

STUDIES ON BIOCHEMICAL PATHOLOGY IN TRICHINOSIS. II. CHANGES IN LIVER AND MUSCLE GLYCOGEN AND SOME BLOOD CHEMICAL PARAMETERS IN MICE

by George L. Stewart

ABSTRACT

Glycogen content of mouse diaphragm muscle infected with *Trichinella spiralis* increased above that of uninfected mouse muscle early during a 34-day period following infection, and subsequently decreased to levels found in control muscle after day 24 post-infection (PI). There was no significant difference between the ratio of infected and uninfected mouse liver wet weight : dry weight, total liver protein, ratio of liver wet or dry weight : mouse body weight, or liver glycogen. Total body weight of trichinella-infected mice was less than that of uninfected mice after day 10 PI. The level of serum lactate in trichinosis mice was above that of uninfected mice between days 6 and 12 PI. Serum glucose levels in infected animals were less than those in uninfected control mice between days 4 and 14 PI, and there was no significant difference in serum insulin levels between infected and uninfected mice over a 25-day period of study. There was a significant decrease in the concentration of blood urea nitrogen of trichinella-infected mice below that of control mice between days 4 and 24 PI.

INTRODUCTION

On the light and electron microscopic level, a number of workers have observed an increase in the amount of glycogen present in skeletal muscle infected with *Trichinella spiralis* (Zarzycki, 1956; Fasske and Themann, 1961; Beckett and Boothroyd, 1962; Karpiak et al., 1963). These reported increases in muscle glycogen occurred before day 20 post-infection and were followed by a decrease in muscle glycogen to levels similar to, or below, those of uninfected muscle. In the present study these alterations in muscle glycogen

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were demonstrated by chemical means and a number of associated physical and chemical parameters in blood and liver from mice infected with *T. spiralis* were observed.

MATERIALS AND METHODS

The source of trichinella, method of excystment of muscle larvae and procedure for infection of mice were those used in previous studies (Stewart and Read, 1972a). Male or female 6-week-old Swiss white mice (Texas Inbred Mice Co., Houston, Texas) were used in all experiments. Mice were infected with 1000 larvae.

The anthelmintic methyridine was given to all infected and uninfected mice on day 11 PI, unless otherwise stated in context (Stewart and Read, 1972a).

For extraction of glycogen from diaphragm muscle and liver, tissue samples were removed at 2-minute intervals, rapidly weighed, and immediately immersed in 2 ml of 30% KOH at 100°C. All samples were removed from the boiling water bath after 15 minutes and cooled to room temperature. Glycogen was extracted and determined by the method of Montgomery (1957).

In determination of glycogen in trichinella larvae, worms were excysted on the days stated in context, washed 3 times in 0.85% NaCl (saline), separated into three equal samples and counted. Following centrifugation and removal of the supernatant (saline), 1 ml of 30% KOH at 100°C was pipetted onto the larval pellet. Each suspension of larvae was treated as above for extraction and determination of glycogen.

For removal of worms from muscle prior to encystment (before day 24 PI), the total body musculature of infected mice was run through a meat grinder, placed in saline solution in stoppered flasks with glass beads and shaken for 15 minutes at medium speed. The resulting suspension was strained through several thicknesses of cheesecloth and collected larvae were washed three times in saline.

The number of larvae/mg dry weight of diaphragm muscle was determined on 20 mice from the group of animals used in the study on muscle glycogen by methods previously outlined (Stewart and Read, 1972a).

For determination of serum lactate and serum glucose, 1 ml of blood was drawn, by direct heart puncture, from each experimental animal, and immediately placed in 2 ml of 0.3N BaOH. Two ml of 5% ZnSO₄ were subsequently added and the resulting precipitate was centrifuged at 2000 rpm for 15 minutes. One ml of the supernatant was used for serum lactate determination by the method of Barker-Summerson (1941), and 0.5 ml of the supernatant was used in the determination of serum glucose by the "Glucostat Special" method (Worthington Biochemical Corp., Freehold, N.J., 07728).

In determination of mouse liver protein, a sample of liver was removed, rinsed briefly in cold saline, blotted dry on filter paper, weighed, and homogenized at 0°C in 2 ml of glass-distilled water. Proteins were precipitated at 5°C by addition of an equal volume of 10% trichloroacetic acid. After centrifugation the protein pellet was dissolved in 1N NaOH and total protein was determined by the method of Lowry et al. (1951).

