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

I. CHANGES IN THE WATER CONTENT, DRY WEIGHT, AND TISSUES OF THE SMALL INTESTINE

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Summary

The fresh weight of the rat's small intestine together with its contents, as well as the intestinal tissue alone, was doubled during the 15 days following infestation by the nematode *N. muris* (Yokogawa, 1920). This increase, which was independent of the growth of the rat, was largely due to a two-fold increase in the water content of both the lumen and the tissues. The dry weight of the tissues was increased by half.

Associated with these changes was a two-fold increase in the tangential diameter of the jejunum which was the site of the infestation. This was not due solely to a dilatation but also to the increase in the amount of intestinal tissue. The width of the circular layer of the muscularis externa of the jejunum was doubled and the longitudinal layer was increased by half. Histologically, the increase of the circular layer appeared to be due to hypertrophy, but an increase in the number of the smooth-muscle nuclei suggested that hyperplasia might also be present.

I. Introduction

The gross and microscopic pathology of the small intestine of rats infested by the nematode *Nippostrongylus muris* (Yokogawa, 1920) has been described by Porter (1935) and Taliaferro and Sarles (1939). The latter workers reported that the jejunum may be dilated to two or three times its normal diameter, and that the dilatation was associated with an accumulation of fluid and gas. No mention was made in either of these papers of an apparent hypertrophy of the tissues of the infested jejunum.

Experiments were done to show the distribution of water and dry matter between the contents and tissues of the infested small intestine, and to determine the changes occurring in these relationships during the course of the infestation which persists for about 2 weeks after the subcutaneous introduction of infective larvae (Africa 1931). Measurements were made of the tangential diameters of the jejunum and of the thickness of the muscularis externa because any quantitative changes in the tissues would affect the weights of water and solid matter. The number of nuclei in sections of the circular layer of the muscularis externa were estimated to determine whether hyperplasia was present.

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II. Methods

(a) *Water and Dry Matter Content*

(i) *Changes in the Small Intestine together with its Contents.*—This experiment was planned to measure the water content and dry weight of the intact small intestine daily during the 15 days of infestation. Sixteen groups of five female albino rats were randomly allotted to days 0–15. The groups representing days 1–15 were infested by a subcutaneous injection of 3500–4000 infective larvae of *N. muris*. Four rats from each group were killed in an ether jar after a fast of about 6 hr. Some ingesta remained in the small intestine after this fast, and undoubtedly affected the intestinal weights recorded. On the other hand, a fast for as long as 24 hr failed to empty completely the small intestine and may have affected the volume of water in the lumen. The intact small intestine, which was separated rapidly from the stomach, caecum, and mesentery, was lightly blotted and weighed in a covered petri dish. It was then dried to constant weight at about 100°C.

The severity of the infestations were judged subjectively. No worm counts were possible as the intestinal contents were not removed.

(ii) *Changes in the Small-intestinal Tissue after Removal of the Contents.*—Preliminary examination of the results of the first experiment indicated that groups of five rats killed at intervals of 3 days would be sufficient to provide the information required. Accordingly, six groups to represent days 0, 3, 6, 9, 12, and 15 were used. The small intestine, after its separation from the other organs, was slit open longitudinally, the contents were lightly scraped off, and the mucosa lightly blotted. Weighing and drying were carried out as before.

In each experiment the data were corrected to the mean body weight at death, on the grounds that a linear relationship exists between intestinal weight and body weight in the growing rat (Brody 1945).

(b) *Measurements of the Diameter of the Jejunum and the Width of the Muscularis Externa*

Eighteen female albino rats weighing from 100 to 150 g were paired according to weight. One of each pair was randomly selected for infestation by the subcutaneous injection of about 4000 infective larvae. The pairs were then randomly divided between three groups. There were, therefore, three infested and three non-infested rats in each group. One group was killed 7 days after infestation, the second after 10 days, and the third after 14 days. By the end of the first week after skin penetration the parasites had been in the small intestine for 4 or 5 days (Yokogawa 1922). The tenth day was chosen because Taliaferro and Sarles (1939) reported that the most marked histological reactions of the host occur about that time, and the fourteenth day chosen because Africa (1931) showed that the infestation begins to decline at about the end of the second week.

The rats were killed in an ether jar. The small intestine was removed, a specimen about 1 in. in length was taken from the jejunum about 6 in. below the pylorus, and fixed in corrosive formalin so as to reduce shrinkage to a minimum.

