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STUDIES ON CHEMICAL PATHOLOGY
IN TRICHINOSIS

BY

GEORGE LOUIS STEWART

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Thesis Director's signature:

Houston, Texas

May 1973
"From goblins and ghoulies
and long-legged woolies
and things that go bump in
the night, dear Lord,
deliver us."

(Author unknown)
Studies on Chemical Pathology in Trichinosis

by

George Louis Stewart

Trichinella migratory larvae were found in greatest numbers in the peritoneal washings and blood of mice harboring adult worms between days 9 and 14 postinfection (PI). Chemical abbreviation of the stay of adult trichinella in the intestines of experimental mice with the anthelmintic methyridine (when given on days 11 or 12 PI) resulted in heavy infection of muscle while restricting the age difference between larvae to 5 or 6 days, respectively.

The ribonucleic acid (RNA) per unit protein of trichinosed mouse diaphragms rose above that of uninfected after day 13 PI. The RNA per unit protein of trichinosed muscle increased over that of controls as the total number of muscle larvae present rose. There was a minimum number of trichinella-infected fibers required for detection of the full pattern of change known to occur in trichinosed muscle RNA per unit protein. When the lives of adults were terminated with methyridine the full pattern of change in RNA per unit protein of infected muscle was discernable only when the drug was given after day 9 PI. When total RNA of infected muscle was related to wet weight, dry weight or protein a similar magnitude and pattern of change was observed. Methyridine was shown to have no influence over the RNA per unit protein of mouse muscle. Infected mice given cortisone exhibited a pattern and magnitude of change in diaphragm RNA per unit protein similar to that of diaphragm muscle from infected mice not given cortisone. Infected
mouse diaphragms showed an increase in the in vitro incorporation of 
$^{14}$C-uridine into RNA over control muscle. Larval RNA and protein were 
shown to account for a small percentage of those of the host parasite 
system. RNA turnover in infected diaphragms was greatest during the 
early stages of infection, and dropped below that of controls later in 
the study.

The wet weights and dry weights of infected muscle rose above that 
of controls between days 28 and 40 PI. The per cent water of trichini-
ella-infected diaphragm rose above that of uninfected between days 
10 and 40 PI. The per cent protein of trichinosed diaphragm muscle 
dropped below that of controls about day 15 PI and remained below that 
of uninfected diaphragms throughout 57 days of infection.

The RNA metabolism of liver tissue from infected and uninfected 
mice was similar throughout 40 days of infection. Whole cardiac muscle 
from trichinella-infected animals showed no difference in RNA per unit 
protein from that of control mouse myocardium over 40 days of infection.

Trichinosed mouse diaphragms showed an increase in DNA per unit 
protein, and in the incorporation rate of thymidine over that of control 
muscle. The total RNA, total protein, total DNA, $\mu$g RNA per mg 
protein of larvae, and the rate of incorporation of thymidine by larvae 
were determined. The rate of incorporation of thymidine by trichinella-
infected diaphragms was adjusted for the larval component.

The incorporation of $^{14}$C-proline, $^{35}$S-methionine, $^{3}$H-tryptophan, 
alanine and glycine by trichinosed diaphragm muscle rose above that 
of uninfected between days 13 and 40 PI. The total hydroxyproline 
(as a measure of total collagen) was above that of uninfected between
days 26 and 40 PI. Amino acid analysis of hydrolyzed protein samples from infected and uninfected diaphragm showed the $^{35}$S label to occur only in methionine sulfone (an hydrolysis product of methion); the $^{14}$C label was found in proline and hydroxyproline.

These biochemical changes are discussed in terms of the morphological alterations trichinosed muscle is known to undergo, and a model for biochemical interaction between the host and the parasite is proposed.
ACKNOWLEDGEMENTS

I take great pleasure in expressing my most sincere appreciation of the patient guidance given me by Dr. Clark P. Read, under whom I have had the extreme privilege to work for the past four years.

I am deeply indebted to Drs. Franklin Sogandares-Bernal and Richard D. Lumsden for their confidence, help and invaluable friendship. They were instrumental in my decision to study parasitology.

I dedicate this dissertation to my lovely wife, Breck, for her devotion, and for the patience she displayed during the many hours I spent with my "bugs".

