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

II. CHEMICAL CONSTITUENTS OF THE JEJUNUM AND DRY WEIGHT OF THE MUCOSA

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Summary

There was a 50 per cent. gain in the dry weight of the mucosa relative to the unit length of the jejuna of rats infested by the nematode *Nippostrongylus muris*. The importance of this to measurements of the rate of intestinal fluxes is discussed.

The concentrations of the electrolytes sodium and chloride were increased in total jejunal tissue on both a wet weight and a fat-free dry weight basis. Potassium concentration increased on the dry weight basis but was unchanged in wet tissue. The water content of this tissue was also greater in the infested rats but the fat content fell. There was a twofold increase in the volume of whole blood in the jejunum. The physiological significance of these changes are not clear but their possible association with undernourishment is suggested.

I. Introduction

In Part I of this series (Symons 1957) it was reported that the dry weight of the tissues of the small intestine of the rat infested by the nematode *Nippostrongylus muris* (Yokogawa, 1920) was increased by about 50 per cent. In histological sections there was a twofold increase in the thickness of the muscularis externa which appeared to be hypertrophied. Although no quantitative measurements were made it also appeared that the bulk of the musoca was increased. Not all of the latter could be accounted for by the oedema of the villi.

The hypertrophy of the muscularis externa and retention of ingesta in the infested jejunum (Symons 1959) were evidence of changes in the motor function of the gut. Black (1955) has stated that potassium depletion has been demonstrated in a number of alimentary disorders, including gastro-enteritis. Severe potassium depletion can cause intestinal dilatation and even paralytic ileus (Schlesinger, Payne, and Black 1955). These changes are believed by Streeten and Vaughan Williams (1952) to be due to a loss of intracellular potassium, although their dogs depleted of sodium and chloride had elevated plasma potassium levels. Daniel and Bass (1956), who investigated the effect of electrolyte depletion upon gastro-intestinal motility of the rat, concluded that the tract resisted potassium depletion and that the mechanism controlling its electrolyte distribution probably differed from that in skeletal muscle. They did, however, show that electrolyte deficiencies could be reflected in the gut and may decrease propulsion. It was postulated therefore that there might be alterations of electrolyte concentration in the jejunal tissue of infested rats.

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It was also necessary for subsequent experiments to arrive at a satisfactory means of expressing the rate of absorption or net flux from the intestine. The rate per unit length of intestine would be unsatisfactory because, as has already been stated, the ratio mucosal area per centimetre length was probably not the same for both normal and infested rats. Dry weight of total intestinal tissue, by which is meant mucosal and muscular tissue together, would not be satisfactory because of the hypertrophy of the muscularis externa in the infested animals.

It was therefore decided to express fluxes in terms of the dry weight of the jejunal mucosa. A measurement of this fraction of the intestinal tissue would not only allow fluxes from the jejunum to be referred directly to the functional tissue concerned, but would also provide a quantitative confirmation of the increase already reported (Symons 1957).

In this part, therefore, are included electrolyte, water, whole blood, and fat estimations, as well as the dry weight of the jejunal tissue of normal and infested rats.

II. Methods

The rats weighed between 120–200 g. Infestations were produced by the method already described (Symons 1957) and the rats were killed on the tenth day of the infestation for reasons already given (op. cit. p. 375).

(*a*) *Dry Weight of Mucosal Tissue per Centimetre Length of Jejunum*

(i) *Dry Weight of Total Jejunal Tissue* (*mucosa plus muscle layers*).—The jejuna of 18 normal and 18 infested rats, which were fasted and anaesthetized with urethane, were washed out with warm isotonic saline solution. The rats were killed and bled by severing the great vessels in the thoracic cavity. The jejuna were carefully freed from the mesentery and removed to an enamel tray. After a segment of 8 cm had been isolated from below the the duodeno-ileal flexure it was opened longitudinally and lightly scraped to remove all mucous and debris. These segments were placed in groups of three in small weighed petri dishes and dried to constant weight by heating for 24 hr at 100°C. Six samples of both normal and infested rats, each containing three segments of 8 cm, were therefore obtained by this method.

(ii) *Dry Weight of Jejunal Smooth Muscle.*—Eighteen rats of each group were treated as above except that the mucosal layer was removed by scraping with the edge of a microscope slide. Histological examination showed that this removed all the mucosa except that in the depths of the crypts.

The dry weight of the mucosa per centimetre length of jejunum could then be calculated for both normal and infested rats from the difference between the mean dry weights of the six samples of total and muscular tissue.

(*b*) *Jejunal Tissue Constituents*

(i) *Sodium, Potassium, Chloride, Water, and Fat.*—Except for blood content, which was estimated separately, extractions were made for fat and for the electrolytes sodium, potassium, and chloride from 45 male rats in both the control and infested groups. They were grouped in samples of three, for accuracy and convenience, so that finally there were 15 estimates for comparison. In addition it

was necessary to divide the work into three separate batches spread over about as many weeks. At all times, however, three infested rats were killed alternately with three controls.

