

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

III. JEJUNAL FLUXES IN VIVO OF WATER, SODIUM, AND CHLORIDE

By L. E. A. SYMONS*

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Summary

The net fluxes of water, sodium, and chloride were measured *in vivo* by perfusion of the jejunum. There was a net absorption of these three substances from isotonic saline solutions in normal rats, but a net influx to the lumen in each instance in rats infested with the nematode *Nippostrongylus muris*. The unidirectional fluxes of sodium and the net fluxes during perfusion with hypo- and hypertonic saline solutions indicated that this was fundamentally due to a derangement of efflux while influx was unaffected. The gross effect, however, was also due to an increase of influx because of the greater weight of mucosal tissue per centimetre of jejunum in the infested animal. The unidirectional fluxes of water did not support these conclusions unequivocally. The fluid which accumulates in the infested small intestine can be explained by these results.

I. INTRODUCTION

It has been shown in Part I of this series (Symons 1957) that there was a considerable increase in the fluid in the lumen of the small intestine of rats infested by the nematode *Nippostrongylus muris* (Yokogawa, 1920). This suggested that there was either a hypersecretion into the gut, a failure of absorption, or a combination of both these factors.

Baker (1955) has suggested that one cause of the anaemia of mice associated with the infestation with *Nematospiroides dubius* may be a deficient absorption as a result of the atony, dilatation, and inflammation which occur in the small intestine. Lesions of this nature occur in nippostrongylosis of rats (Symons 1957).

Indirect measurements by means of balance studies had indicated that intestinal infestations in various hosts may affect absorption. Stewart (1933), Shearer and Stewart (1933), and Franklin, Gordon, and Macgregor (1946) have shown a decrease of crude protein digestion and net mineral absorption by sheep infested with a number of species of nematode. Rogers (1942) found impairment of mineral absorption by rats infested with *Trichinella spiralis* in the intestinal phases. Andrews, Kauffman, and Davis (1944) could not confirm that absorption was affected in sheep infested with *Trichostrongylus colubriformis*.

Direct measurement of movements across the jejunal mucosa, the site of the infestation, was therefore considered to be an important part of the investigation of the pathology of nippostrongylosis. The net fluxes of water, sodium, and chloride, as well as the unidirectional fluxes of the first two substances were measured *in vivo* by a perfusion technique.

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

II. METHODS

(a) *Net Jejunal Fluxes*

(i) *Animals*.—Albino rats from a colony inbred for many generations were used in all experiments. They were infested when they weighed about 120–150 g by the subcutaneous injection of about 3500 larvae of *N. muris*. Most experiments were carried out on the tenth day of the infestation for the reasons given in Part I (Symons 1957). Some perfusions were performed on the ninth or eleventh days as it was not always possible to perfuse sufficient rats from one batch on one day. There was no reason to believe that this introduced any variation into the results. Non-infested controls were perfused in every experiment.

All rats were fasted overnight and anaesthetized with urethane given intraperitoneally at approximately 0.1 mg/100 g body weight. Not only was this anaesthetic easy to administer and long acting, but Alvarez and Hosoi (1929) have shown that it had a negligible effect upon the motility of the gut.

The severity of the infestation was judged subjectively from the appearance of the rat and its jejunum.

(ii) *Perfusion*.—A modified version of the *in vivo* perfusion technique of Curran and Solomon (1957) was used to measure the net fluxes of water and of the sodium and chloride ions across the jejunum of normal and infested rats from various solutions of sodium chloride.

Haemoglobin could not be used as a marker for the volume of solution perfused as it was possible that blood is lost into the lumen of the intestine of infested rats. A high quality burette from which the tap had been removed was supported in a "Perspex" tank. At the end of the burette was attached a No. 25 gauge hypodermic needle which pierced a rubber stopper in the side of the tank. To the outer end of this needle was attached a cannula made of "Polythene" tubing with an original internal diameter of 0.5 mm. This was reduced by stretching. This cannula ended in another No. 25 or 26 gauge hypodermic needle from which the base had been removed. This needle was inserted in the loop of intestine to be tested.

Water in the tank was mixed and heated to 40°C by a constant-temperature heater, which was also used to circulate water through a small copper operating table upon which the rat was laid during the experiment. Also in the tank was a glass vessel to wash out the intestinal loop with the solution to be perfused.