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I

INTRODUCTION

Since shortly after the discovery of *Trichinella spiralis* in 1835 in the skeletal musculature of a human, both the adults and muscle larvae have been the subjects of a great amount of scientific research. Although numerous studies have been conducted on various aspects of the life cycle, development, and morphology of this helminth, and, in the past 25 years, several *in vitro* studies have been carried out on the adults and the larvae, only a small amount of research has been directed to the biochemistry of the host muscle-larva relationship.

Light and electron microscopic, as well as histochemical, studies have revealed this host-parasite relationship to be a very intimate one involving complex morphologic and chemical changes in infected host muscle fibers.

Studies on the light microscopic level have demonstrated that trichinosed muscle fibers undergo several morphological changes. In 1896 Ehrhardt observed that one of the most salient characteristics of trichinosed muscle was an increase in the size and number of fiber nuclei between days 1 and 5 postinvasion of the muscle fiber. Drachman and Tunchay (1965) found that the earliest alterations in infected guinea pig muscle occurred between days 5 and 10 postinfection (PI) with loss of myofilamentous structure, an increase in the size of fiber nuclei, and an increase in the basophilia of infected muscle sarcoplasm. By day 15 PI these same workers noted a marked increase in the number of fiber nuclei, with displacement of the same from their normal subsarcolemmal position to a more central location within the infected fiber.
In 1913 Flury showed that the amount of glycogen in heavily
trichinosed muscle was less than that of uninfected muscle. However,
Zarzycki (1956) and Beckett and Boothroyd (1962) observed an increase
in the histochemically demonstrable glycogen of infected fibers between
the time larvae first invaded host muscle and day 20 PI. Thereafter
there was a rapid decrease in the glycogen of infected fibers.
Zarzycki (1963) demonstrated a marked increase in succinic acid dehydro-
genase activity in infected fibers, Kedrowa (1965) observed an increase
in creatine phosphokinase activity during the first 3 weeks after
invasion, and Bullock (1953), and Stoyanov and Nenov (1965) showed an
enhanced alkaline phosphatase activity of infected muscle. Maier and
Zaiman (1966) histochemically demonstrated an increase in the ribonucleic
acid and in the activity of the Golgi of infected muscle, and demo-
strated acid phosphatase, non-specific esterase and amino-peptidase
activity associated with lysosome-like bodies. Gabryel and Gustowska
(1967) were also able to correlate an increase in RNA with the augmented
basophilia of infected muscle. In addition, these same workers were
able to demonstrate, by autoradiographic means, the incorporation of
$^{3}H$-thymidine by infected muscle nuclei.

Fasske and Themann (1961) and Ribas-Mujal and Rivera-Pomar (1968)
conducted electron microscopic studies of rat muscle fibers during the
early stages of infection with trichinella and found: a) an increase
in the size of infected fibers; b) an increase in the size and number of
nuclei, with a concomitant increase in the size of nucleoli; c) loss
of myofilamentous elements; d) an early increase in the number of free
ribosomes and in the ribosome-studded membranes of the endoplasmic
reticulum; e) positional changes in the triads followed by several phases of alteration undergone by the tubules of the T system; f) a marked increase in the size and number of mitochondria; and g) an increase in the number of Golgi complexes. More recently, Despommier (personal communication) has conducted a similar study on trichinized mouse muscle fibers and observed the same morphological changes, except he was unable to detect an increase in the number of infected fiber nuclei.

Karpiak et al. (1963) observed an increase in fat synthesis around day 20 PI, and Kozar et al. (1965) demonstrated an increase in oxygen consumption of infected muscle in the presence of succinate or propionate (but not with other Krebs cycle intermediates). These observations coincide with an increase in the number and size of mitochondria occurring after day 12 PI (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968; Despommier, personal communication).

Beckett (1961) histochemically demonstrated a decrease in the amount of protein in infected fibers between days 5 and 28 PI, a finding which is in chronological agreement with the destruction and loss of myofilamentous elements of infected fibers (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968).

Teppema et al. (in press) have conducted a light and electron microscopic study of trichinella-cyst formation, and from their observations have concluded that the host fiber is intimately involved in the production of cyst substance. Ritterson (1966) demonstrated that the parasite-cyst is composed primarily of collagen.