The rats which were not fasted were killed by a blow on the head under light ether anaesthesia. They were not bled. The alimentary tract was quickly removed and a segment about 9 cm distal to the pylorus and about 30 cm long was isolated. This length of segment included all that part of the small intestine which was infested by the parasites.

After the external blood had been washed off with distilled water, the segment was laid on a sheet of blotting paper, slit open longitudinally, and the contents lightly scraped off with a scalpel. Most of the parasites were removed by this procedure. The intestine was then blotted to remove any surface fluid and placed in a closed petri dish of known weight together with two similar segments to complete the group of three. After weighing, the samples were then dried to constant weight at about 100°C. It was found that 24 hr drying was sufficient. Before reweighing after drying, the petri dishes were allowed to equilibrate with room temperature over calcium chloride in a desiccator.

The subsequent extractions were based on those described by Lowry and Hastings (1942). The samples were ground with a mortar and pestle, transferred to weighed and stoppered 100-ml Erlenmeyer flasks, redried, and shaken with ether. After standing overnight the ether was drawn off and more added with further shaking. This ether was drawn off after a further 3–4 hr and the sample redried, first in a draught through a fume cupboard and finally at about 100°C. The percentage of fat on a dry weight basis was calculated by difference.

The defatted tissue was stored in test tubes at about −10°C until extracted for electrolytes. About 550 mg of this tissue was weighed into 300-ml Erlenmeyer flasks and redried. 20 ml 0·75N HNO_3 per 500 mg tissue was added and, to allow for evaporation which had been calculated previously, a further 0·35 ml HNO_3 added. Extraction was by the open Carius method described by Castor, MacDonald, and Armstrong (1955) without the addition of the reagents for chloride estimation. The extraction was carried out in an autoclave for 2 hr at 15 lb/sq.in. pressure. A watch-glass was placed over the mouth of the flask during this process. After cooling, the mixture was centrifuged and the supernatant was drawn off, quick-frozen in test tubes immersed in dry ice and ethanol, and stored at about −10°C.

For sodium and potassium analyses, two 5-ml aliquots of this supernatant were evaporated in 100-ml Erlenmeyer flasks over a hotplate to remove all nitric acid. The evaporation was repeated once by adding a little distilled water. The residue was finally diluted to 50 ml and the concentration of these two electrolytes then determined using the flame photometric attachment to a Beckman DU spectrophotometer and expressed as m-equiv/kg fresh tissue and m-equiv/100 g of dry fat-free tissue.

Chloride analyses were made in duplicate without prior evaporation of the supernatant by the Volhard method as modified by Lowry and Hastings (1942), and expressed on the same fresh weight and dry fat-free bases as were the other electrolytes.

(ii) *Whole Blood.*—The whole blood content of control and infested tissue was calculated using slight modifications of the acid haematin method of Goodman, Lewis, and Schuck (1951).

Segments of small intestine were prepared by the same method as described above. Eighteen infested rats were used to prepare nine paired samples, but it was necessary to use four animals per sample for the normal rats, i.e. 36 rats were used. As each sample was prepared it was transferred to a cold room at about 4°C. All subsequent operations were carried out in this room with cold glassware and reagents.

The samples were homogenized in 100 ml of distilled water in a Waring Blendor and 3 ml homogenate added, in duplicate, to 3 ml of the phosphotungstic acid reagent of Crooke and Morris (1942) in 15-ml graduated centrifuge tubes. 5 ml ether and then 2 ml distilled water were added. The mixture was thoroughly shaken between each step. After centrifuging for 5 min at 3500 r.p.m. the volume of the ether phase was recorded before it was carefully transferred to stoppered cuvettes and the transmission read at 400 mμ in a Bausch and Lomb spectrometer against ether as a blank.

Standard curves were prepared separately for the control and infested groups. Rats were bled in the cold from the carotid arteries and jugular veins into a drop of heparin. From this blood 1·25 ml was diluted to 500 ml to give a concentration of 0·25 ml/100 ml. Further dilutions were made from this to give a series from 0·05 ml to 0·25 ml/100 ml in steps of 0·05 ml. These standards were then treated as described above.

All photometry was carried out at room temperature. It was convenient to correct the percentage transmission to that for 5 ml of ether phase, assuming a linear relationship between transmission and concentration. The volume of the ether phase never varied by more than $\pm$0·3 ml and usually by less than this so that any error introduced by this practice would be negligible.

This method does not make an absolute measurement of acid haematin but it does make a valid estimation of blood volume which was expressed as ml/kg fresh tissue.

