PATHOLOGY OF INFESTATION OF THE RAT WITH *NIPPOSTRONGYLUS MURIS* (YOKOGAWA)

V. PROTEIN DIGESTION

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Summary

The digestion of protein by rats infested with the intestinal nematode *Nippostrongylus muris* (Yokogawa, 1920) was measured directly by feeding by stomach tube egg albumin labelled with radioiodine. One hour later nearly 70 per cent. was recovered from the gastro-intestinal tract of infested rats, but only 50 per cent. from that of normal rats. This difference was due largely to a depression of digestion in the small intestine, although absorption was also slightly reduced. Gastric digestion was not affected, nor was there any difference between the rates of gastric emptying by normal and infested rats.

I. INTRODUCTION

General texts on parasitology commonly refer to the possible effects of intestinal nematode infestation upon digestion by the host. Lapage (1956), for instance, mentions the possible effect of *Trichostrongylus* spp. upon digestion and absorption but does not refer to observations or experiments. Several investigators have made indirect assessments by balance studies. Stewart (1933), Shearer and Stewart (1933), and Franklin, Gordon, and Macgregor (1946) have shown by this method that there was decreased digestion of crude protein and decreased net mineral absorption by sheep infested with various species of nematodes. Spedding (1954) and Shumard, Bolin, and Eveleth (1957) confirmed that the digestibility of crude protein was decreased by infestation, but Andrews, Kauffman, and Davis (1944) could not confirm that either digestion or absorption was affected in sheep infested by *T. colubriformis*. Rogers (1941, 1942) has shown that rats infested with *Trichinella spiralis* experienced an impaired protein digestion during the intestinal phase.

It is not possible to separate digestion from absorption, nor to allow for secretion into the lumen of the gut when digestion is measured by means of balance studies. It is conceivable that only one of these may be deranged. For these reasons a direct measurement of protein digestion was of considerable interest in nippostrongylosis of rats and in intestinal infestations generally.

Egg albumin labelled with radioiodine was used to measure protein digestion. The efficacy of this method has been discussed by Borgstrom *et al.* (1957). The rate of gastric emptying and absorption from the small intestine was also estimated. An approximate comparison between normal and infested rats was also made of gastric digestion as distinct from digestion in the stomach and small intestine together.

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II. Methods

The type of rats used, the method of infesting them, and the reasons for carrying out the experiments on the tenth day of the infestation have been stated earlier by Symons (1957). A 1 per cent. (w/v) solution of a technical grade of egg albumin (E.A.) in 0·9 per cent. NaCl was labelled with ^{131}I by the ammoniacal method of Francis, Mulligan, and Wormall (1959). Before being fed to the rats, sufficient carrier E.A. was added to this solution to increase the protein concentration to about 5 per cent. The rats which were fasted overnight were fed 2 ml of this solution by stomach tube, without anaesthesia, using the gagging device of Gillespie and Lucas (1957). They were then returned to their cages for 1 hr, after which they were killed by a blow on the head. Infested rats were fed and killed alternately with normal controls.

The stomach and small intestine were isolated by ligatures. The stomach was opened and washed directly into a 25-ml volumetric flask with cold 0·25 per cent. E.A. The small intestine was opened longitudinally into a 100-ml conical flask and washed six times. The washings were transferred successively to a 100-ml volumetric flask. The caecum and large bowel were discarded, as it was determined previously that less than 1 per cent. of the radioactivity of the preparation administered reached these organs in either infested or normal rats 1 hr after feeding. The flasks were stored at about 4°C for 1–2 hr, after which 5-ml samples were transferred to graduated centrifuge tubes and the protein precipitated by the addition of 5 per cent. phosphotungstic acid to a final concentration of 2·5 per cent. (Borgstrom *et al.* 1957). After a further 1–2 hr at about 4°C the precipitates were spun down. When washings from the stomach or small intestine were added to the labelled E.A. solution and the protein precipitated, less than 1 per cent. of the radioactivity remained in the supernatant.

After the volume of the supernatant was recorded, it was separated from the precipitate, which was drained. Triplicate 2-ml aliquots of the supernatant were placed in suitable test tubes for radio-assay. The precipitates were dissolved in about 5 ml of 0·4N NaOH, then diluted with water to 10 ml and 2-ml aliquots taken for counting. The radioactivity was expressed as counts/sec in all instances.

