THE UPTAKE OF RADIO-ACTIVE PHOSPHATE BY NEMATODE PARASITES AND BY TISSUES OF THE SHEEP

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Summary

The relative uptake of radio-active phosphate by the tissues of the sheep and by the parasites *Trichostrongylus* spp., *Haemonchus contortus*, and *Oesophagostomum columbianum* has been investigated.

After injections into the abomasum of the host, *Trichostrongylus* accumulated phosphate very rapidly and in excess of the absorption by the small intestine of the host, but after intravenous injections the uptake by the parasites was similar to that of the small intestine.

The uptake of labelled phosphate by *H. contortus* showed an appreciable rise 8 hr. after both intra-abomasal and intravenous injections, whereas by this time the phosphate content of the host's abomasal tissues was decreasing.

O. columbianum absorbed less phosphate than the tissues of the host's rectum up to 4 hr. after both methods of dosing, but from that period showed a rise that was somewhat variable.

The significance of these results is discussed. It is suggested that the parasites feed on the tissues of the host and not on the contents of the alimentary tract.

I. INTRODUCTION

Wright (1950) has emphasized the necessity for further knowledge of the physiology of parasites as a basis for the development of more efficient anthelmintics. An understanding of the feeding habits of nematode parasites of the alimentary tract is of some importance in determining the mode of entry of anthelmintics into these parasites. It is of particular importance in assessing the mode of uptake of phenothiazine by parasites of the alimentary canal of sheep, and the present work has been undertaken partly in this connection.

Isotopic tracers have been used to follow the rates of absorption of substances by parasites attached to the mucous membrane of the host and by those free in the alimentary tract. Rogers and Lazarus (1949) have used labelled phosphate to show that *Ascaridia galli* feeds on the gut contents of the chicken, whereas *Nippostrongylus muris*, a parasite of the small intestine of the rat, is a tissue feeder. Read (1950) has used radio-active phosphorus in studying the uptake of phosphate and the carbohydrate metabolism of the cestode *Hymenolepsis diminuta*.

Radio-active phosphorus has therefore been selected as a tracer for following the uptake of labelled phosphate by nematode parasites of the sheep and as a means of determining the nature of their feeding habits.

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II. Materials and Methods

The nematodes examined in this investigation, *Haemonchus contortus, Trichostrongylus* spp. (very largely *T. colubriformis*), and *Oesophagostomum columbianum,* were obtained from naturally infested sheep.

Radio-active phosphorus was used as sodium dihydrogen phosphate in phosphate buffer at pH 7.3. The material was prepared for estimation by ashing with perchloric and nitric acids and precipitating the phosphate as magnesium ammonium phosphate with a magnesium citrate reagent (Mathison 1909) according to the method of Lohmann (1928). The precipitates were washed with 1 per cent. ammonia, transferred quantitatively to standard glass counting dishes, dried at 110°C., and the radio-activity estimated with a Geiger-Müller counter and scale of eight. Aliquots of diluted intestinal fluid were placed directly on the counting dishes, dried in a thin layer, and the ^{32}P content determined.

The mean error in the determination of radio-activity was ± 14 per cent. Corrections for self-absorption and resolving time were found to be unnecessary.

III. Procedure and Results

Infested sheep, which had been fed on the laboratory stock ration, were each given intravenous or intra-abomasal injections of 10 ml. of a solution of sodium dihydrogen phosphate containing about 1 mc. of ^{32}P. After intervals of 2, 4, and 8 hr. animals were killed and samples of *Haemonchus contortus, Trichostrongylus* spp., and *Oesophagostomum columbianum* were collected from the abomasum, small intestine, and rectum respectively. Samples of gut mucosa from selected sites in the abomasum, small intestine, and rectum of the host were also taken. Samples of intestinal fluid were taken at a distance of 15-20 ft. from the pylorus. Some *Haemonchus* were found floating in the abomasal contents and others were picked off the abomasal mucosa. *Trichostrongylus* were removed from the intestinal mucosa with fine forceps under a dissecting microscope; *Oesophagostomum* were collected from the walls of the rectum and by washing the contents of the rectum through a sieve.

The parasites and the tissues of the host were washed repeatedly in 0.9 per cent. saline, the excess fluid removed with filter paper, and the wet weight determined. The weight ranged from 0.05 to 0.1 g. The material was then ashed and prepared for counting. Two samples were prepared for each ^{32}P determination and each experiment was carried out twice.