In studies on muscle and liver glycogen, blood glucose, lactate, urea nitrogen, and insulin, all animals were starved for 12 hours before killing. All animals in experiments dealing with chemical determinations were killed between 10:00 A.M. and 11:00 A.M. on the days indicated in context, and death was induced by intraperitoneal injection of 0.5 ml of 0.75% chlorobutanol (Eastman Kodak Co., Rochester, N.Y.).

Adult trichinella were collected by the 0.05% NaOH method of Larsh et al. (1952).

Blood urea nitrogen was determined by a modification of the Gentzkow-Masen (1942) method (Sigma Chemical Co., P. O. Box 14508, St. Louis, Mo., 63178).

Blood insulin was determined using an "Insulin Immunoassay Kit" (Amersham/Searle Corp., 2636 S. Clearbrook Dr., Arlington Heights, Illinois, 60005).

For determination of liver dry weights, whole livers were removed, placed in preweighed aluminum foil cups and put in a 90°C oven for 72 hours.

All chemicals were reagent grade. Student's "t" test was employed to evaluate the significance of differences.

RESULTS

Experiment I

Total glycogen was determined every third day between days 4 and 16 post-infection (PI) and on days 18, 20, 24, 28, 32, and 34 PI in diaphragm muscles from mice infected with *Trichinella spiralis* and uninfected mice. Total μg glycogen of diaphragm muscles from trichinella-infected mice was an average of 55.26% (SE \pm 3.10) above that of uninfected after day 13 PI (figure 1). Total glycogen was determined for trichinella larvae (table 1) between days 16 and 34 PI and subtracted from total glycogen of infected diaphragm muscle (figure 1, line A). Results are presented in figure 1, line B. Total glycogen from infected mouse diaphragm muscles adjusted for the larval component is significantly different from that of uninfected mice between days 16 and 24 PI (an average increase of 38.95%; S.E. \pm 4.90).

Experiment II

Mg% glucose in blood from infected and normal mice was determined every other day between days 2 and 24 PI (samples from day 22 PI were lost).