Care was taken to ensure that the pairs of sections, each section about $\frac{1}{2}$ in. apart, subsequently cut from these specimens, were at right angles to the longitudinal axis of the gut. One pair of sections from each rat was stained with haemotoxylin and eosin and the other with celestine blue and eosin to differentiate the nuclei.

Because the sections, particularly those taken from the infested rats, were not usually circular after fixation, a comparison of diameters was made by measuring two tangential diameters perpendicular to each other. The initial diameter was chosen in a random manner. A grid in an ocular of the microscope was used to measure the diameters of a pair of sections from each rat.

The widths of the circular and longitudinal layers of the muscularis externa were measured with an ocular micrometer. Four measurements approximately 90° apart were made on each section. Pairs of sections were again used; thus eight measurements were made on the jejunum of each rat.

The number of nuclei in the circular layer was estimated to determine if hyperplasia was present. A radial grid of eight divisions was made on a photographic negative, and the number of nuclei in four divisions was counted when the grid was centrally placed over the section with a drop of immersion oil between the grid and the cover-slip. The actual divisions counted were decided for each section by random numbers. This random distribution was not always possible as occasionally a tear in the muscle layers meant that a particular division had to be abandoned for another. Counts were made on pairs of sections from each rat.

III. Results

(a) *Water and Dry Matter Content*

The infestations, as judged by the size and congestion of the small intestines, were moderate rather than heavy. In the early stages of the intestinal phase the apparent dilatation, which appeared on about the fifth day, was confined to the upper jejunum, and was well defined at both its proximal and distal ends. The infested section was markedly congested, and the associated blood vessels in the mesentery were engorged. Although there may have been an increase in the water content of the ileum at this stage, this section of the bowel was not obviously distended until about the eighth day after the injection of the larvae. The increased diameter of the ileum which, however, was never as pronounced as that of the upper jejunum, appeared to be solely due to a mechanical distension by fluid, whereas that of the jejunum itself was also associated with a marked thickening of the intestinal tissue. Some time after the tenth day the dilatation began to disappear. Towards the fifteenth day the jejunal wall appeared flabby and thickened rather than distended. The mucosal surface of the intestine, particularly that of the jejunum, felt rough when lightly stroked by a scalpel blade.

(i) *The Intact Intestine.*—In Figure 1 (line *A*) are shown the corrected mean fresh weights of the small intestines at daily intervals. In Figure 2 are shown the corrected mean water and dry matter weights, also at daily intervals. In both instances each point represents the mean of four rats.

If orthogonal polynomials are fitted to the data for water content, the quadratic curve is significant at the 5 per cent. level, but not at the 0·1 per cent. level. This curve suggests that the water content reached a maximum on about the thirteenth to fourteenth day after infestation by injection. On the other hand, the linear increase of both the dry matter and fresh weight was significant at the 0·1 per cent. level, whereas the quadratic component was non-significant. These fitted curves