Similar trends were observed in duplicate experiments, but the values of the actual uptake of ^{32}P by the host tissues and by the parasites showed some variation. This variation was probably due to differences in such factors as intestinal motility, the rate of absorption, and the amount of food previously consumed by the sheep. The general physiological condition of the animals and the degree of parasitic infestation might also have affected the results obtained.

The values for the relative ^{32}P concentration as shown in the tables and graphs were calculated as the ratios of counts/min./g. wet wt. of tissue to dose (disintegrations/min.); the highest value recorded was taken as equivalent to 100 and the other values were appropriately adjusted.

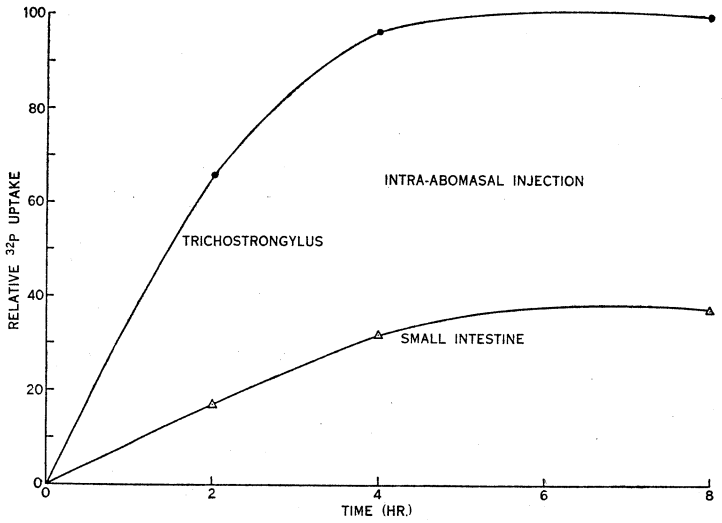

Fig. 1.—Relative amounts of radio-active phosphorus found in *Trichostrongylus* spp. and host gut tissues at different periods after dosing infested sheep intra-abomasally with sodium dihydrogen phosphate containing ^{32}P.

Figure 1 shows that *Trichostrongylus* taken from sheep that had been given an intra-abomasal injection absorbed approximately three times as much ^{32}P as the small intestine. The radio-activity of *Haemonchus* showed a considerable rise 8 hr. after the same type of dose whereas by this time the ^{32}P content of the abomasal tissue was decreasing (Table 1). The absorption of

TABLE 1

RELATIVE 32P UPTAKE BY HOST TISSUES AND PARASITES AFTER INTRA-ABOMASAL INJECTION OF THE HOST

Sample	2 Hr.	4 Hr.	8 Hr.
Abomasum	1.5	25.4	21.5
H. contortus	17.7	18.2	42.0
Rectum	8.0	27.5	14.8
O. columbianum	2.3	4.5	No value available

The figures refer to the relative ^{32}P concentration, calculated as the ratio of counts/min./g. wet wt. to dose (disintegrations/min.).

^{32}P by *Oesophagostomum* following an intra-abomasal injection of the host could not be estimated, as there were not enough worms present in either of the sheep used for the 8-hr. experiments.

The results obtained after intravenous injections are shown in Table 2. The absorption of ^{32}P by *Trichostrongylus* followed a course parallel with the absorption by the small intestine; *Haemonchus* again appeared to be taking up an increasing amount of ^{32}P when the amount in the abomasal tissue was falling off. Variable results were obtained with *Oesophagostomum*; in one experiment the worms absorbed a much greater amount of ^{32}P than did the rectum, and in the other experiment, the ^{32}P uptake by the parasites and by the tissues of the rectum was of the same order.

TABLE 2

RELATIVE ^{32}P UPTAKE BY HOST TISSUES AND PARASITES AFTER INTRAVENOUS INJECTION OF THE HOST

Sample	2 Hr.	8 Hr.
Abomasum	68.4	46.9
H. contortus	49.7	74.0
Rectum	43.2	36.5
O. columbianum	5.3	79.0
Small intestine	53.0	78.8
Trichostrongylus spp.	59.7	85.2

The results refer to the relative ^{32}P concentration, calculated as the ratio of counts/min./g. wet wt. to dose (disintegrations/min.).

Figure 2 shows the relatively high ^{32}P content of the intestinal fluid of the host after an intra-abomasal injection compared with that obtained after an intravenous injection; no values were recorded for the latter at an 8-hr. period.

