT DIET RATS AFTER AN ORAL FEED OF WHEAT PROTEIN AND AFTER A PERIOD OF ONE MONTH ON NON-WHEAT DIET. (Average of many sections examined). Number of rats 8.

Proximal Intestine	**26.4mm^2 ± 1.4mm**	**26.5mm^2 ± 1.7mm**
Distal Intestine	**24.0mm^2 ± 1.0mm**	**22.4mm^2 ± 2.0mm**

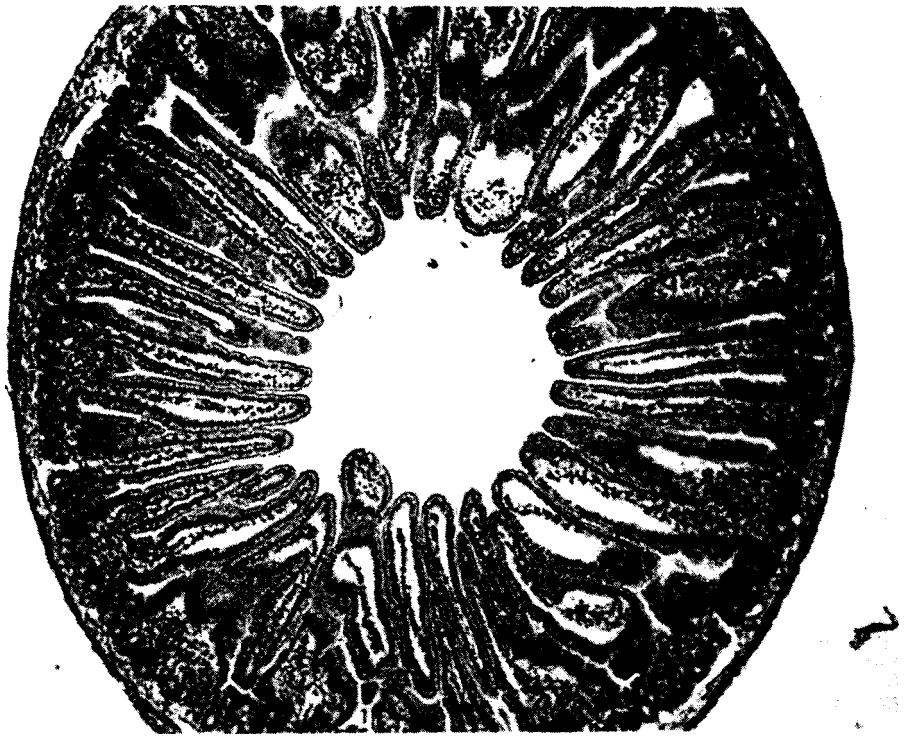

FIGURE 1
A duodenal section from an 18 day old rat reared from a mother on normal diet. Note the numerous long slender villi. Mag x200.

FIGURE 2
A duodenal section from the intestine of an 18 day old rat from a mother on special diet. It is very similar to the previous. Mag. x200.