The intimate nature of the muscle fiber-trichinella relationship is indicated by observed changes induced by the larva in host-muscle fiber
morphology and chemistry. It was felt that this was an excellent model for the study of host-parasite interaction on the biochemical level. The present investigation is concerned with: a) studies preliminary to biochemical investigation; b) findings on changes in RNA per unit protein of infected mouse diaphragm muscle, liver and heart; c) alterations of DNA metabolism in trichinella-infected mouse muscle; d) changes in protein synthesis of infected diaphragm muscle; and e) the chronology of trichinella-cyst formation.
II

MATERIALS AND METHODS

A. Biological

Male, 5 to 6-week-old Swiss white mice (Texas Inbred Mice Co., Houston, Texas) were used in all experiments. The strain of *Trichinella spiralis* used in this study has been maintained in mice in this laboratory since 1969, at which time it was obtained from Drake University where it had been maintained since 1944.

Trichinella larvae for infecting experimental animals were obtained by digestion of skinned, eviscerated trichinosed-mice in a 1% pepsin-0.5% HCl solution at 37°C in a shaking water bath for 2 hr. Excysted larvae were washed 5 times by sedimentation in 0.85% sodium chloride, and 10-0.5 ml aliquots were counted on a glass counting slide. The number of larvae per ml was adjusted to the desired inoculum per mouse. The larvae were suspended by a magnetic stirring device in 0.85% sodium chloride and 1 ml aliquots were given to experimental mice by gavage. Mice were infected with 1000 larvae each unless otherwise stated in context.

For determination of the number of migratory larvae present in the blood and peritoneum, 2 mice on every day between days 5 and 17 PI and on days 20 and 25 PI were sacrificed. Each mouse was exsanguinated by direct heart puncture and the peritoneum was washed 4 times with 5 ml of 0.85% sodium chloride. The blood and peritoneal washings from the same mouse were combined and centrifuged to pellet the migratory larvae. Larvae were suspended in 10 ml of 0.85% sodium chloride by means of a magnetic stirring device and counted in 10 - 1 ml aliquots on a glass counting slide.
B. Experimental procedure

The anthelminthic methyridine (2-(B-methoxyethyl) pyridine, K & K Laboratories, Inc., Plainview, New York) was given at a dose level of 500 mg/kilo (Denham, 1965) on day 11 PI unless otherwise indicated in context. In all experiments 2 uninfected mice were given methyridine on the same day on which all infected mice were injected with the anthelminthic.

In the experiment in which the effect of suppression of the inflammatory response upon changes in the RNA per unit protein of diaphragm muscle was investigated, cortisone acetate ("Cortone") was given to 1 of the 2 groups of infected animals and 1 of the 2 groups of uninfected mice. After the method of Coker (1955), mice were given daily injections of 0.5 mg cortisone acetate suspended in 0.05 ml of sterile 0.85% sodium chloride. Animals from the infected and uninfected control groups were given 0.05 ml of sterile 0.85% sodium chloride each day.

In experiments involving the extraction of total protein, and total RNA and/or total DNA from whole diaphragm or cardiac muscle or a piece of the left lobe of the liver, the appropriate tissue was removed from 3 infected and 3 uninfected mice, rinsed twice in Krebs-Ringers solution containing 25 mM tris-maleate buffer at pH 7.4 (KRT) at 5°C, blotted on filter paper, weighed on a torque balance, placed in dry test tubes immersed in a dry ice-acetone bath, homogenized in 2 ml of cold (5°C) 0.5 N perchloric acid in a Pyrex homogenizer and submitted to the Schmidt-Thannhauser (1945) extraction procedure as modified by Hutchison and Munro (1961).
For analysis of total protein, total RNA and/or total DNA of larval tissue the pepsin-HCl digestate was strained through a double layer of cheese cloth and then washed 10 times in 0.85% sodium chloride. Ten 0.5 ml aliquots of the magnetically stirred larval suspension were counted. The maximum variation in replicate counts was 33%. The larvae were homogenized in 2 ml of 0.5 N perchloric acid and treated as above.

In experiments in which changes in the wet weight, dry weight and total water of trichinosed and uninfected muscle was determined, the diaphragms of 3 infected and 3 control animals were removed, rinsed twice in KRT, blotted dry, weighed in tared, aluminum cups on a Mettler balance and placed in a 90°C oven for 48 hr. In the experiment in which the percent protein of muscle dry weight was determined, the dried diaphragms were homogenized in 1 N NaOH.

C. Chemical determinations

In all experiments, total protein was determined by the method of Lowry et al. (1951), total RNA was assayed for by the orcinol method of Dische (1955), and total DNA was determined by the method of Burton (1956).