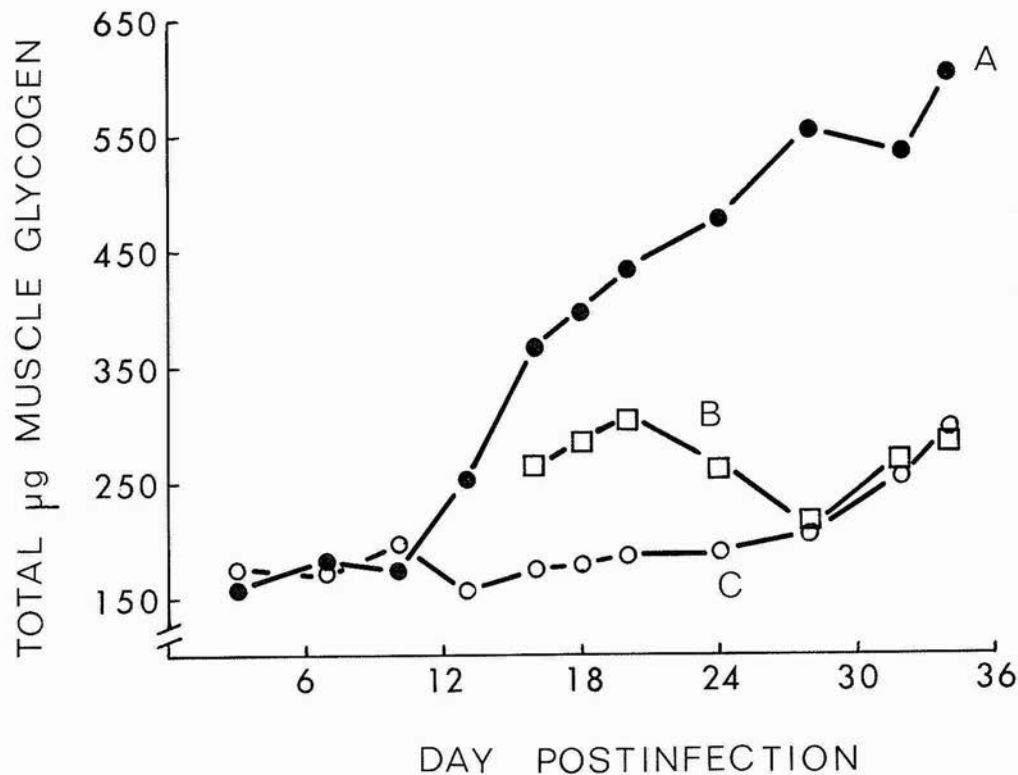


FIG. 1. TOTAL μ g GLYCOGEN IN NORMAL MOUSE DIAPHRAGM MUSCLE (C) AND TRICHINELLA-INFECTED MOUSE DIAPHRAGM MUSCLE with (B) and without (A) glycogen from trichinella larvae subtracted. All points in lines A and B are samples from 5 infected mouse diaphragms and all points in line C are samples from 3 uninfected mouse diaphragms. Between days 13 and 34 PI, points in lines A and C differ significantly, and between days 16 and 24 PI points in lines B and C differ significantly. $p < 0.05$.

TABLE 1

TOTAL μg GLYCOGEN OF TRICHINELLA LARVAE
ON DAYS 16, 18, 20, 24, 28, 32, AND 34 PI

Standard errors are listed in adjacent column. Determinations were made on 3 samples of larvae on each day indicated.

Day PI	Average μg glycogen per larva	S.E.
16	0.0251	± 0.0017
18	0.0283	± 0.0017
20	0.0291	± 0.0023
24	0.0398	± 0.0014
28	0.0541	± 0.0023
32	0.0590	± 0.0013
34	0.0595	± 0.0017

TABLE 2

TOTAL NUMBER OF ADULT TRICHINELLA RECOVERED FROM THE
GUTS OF MICE NOT GIVEN METHYRIDINE

Data presented as percentage of total number of worms in inoculum (1000). Standard errors are in an adjacent column. Determinations were made on 3 mice on each day in the experiment.

Day PI	Number of worms recovered \div 1000(%)	S.E.
8	87.9%	± 2.69
12	55.4%	± 4.32
14	46.4%	± 4.08
16	31.2%	± 5.26
18	17.1%	± 3.09
20	9.4%	± 0.91
24	0.3%	± 0.058