Into the distal end of the loop being tested was inserted a "Polythene" cannula with an internal diameter of 2 mm. This drained into a high quality 10 ml graduated centrifuge tube. The centrifuge tubes used were all calibrated against the volume delivered by the burette between the 14 and 19 ml marks. The volumes perfused were therefore measured directly from the burette. Perfusion was always commenced from the 14.0 ml mark and proceeded under hydrostatic pressure at the rate of about 0.2–0.4 ml/min. This pressure varied from approximately 27 cm of water at the commencement, to 21 cm at the end of each perfusing period of 15 min.

After the abdomen of the rat was clipped the jejunum was exteriorized through a laparotomy. A ligature was tied just distal to the duodeno-ileal flexure and a transection of the intestine made at the suitable distance below this. The aim was

to include the section of maximum infestation in this loop. After the loop had been washed out with the appropriate solution, the distal cannula was tied into the transected end and the proximal cannula inserted through the wall of the intestine below the initial ligature. It was not necessary to ligate this cannula as the intestine did not leak.

Kinking of the segment was avoided by laying it on the abdominal wall rather than by returning it to the cavity. A thermometer was inserted beneath it after which it was covered with a piece of tissue moistened with the perfusing solution. Over this was placed a thin "Polythene" sheet. The preparation was kept warm by placing an electric blanket over all.

About 10–15 min of perfusion was allowed as an equilibrium period during which time the temperature of the loop was raised and kept at $38^{\circ} \pm 0.5^{\circ}\text{C}$. After this interval the loop was perfused for three periods of 15 min.

At the completion of the perfusion the rat was killed by inserting scissors into the thoracic cavity to sever the great vessels. This bled the segment which was carefully freed from its mesentery and its length measured to the nearest 0.5 cm. The segments were usually about 7 cm long. Care was taken to standardize this procedure. Peristalsis was still present at the end of the perfusion.

Duplicate aliquots of the perfusate were diluted 1 in 100 and 1 in 25 for sodium and chloride analyses respectively. The diluted samples together with samples of the perfused solution were then quick-frozen in dry ice and ethanol and stored. The sodium concentration was estimated by the flame photometric attachment to a Beckman DU spectrophotometer and the chloride by the method of Schales and Schales (1941) without protein precipitation.

The calculations of the net fluxes were essentially those of Visscher *et al.* (1944) except that they were expressed as millilitres of water or milliequivalents of sodium and chloride per centimetre length of jejunum and per gram of mucosal tissue per hour. The ratio of length of jejunum to dry weight of mucosa for normal and infested rats had been calculated previously (Symons 1960a). The rate of net flux was calculated for each 15-min period and the mean of the three taken as the rate for each rat. Six normal and six infested rats were used in each experiment.

(b) *Unidirectional Fluxes*

(i) *Sodium*.—The procedure was identical with that described above except that an approximately isotonic saline solution containing ^{24}Na prepared by the Australian Atomic Energy Commission was perfused. The solution when freshly prepared contained about $6\text{ }\mu\text{C/l}$. These perfusions were carried out in two batches with freshly irradiated sodium in each and with an infested rat alternating with a normal control. Six rats of each group were again used. Net sodium and water fluxes were calculated as before.

Duplicate 1-ml aliquots of the perfusate were placed in aluminium planchettes to which a drop of detergent had been added. These were carefully dried on a hotplate and the number of counts made in 10 min was recorded. A Geiger-Müller counter with a halogen-quenched end-window tube was used for this procedure. Corrections were made for background and decay, but self-absorption was ignored

as it was estimated to make a negligible difference to the subsequent calculations of efflux which were made according to the formula of Visscher *et al.* (1944). Some of these calculations were checked using the formulae of Curran and Solomon (1957) but were found to be identical except in the special instance where the net water flux was zero. In this instance the Curran and Solomon formulae would also give a zero sodium efflux which would not necessarily be true. Influxes were estimated by the difference between net fluxes and effluxes.