For the determination of hydroxyproline of infected and uninfected mouse diaphragms, samples from the total protein fraction of each diaphragm (dissolved in 1 N NaOH) were hydrolyzed in 6 N HCl at 125°C for 1 1/2 hr, evaporated to dryness, desalted on a column (Heathcote et al., 1971) containing ion retardation resin (AG11A8, 50-100 mesh; Bio-Rad Laboratories, Ltd., St. Albans, England) and assayed for hydroxyproline by the method of Bergman and Loxley (1963).
D. Radioisotopic methods

In experiments dealing with changes in the rates of incorporation of \(^{14}\)C-uridine, -proline or \(^{35}\)S-methionine, the diaphragms of trichinosed and control mice were removed, washed briefly in KRT, and placed in 50 ml Erlenmeyer flasks. These flasks contained the appropriate label (1.25 \(\mu\)M \(^{14}\)C-uridine, sp.act. = 0.8 \(\mu\)Ci/\(\mu\)M; 1.25 \(\mu\)M \(^{14}\)C-proline, sp.act. = 1.6 \(\mu\)Ci/\(\mu\)M; or 2 \(\mu\)M \(^{35}\)S-methionine, sp.act. = 5 \(\mu\)Ci/\(\mu\)M) and 10 mM glucose in 25 ml of KRT. Incubations were carried out for 1 hr, at 37°C with oxygen bubbled directly into the medium. After 1 hr the diaphragms were removed from the incubation medium, rinsed twice in cold KRT, blotted on filter paper, placed in dry test tubes immersed in a dry ice-acetone bath and treated as outlined above for the extraction of total protein and total RNA.

In experiments dealing with changes in the rates of incorporation of \(^{3}\)H-thymidine, -uridine, -tryptophan, -glycine or -alanine by trichinealla infected and control diaphragm muscle, mice were sacrificed 24 hr after intraperitoneal injection of the appropriate label (15 \(\mu\)Ci of \(^{3}\)H-thymidine, sp.act. = 23 \(\mu\)Ci/mM; 15 \(\mu\)Ci of \(^{3}\)H-uridine, sp.act. = 6.4 \(\mu\)Ci/mM; 5 \(\mu\)Ci of \(^{3}\)H-tryptophan, -glycine or -alanine, sp.act. = 1 \(\mu\)Ci/mM; 2.2 \(\mu\)Ci/mM; and 2.6 \(\mu\)Ci/mM, respectively) and their diaphragms were treated as above.

In studies on the incorporation of \(^{14}\)C-uridine, -proline and \(^{35}\)S-methionine into trichinosed and control mouse diaphragm, 2-0.5 ml aliquots of the RNA fraction of muscle (uridine study) or the protein fraction (proline and methionine study) were dried on planchets and
radioactivity determined on a gas-flow counter. In experiments in which the incorporation of $^3$H-thymidine, $^3$H-uridine, $^3$H-tryptophan, $^3$H-glycine, and $^3$H-alanine by infected and uninfected mouse muscle and by larvae (thymidine study) was determined, 0.5 ml aliquots were placed in 10 ml of Beckman scintillation fluid and the radioactivity was determined in a Packard Tri-Carb scintillation spectrometer.

In the experiment involving determination of $^3$H-thymidine incorporation by larvae, a control was run for the possible uptake of label by the larvae during the 2-hr period of pepsin-HCl digestion. Trichinosed mice which had not been given an i.p. injection of label were digested along with uninfected mice each of which had been given an i.p. injection of 15 $\mu$Ci $^3$H-thymidine and the larvae were treated in the same manner as larvae which had undergone in vivo exposure to label.

In studies on the incorporation of methionine and proline by trichinosed and control diaphragms, 2 ml aliquots from the protein fraction from each diaphragm were hydrolyzed in 6 N HCl at 125$^\circ$C for 1 1/2 hr in sealed ampoules, evaporated to dryness, desalted on ion retardation resin, again evaporated to dryness, dissolved in 0.1 N HCl and the disposition of the radioactivity was determined on a Technicon amino acid analyzer and a Packard Tri-Carb analyzer. Samples submitted to the above procedure in the proline and in the methionine study were taken from the muscle protein fraction of mice sacrificed on days 12, 21, and 28 PI.