(iii) *Cleaning Glassware for Electrolyte Analyses.*—All test and centrifuge tubes were cleaned with fuming nitric acid and absolute ethanol in a fume cupboard. They were then given several washings in tap water and then distilled water and dried. Other glassware was soaked in equal parts of concentrated nitric acid and water before washing in tap and distilled water. Pipettes were washed by drawing through them successively nitric acid, ethanol, and ether.

III. Results

(*a*) *Dry Weight of Mucosal Tissue per Centimetre Length of Jejunum*

The dry weight of the mucosal tissue per centimetre of jejunum together with the dry weight of total jejunal tissue per centimetre are set out in Table 1. In infested animals both ratios are increased by 50 per cent. and these differences are highly significant ($P<0·001$). This increase in the dry weight of total tissue of

the jejunum confirms the earlier finding of Symons (1957). The increase in the weight of the mucosal tissue is a quantitative confirmation of the observation made in the same report. The significance of this to measurements of fluxes from the jejunum is discussed below.

TABLE 1

DRY WEIGHTS OF TOTAL AND MUCOSAL TISSUE PER CENTIMETRE LENGTH OF JEJUNUM OF NORMAL AND INFESTED RATS

	Total Tissue (g/cm)	Mucosal Tissue (g/cm)
Normal rats	$0{\cdot}021 \pm 0{\cdot}003$	$0{\cdot}015 \pm 0{\cdot}003$
Infested rats	$0{\cdot}033 \pm 0{\cdot}002$	$0{\cdot}023 \pm 0{\cdot}002$

(*b*) *Jejunal Tissue Constituents*

The water content of the jejunal tissue on a fresh weight basis increased from $78{\cdot}1 \pm 0{\cdot}8$ g/100 g tissue for normal rats to $80{\cdot}6 \pm 0{\cdot}4$ g/100 g tissue for infested rats. Fat content on a dry weight basis decreased from $16{\cdot}1 \pm 3{\cdot}3$ to $12{\cdot}2 \pm 1{\cdot}9$ g/100 g tissue for normal and infested rats respectively. Both differences are highly significant ($P < 0{\cdot}001$).

TABLE 2

ELECTROLYTE CONSTITUENTS OF JEJUNAL TISSUE IN NORMAL AND INFESTED RATS

	Sodium	Potassium	Chloride
	m-equiv/kg tissue on wet weight basis		
Normal rats	$43{\cdot}8 \pm 3{\cdot}4$	$96{\cdot}3 \pm 5{\cdot}7$	$39{\cdot}1 \pm 1{\cdot}7$
Infested rats	$48{\cdot}6 \pm 2{\cdot}8$ $P < 0{\cdot}001$	$95{\cdot}4 \pm 7{\cdot}0$ n.s.	$45{\cdot}7 \pm 2{\cdot}0$ $P < 0{\cdot}001$
	m-equiv/100 g fat-free tissue on dry weight basis		
Normal rats	$23{\cdot}8 \pm 2{\cdot}2$	$52{\cdot}4 \pm 2{\cdot}6$	$21{\cdot}2 \pm 1{\cdot}0$
Infested rats	$28{\cdot}6 \pm 2{\cdot}0$ $P < 0{\cdot}001$	$56{\cdot}1 \pm 3{\cdot}8$ $P < 0{\cdot}01$	$26{\cdot}9 \pm 1{\cdot}6$ $P < 0{\cdot}001$

In Table 2 are shown the sodium, potassium, and chloride concentrations in the jejuna of both normal and infested groups expressed as m-equiv/kg fresh weight and as m-equiv/100 g fat-free solids. The standard deviations and the results of *t*-tests of the differences between normal and infested rats are included.

These values for normal tissue are in close agreement with those of Grollman (1954) who found 43·5 m-equiv. Na^+/kg blood-free, fat-free wet tissue and 103·1 m-equiv. K^+/kg "gut" tissue of dogs. If these are corrected to fresh tissue on the

basis of the concentrations of fat and blood found in the present work these values become *c.* 42 m-equiv. Na^+/kg and 99 m-equiv. K^+/kg respectively. Manery and Bale (1941) reported 40·7 m-equiv. Cl^-/kg and 47·7 m-equiv. Na^+/kg fresh weight in rabbit small intestine.

It can be seen from Table 2 that there was an increase in the concentration of the sodium and chloride ions when measured on both a fresh weight and dry weight, fat-free basis. There is no difference in the potassium content when measured on a fresh weight basis but it is increased significantly when expressed as concentration per 100 g fat-free tissue. This latter result is probably due to the fact that the increased water content, which histologically can be seen to be due largely to oedema of the mucosa and is therefore extracellular fluid presumably containing little potassium, would tend to mask any increase of that element when measured in fresh tissue.