(ii) *Water*.—The procedure was again identical with that used before except that D₂O (99.7 per cent. pure) was added to isotonic saline to give a concentration of D₂O of about 3 per cent. (w/w). During each half of the experiment a freshly made solution was kept in a stoppered measuring cylinder from which the burette of the perfusing apparatus was replenished before each perfusion. An aliquot was also taken for analysis after the perfusion of each rat. The experiment was again divided into two daily periods in which six rats were perfused in each and an infested rat alternated with a normal rat.

To reduce exchange of D₂O with the atmosphere a rubber cap with a hole just large enough to take the distal cannula was fitted over the top of the centrifuge tube in which the perfusate was collected. This tube was closed with a rubber stopper immediately after perfusion. Shortly afterwards duplicate samples were quickly withdrawn for sodium and chloride analyses. The remainder was transferred to small vaccine bottles with special close-fitting rubber caps and stored at about 4°C. Analysis of D₂O concentrations in these samples was carried out by the Coal Research Section, C.S.I.R.O., using a Perkin-Elmer infra-red spectrometer, No. 112. The percentage D₂O (w/w) was estimated by measuring the intensity of the HOD band ($\text{H}_2\text{O} + \text{D}_2\text{O} \rightleftharpoons 2 \text{HOD}$) at 3.98μ with a slit width of 0.01 mm (Gaunt 1956). The cell used had calcium fluoride windows with a fixed light path of about 0.02 mm.

Efflux was calculated by the formula of Visscher *et al.* (1944) and influx by difference from the net flux.

All tests of significance were made using the *t*-test and standard deviations were calculated.

III. RESULTS

(a) *Net Jejunal Fluxes*

In Table 1 are shown the net fluxes of water, sodium, and chloride for six normal and six infested rats perfused with solutions containing 72, 142, and 280 m-equiv. NaCl/l. The statistical differences refer to comparisons of the hypo- and hypertonic perfusions with that of 142 m-equiv. NaCl/l. All results are expressed in terms of net flux/hr/g of dry mucosal tissue. For reasons given by Symons (1960a) this provides a measure of flux per unit of functional tissue. The plus sign indicates net absorption from the lumen and the minus sign a net influx into the lumen.

There was a net absorption of water and of both ions from the jejunum of normal rats perfused with a solution containing 142 m-equiv. NaCl/l. The infestation, on the other hand, reversed the direction of movement so that there were net influxes of water and electrolytes. These differences between normal and infested rats are highly significant.

It has been shown by Visscher *et al.* (1944) and confirmed by Curran and Solomon (1957) that water from a hypotonic saline solution is very rapidly absorbed in response to the osmotic gradient between the lumen and plasma. Conversely water was found to enter the lumen during absorption from a strongly hypertonic solution. In these experiments this was again found to be true for normal rats, when the rates were highly significantly different from those for perfusion with an approximately isotonic saline solution. The absorption of the sodium ion was not

TABLE 1
NET FLUXES PER HOUR PER GRAM DRY MUCOSAL TISSUE FROM JEJUNAL LOOPS OF NORMAL AND INFESTED RATS PERFUSED IN VIVO WITH SALINE SOLUTIONS
+ = net absorption from the lumen; - = net influx to the lumen. Statistical differences refer to comparisons of isotonic with hypo- and hypertonic saline perfusions

Saline Concn. (m-equiv/l)	Water (ml)	Sodium (m-equiv.)	Chloride (m-equiv.)
Normal rats			
72	$+21.03 \pm 4.53$	$+0.25 \pm 0.36$	$+0.77 \pm 0.39$
142	$+7.58 \pm 2.87$	$+0.63 \pm 0.62$	$+1.18 \pm 0.61$
280	-8.11 ± 5.44	$+0.70 \pm 1.56$	$+3.58 \pm 1.90$
	$\left. \begin{array}{l} P < 0.001 \\ P < 0.001 \end{array} \right\}$	$\left. \begin{array}{l} \text{n.s.} \\ \text{n.s.} \end{array} \right\}$	$\left. \begin{array}{l} \text{n.s.} \\ P < 0.05 \end{array} \right\}$
Infested rats			
72	-7.39 ± 2.95	-2.10 ± 0.28	-1.57 ± 0.25
142	-5.50 ± 3.82	-1.10 ± 0.41	-0.51 ± 0.68
280	-12.70 ± 3.20	-0.95 ± 1.45	$+0.95 \pm 0.69$
	$\left. \begin{array}{l} \text{n.s.} \\ P < 0.01 \end{array} \right\}$	$\left. \begin{array}{l} P < 0.001 \\ \text{n.s.} \end{array} \right\}$	$\left. \begin{array}{l} P < 0.01 \\ P < 0.01 \end{array} \right\}$