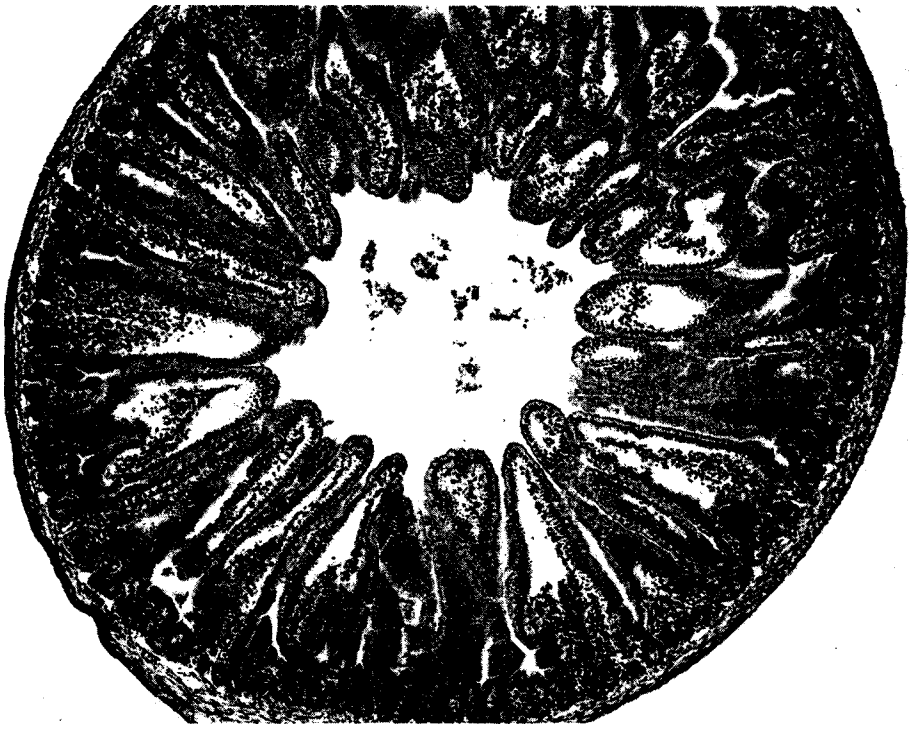

FIGURE 3
An ileal section of an 18 day old rat reared on normal diet. Villi are numerous and slightly blunted at their tips. Mag. x 200.

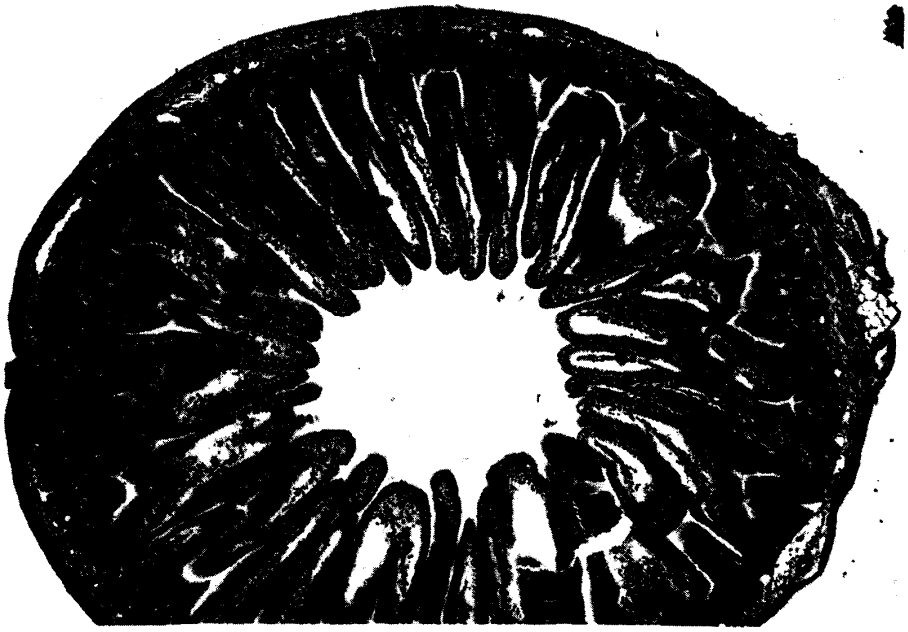

FIGURE 4
Ileal section of a special diet 18 day old rat, showing it to be very similar to Fig. 3. Mag. x 200.

FIGURE 5
A duodenal section from an adult rat reared on normal rat diet showing
numerous villi with broad shape and blunted ends. Mag. x 20fX

FIGURE 6
Duodenal section from a special diet rat; the villi are quite different, being
long and slender and appearing quite healthy. Mag. x 200.