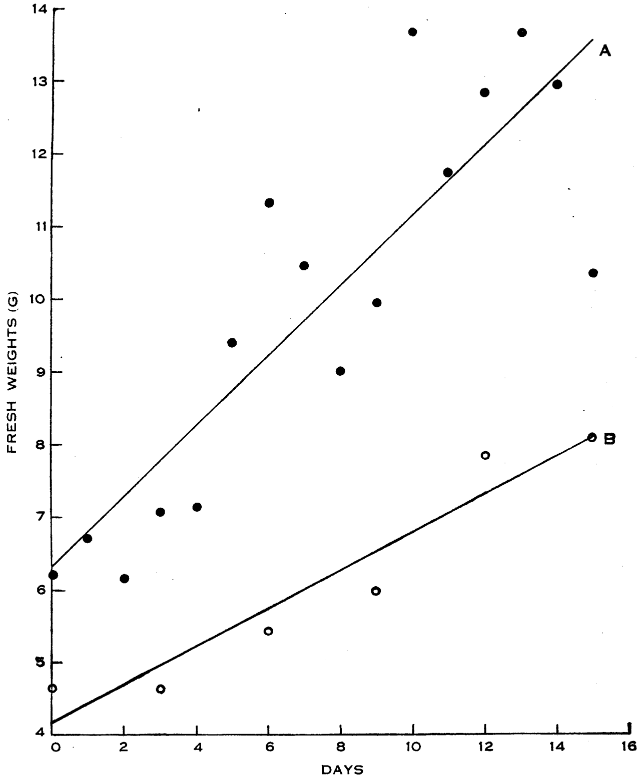

Fig. 1.—Changes in the fresh weights of the rat's small intestine. ● Daily fresh weights of the small intestine together with its contents. Each point represents the mean of four rats. ○ The fresh weights at 3-day intervals of the small intestine from which the contents had been removed. Each point represents the mean of five rats. Line *A*, intact intestine. Regression fresh weight on time: $b = 0{\cdot}4798 \pm 0{\cdot}0776$ ($P < 0{\cdot}001$). Line *B*, intestinal tissue. Regression fresh weight on time: $b = 0{\cdot}7892 \pm 0{\cdot}1220$ ($P < 0{\cdot}05$).

should, however, be accepted with caution as the variability of the observations does not allow a more precise determination of the relationship of time with the changes that occurred throughout the infestation.

On the other hand, if the linear regressions of the fresh weight, water, and dry matter content on time be taken as a measure of the changes, there were increases which were all significant at the 0·1 per cent. level. These regression coefficients are given in the legends to Figures 1 and 2. From Figure 1 it can be seen that the mean fresh weight of the small intestine increased two-fold over the duration of the experiment. Similarly, from Figure 2, it is apparent that the water content was slightly more than doubled, whereas the dry matter was increased by half.

(ii) *Tissue of Small Intestine*.—The change, at intervals of 3 days, of the fresh weight of the small intestine from which the contents were removed is shown in Figure 1 (line *B*). The changes in water and dry matter content are shown in Figure 3. Each point represents the mean of five rats.

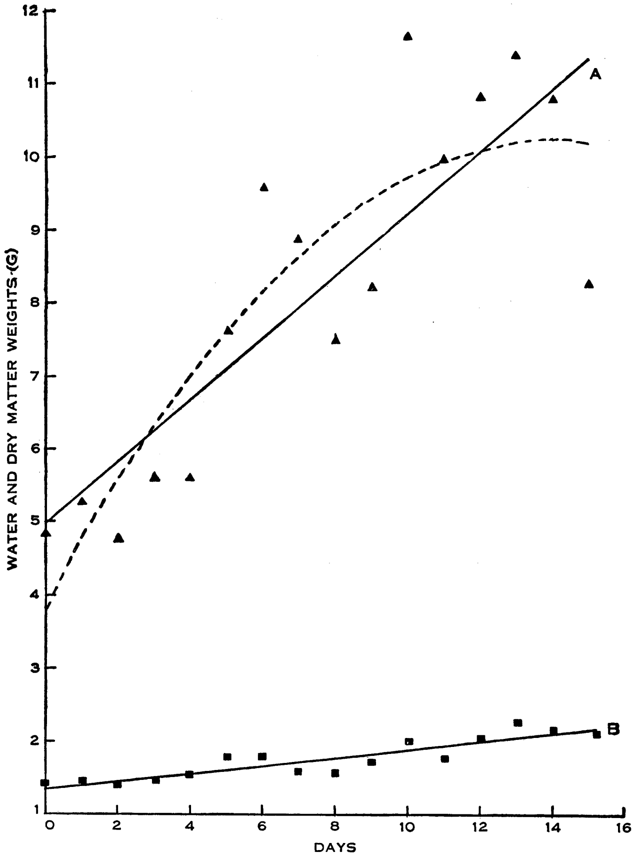

Fig. 2.—Daily water and dry matter weights of the rat's small intestine together with its contents. Each point represents the mean of four rats. ▲ Water content. ■ Dry matter. Line *A*, regression water content on time: $b = 0{\cdot}4252 \pm 0{\cdot}0742$ ($P < 0{\cdot}001$). – – – – Quadratic curve ($P < 0{\cdot}05$). Line *B*, regression dry matter on time: $b = 0{\cdot}0546 \pm 0{\cdot}0069$ ($P < 0{\cdot}001$).

If orthogonal polynomials are fitted there appears to be a linear increase in each instance, all significant at the 5 per cent. level, but the significance of these curves should also be accepted with caution. As before, the linear regression coefficients of the weights on time are shown in the legends to Figures 1 and 3. They are significant at the 5 per cent. level. Again, there was a two-fold increase in the weight of the fresh intestine; the water content was slightly more than doubled and the dry matter increased by half.