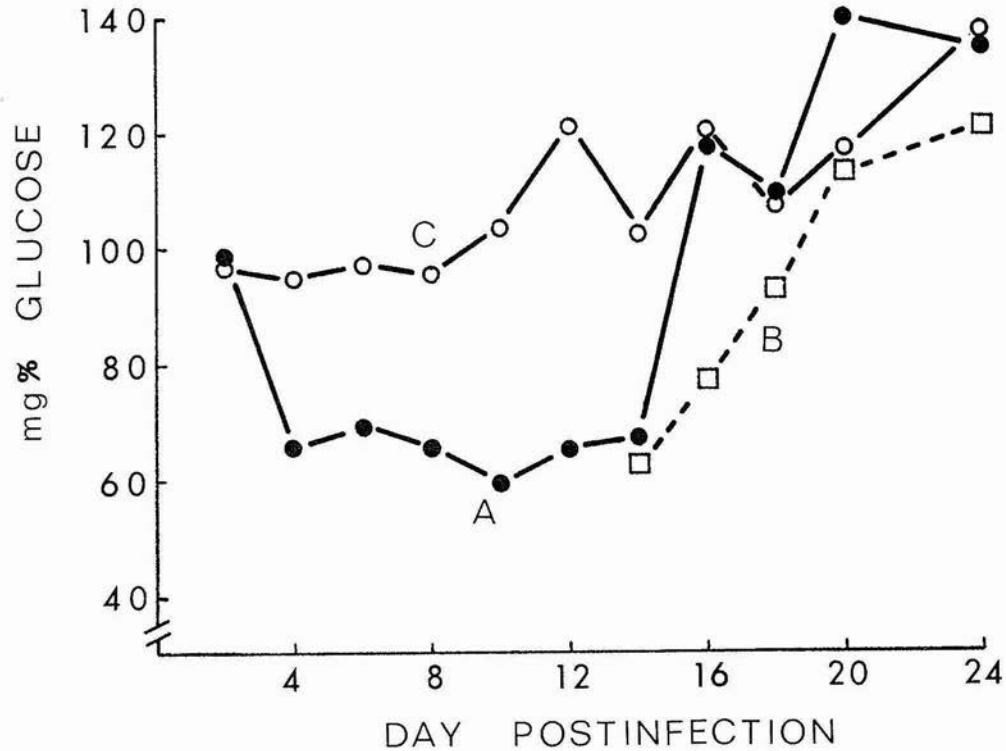


FIG. 2. Mg% BLOOD GLUCOSE FROM TRICHINELLA-INFECTED MICE not given methyridine (B), trichinella-infected mice given methyridine on day 11 PI (A), and uninfected animals (C). All points in line A between days 4 and 14 PI are significantly different from points from the same days in line C, and points on days 14, 16, and 18 PI in line B differ significantly from points on these days in line C. All points are the average of samples from 3 mice. $p < 0.05$.

Mg% blood glucose in infected mice given methyridine (figure 2, line A) on day 11 PI decreased between days 4 and 14 PI an average of 38.6% (S.E. \pm 5.27) below that of uninfected animals (figure 2, line C). After day 14 PI, Mg% blood glucose in infected mice not given methyridine (figure 2, line B) remained below that of uninfected mice (figure 2, line C) through day 18 PI. No adult trichinella were found after day 12 PI in the guts of infected mice given methyridine. Percentage of total worms in the infecting dose recovered from mice not given methyridine is shown in table 2.

Mg% lactate in blood from infected and uninfected mice was determined in blood samples from the same mice used in blood glucose determinations. Mg% blood lactate in infected mice given methyridine on day 11 PI (figure 3, line A) rose between days 6 and 12 PI an average of 37% (S.E. \pm 1.73) above that of control animals (figure 3, line C). After day 12 PI, blood lactate in infected animals not given methyridine remained above that of uninfected mice between days 14 and 18 PI (average increase = 20.2%; S.E. \pm 3.96).

Experiment III

There was no significant difference between serum insulin in trichinella-infected and uninfected mice over a 24-day period of study (range: 19-33 μ units/ml).

Mg% urea nitrogen in blood from infected mice was significantly less than that of uninfected from day 4 through day 24 PI (figure 4), with an average depression of 24.5% (S.E. \pm 2.24).

Experiment IV

Total g body weight of trichinella-infected mice was an average of 22.5% (S.E. \pm 2.14) below that of uninfected animals between days 8 and 30 PI (figure 5). Mg whole liver wet weight and dry weight per g mouse body weight in infected mice was similar to that of uninfected animals between days 4 and 30 PI (samples taken every 3rd day). Liver wet : dry weight ratio was similar in infected and uninfected mice over the same time period as above, and there was no significant difference in Mg liver protein per mg liver wet weight in infected and uninfected mice over a similar period of study (average = 208.18; S.E. \pm 3.91).

In preliminary experiments it was found that although methyridine had no effect on muscle glycogen, serum insulin, or blood glucose, this drug caused a 30% increase in the liver glycogen of both infected and uninfected mice. For this reason mice in the following experiment were not given methyridine. There was no significant difference between μ g liver glycogen per mg wet weight of liver tissue from infected and uninfected mice (average = 72.01; S.E. \pm 1.03).