The radioactivity in the 2 ml of E.A. solution fed to the rats was estimated by diluting samples with 0·25 per cent. E.A. and taking 2-ml aliquots for counting. In addition, 5-ml aliquots of these diluted solutions were precipitated with phosphotungstic acid to estimate any free ^{131}I which was present in the original solution and allowance was made for this in the final calculations.

All count rates were determined with a well-type EKCO N55OA scintillation counter, and an EKCO N530 automatic scaler, and were corrected for background and decay.

Calculations were made assuming that labelled and carrier protein were not digested or absorbed differently, and that all the radioactivity in the supernatant represented completely or partially hydrolysed protein.

The radioactivity in the worms was estimated to ensure that the amount of protein or the products of hydrolysis, which was taken up by the parasites themselves, was not of significance to the experiment. The parasites were recovered

from three rats, 1 hr after feeding 2 ml of labelled E.A., by opening the intestines longitudinally into warm physiological saline in conical flasks. The intestines were allowed to remain in the flasks for a few minutes before removal. The parasites were then allowed to settle. They were washed at least seven times with saline, the excess water was removed on filter paper, and the worms spread thinly on planchettes. In this instance the radioactivity present in the parasites per mg of wet weight, and in an appropriate dilution of the E.A. solution fed to the rats, was then determined using a Geiger–Müller tube with a thin end-window.

III. Results

The activity taken up by the parasites was found to be less than 0·01 per cent. of the total activity fed to the rats and was therefore ignored in all calculations.

There were nine rats in both the normal and in the infested groups, but one of the latter had to be discarded as a tear was found in the small intestine. The severity of the infestations, judged subjectively, was found to range from light to heavy; four infestations were heavy.

The sum of digested and undigested protein recovered from the gastro-intestinal tract, expressed as a percentage of the total radioactivity fed to the rats, was calculated from the equation

$$R_a = [(S_a+I_a)/T_a] \times 100,$$

where

R_a = percentage radioactivity recovered,

S_a+I_a = sum of the activities of the supernatant and precipitated protein from the stomach and small intestine respectively, and

T_a = total radioactivity fed to each rat.

The proportion digested in the stomach and small intestine together was expressed as a percentage of the total radioactivity fed to an animal and was calculated from the equation

$$\text{Percentage digestion} = \{[T_p-(S_p+I_p)]/T_p\} \times 100,$$

where S_p, I_p, and T_p are the activities in the precipitated protein from the stomach, small intestine, and meal fed to the rats respectively. The last of these three quantities was smaller than the total activity fed to the rats (T_a) because allowance was made for free ^{131}I in the solution.

The radioactivity available for absorption from the small intestine (expressed in terms of the symbols used above) was $T_a-(S_a+I_p)$ and the radioactivity actually absorbed was $T_a-(S_a+I_a)$. The percentage absorbed from the small intestine was then calculated from the equation

$$\text{Percentage absorbed} = \frac{(T_a-S_a)-I_a}{(T_a-S_a)-I_p} \times 100.$$

The results of the experiment are shown in Table 1. Fifty per cent. of the meal was recovered from the normal and 68·9 per cent. from the infested rats. The difference between these two, the 95 per cent. confidence limits of which are also

shown in the table, is due mainly to a depression of digestion, although absorption from the small intestine was also reduced. It is emphasized that digestion here refers to the sum of digestion in the stomach and small intestine. In normal rats 58·6 per cent. of the meal was digested while only 35·8 per cent. was digested by the infested animals. The difference within the 95 per cent. limits of confidence indicates that this is a well-marked depression. The lower percentage of absorption by the small intestine, as can be seen from the table, is not so clearly significant.

Gastric emptying was not affected by the infestation as about 90 and 91 per cent. of the meal left the stomach in 1 hr in normal and infested rats respectively. An approximate comparison of gastric digestion as distinct from digestion in the stomach and small intestine together could be made by calculating the ratio of hydrolysed protein to undigested protein that remained in the stomach at the time of death. There was no statistical difference between gastric digestion by normal and infested rats.