(*b*) *Measurements of the Diameter of the Jejunum and the Width of the Muscularis Externa*

(i) *General.*—In Plate 1, Figures 1 and 2, are photographs of cross sections of typical normal and infested jejuna respectively. The cellular changes during an initial infestation have been described in detail by Taliaferro and Sarles (1939). Attention must be drawn, however, to the apparent increase in the bulk of the mucosa of the infested intestine. This did not appear to be due solely to the oedematous conditions of the irregularly shaped villi which replaced the regular finger-like villi of the normal animal.

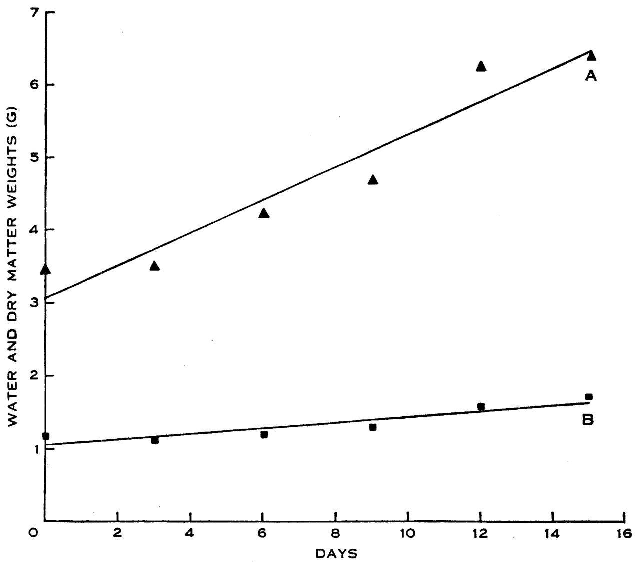

Fig. 3.—Water and dry matter weights at 3-day intervals of the rat's small intestine from which the contents had been removed. Each point represents the mean of five rats. ▲ Water content. ■ Dry matter. Line *A*, regression water content on time: $b=0{\cdot}6737 \pm 0{\cdot}0986$ ($P<0{\cdot}05$). Line *B*, regression dry matter on time: $b=0{\cdot}1154 \pm 0{\cdot}0262$ ($P<0{\cdot}05$).

(ii) *Tangential Diameters.*—The mean tangential diameters of all the infested intestines together was 4·408 mm ($\pm 1{\cdot}162$), whereas the mean of the non-infested rats was 2·266 mm ($\pm 0{\cdot}440$). This difference was significant at the 0·1 per cent. level. The diameter of the jejunum in the infested rat was, therefore, almost doubled under the conditions of this experiment. The comparative sizes of a typical infested and non-infested jejuna are shown in Plate 1, Figures 1 and 2.

(iii) *Width of the Muscularis Externa.*—In Table 1 are shown the mean widths of the circular and longitudinal layers of the muscularis externa in both infested and non-infested rats. The mean width of the circular layer, when all the measurements of the infested rats were taken together, was 156·2 μ. This was significantly greater at the 0·1 per cent. level than the mean width of the circular layer in the non-infested rats, which was 76·0 μ. Similarly the mean width of 71·5 μ for the longitudinal layer in the infested rats was significantly greater at the 5 per cent. level than the mean width of 45·7 μ in the non-infested rats. It can be seen, therefore, that the circular layer of the muscularis externa was doubled in width whereas the longitudinal layer was increased by nearly half.

There was insufficient evidence to determine if the rate of increase in the width of the muscularis externa was greater before the seventh day or between the seventh and tenth days, but there was little change after the tenth day.

Plate 1, Figures 1 and 2, provides a comparison of the widths of the complete muscularis externa in the infested and non-infested rat. In Plate 2, Figures 1 and 2, are compared the more detailed appearances of the circular layer of this muscle.

Table 1

MEAN WIDTHS OF THE MUSCULARIS EXTERNA IN JEJUNAL SECTIONS FROM INFESTED AND NON-INFESTED RATS

Day after Infestation	Width of Circular Layer (μ)		Width of Longitudinal Layer (μ)	
	Infested	Non-infested	Infested	Non-infested
7	110·9	68·6	63·6	39·5
10	173·5	82·2	66·7	53·6
14	184·4	77·2	84·0	44·0
All rats	156·2	76·0	71·5	45·7

The magnification is the same in both figures. It can be seen that the muscle in Plate 2, Figure 2, increased in width due to a thickening of the individual fibres. The nuclei, which are less densely stained, are also thicker in cross section, and possibly shorter. This appearance is similar to the hypertrophy of cardiac muscle as described by Wright (1951).