$^3$H-alanine was obtained from ICN (Irvine, Calif.). All other radioisotopes used were purchased from Amersham/Searle Corp., Houston, Texas. Other chemicals used were of reagent grade.
E. Statistical methods

Student's "t" test was used to evaluate the significance of differences.
III

RESULTS

A. Work preliminary to biochemical investigation

Figure 1 shows the number of migratory larvae obtained from the combined peritoneal washings and blood from mice (infected with 500 adults) every day between days 5 and 17 PI and on days 20 and 25 PI. The greatest number of migratory larvae was found between days 9 and 14 PI. Before and after this period few larvae were found in the peritoneal washings and blood of infected mice.

The anthelminthic methyridine was used to chemically abbreviate the stay of adult trichinella (Denham, 1965) in the intestines of experimental mice used in biochemical studies. This was done to halt production of migratory larvae on a given day, thereby limiting the age disparity between muscle larvae.

An experiment was conducted to insure that methyridine would eliminate all of the adults from the intestines of experimental mice, and to determine how long it would take for the drug to act on the worms. Ten mice were infected with 440 larvae each, and divided into 2 groups. Group I mice were given 500 mg/kilo of methyridine on day 12 PI and group II animals were given no anthelminthic. On day 10 PI the intestines of 2 mice from each group were removed and the adults present were counted. This was repeated for 3 mice from each group on day 13 PI. The results of this experiment are shown in Table I and reveal that methyridine is effective in eliminating all adult worms from the intestines of animals within 24 hr.
Figure 1. The number of trichinella migratory larvae obtained from the combined peritoneal washings and blood of mice.
Table I. The number of adult *trichinella* recovered from the intestines of group I and group II mice before group I mice received an anthelmintic (day 10 PI) and after methyridine was given on day 12 PI.
<table>
<thead>
<tr>
<th></th>
<th>Day 10 PI (total # of adults recovered)</th>
<th>Day 13 PI (total # of adults recovered)</th>
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<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mouse 1)</td>
<td>264</td>
<td>mouse 1)</td>
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<tr>
<td>mouse 2)</td>
<td>300</td>
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<td>mouse 3)</td>
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<tr>
<td><strong>Group II</strong></td>
<td></td>
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<tr>
<td>mouse 1)</td>
<td>308</td>
<td>mouse 1)</td>
</tr>
<tr>
<td>mouse 2)</td>
<td>320</td>
<td>mouse 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mouse 3)</td>
</tr>
</tbody>
</table>
B. Changes in the RNA per unit protein of trichinosed muscle

Changes in the RNA per unit protein of the diaphragm muscles of trichinosed and uninfected mice were compared on days 10 PI and every 5 days between day 17 and 72 PI. Each point in both the upper (infected) and lower (uninfected) curves of Figure 2 is the average of samples from the diaphragms of 3 mice. Methyridine was given on day 12 PI against an infective dose of 600 larvae/mouse. In Figure 2 all points (except day 10 PI) show the RNA content of infected diaphragms to differ significantly from that of uninfected controls.

The RNA per unit protein of diaphragms of mice infected with varying numbers of larvae was compared with that of controls (Fig. 3). Group I mice were given an infective dose of 1000 larvae; group II mice were infected with 500 larvae; group III animals were given 200 larvae; and group IV animals were given 50 larvae. The diaphragms of 10 mice from group I were shown to contain 32 larvae per mg wet weight of tissue (S.E.=2.27); those from group II mice contained 24.7 larvae per mg wet weight of tissue (S.E.=2.02); those from group III mice had 20.7 larvae per mg wet weight of tissue (S.E.=1.93); and muscle from group IV mice showed 17.1 larvae per mg wet weight of tissue (S.E.=1.66). Figure 3 compares the changes in RNA per unit protein of the 4 groups of trichinella-infected mice and that of control animals on every 5th day, beginning with day 5 PI (group II muscle RNA was that of mice killed in a separate experiment on every 4th day between days 4 and 32 PI, and on day 38 PI). The RNA per unit protein of the diaphragms of group I and group II mice was significantly different from that of controls between days 15 and 40 PI;
Figure 2. Changes in RNA per unit protein of trichinosed (■) and uninfected (●) mouse diaphragms. Standard errors are shown for those points which differed significantly ($P < 0.05$).
Figure 3. Changes in RNA per unit protein of uninfected mouse diaphragms (▲) and those of the diaphragms of mice infected with 1000 trichinella larvae (○), 500 larvae (□), 200 larvae (■) and 50 larvae (●).
the RNA per unit protein of diaphragms from mice infected with 200 larvae (group III) significantly differed from that of uninfected muscle between days 15 and 35 PI; while the RNA per unit protein of mouse muscle from group IV animals differed from that of controls only on day 15, 25 and 35 PI.