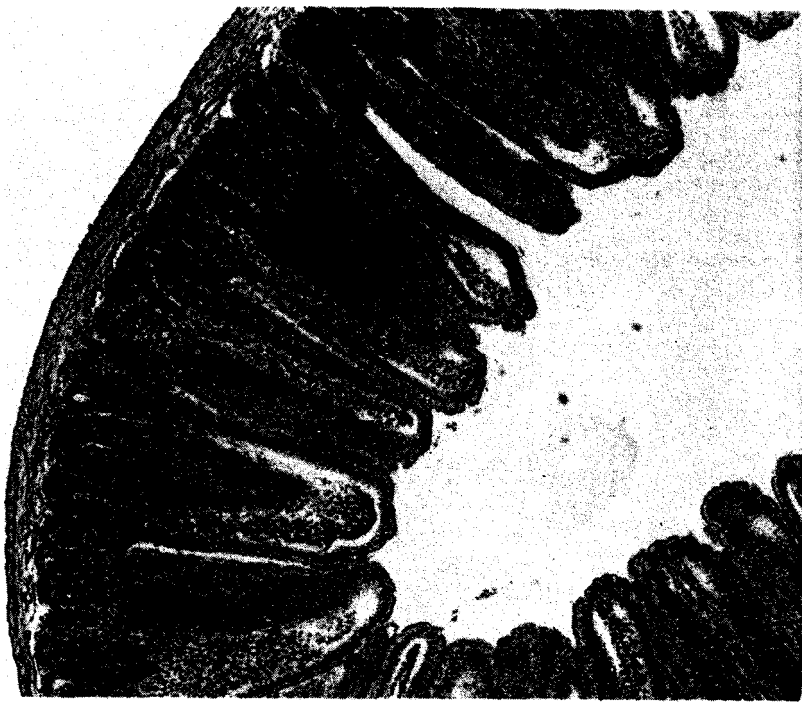

FIGURE 7
Duodenal section of a normal diet rat after an oral dose of wheat protein;
little difference is seen from that of fig. 5. Mag. x 200.

FIGURE 8
Duodenal section of a special diet rat after an oral dose of wheat protein; it
is different from that of Fig. 6 inasmuch as it shows slight broadening and
blunting of the villi. Mag. x 200.

FIGURE 9
Shows a section from the ileal region of a rat diet fed animal, villi are broader with blunted ends, and appear healthy. Mag. x 200.

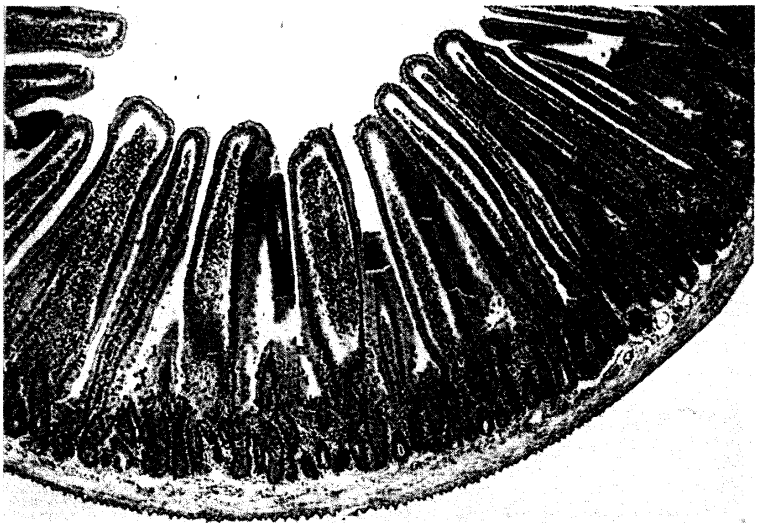

FIGURE 10
An ileal section from a special diet reared rat. Note the difference in the shape of the villi; they are much longer and have a narrow appearance; the villi are not broad and blunt. Mag. x 200.

FIGURE 11
Ileal section of a rat diet animal after an oral dose of wheat protein; it is not very different to Fig. 9. It could be said that the villi were of a more blunt appearance. Mag. x 200.