significantly changed by perfusion with hypo- and hypertonic solutions. This is again in conformity with the results of Curran and Solomon's experiments when they found a poor response by sodium to osmotic gradients. The small changes that were recorded were, however, in the directions that could be predicted. The changes in the net fluxes of chloride were also in the predicted directions, but only the increase in response to hypertonic perfusion reached statistical significance ($P < 0.05$).

There was no significant change in the rate of net water influx during perfusion of infested jejunum with hypotonic solution but the increase during hypertonic perfusion was in the predicted direction and was statistically significant ($P < 0.01$). This strongly suggests that efflux was affected rather than influx. The rates of net electrolyte fluxes were not quite so unequivocal, although the significant increase in net influx of sodium during hypotonic perfusion did support the evidence from the net water fluxes. The net chloride influxes were also increased during hypotonic

perfusion but they became net effluxes during hypertonic perfusion, which did not indicate any marked inability to absorb. The response in this instance was not as great as that recorded for normal rats.

(b) *Unidirectional Fluxes*

(i) *Sodium*.—The results of experiments in which jejunal loops of normal and infested rats were perfused with solutions containing ^{24}Na -labelled NaCl at a concentration of about 140 m-equiv/l are shown in Table 2. These are expressed as fluxes/hr/cm length of intestine as well as per gram of dry mucosal tissue. The

TABLE 2
SODIUM FLUXES FROM JEJUNAL LOOP OF NORMAL AND INFESTED RATS PERFUSED WITH SODIUM CHLORIDE SOLUTION LABELLED WITH RADIOSODIUM
Sodium chloride concentration 140 m-equiv/l

	Net Flux*	Influx	Efflux
m-equiv. sodium/hr/g dry mucosal tissue			
Normal	$+1.7 \pm 0.8$	3.3 ± 1.4	5.0 ± 1.7
Infested	-1.4 ± 0.7	3.3 ± 1.0	1.9 ± 0.9
	$P < 0.001$	n.s.	$P < 0.01$
m-equiv. sodium/hr/cm jejunum ($\times 10^2$)			
Normal	$+2.56 \pm 0.67$	5.01 ± 0.58	7.57 ± 1.20
Infested	-3.15 ± 1.52	7.65 ± 1.74	4.51 ± 1.45
	$P < 0.001$	$P < 0.01$	$P < 0.01$

* + = net efflux; - = net influx.

latter is a measure of the rate of flux per unit functional tissue, while the former allows for the fact that there is actually more mucosal tissue per centimetre of jejunum in the infested rat.

It can be seen that per unit weight of tissue the rate of influx was the same in both normal and infested animals. On the other hand, the rate of efflux in the infested rat was reduced to nearly a third of that in the normal rat. This confirmed the indications reported in the previous section that a derangement of efflux accounted for the net influx found for isotonic perfusion. Once again the net fluxes were in the same directions as previously found although they differed in magnitude. The net efflux in normal rats especially was considerably greater.

The sodium fluxes per centimetre of jejunum are important as they are measurements of what actually happens in the jejunum as a whole. The influx of sodium was then greater in the infested animal. For the same reason the total efflux per centimetre was not so markedly reduced by the infestation when only the effluxes per unit of mucosal tissue were considered.

(ii) *Water*.—The rates of net flux, influx, and efflux of water in normal and infested rats are set out in Table 3 where they are again expressed in terms of dry weight of mucosal tissue and centimetre length of jejunum.

It was not possible to measure the unidirectional movement of water concurrently with sodium because the radiosodium and the infra-red spectrometer for deuterium analysis were not available at the same time.