The concentrations of whole blood in the jejunal tissue increased from $13 \cdot 2 \pm 2 \cdot 7$ ml/kg tissue for normal rats to $22 \cdot 5 \pm 2 \cdot 0$ ml/kg tissue for infested rats on a fresh weight basis. The difference, which is again highly significant ($P < 0 \cdot 001$), shows that there is nearly a twofold increase in the concentration of blood in infested tissue. The obvious congestion of the intestine certainly suggested that this might be so.

IV. Discussion

The 50 per cent. increases of the dry weights of jejunal tissue and of mucosal tissue alone from the same organ is in agreement with that reported earlier for the entire small intestine (Symons 1957). The ratio mucosal dry weight per centimetre length of jejunum also provides a means of expressing fluxes or absorption from this section of the gut provided the length is known. It must be emphasized that this ratio refers to the jejunum alone. Fisher and Parsons (1950) have shown that the ratio of surface area of mucosa to length of intestine in the rat increases with the distance proximal to the ileo-caecal valve. These authors described a method of measuring mucosal area but it was not possible to apply it to these infested rats because of the irregular shape of the villi.

It has already been pointed out (see Section I) that neither total dry weight of the total small intestine nor its length would provide a basis of comparison for absorption rates between normal and infested rats. These results bear this out. The serosal area can be measured quite simply, but this would provide only an artificial measure of absorptive rate and would depend upon a similar relationship between serosal and mucosal areas in both groups of rats. It might be objected that mucosal dry weight is not a precise measure of functional tissue in an infested animal if there is damage to some epithelial cells but not to others. It is, however, difficult to envisage any other method which would more accurately relate function to the tissue concerned.

The consequence of the difference in this ratio of mucosal dry weight to length between normal and infested rats is of some importance to absorption studies. Absorption expressed per unit of dry weight would provide a comparison of function per unit of mucosa. On the other hand, the rate of absorption per centimetre length

would compare what might be called the gross rate of absorption from identical lengths of intestine bearing different areas of mucosa. In fact these differences were subsequently found to be important (Symons 1960).

The increase in the water content of the jejunum confirms the earlier report of a similar increase in the small intestine as a whole. The fall in fat content of the same organ has also been found to be true of the carcass as a whole (Symons 1959).

It is highly unlikely that the increases in electrolyte concentrations are due to the inclusion of parasites in the dried and ground tissue. The great majority of these were removed. The few that remained would constitute a very small fraction of the total weight of tissue for they are hair-like and 4–5 mm in length. Calculations were made to ascertain whether the different proportions of water, blood, and solids in the infested tissue could possibly account for these electrolyte changes. It was assumed that in these calculations the additional water was extracellular fluid containing little potassium, and that whole blood contained the normal proportions of the electrolytes. It has been reported by Symons (1959) that the serum electrolytes are not affected by the infestation. When these estimations were made, it was found that unless the actual concentrations of electrolytes in the tissue were increased, the altered proportions of water, blood, and solids would have produced an increase of only 1·2 m-equiv. Na^+/kg and losses of 3·1 m-equiv. K^+/kg and 0·3 m-equiv. Cl^-/kg of fresh weight.

There is no reasonable doubt that the increases reported are real but it is difficult to assess their physiological significance. Certainly it can be stated confidently that there is no loss of potassium which might cause a loss of motor activity of the gut. The concentrations of electrolytes shown in Table 2 refer to the mucosal and muscular layers together. No attempt has been made to measure them separately nor to divide them compartmentally between the extra- and intracellular fractions. An assessment of the physiological significance of the changes in the infested rats would depend upon such examinations.

It is interesting, however, to compare these results with those of Widdowson and McCance (1956) and Huth and Elkington (1959) who measured electrolyte levels in various tissues in starved or otherwise undernourished rats. The former authors found that there was a gain in the chloride and sodium content of the liver in both starved and chronically undernourished rats. The change in the potassium level was indefinite. The total body chloride and sodium levels on a fat-free wet basis were found by the latter two investigators to have increased in starved rats, while the potassium level was significantly decreased. Both groups assumed that there was an increase of extracellular water if this can be defined by the chloride space. Huth and Elkington, however, suggested that the additional chloride could be due alternatively to a sequestration of that electrolyte. These two also reported that there was a fall of potassium relative to total water in the carcasses of starved rats. These ratios for the jejuna of normal and infested rats in the examinations described here were 0·124 m-equiv/ml and 0·118 m-equiv/ml respectively, which are not significantly different. It has been shown by Symons (1959) that undernutrition, if not starvation, in severely infested rats is a major factor in the symptomatology of infestation by *N. muris*. The loss of fat from the jejunum supports

this observation. There can be a marked loss of appetite. It is possible, therefore, that the electrolyte changes in the jejunum reported here are a reflection of the same changes found in other tissues both by Widdowson and McCance and by Huth and Elkington and that they are due to undernourishment.

V. Acknowledgments

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