

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

VI. ABSORPTION IN VIVO FROM THE DISTAL ILEUM

By L. E. A. SYMONS*

[Manuscript received July 19, 1960]

Summary

The net fluxes of water, sodium, and chloride and the absorption of glucose were measured *in vivo* in the distal ileum of rats infested in the jejunum by the nematode *Nippostrongylus muris*. The rate of net fluxes of water and chloride was not affected, but the rate of absorption of glucose and probably sodium was increased in infested rats. The dry weight of the distal ileum per unit length was unchanged by the infestation.

These results are discussed in relation to the previously reported deranged function of the infested jejunum, and it is concluded that malabsorption is not of major importance in nippostrongylosis of rats.

I. INTRODUCTION

In Part III of this series it was reported that the efflux of sodium per gram of dry mucosa from the lumen of the jejunum of rats infested with the nematode *Nippostrongylus muris* (Yokogawa, 1920) was reduced to about one-third of that of normal rats (Symons 1960*b*). The rate of absorption of glucose from the jejunum was similarly depressed. On the other hand, no reduction of the rate of absorption of glucose or of histidine was observed when absorption was measured from the entire small intestine (Symons 1960*c*). It was postulated, therefore, that absorption from the worm-free ileum below the jejunum, the site of the infestation, was not affected by the parasites.

The present paper is an account of measurements in the distal ileum of the net fluxes of water, sodium, and chloride and of the absorption of glucose.

II. METHODS

The preparation of the rats and the method of anaesthetization have been described in Part III (Symons 1960*b*).

(a) Net Fluxes

The *in vivo* technique of Curran and Solomon (1957) as modified by Symons (1960*b*) was used to perfuse loops of the distal ileum of infested and normal rats. The distal cannula was tied into the ileum 3–5 cm proximal to the ileocaecal valve and the proximal cannula about 7 cm above it. The loop was perfused for three periods of 15 min with a solution containing 140 m-equiv. NaCl/l. After perfusion the length of the loop was measured, it was then opened longitudinally and the

* Division of Animal Health, C.S.I.R.O., McMaster Laboratory, Glebe, N.S.W.

mucus and debris were carefully removed. All the loops from any one experiment were stored at about -10°C until they were dried together at about 100°C .

The net fluxes were expressed as ml or m-equiv/hr/g dry weight tissue. The mean of the three perfusion periods was taken as the rate for each rat.

Sodium concentrations were determined by flame photometry with a Beckman DU spectrophotometer, and chloride concentrations were estimated without protein precipitation, by the method of Schales and Schales (1941).

(b) *Glucose Absorption*

The rate of glucose absorption from the distal ileum was measured by the following methods:

- (1) Nelson-Somogyi: the loops were prepared and perfused as before with a solution of 0.25M D-glucose in Krebs-Henseleit bicarbonate buffer (Umbreit, Burris, and Stauffer 1949). After suitable dilutions of the perfusates, the glucose was determined by the Nelson-Somogyi method (Nelson 1944). This experiment was carried out twice with six normal and six infested rats on each occasion.
- (2) Schaffer-Hartmann: the loops were perfused as before with 0.25M D-glucose in bicarbonate buffer, but the glucose analyses were made by the method of Schaffer and Hartmann (1921).
- (3) One hour perfusion: the loop was prepared as before, except that it was returned to the abdominal cavity and the distal cannula brought to the exterior through a stab wound in the flank. The proximal cannula entered the abdominal cavity through the laparotomy wound which was closed around it. The temperature of the abdomen was raised to 38°C before perfusion was begun with 0.25M D-glucose in 145 m-equiv. NaCl/l. There was no further equilibration period but the rate of perfusion was reduced to 0.1–0.2 ml/min and all the fluid in the collecting tubes and the loop was recovered for glucose estimation by the method of Schaffer and Hartmann. An estimation of water absorption was not possible in this experiment.

Tests for the presence of reducing substances, which might have entered the loop during perfusion, were made by perfusing loops in normal and infested rats with bicarbonate buffer alone. Glucose analyses were made in two separate experiments by the Nelson-Somogyi and Schaffer-Hartmann methods.

The rates of absorption of glucose and water were expressed as mm/hr/g and ml/hr/g of dry ileal tissue respectively.

III. RESULTS

(a) *Dry Weight of Distal Ileum per Centimetre*

The dry weight of the distal ileum per centimetre, measured with the tissue from 12 normal and 15 infested rats, was found to be 0.21 ± 0.003 g in both instances. This result supported the histological evidence reported previously

(b) *Net Fluxes*

In the experiments reported earlier (Symons 1960*b*), it was found that there was a net influx into the infested jejunum, but in the present experiments there was a net efflux from the ileum of infested rats of sodium, chloride, and water. In