When the RNA per unit protein of muscle from mice from the 4 infected groups of animals was compared it was found that group I animals differed from group II mice on days 15 through 40 PI; the RNA per unit protein of diaphragms from group II mice was significantly different from that of group III on days 15-40 PI; and that of group III animals differed significantly from group IV mice only on days 20 and 25 PI.

Methyridine was given on days 8, 9 or 11 PI to infected mice, and the changes in the total RNA of muscles from these 3 groups of experimental mice were compared with those of controls (Fig. 4). The RNA per unit protein of muscle from group A mice (given methyridine on day 11 PI) differed significantly from that of controls on days 14 through 39 PI; that of group B mice (given methyridine on day 9 PI) differed from that of uninfected animals on days 19 to 39 PI; and that of group C mice (given methyridine on day 8 PI) was significantly different from that of controls between days 24 and 39 PI. A comparison of the changes in RNA per unit protein of the 3 groups of experimental mice revealed that that of group A mouse muscle differed significantly from that of group B from day 14 PI to the end of the experiment; and that of group B differed significantly from that of group C animals between days 19 and 39 PI.

The diaphragms of 10 mice from group A contained 30.8 larvae per mg wet weight of tissue (S.E.=3.83); those of group B had 24.5 larvae per mg wet weight of tissue (S.E.=1.67); and those from group C mice showed 19.7 larvae per mg wet weight of tissue (S.E.=1.57).
Figure 4. Changes in the RNA per unit protein of the diaphragms of uninfected mice (■) and trichinosed mice given methyridine on day 11 (○), 9 (●) and 8 (▲) PI.
The RNA per unit protein of trichinella-infected (inoculated with 660 larvae/mouse with methyridine given on day 12 PI) mouse muscle and uninfected mouse diaphragms was determined on day 10 PI and every 5th day between days 17 and 72 PI. When the changes in RNA of trichinosed and uninfected tissue in terms of protein (Fig. 5) were compared when total RNA was related to tissue wet weight (Fig. 6), and tissue dry weight (Fig. 7), it was found that there was a similar pattern and magnitude of change in the total RNA of infected muscle over that of controls.

Trichinosed muscle fibers are often infiltrated by inflammatory cells (i.e., eosinophils, neutrophils, histiocytes and macrophages). To determine if the elevated RNA content of infected muscle might be due, in part, to the presence of these cell types, cortisone acetate (a known suppressor of the cellular inflammatory response) was given to infected mice, and the changes in the RNA per unit protein of their diaphragms was compared with that in trichinella-infected mice not treated with cortisone and that in uninfected controls. The RNA per unit protein of the diaphragms of 3 cortisone-injected, infected mice, 3 trichinosed mice not given cortisone, 3 uninfected controls (given sterile saline injections), and 3 uninfected mice given cortisone was determined every 6th day between days 4 and 40 PI. The data, plotted in Figure 8, show that the changes in RNA per unit protein in cortisone-injected, infected mice follow a similar pattern and magnitude to that seen for infected mice not given cortisone. In addition, the RNA per unit protein of uninfected mouse muscle (given saline injections) is similar to that of uninfected mice given cortisone.
Figure 5. Changes in the total RNA in terms of milligrams protein of infected (●) versus uninfected diaphragm (■).
Standard errors are shown for all points which differed significantly. (P < 0.05).
Figure 6. Changes in the total RNA related to milligrams wet weight of trichinella-infected (●) versus control (■) diaphragms. Standard errors are shown for all points which differed significantly (P < 0.05).
Figure 7. Changes in the total RNA related to milligrams dry weight of trichinosed (●) versus uninfected (■) muscle. Standard errors are shown for all points differing significantly (P < 0.05).
Figure 8. The changes in RNA per unit protein are presented for trichinosed mice not given cortisone (○), trichinosed mice given cortisone (●), control mice given cortisone (■) and control mice not given cortisone (□). Both infected groups of mice showed muscle RNA per unit protein significantly different from that of controls after day 13 PI.
The RNA per unit protein of diaphragms from uninfected mice given methyridine was compared with that of muscle from uninfected mice not given methyridine every 10 days between days 0 and 40 postinjection. Figure 9 reveals that there was no significant difference between muscle RNA content of the 2 groups of mice during the period of observation.