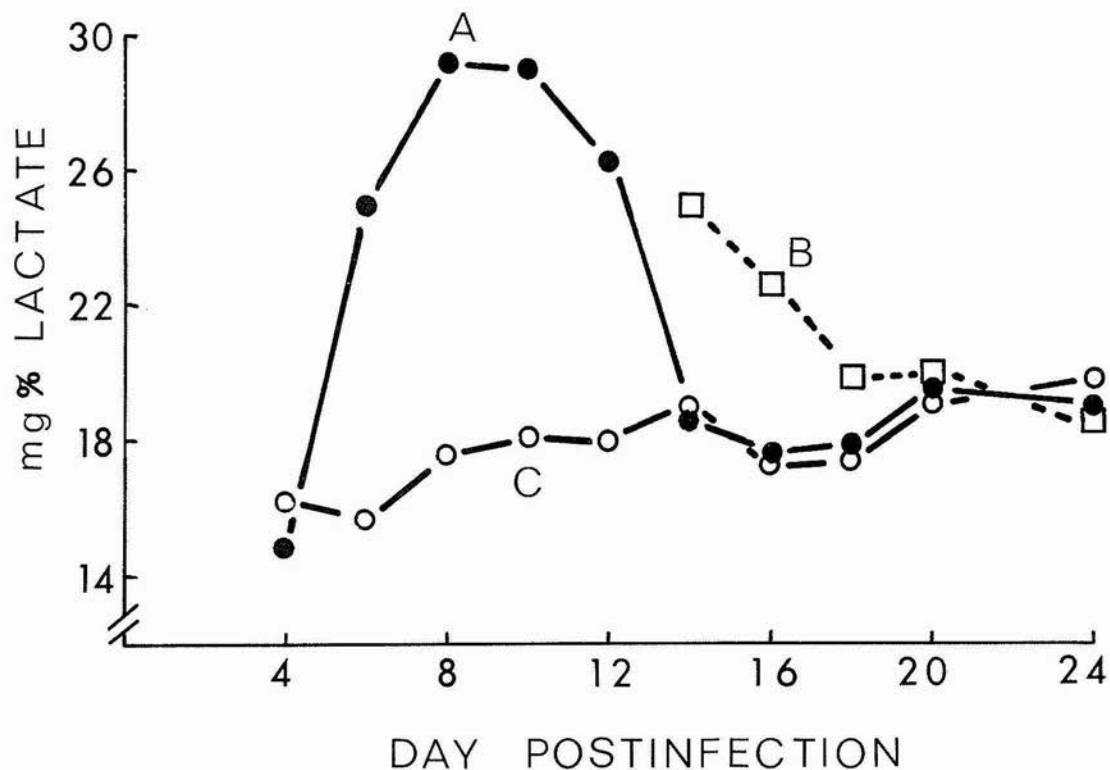


FIG. 3. Mg% BLOOD LACTATE FROM TRICHINELLA-INFECTED MICE not given methyridine (B), trichinella-infected mice given methyridine on day 11 PI (A) and uninfected animals (C). All points in line A between days 6 and 12 PI differ significantly from points on the same days in line C, and points on days 14, 16, and 18 PI in line B are significantly different from points on those days in line C. All points are the average of samples from 3 mice. $p < 0.05$.