NET FLUXES FROM DISTAL ILEUM OF NORMAL AND INFESTED RATS PERFUSED IN VIVO WITH SODIUM CHLORIDE

	Water Flux (ml/hr/g dry tissue)	Sodium Flux (m-equiv/hr/g dry tissue)	Chloride Flux (m-equiv/hr/g dry tissue)
Normal rats	$+7.14 \pm 3.41$	$+1.14 \pm 0.50$	$+2.12 \pm 0.73$
Infested rats	$+4.48 \pm 4.84$	$+1.62 \pm 0.82$	$+2.09 \pm 0.94$
	$\left. \begin{array}{l} \text{ } \end{array} \right\} \text{n.s.}$ $\left. \begin{array}{l} \text{ } \end{array} \right\} P < 0.05$ $\left. \begin{array}{l} \text{ } \end{array} \right\} \text{n.s.}$		

(c) *Absorption of Glucose and Water*

No reducing substances were detected by either method of glucose analysis in the perfusates from the ileum of normal or infested rats perfused with bicarbonate buffer alone. In the first experiment it was found that the distal ileum of infested rats absorbed glucose at a faster rate than did the ileum of the controls, but the difference was small. Furthermore, the results suggested that either the rate of absorption by the normal rats was negligible, or that possibly glucose actually entered the lumen from the tissue during perfusion. For these reasons, the experiment was repeated with the identical technique and with perfusing solutions of the same concentration. In the second experiment, there was a positive absorption of glucose from the ileum of the normal rats, and the rate of absorption was again faster in the infested rats.

Because the large variation of the rate of absorption between individual rats for these two experiments was considered to be due mainly to the Nelson-Somogyi method of glucose determination, the experiment was repeated using the Schaffer-Hartmann method. Again, there was found to be a greater rate of absorption from the ileum of infested rats, and the Schaffer-Hartmann method of glucose analysis reduced the experimental error considerably.

Because the absolute amount of glucose absorbed in one perfusion period of 15 min was small, the experiment was repeated by perfusing for 1 hr and estimating all the glucose in the perfusate and in the loop at the end of this period. By this method it was found again that the infested rats absorbed glucose at a faster rate. The statistical significance of these four experiments is discussed below.

TABLE 2

ABSORPTION OF D-GLUCOSE AND NET FLUXES OF WATER FROM THE DISTAL ILEUM OF NORMAL AND INFESTED RATS PERFUSED IN VIVO WITH D-GLUCOSE

D-Glucose concentration 0.25M. + = net absorption; - = net influx to the lumen

Expt. No.	Methods of Glucose Analysis	No. of Rats Used	Glucose Flux (mm/hr/g dry tissue)	Water Flux (ml/hr/g dry tissue)
1	Nelson-Somogyi	6 normal 6 infested	-0.97 ± 0.98 $+1.06 \pm 1.30$	-3.36 ± 1.49 -2.29 ± 1.38
2	Nelson-Somogyi	6 normal 6 infested	$+2.67 \pm 1.30$ $+4.00 \pm 1.80$	-1.32 ± 0.91 -1.55 ± 1.60
3	Schaffer-Hartmann	7 normal 6 infested	$+0.07 \pm 0.88$ $+0.81 \pm 0.25$	-3.17 ± 3.02 -3.21 ± 2.00
4	Schaffer-Hartmann after 1 hr perfusion	6 normal 6 infested	$+1.83 \pm 0.60$ $+2.84 \pm 1.38$	—

* Analysis of variance: see text for details of statistical analyses.

The net fluxes of water which were measured in the first three of these experiments are also shown in Table 2. In every instance, there was a net influx into the lumen and in no instance was there any statistically significant difference between normal and infested rats.

IV. DISCUSSION

The finding that the dry weight of the ileal tissue per unit length of normal and infested rats was identical contrasts with the dry weight of jejunal tissue which was increased by 50% per centimetre in the infested rats (Symons 1960a). The similarity of the dry weights of the ileum of normal and control rats justified the decision to express the rates of absorption per unit dry weight of entire tissue in the present experiments. It was not necessary to separate the mucosa from the muscle layers as was done in the jejunal experiments.

A comparison can be made between the net fluxes of water and sodium found in the present experiments and those reported by Curran and Solomon (1957) who perfused the distal ileum of rats by the *in vivo* technique upon which these experiments were based. The net fluxes of water and sodium for normal rats in Table 1, when converted by the ratio of dry weight/cm to 0.15 ml and 0.023 m-equiv/hr/cm respectively, are within the upper limits of the wide range shown by Curran and Solomon for perfusion with a solution containing 150 m-equiv. NaCl/l. They did not state a similar range for net chloride fluxes, but approximate net rates derived from other data in their paper suggest that the net fluxes in the present experiment were higher than they obtained. On the other hand, the fluxes presented now tend to support the finding of Curran and Solomon that the net chloride flux is greater than the net sodium flux.

A comparison of the rate shown in Table 2 for glucose absorption from the distal ileum of normal rats with the rate of absorption from the jejunum of normal rats reported earlier (Symons 1960c), suggests that the former is lower. This confirms the reports of *in vitro* experiments with the small intestines of rats by Fisher and Parsons (1950) and hamsters by Korelitz and Frank (1959), who found that the rate of absorption increased with the proximal distance from the ileocaecal valve.