(iv) *Number of Nuclei in the Circular Muscle*.—The estimated mean number of the nuclei of the smooth muscle in one division ($\frac{1}{8}$ of the cross section) of the circular layer of the muscularis externa of the jejunum of infested rats was 241, and in the non-infested rats the number was 175. The 95 per cent. confidence limits were within the range of 262–221 and 188–161 respectively. This difference was significant at the 5 per cent. level, and appears to indicate that part at least of the increase in width of this layer was due to hyperplasia. It was noticed, however, that the number of nuclei was markedly increased in those areas of the sections where the fibres were contracted, and that these contracted areas occurred more frequently in the sections from infested rats. Mitotic figures were not seen.

IV. Discussion

The effect of growth on intestinal weight during the 15 days of infestation in both water and dry matter content experiments can be ignored because all weights were adjusted to the mean body weight at death.

The marked gains in the fresh weights of the intact intestine and intestinal tissue alone were associated with increases in both water and dry matter content. It was not possible, however, to make a precise statement relating these changes to the course of the infestation. They appeared to be linear with time from day 0, except that if the quadratic curve of Figure 2 can be accepted as truly descriptive, the water content of the intact intestine may have reached a peak on about the thirteenth or fourteenth day, and thereafter declined. From observation of many infested rats, a decline in water content, and probably also of fresh weight, was expected at the end of 2 weeks, but this was not definitely established by these experiments. Furthermore, it seems unlikely that there would be a linear increase of weights over the first 2 or 3 days. The parasites do not begin to enter the small intestine until about 48 hr after the subcutaneous injection of the larvae because there is an intervening passage through the lungs. It seems probable that a precise curve relating weight changes to time would be sigmoid in form. There is, in fact, a suggestion in both Figures 1 and 2 that the fresh weight and water content of the intact intestine did not begin to increase until the third or fourth day, but there were insufficient data to confirm this.

The increase in the fresh weight of the intact intestine was not due to the greater retention of ingesta as the infestation progressed, since there was also a progressive increase in the weight of the intestinal tissue alone. It is possible, however, that the greater scatter of the daily mean weights in the experiment concerned with the intact intestine was due in part to variations in the amount of retained ingesta. The weight of the parasites was ignored because of their small size.

It is clear that, since the water content of both the intact intestine and of intestinal tissue alone was slightly more than doubled whereas the dry matter increased by only half, the former was largely responsible for the gain in fresh weight. The increase in water content was not confined solely to the intestinal tissue since it was observed to accumulate in the lumen. This is confirmed in Figures 2 and 3 from which it can be estimated that there was an increase of about 6·5 g of water in the intact intestine, and about 3·5 g in the intestinal tissue. The subsequent histological examination of the jejunum confirmed the accumulation of water in the tissues, which was largely confined to an oedema of the mucosa. The submucosa and the muscularis externa did not appear to be oedematous, although any increase in the absolute amount of tissue would account for some of the increase in water content.

The increase in size of the muscularis externa undoubtedly accounted for some of the gain in dry matter of the intestinal tissue. The circular layer of this intestinal muscle was doubled in thickness, whereas the longitudinal layer increased by half. No attempt was made to determine quantitatively if there was an absolute increase in the amount of mucosal tissue although examination of sections such as those shown in Plate 1, Figure 2, suggested that there was an increase.

Histologically the changes in the circular muscle were consistent with hypertrophy. Although the evidence for hyperplasia of this muscle was doubtful, it was difficult to account for the apparent increase in the mucosal tissue by any other means. It was also difficult, however, to determine if there was a difference between the density of mitotic figures in the epithelial cells of the normal and infested mucosa.

The enlargement of the infested jejunum was, therefore, not due solely to a dilatation by fluid or gas, but, in part at least, to an increase in the bulk of tissue. Had a dilatation been solely responsible, the intestinal tissue would have been thinner. On the other hand, the accumulation of fluid in the lumen must have been partly responsible for the increase in size since, in the terminal stages of an infestation, when the water was leaving the gut, it was thick and flabby rather than distended. Damage to the epithelium may have accounted for the accumulation of the fluid in the lumen of the gut, but further work is required to determine if a more active process was involved.