C. Changes in RNA metabolism in trichinosed muscle

It seemed desirable to investigate in greater detail that portion of the infection during which the RNA per unit protein of trichinosed muscle underwent maximum change. Changes in the RNA per unit protein and rates of synthesis of RNA were investigated in trichinosed versus control diaphragm muscle every other day between days 4 and 24 PI and every 3rd day between days 24 and 39 PI (samples from day 12 PI were lost). Methyridine was administered to all infected mice and 3 uninfected control mice on day 11 PI. The rates of RNA synthesis in infected and uninfected mouse muscle were determined by measuring the incorporation of $^{14}$C-uridine into diaphragm RNA in vitro. The data are presented in Figure 10 and show that the rates of uridine incorporation by trichinella-infected muscle were greater than uninfected tissue on most days after day 8 PI.

Changes in the RNA per unit protein were also determined and the results corroborated the changes observed in the experiment shown in Figure 2. After day 10 PI, the RNA content of all infected diaphragms differed significantly from that of corresponding uninfected muscle (Fig. 11).
Figure 9. Changes in the RNA per unit protein of uninfected mice (●) given methyridine versus uninfected mice (□) not given methyridine. None of the points differed significantly.
Figure 10. **In vitro** incorporation of $^{14}$C-uridine into RNA in trichinosed and uninfected diaphragm muscles. Infected = ⭕ uninfected = ●. Standard errors are shown for significantly different points ($P < 0.05$).
Figure 11. Changes in RNA per unit protein in trichinosed versus uninfected diaphragms. Standard errors are shown for points which differed significantly ($P < 0.05$).

Infected = ■; uninfected = ●.
The data presented in Figures 10 and 11 were used to calculate specific activity (nanomoles uridine incorporated into RNA per hour per microgram total RNA). The data, presented in Figure 12, reveal a sharp increase in specific activity occurring between days 6 and 14 PI in infected muscle over that of uninfected, followed by a decline to specific activities in infected muscle which were lower than those observed for control tissue.

In order to determine the magnitude of the role played by the larval RNA and protein components in the observed elevation of infected muscle over that of uninfected, larvae were analyzed for RNA and protein content. It was found that the larvae contained similar quantities of RNA and protein on days 37, 42, 47 and 57 PI. Analysis of larvae showed an average RNA content of 0.0109 µg RNA/larva (S.E. = 0.00034) and an average protein content of 0.3346 µg protein/larva (S.E. = 0.017). The excystment of larvae from the diaphragms of mice used in the total RNA experiment shown in Figure 2 revealed an average of 38.7 larvae per mg wet weight of tissue (S.E. = 9.78). These data allowed the subtraction of larval RNA and protein from that of the infected muscle providing µg RNA/mg protein of trichinella-infected host tissue without the parasite component on days 32, 37, 42, 52, 57, 62, 67, and 72 PI. From Figure 13 it may be seen that, although the larvae account for a substantial amount of the host-parasite RNA, all of the adjusted values for infected diaphragms (except day 72 PI) differ significantly from those of uninfected controls on corresponding days.
Figure 12. Specific activity of diaphragm RNA after 60 min incorporation of $^{14}$C-uridine in vitro during a 39-day period of infection with T. spiralis. Standard errors are shown for all significantly different points (P < 0.05). Infected = ■; uninfected = ●.
Figure 13. Changes in the RNA per unit protein of trichinosed and uninfected diaphragm after correction of infected muscle RNA and protein for the parasite component. The parasite RNA and protein were subtracted from that of the host-parasite complex on days 32, 37, 42, 47, 52, 57, 62, 67 and 72 PI. Standard errors are shown for those points that differ significantly (P < 0.05). Infected diaphragms = ■; uninfected diaphragms = ●.
D. Changes in the total protein, total water, wet and dry weights of trichinosed muscle.

Changes in the wet and dry weights, and water content of trichinosed versus uninfected mouse diaphragm muscle was determined every 6th day between days 4 and 40 PI.

Figure 14 reveals that the wet weights of infected diaphragms began to rise above those of uninfected about day 28 PI and remained elevated throughout the remainder of the 40-day experimental period. The dry weights of infected diaphragm muscle rose and remained above those of controls between days 28 and 40 PI (Fig. 15).

The water content of infected diaphragms differed significantly from that of uninfected between days 10 and 40 PI (Fig. 16).