There was no evidence in the present experiments that the net influx of water, sodium, and chloride and the depression of absorption of glucose that occurred in the jejunum of infested rats occurred in the distal ileum. There was, in fact, a strong indication that a compensatory increase in the rate of absorption occurred. The net efflux of chloride from the ileum of infested rats did not differ significantly from that from the ileum of normal rats, but there was evidence of an increase in the rate of net sodium efflux, although slight, and significant only at the 5% level. An increase in the rate of net efflux of sodium is, however, not necessarily evidence of increased rate of absorption. The increase found could have been due to a reduction in the rate of influx into the lumen without any change in the rate of efflux. Only measurement of the unidirectional fluxes could decide this point.

The results of the glucose absorption experiments do, however, indicate a compensating faster rate of absorption from this region of the small intestine. No single experiment provided unequivocal evidence that glucose was absorbed faster from the distal ileum of infested rats, but the fact that in each experiment the rate was greater in these animals strongly suggested that this was so. It was not possible to analyse the four experiments together, but an analysis of variance showed that although the variation between the two Nelson-Somogyi experiments was highly significant ($P < 0.001$), the variation between the normal and infested rats was also significant ($P < 0.01$). This result refuted the original hypothesis that absorption from the distal ileum was not affected by the infestation, and provided evidence that the infestation increased the rate of absorption from this section of the small intestine. One-tailed *t*-tests of the hypothesis that the mean rates of absorption in normal and infested rats were equal, against the alternative hypothesis that the infested rats absorbed at a faster rate, were then made on the two Schaffer-Hartmann experiments. These tests were found to be significant at the 5 and 7% levels respectively. In order to summarize the results of the three independent statistical tests

which were made of the data of the four experiments, the exact probabilities associated with each test were calculated and compounded according to Wallis (1942). The resulting compounded probability was less than 0.001. This indicates that there is no reasonable doubt that the infestation increased the rate of absorption of glucose from the distal ileum. The extent of this increase cannot be estimated with any accuracy from the results available, but may be only slight.

The net movement of water across the epithelium of the distal ileum was measured under two conditions. From a solution containing 140 m-equiv. NaCl/l only, there was a net efflux of water from both normal and infested rats, the rates of which were not statistically significantly different, although the mean rate from the infested ilea was lower. In the glucose-absorption experiments the solutions were hypertonic because the bicarbonate buffer in the first three, and the sodium chloride in the last experiment were not adjusted osmotically to allow for the added glucose. This accounts for the net influx which occurred in all instances, and the results accord with the findings of Curran and Solomon (1957). Symons (1960c) showed that hypertonic solutions also cause a net influx into the infested jejunum; therefore, the present experiment does not exclude the possibility of the infestation affecting water fluxes in the ileum.

In the earlier experiments, an apparent contradiction was found between the deranged function of the infested jejunum and the unaffected rate of absorption of glucose and histidine from the small intestine as a whole (Symons 1960b, 1960c). This may now be explained by the compensating increase in the rate of absorption of glucose from the ileum. This explanation is supported by the probable increase in the rate of absorption of sodium.

Alternatively, however, it is now possible to envisage normal physiological responses which would ensure that the products of digestion, which are not absorbed by the infested jejunum, would be absorbed by the unaffected ileum. Borgström *et al.* (1957) have shown that all fat and carbohydrate and the greater part of protein fed to rats was absorbed before the ingesta reached the lower ileum, chiefly from the duodenum and jejunum. Schlüssel and Sunder-Plassmann (1953) also found that the greater part of protein absorption occurred in the first half of the small intestine. Reynell and Spray (1956) have shown that as much as the upper two-thirds of the small intestine of the rat may be resected without loss of weight or demonstrable defects of absorption. These findings indicate a large functional reserve in the small intestine. Furthermore, several workers have shown that, within limits, there is a direct, though not necessarily linear, relationship between concentration in the lumen and rate of absorption (Groen 1937; Fullerton and Parsons 1956; Acland and Illman 1959; Jervis and Smyth 1959). It can be postulated, therefore, that the products of digestion will reach the ileum of the infested rats at higher concentrations than will occur normally, and that the rate of absorption will be correspondingly faster. It must be emphasized that in the experiments presented in this paper, the normal and infested rats were perfused with similar solutions, so that the increased rate of glucose absorption which is reported is not due to a higher concentration in the lumen of the ileum. It is not known whether the increased rate of absorption due to the infestation will alter the response of the ileum to different concentrations in the lumen.

It was shown in Part V of this series (Symons 1960*d*) that the digestion of protein was considerably depressed by the infestation and absorption of the products may have been slightly reduced. Because it can now be stated that the absorption of carbohydrate at least is not affected, it is concluded that a relative failure of digestion rather than malabsorption of its products is the more important aspect of nippostrongylosis of the rat.

V. ACKNOWLEDGMENTS

The author wishes to thank Dr. G. M. Tallis, Division of Animal Genetics, C.S.I.R.O., for discussion of the statistical analysis of the glucose-absorption experiments, and Miss Mary Bartle, Division of Mathematical Statistics, C.S.I.R.O., who carried them out.

The technical assistance of Miss Marian Carpenter is also gratefully acknowledged.

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