TABLE 1

RECOVERY, DIGESTION, AND ABSORPTION OF EGG ALBUMIN IN RATS INFESTED WITH N. MURIS

	Normal	Infested	Difference*
Total recovery from gastro-intestinal tract (as % of total radioactivity fed)	50·0	68·9	−18·9±10·6
Digestion in stomach and small intestine (as % of radioactivity fed as labelled protein)	58·6	35·8	22·8±13·9
Absorption from small intestine (as % of radioactivity available for absorption)	89·6	80·3	9·3±8·7
Gastric digestion $\left(\text{ratio: } \frac{\text{hydrolysed protein}}{\text{undigested protein}}\right)$	0·64	1·21	−0·57±1·05
Gastric emptying (as % of radioactivity fed)	89·5	90·7	−1·2±11·1

* Differences are given with their 95 per cent. confidence limits.

IV. DISCUSSION

Advantages of this method are that the labelled protein can be distinguished from the endogenous protein secreted into the gut during digestion, and absorption and digestion can be separated. Dreisbach and Nasset (1954) have demonstrated that the amount of nitrogenous material recovered from the gastro-intestinal tract of rats was equal to, or even greater than, the amount of protein ingested. Pisano, Paine, and Taylor (1959) found that the nitrogen secreted into the duodenal loop of chickens obscured the measurement of absorption of protein from that region.

In the experiment reported here, about 70 per cent. of the radioactivity was recovered from the gastro-intestinal tract of the infested rats, while only 50 per cent. was recovered from controls. A reduced ability to digest the egg albumin was largely responsible for this difference, but there also appeared to be a derangement of absorption. The impairment of egg albumin digestion supports the findings of the earlier workers mentioned, who by balance studies concluded that nematode infestations in sheep or rats could depress protein digestion. Because there was no impairment of gastric digestion it appears that this depression of digestion associated

with nippostrongylosis occurs entirely in the small intestine, which is the site of infestation. The secretion of anti-enzymes by the parasite was postulated by Shearer and Stewart (1933) to inhibit the action of pepsin, but the present experiment offers no evidence as to the cause of the depression.

The possible reduction of absorption of nitrogen from the small intestine as a whole is interesting. The values reported here have not strong statistical support, but they tend to contradict the experiments reported in Part IV of this series (Symons 1960). It was then found that neither glucose nor histidine absorption was retarded in the complete small intestine of the infested rats; on the other hand, perfusion of the infested jejunum alone did show that glucose absorption from that section was reduced to one-third or less of the normal rate, but histidine absorption was not tested in the jejunum alone. Borgstrom *et al.* (1957) found that, in man, although absorption of fat, carbohydrate, and protein began in the duodenum, protein absorption was not complete even at the distal end of the small intestine; furthermore, the absorption of glucose was completed in the proximal half of that organ. These findings support those of Schlüssel and Sunder-Plassmann (1953) who reported that some of the protein labelled with radiosulphur and fed to rats was recoverable from the rectum, although the greatest degree of absorption occurred in the duodenum and upper jejunum. This slower rate of protein than glucose absorption may mean that the jejunum, which is the site of the infestation, is relatively more important to the former and could account for the discrepancy between this and the earlier experiment. In addition, there may be a complex interrelationship between the rate of digestion, the concentration of the products of hydrolysis, and absorption, which was not present in the earlier experiments.

Neither the depression of digestion nor of absorption was likely to be due to increased motility of the upper part of the small intestine as Symons (1959) has shown that there is, in fact, a significant decrease in the rate of passage through this part of the gut of infested rats.

Both groups of animals passed about 90 per cent. of the meal from their stomachs in 1 hr. This confirms earlier experiments (Symons 1959, 1960) when it was found that infested rats emptied a solid meal or solution of glucose or histidine from the stomach at the same rate as the controls. The estimate of the rate of gastric emptying makes no allowance for the fact that some of the meal fed by stomach tube is forced past the pylorus nor for any regurgitation into the stomach that may occur. This regurgitation which, because of the greater volume of ingesta in the upper small intestine (Symons 1957), could conceivably be more pronounced in the infested rats, would also interfere with the measurement of protein digestion in the stomach. For this reason, the estimates of gastric emptying and digestion must be accepted with caution.

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