

Oxygen Uptake of Liver Slices from Rabbits Infected with *Eimeria stiedae*.*

(23981)

W. M. DICKSON, J. S. DUNLAP, VERNER L. JOHNSON, AND D. DUNLAP

Biochemical studies of host-parasite relationship using a protozoan parasite have been concerned with blood parasites such as *Plasmodium* and *Trypanosoma* for the most part (5). Nutritional relationship between *Eimeria neischulzi* and its host, the rat, has been extensively investigated with particular regard to vitamin content of host diet (1-4). *Eimeria stiedae*, the liver coccidium of rabbits, has received little attention from those interested in biochemical host-parasite relations. Smith and McShan (6) found that succinic dehydrogenase activity of rabbit liver homogenates was markedly increased after 10 and 15 days of *E. stiedae* infection, and decreased to a slightly elevated level by the twentieth day. They also found a decrease in percentage dry weight of infected liver, and interpreted this finding to be a result of liver edema.

This report deals with the oxygen uptake of infected liver slices incubated in a non-nutrient medium.

Material and methods. Crossbred white rabbits obtained from local dealer were used. These rabbits were examined for coccidiosis by fecal examinations before use. Animals between 2 and 5 months of age were employed. They were infected orally with a sublethal dose of sporulated oocysts. One-half of each litter was infected. An infected and a non-infected litter mate were sacrificed on third, sixth, tenth, thirteenth, seventeenth, twentieth and twenty-fourth days after infection. Three replications, consisting of 7 infected and 7 control animals, were made. Liver slices were prepared in Berkeley-type glove box, at low temperature (10-14°C). Each slice weighed approximately 100-150 mg (wet weight). Individual slices were suspended in Warburg flask containing 1.5 ml of pH 7.4 Krebs-Ringer phosphate buffer. The center well of flasks contained 0.15 ml of 20% potassium

hydroxide to absorb the CO₂. Duplicate flasks were prepared for each liver. Flasks were equilibrated in water bath at 38°C for 20 minutes. Readings were taken, thereafter, at 5 minute intervals for 30 minutes, and at 15 minute intervals for 2 hours. The QO₂ ww (oxygen uptake/mg wet weight/hr) was obtained by plotting cumulative microliters of oxygen used against time and drawing the best straight line for initial linear uptake. Pieces of liver tissue were dried in desiccator at room temperature over concentrated sulfuric acid to determine dry weight. Other pieces were analyzed for nitrogen by micro-Kjeldahl procedure. These data were used to calculate oxygen uptake/mg dry weight/hour (QO₂ dw) and oxygen uptake/mg N/hour (QO₂ n).

Results are summarized in Table I. Oxygen uptake in the infected livers was very significantly increased over that of non-infected controls ($P < .01$). This increased uptake became noticeable at 6th day, increased to a peak at 17th day, and declined slowly thereafter. Seventeen to 21 days after infection with *E. stiedae*, the oocysts make their first

TABLE I. Effect of *E. stiedae* Infection on Rabbit Liver.

	Days infected	QO ₂ dw*	% dry wt	QO ₂ n	% N
Control		1.35	29.3	17.60	13.89
Infected	3	1.48	30.6	23.09	15.72
Control		1.30	29.4	19.14	15.30
Infected	6	2.01	27.9	24.24	15.88
Control		1.37	30.3	18.86	13.88
Infected	10	2.04	26.2	24.34	13.76
Control		1.19	29.5	15.62	16.94
Infected	13	2.65	21.0	28.36	15.67
Control		1.80	26.9	18.56	17.31
Infected	17	3.92	20.7	36.28	16.01
Control		1.62	30.8	17.76	18.47
Infected	20	2.72	23.4	28.69	14.43
Control		2.23	27.0	22.40	15.37
Infected	24	3.97	20.4	32.25	14.96

* This work supported by N.I.H. Grant 879. Scientific Paper No. 1714 Washington Agric. Exp. Stations, Pullman, Wash.

* Each value represents duplicate determinations in 3 replicates; thus, each figure is the mean of 6 determinations.

appearance outside the epithelial cells of bile ducts and there is a disappearance of schizogony. Further study is necessary to determine whether the increase in liver metabolic activity is due to a change in the parasite or whether the parasite change results from a metabolic alteration in the host. In a few experiments, the medium was enriched with adenosine triphosphate; this addition neither accentuated nor diminished the oxygen uptake.

Infection appeared to have little effect upon percentage of protein (nitrogen $\times$ 6.25) but percentage dry weight of infected livers was very significantly lower ($P < .01$) than that of the non-infected. This is in agreement with findings of Smith and McShan (6). These authors suggested that decrease in dry weight percentage was due to liver edema. Although total liver weights were not obtained in our experiments, an 8 to 9% decrease in dry weight (Table I) accompanied by 1 to 2% decrease in protein would imply either a considerable increase in total protein or a decrease of some other component. Since rabbit liver contains very little fat (7), glycogen depletion seems to be a logical explanation. Our oxygen uptake findings tend to confirm this possibility.

Summary. Oxygen uptake of liver slices

from rabbits infected with *Eimeria stiedae* was measured at various times after infection with a sublethal number of organisms. Oxygen uptakes of slices from non-infected litter mates were determined concurrently. Measurements were made in a medium consisting of Krebs-Ringer phosphate buffer at pH 7.4 in a water bath at 38°C. Oxygen uptake of infected livers was significantly greater than that of non-infected controls. There was a significant decrease in percentage dry weight of infected livers while percentage protein was little changed.

1. Becker, E. R., Dilworth, R. I., *J. Infectious Diseases*, 1941, v85, 230.
2. Becker, E. R., *Proc. Soc. Exp. Biol. and Med.*, 1941, v46, 494.
3. Becker, E. R., Smith, L., *Iowa State College J. Sci.*, 1942, v16, 237.
4. Becker, E. R., Manresa, F., Smith, L., *ibid.*, 1943, v17, 431.
5. vonBrand, T., *Chemical Physiology of Endoparasitic Animals*, 1952, Part III. Academic Press, Inc., N. Y.
6. Smith, B. F., McShan, W. H., *Annals of N. Y. Acad. Sci.*, 1949, v52, 496.
7. Spector, W. S., *Handbook of Biological Data*, 1956, p74, W. B. Saunders Co., Philadelphia.

Received March 25, 1958. P.S.E.B.M., 1958, v98.

Differential Excretion of Cobalt-containing Products Following Administration of Co⁶⁰-labelled Vit. B₁₂ to Rats. (23982)

S. WIDER,* J. A. WIDER AND E. P. REINEKE

Department of Physiology and Pharmacology, Michigan State University, East Lansing

Radioactivity excreted *via* the urinary tract after administration of Co⁶⁰-labelled Vit. B₁₂ has been demonstrated by microbial and radio-metric(1), chromatographic(2), and extraction(3) studies to be the intact vitamin. While similar studies conducted on feces, following both oral and parenteral administration of radioactive vitamin, reveal a considerable amount of ionic Co⁶⁰, the results have been attributed(1) to microbial action on the

unabsorbed vitamin as it passes through the intestine.

Our data show that after parenteral administration of radioactive Vit. B₁₂ there is a differential excretion pattern between urine and feces that indicates alteration of the vitamin prior to its entry into the intestine. Neither sex nor testosterone propionate administration influences excretion of the radioactive substance.

Methods. A. Four male litters born to mothers maintained during pregnancy and lactation on a diet low in Vit. B₁₂, were used.

* Present address: Medical School, University of Basel, Switzerland.