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INHIBITION OF 3-O-METHYL GLUCOSE TRANS-
PORT IN 'ASCARIS SUUM' MIDGUT BY 'ESCHERICHIA
COLI' ENDOTOXIN

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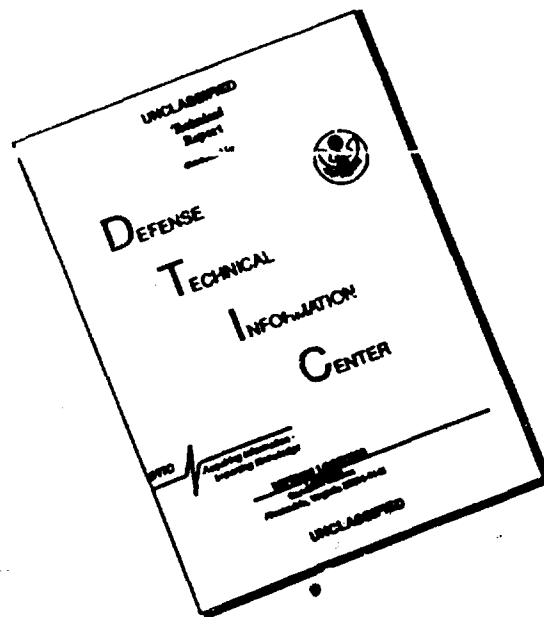
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13. ABSTRACT Movement of 3-0-methyl glucose across the midgut of <u>Ascaris</u> was inhibited by endotoxin. Inhibition was dependent on endotoxin concentration. Despite the fact that the mechanism by which the transport is inhibited is not known, the knowledge that absorptive processes <u>per se</u> can be impaired may lead to a better understanding of the mechanism of endotoxin action.			

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INHIBITION OF 3-O-METHYL GLUCOSE TRANSPORT
IN ASCARIS SUUM MIDGUT BY ESCHERICHIA COLI ENDOTOXIN

John H. Migliaccio

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Dogs were administered intravenous injections of endotoxin during septic shock studies conducted at the Veterans Administration Hospital in Oklahoma City, Oklahoma. Upon necropsy of animals that died of endotoxin shock, helminths found in the intestine were nonmotile and insensitive to mechanical stimulation. Following these observations two dogs, which were naturally infected with hookworms, Ancylostoma canum, and tapeworms, Taenia Faeniaeformis, were given endotoxin orally. Both animals passed parasites in their stools during the following 48 hours. These dogs were killed and at necropsy worms recovered from the gut were nonmotile.

The biological effects of endotoxins have been studied extensively. However, the biochemical basis for endotoxic action has not been determined unequivocally (5). In view of this and the above observations in dogs, studies were carried out in an attempt to establish a specific mechanism of endotoxin action by investigating its effect on a helminth parasite in vitro. Ascaris suum was used as a model to study the effect of endotoxin on intestinal transport of 3-O-methyl glucose by parasitic worms. The intestine appears to be the principal route for nutrient uptake by nematodes (1-3). Beames determined the movement of several hexoses across the midgut of Ascaris and observed that movement of 3-O-methyl glucose from luminal to abluminal surface occurs against a concentration gradient when glucose is present in the incubation medium (1).

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The thesis of this study was that the observed effects of endotoxin on intestinal helminths are due to altered nutritional status of the parasite.

The procedures followed in the experiments were patterned after those of Beames(1). Adult ascarids were collected at a local slaughter house and transported to the laboratory in saline (4), maintained between 32° and 39° C and were used within six hours. Individual worms were incised longitudinally with scissors. The reproductive system was pulled aside to expose the intestine which was then sectioned at the posterior portion. A Pasteur pipette was inserted into the lumen and secured with a silk ligature. Isolation of the middle third of the intestine was achieved by placing loops of silk thread around the intestine just beyond the tip of the Pasteur pipette and in the anterior region of the midgut. Material was introduced into the lumen of the intestine with a syringe fitted with a piece of polyethylene tubing which was inserted into the pipette. Fluid was forced into the intestine until it had moved past the most distant loop of thread. In this way the residual material in the intestine was flushed out of the preparation. The loops of thread were pulled tight and the resulting sac was cut free. The sac was blotted and placed in a vial containing 1.0 ml of glucose solution. The vial was stoppered with a 2-hole rubber stopper and flushed for 8-10 minutes with 95% N₂:5% CO₂, sealed and placed in a metabolic shaker for one hour at 37°C. Extra-luminal and intra-luminal solutions are shown in Table I. Endotoxin used in these experiments was a lipo-

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REFERENCES

1. Beames, 1971, J. Parasit. 57: 97-102.
2. Castro and Fairbairn, 1969, J. Parasit. 55: 13-16.
3. Fairbairn, 1957, Exp. Parasit. 6: 491-554.
4. Harpur, 1963, Can. J. Biochem. Physiol. 41: 1673-1689.
5. Nowtny, 1971, Naturwissenschafter 58: 397.

TABLE I. Effect of Endotoxin on Transport of 3-0-Methyl Glucose

Across Ascaris Suum Midgut

<u>Luminal Solution</u>	<u>Body Fluid</u>	<u>μmoles 3-0-methyl glucose</u> <u>cm²/hr</u> (\pm sx)	<u>(n)</u>
0 μg endotoxin (+)*	0.04M glucose	1.40 \pm 0.32	6
75 μg endotoxin (+)*	0.04M glucose	0.50 \pm 0.05	5
100 μg endotoxin (+)*	0.04M glucose	0.29 \pm 0.02	3

*0.04M C¹⁴ 3-0-methyl glucose (+)
 0.04M 3-0-methyl glucose (+)
 6mM glycochenodeoxycholic acid

All solutions were prepared in Ascaris saline. The solution on the luminal side of the gut contained 0.04M C¹⁴ 3-0-methyl glucose (8985 dpm/μmole). Gas phase 95% N₂: 5% CO₂, temperature 37°C, time in metabolic shaker bath 60 min. (n) above indicates the number of replicates.