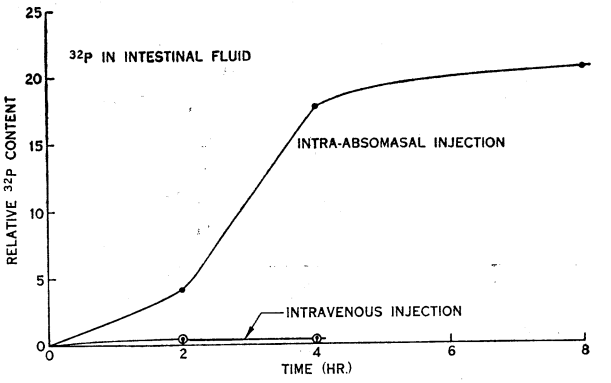

Fig. 2.—Relative amounts of radio-active phosphorus found in the intestinal fluid of sheep at different periods after dosing the sheep intra-abomasally and intravenously with sodium dihydrogen phosphate containing ^{32}P.

IV. DISCUSSION

As the fluid content of the host's intestine was found to be very low in radio-activity after the intravenous injection of labelled phosphate, the rapid

absorption of ^{32}P by *Trichostrongylus* spp. after such injection suggests that the parasites were feeding on the tissues of the host and not on the ingesta.

However, this method of feeding could not alone account for the great uptake of ^{32}P by the worms when labelled phosphate was given by injection into the abomasum. It seems, therefore, that the ^{32}P taken up by feeding on the tissues of the host may have been supplemented by phosphate absorbed through the cuticle.

Evidence that these nematodes are closely associated with the mucosa of the host gut has been discussed by Ackert and Whitlock (1940). Davey (1938), as a result of his *in vitro* experiments with nematode parasites of sheep, concluded that the forms with rudimentary buccal capsules probably feed on tissue elements at, or in, the mucosa. The results of the present investigation are in agreement with these suggestions.

Ransom (1911) classed *Haemonchus contortus* as a blood-sucking parasite; Broughton and Hardy (1935) observed the worms sucking blood from the wall of the abomasum. Haematological observations by one of us (P.M.S.), carried out over an extended period on sheep infested with *Haemonchus*, showed a definite correlation between decrease in red cell count and the degree of infestation. Ackert and Whitlock (1940) described these nematodes as being unattached to the mucous membrane but closely associated with it.

The relatively high absorption of ^{32}P by *Haemonchus contortus* when the sheep were given either intravenous or intra-abomasal injections of labelled phosphate suggested that the worms were feeding on the tissues of the host. Uptake through the cuticle did not appear to be important, as the relative amounts of ^{32}P in both the abomasum and its parasites were of the same order.

The feeding habits of nematodes are closely linked with the degree of the parasites' attachment to the tissues of the host. Very little information on the feeding habits of *Oesophagostomum columbianum* is available, but according to Ransom (1911), immature *Oesophagostomum* feed on the material in the nodules. From our observations, the adult worms appeared mainly to be lying on the walls or free in the contents of the rectum and not to be attached to the mucous membrane. Hoeppli (1927) emphasized the importance of examining the mode of attachment of parasitic nematodes to the host animal as soon as the host has been killed, as many parasites relinquish attachment shortly afterwards. However, in all our experiments the worms were collected immediately the sheep had been slaughtered.

In one series of experiments involving intravenous injections, *Oesophagostomum columbianum* showed a much higher ^{32}P uptake than did the tissues of the rectum, but when the experiments were repeated, the level of ^{32}P in the parasites was found to be similar to that in the tissue. These results, though variable, do show that absorption occurred quite rapidly. It is unlikely that appreciable amounts of labelled phosphate would have reached the contents of the rectum by 2-4 hr., or even 8 hr., after an intravenous injection, so that the high levels found in the parasites would appear to have been due to tissue

feeding. However, in the absence of any determinations on the radio-activity of the rectal contents, the results with regard to *Oesophagostomum* must be considered inconclusive.

In general it appears that the nematodes examined feed on the tissues of the host and not on the contents of the alimentary tract. Thus it seems unlikely that the parasites would take up an anthelmintic *per os* from the gut contents of sheep that have been dosed with the drug. Either the parasites absorb the anthelmintic via the cuticle or by the ingestion of the tissues of the host that contain the drug. With phenothiazine, the amount taken up by the intestinal mucosa of the host is small (Lazarus and Rogers 1950), so it seems probable that the major route of entry of the drug into the parasites would be via the cuticle.

V. Acknowledgment

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