The undoubted hypertrophy of the muscularis externa together with the possible increase in the mucosal tissue suggested hyperactivity of the gut. Whether the presence of the parasites or the fluid in the lumen or both, stimulated this activity cannot be decided at this stage. There is, however, some evidence that the parasites themselves are the primary cause, for in the lightly infested rat they were collected together in reddened and thickened pockets which bulged outwards from the serous surface of the jejunum. Work has been planned to investigate changes in muscular activity during the infestation.

V. Acknowledgments

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Explanation of Plates 1 and 2

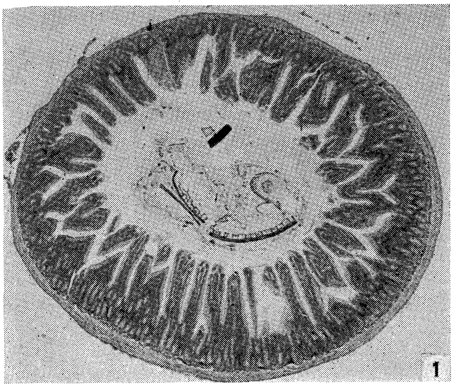
Plate 1

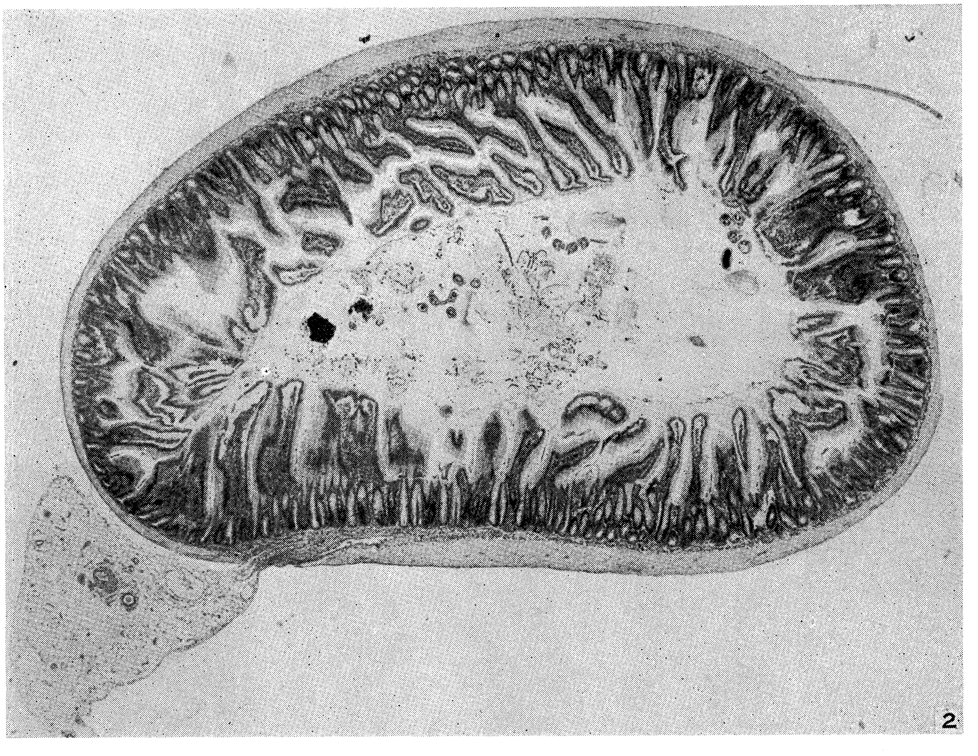
Dilatation of the jejunum

Fig. 1.—Photograph of a cross section of the jejunum of a normal rat.