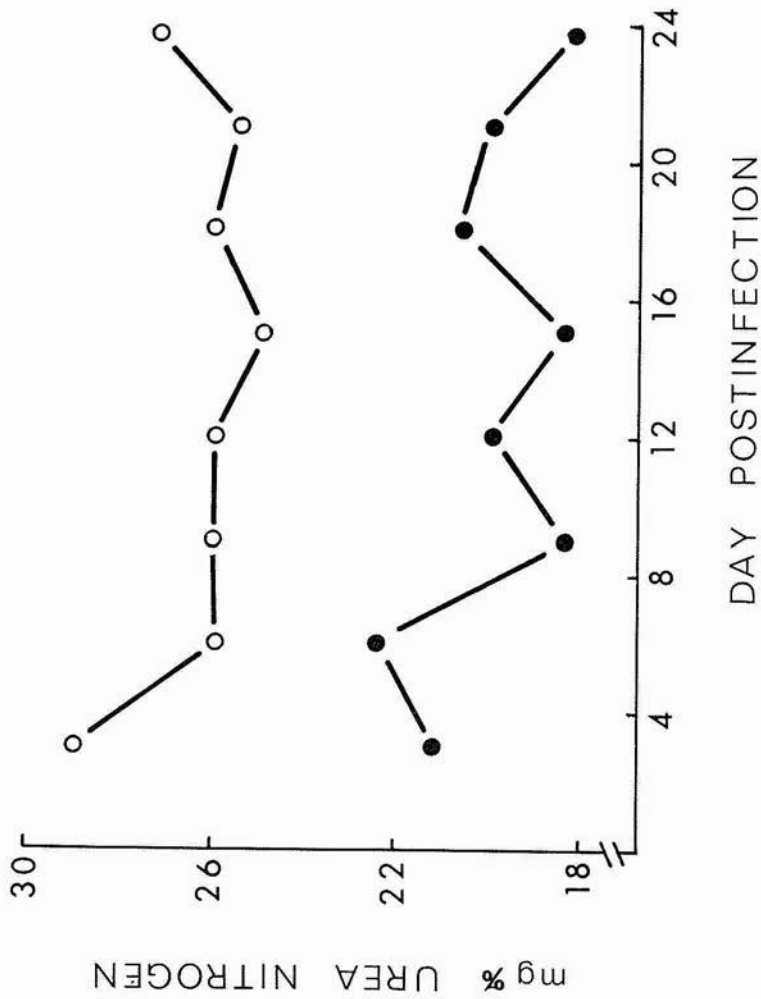


FIG. 4. Mg% UREA NITROGEN IN BLOOD from trichinella-infected (●) and uninfected mice (○). All points between days 4 and 24 PI are significantly different. All points are the average of samples from 3 mice. $p < 0.05$.

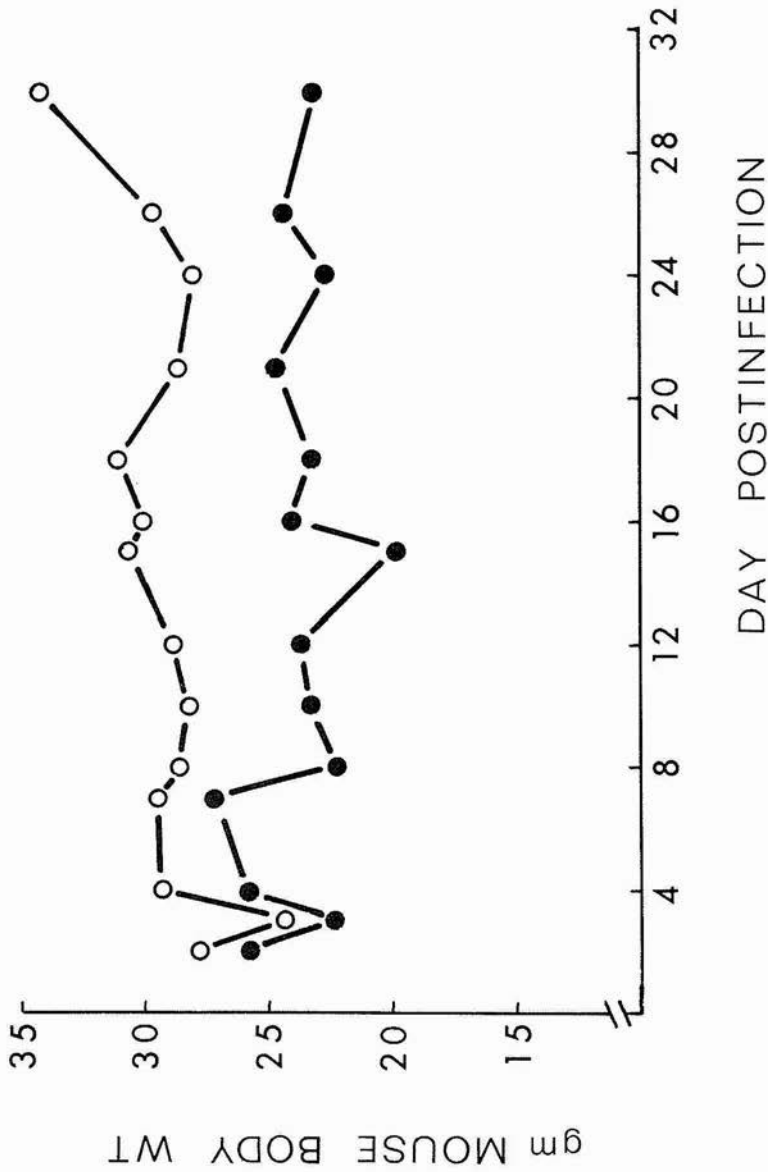


FIG. 5. TOTAL g BODY WEIGHT of trichinella-infected (●) and uninfected (○) mice. All points between days 8 and 30 PI are significantly different. All points are the average of samples from 10 mice. $p < 0.05$.