FIGURE 12
An ileal section of a special diet rat after an oral dose of wheat protein. There is a marked difference in appearance, there is broadening and blunting of the villi to be seen, and it now looks more like the rat diet intestine. Mag. x 200.

Discussion

It has been recognized for some time that wheat proteins have an effect on the villi of the intestine; Strober (1978) suggested that some wheat protein antigens are responsible for much of the histological changes in the gut mucosa, especially if a coeliac condition exists. When there is villus atrophy there is a decreased absorption area in the intestine, but there might be also sites for leakage of ingested proteins allowing access to the circulation which might evoke an allergic response.

Studies carried out previously by Williams (1978, 1979) showed that more wheat protein antigen was present in the blood circulation of non-wheat protein fed animals as compared with animals that had previous experience of those proteins. This study was carried out after feeding wheat proteins to the animals and using the antigen/antibody agar diffusion technique.

This study shows that when a rat is reared on wheat protein diet and further challenged with wheat protein, there is no difference to be observed in the morphology of the intestine, the intestinal villi retain their appearance. However, in rats reared on a non-wheat protein diet the villi are longer and more slender, and when challenged in later life with an oral dose of wheat protein, a marked difference in the appearance of the villi is observed. They become more blunted and broader, similar to those of the rat diet fed animals. When a reversal of this was attempted by placing the animals back on a non-wheat diet, the villi remained as if they had encountered wheat proteins all their life. This suggests that wheat protein does have a non-reversible effect on intestinal villi especially if the first encounter with that protein is in mature life. It is possible that in animals which have been reared on a wheat free diet that changes in the intestinal villi developing three to four days after wheat administration are related to an immune response to that administered antigen. Although the diets could be said to vary considerably in their effect on intestinal motility, there could be factors like bacterial growth affecting changes in the villus size, this was considered and morphological examinations were done throughout the experimental period; it was only after a wheat protein feed that the villi were reduced in size.

There may be changes of an immunological nature, a delayed immune reaction involving secretory IgA or the production of antibodies, with the intestinal villi taking on a different morphological structure as a defense against a foreign protein, thus taking on the appearance of a "normal" villi of perhaps the same immunological status. This could be important in early life, as an encounter with a foreign protein could prepare the intestinal villi with its full immunological complement against ingestion of the "toxic" protein later in life, whereas if a subject is not competent for handling foreign proteins, a detrimental effect can occur in the villi of the intestine. As a non-wheat protein fed rat could be considered abnormal due to their special diet, this might well be the case.

The marked difference between the proximal and distal segments is probably due to the fact that the protein does not stay as long in the proximal region of the intestine as it does in the distal, and by the time that part is reached the protein is broken down by the various intestinal enzymes, and the effect could be due to not only the undigested protein but to the various break-down products, peptides and other potent chemicals resulting from digestion, some of which may be toxic and have a detrimental effect on the villi.

It is interesting to note that only the special diet reared rats show morphological changes. The rat diet animal would be developing from its early stages in a milieu liberally supplied with antigenic material from the mother's diet, and this might be expected to produce tolerance, whereas the special diet animal would not. Thus it seems that both foetus and suckling are normally supplied with a sample of their mother's diet and this may be an essential part of the preparation of the animal's digestive and immunological mechanism.

Studies presented here indicate that wheat proteins have an effect on the intestinal villi of special diet animals, this being more

pronounced in the distal intestine. This fact could have a strong bearing on what is transported into the circulation from the intestine and the transported products could have an effect on behavior patterns elicited by the subject for it is now accepted that foodstuff can have an adverse effect on behavior, especially if a mental condition is present. It could therefore be argued that it is better for an individual to have experience of different proteins early in life and so prepare itself for later life.

Work is in hand to try and ascertain whether the changes in the villi of the intestine are due to immunological or other aspects of the gastro intestinal tract.

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