The results do not show the same clearly defined derangement of efflux from the lumen found with sodium. The reduction of water efflux/hr/g dry mucosal tissue was not statistically significant and suggested that the infestation reduced it by

TABLE 3
WATER FLUXES FROM JEJUNAL LOOPS OF NORMAL AND INFESTED RATS PERFUSED WITH SODIUM CHLORIDE SOLUTION CONTAINING D₂O
Sodium chloride concentration 140 m-equiv/l

	Net Flux*	Influx	Efflux
ml water/hr/g dry mucosal tissue			
Normal	$+10.5 \pm 4.3$	33.2 ± 6.6	43.7 ± 7.0
Infested	-4.3 ± 1.8	37.7 ± 11.1	33.4 ± 10.4
	$P < 0.001$	n.s.	n.s.
ml water/hr/cm jejunum ($\times 10$)			
Normal	$+1.57 \pm 0.64$	4.99 ± 0.99	6.56 ± 1.04
Infested	-0.99 ± 0.40	8.67 ± 2.56	7.68 ± 2.39
	$P < 0.001$	$P < 0.01$	n.s.

* + = net efflux; - = net influx.

about one-third rather than to one-third. The influx was again not affected. It is important to note, however, that the net water fluxes were again in opposite directions and highly significantly different.

The fluxes of water expressed per centimetre of jejunum again indicated the gross effect due to the greater weight of mucosal tissue per unit length of infested intestine, for the influx then became significantly larger ($P < 0.01$). The possible reasons for this lack of conformity between the unidirectional sodium and water fluxes are discussed below.

IV. DISCUSSION

Measurement of the fluxes of water, sodium, and chloride provided strong evidence to account for the fluid which accumulates in the jejunum of the rat infested with *N. muris*. The net fluxes were unequivocally in opposite directions so that there was a net movement of water and of the sodium and chloride ions into the lumen of the infested animal.

Under the conditions of the experiment the jejunum of the normal rat responded to the presence of hypo- and hypertonic solutions in the same manner as did the rats similarly perfused by Curran and Solomon (1957), and the dogs of Visscher *et al.* (1954) in which test solutions were placed in isolated intestinal loops.

In the infested rat the evidence that the net influx during isotonic perfusions was fundamentally due to a derangement of efflux while influx remained constant depended largely upon the unidirectional fluxes of the sodium ion. It was supported by the responses of the jejunum to hypo- and hypertonic solutions. The net water influx was not changed during perfusion of the infested intestine with a solution containing 72 m-equiv. NaCl/l, but it did respond during perfusion with a solution of 280 m-equiv. NaCl/l by increasing significantly the rate of net influx. These differential responses of net water fluxes to osmotic gradients conflict with the unidirectional water fluxes which did not confirm the clearly defined derangement of efflux shown by the radiosodium experiment.

This difference between the two unidirectional flux experiments was rather surprising as Curran and Solomon (1957) had reported a strong correlation between net sodium and water fluxes and had published experimental evidence in confirmation of it. The difference might be explained by the fact that while the rats in the sodium experiment were all heavily to very heavily infested, those in the deuterium experiment were judged to be only moderately to heavily infested. On the other hand, an appreciable loss of deuterium by exchange with the atmosphere would mask any difference between the normal and infested perfusates. However, even without a specially contrived dry box it was calculated that this exchange would be negligible. The collecting centrifuge tube was covered with a rubber cap and the distal cannula was inserted as far down into the tube as possible.

If the results of this experiment are accepted at their face value, the net water influx into the lumen during infestation is fundamentally due to a combination of a small increase of influx and a small decrease of efflux per gram of dry mucosal tissue. The gross effect is due mainly to a large increase of influx because of the greater weight of mucosal tissue per centimetre of jejunum. Furthermore, if these conclusions can be accepted, sodium movement is not a reliable guide to water movement in infested animals, but reasons for doubting these results have been given.

It should be added here that subsequent experiments with glucose (Symons 1960b) supported the evidence that absorption from the jejunum is affected by the infestation.

The assumption of Curran and Solomon (1957) that influx represents a constant diffusion into the lumen from a source of constant concentration on the other side of the membrane can be used to explain the accumulation of fluid in the jejunum even in the fasting state, provided that the efflux is impaired. In Table 2 it can be seen that the efflux of sodium in the infested rat is less than the influx whether measured per unit of mucosal tissue or per centimetre of intestine. Similarly, as can be seen in Table 3 the efflux of water tended to be smaller than the influx.

Finally, these results indicate that nematodes can markedly affect the function of the intestine at the site of the infestation.

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