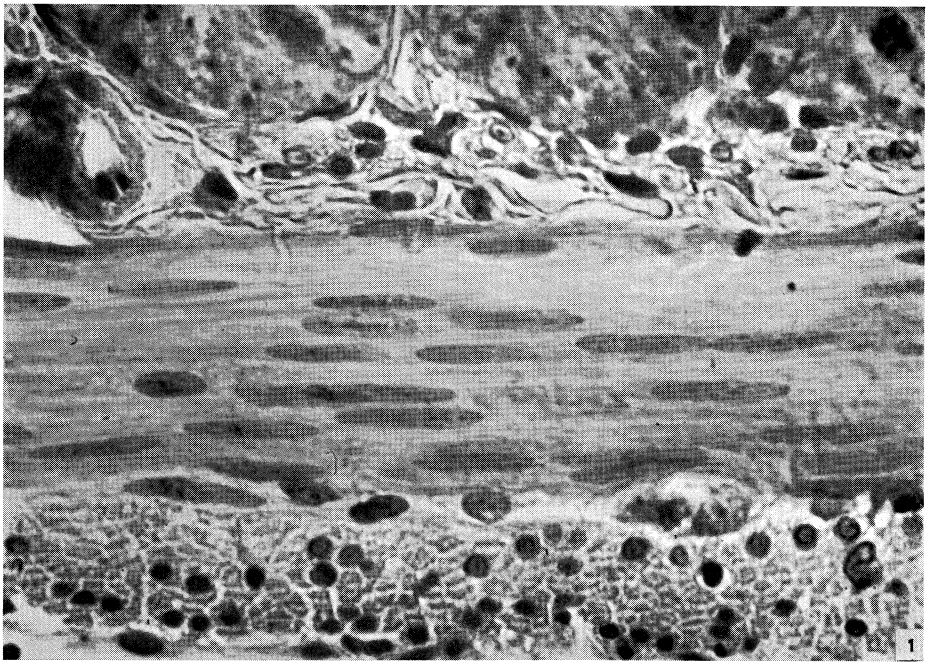
Fig. 2.—Photograph of a cross section of the jejunum of a rat infested by the nematode *N. muris*. The size has been increased. The villi are more irregularly shaped and are oedematous. The muscularis externa is thickened. The break in the integrity of the intestinal wall at the lower left-hand corner is probably due to the penetration of a parasite.

PATHOLOGY OF NIPPOSTRONGYLOSIS OF THE RAT. I





PATHOLOGY OF NIPPOSTRONGYLOSIS OF THE RAT. I



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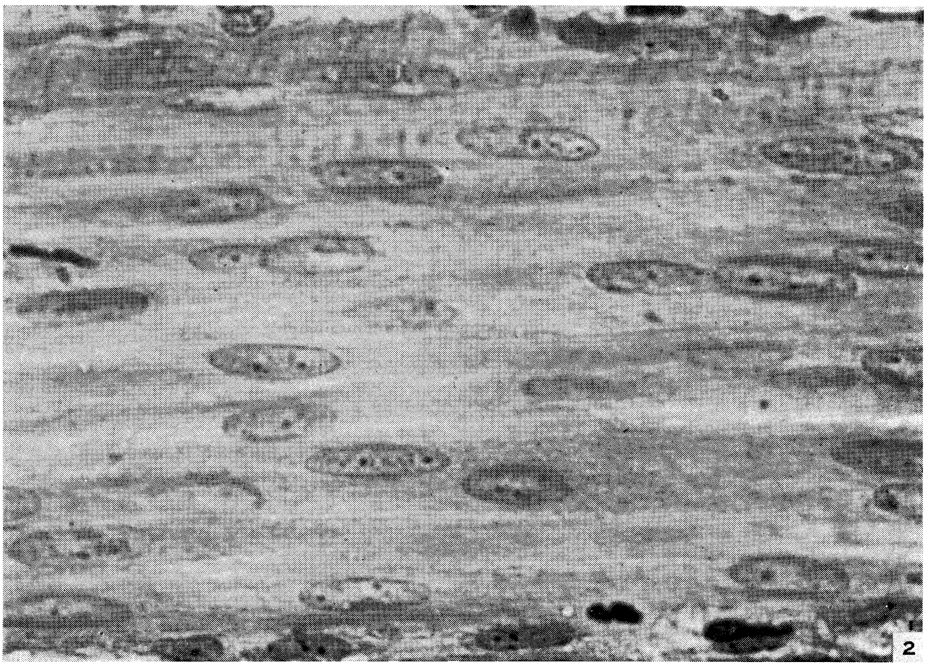

PLATE 2

The circular layer of the muscularis externa

Fig. 1.—Photomicrograph of a section of the circular layer of the muscularis externa of the jejunum of a normal rat.

Fig. 2.—Photomicrograph of a section of the circular layer of the muscularis externa of the jejunum of a rat infested by the nematode *N. muris*. The width of the muscle has been increased due to a thickening of the individual fibres. The nuclei which are less densely stained, are also thicker in cross section.