DISCUSSION

By the use of chemical methods, an increase in glycogen content of mouse diaphragm muscle infected with *Trichinella spiralis* was observed between days 16 and 24 PI (figure 1, line B). These findings agree with those of Zarzycki (1956) and Beckett and Boothroyd (1962), who reported an increase in histochemically demonstrable glycogen in trichinella-infected fibers between days 10 and 20 PI, followed by a decrease in infected-fiber glycogen shortly thereafter to levels similar to, or less than, uninfected fibers.

Immediately after entrance into muscle fibers, trichinella larvae undergo a three-phase growth pattern (Despommier et al., 1975). In phase III of this pattern (days 9-24 PI) muscle larvae undergo their greatest increase in size. In the present study, a dramatic rise in glycogen content of larvae occurred between days 16 and 28 PI (table 1).

Mg% blood glucose in infected mice (methyridine given on day 11 PI) was below that of uninfected animals between days 4 and 14 PI (figure 2, lines A and C), a period during which intestinal malabsorption of glucose was reported in mice infected with *T. spiralis* (days 4-16 PI; Olson and Richardson, 1968). Mg% blood glucose in infected mice not given methyridine (figure 2, line B) returned to normal levels as the number of adult trichinella present in their intestines decreased (table 2).

Mg% blood lactate in infected mice (methyridine given on day 11 PI) was above that of uninfected between days 6 and 12 PI (figure 3, lines A and C). Larsh and Race (1954) observed a mild inflammation in the intestines of mice infected with *T. spiralis*, which began about day 4 PI, entered an acute phase, which peaked around day 8 PI, and then began to subside around day 16 PI. The large numbers of inflammatory cells taking part in this cellular response may make a significant contribution to the observed increase in blood lactate from infected mice (figure 3, line A). Methyridine, given on day 11 PI, may have terminated gut inflammation (figure 3, line A) earlier than usual by removing adult worms from the intestine. On the other hand, blood lactate from infected mice not given methyridine remained elevated above that of uninfected animals until day 18 PI, when all but 17% of the initial worm dose was gone. Another significant source of lactate may be the extreme peristaltic activity of the smooth muscles of the gut. Schanbacher et al. (1976) have shown greatly enhanced gut motility associated with intestinal trichinosis. In addition, the observed decrease in mg% urea nitrogen in blood from trichinella-infected mice below that of control animals (24.5%; S.E. \pm 2.24) throughout the 24-day period of study (figure 4) may imply alterations in nitrogen metabolism in the livers of trichinous mice (Hepler, 1973). Furthermore, this decrease in blood urea nitrogen may indicate a general hepatic insufficiency, including a decrease in the ability of liver tissue from trichinella-infected mice to metabolize lactate. The matter requires further investigation.

I had thought that enhanced insulin production by the pancreatic beta

cells might contribute to the observed increase in trichinella-infected mouse muscle glycogen; I found no significant difference in the levels of serum insulin from infected and uninfected mice.

In agreement with the findings of Castro and Olson (1967) working with guinea pigs, I found the total mouse body weight of infected mice to be below that of control animals between days 10 and 32 PI. The ratios of liver wet or dry weight : mouse body weight, liver protein : liver wet weight, and liver glycogen : liver wet weight were similar in infected and uninfected animals.

Marked alterations occur in the biochemistry (Stewart and Read, 1972a, 1972b, 1973a, 1973b, 1974) and ultrastructure (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1971; and Despommier, 1975) of muscle infected with *T. spiralis*. On the basis of these findings, Stewart and Read (1973b) have suggested that the damage caused by entering trichinella larvae induces muscle fibers to undergo regeneration. Shortly thereafter information from the larva redirects regeneration in host fibers to the synthesis of enzymes and structural proteins for establishing a suitable environment for the growth and development of the larva. This may include, as indicated by the present study, an enhancement of glycogenesis in infected fibers around day 12 PI. The period during which the glycogen content of trichinella larvae rises most dramatically (table I) parallels a marked decrease in the elevated glycogen content of infected muscle between days 20 and 28 PI (figure 1, line B).

These findings lend further support to the hypothesis that trichinella-infected muscle fibers undergo redifferentiation rather than degeneration, and are metabolically active as a "nurse cell" (Purkerson and Despommier, 1974) to the trichinella larva.

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