Changes in the protein/mg dry weight of trichinella-infected versus control diaphragm muscle was examined on day 10 PI and every 5th day between days 17 and 72 PI. As shown in Figure 17, the mg protein/mg dry weight of tissue was significantly less than that of controls on days 17 through 57 PI. Mice in this experiment were infected with 660 larvae and methyridine was given on day 12 PI.

E. Changes in RNA per unit protein of mouse cardiac muscle and the rates of synthesis of RNA and RNA per unit protein in trichinosed mouse liver

Although trichinella larvae undergo no growth or development in the liver or cardiac tissue of their hosts, they do pass through both the heart and liver on the way to the skeletal musculature. In addition, myocarditis commonly occurs in trichinosis. Studies were undertaken
Figure 14. Changes in the wet weights of diaphragms of infected (○) and uninfected mouse muscle (■). Standard errors are shown for those points which differ significantly (P < 0.05).
Figure 15. Changes in the dry weights of trichinella-infected (●) and uninfected mouse muscle (■). Standard errors are shown for all points which differed significantly (P < 0.05).
Figure 16. Changes in the per cent water of trichinella-infected (●) and control (■) diaphragms. Standard errors are shown for all significantly different points (P < 0.05).
Figure 17. Changes in the milligrams protein per milligram dry weight (per cent protein) of experimental (●) and uninfected diaphragm muscle (■). Standard errors are shown for all points which differ significantly (P < 0.05).
to determine any changes in the RNA per unit protein and rates of synthesis of RNA in the livers of infected mice, and changes in the RNA per unit protein of the cardiac muscles of trichinosed animals.

Changes in the RNA per unit protein of, and rates of incorporation of uridine by infected versus uninfected mouse liver tissue were observed every 4th day between days 4 and 40 PI. There was no difference in the RNA per unit protein of infected and that of uninfected mouse liver during the duration of the study (Fig. 18). The rates of incorporation of uridine (Fig. 19) by liver from experimental mice was significantly different from that of controls on days 24 and 36 PI.

Changes in the RNA per unit protein of whole heart muscle of trichinella-infected versus uninfected mice was compared every 4th day between days 4 and 40 PI. The total RNA of infected mouse cardiac muscle did not differ from that of uninfected tissue during the experimental period (Fig. 20).

F. Changes in DNA metabolism of trichinosed mouse muscle.

The DNA per unit protein of trichinosed and uninfected muscle was determined on every other day between days 4 and 14 PI, on every 3rd day between days 14 and 39 PI and on day 40 PI. The DNA per unit protein of trichinosed diaphragm rose above that of controls about day 13 PI (Fig. 21) and continued to rise to about day 23 PI, at which time it was about 3 times that of uninfected muscle. Thereafter, the trichinosed diaphragm DNA per unit protein remained elevated above that of controls.
Figure 18. Changes in the RNA per unit protein of trichinosed (●) versus uninfected (■) mouse liver tissue. No points in the two lines are significantly different.
Figure 19. Changes in the rates of incorporation of $^3$H-uridine by trichinosed and uninfected mouse liver tissue. $V =$ nanomoles uridine incorporated into RNA per gram protein per hour. Points in the two lines on days 24 and 36 PI are significantly different ($P < 0.05$).
Figure 20. Changes in the RNA per unit protein of heart muscle from trichinella-infected (●) and uninfected (■) mice. No points in the two lines differ significantly from each other.
Figure 21. Changes in the DNA per unit protein of trichinosed versus control diaphragm muscle. Standard errors are shown for all significantly different points (P < 0.05).
On the above mentioned days the rate of incorporation of labeled thymidine (Fig. 22) by trichinella-infected and uninfected mouse diaphragm muscle was investigated, and that of trichinosed muscle began to rise above that of control diaphragm around day 10 PI and continued to increase to a level approximately 13 times that of control muscle on day 17 PI. The rate of incorporation of thymidine by the diaphragms of experimental mice remained above that observed in uninfected diaphragms throughout the 40-day period.

The RNA per unit protein of infected and uninfected diaphragm muscle was determined from the same experimental mice used in the two experiments just described. It was found (Fig. 23) that the RNA per unit protein of infected muscle was elevated above that of uninfected about day 13 PI and continued to rise to about day 23 PI at which time it was about 3 times that of control muscle. Thereafter RNA of infected diaphragm remained elevated above that of uninfected diaphragm.

Determination of the total protein, total RNA, μgms RNA/mg protein, and picomoles of thymidine incorporated into DNA per gram larval protein per hour were carried out on days 23, 25, 26, 28, 32, 35, and 38 PI (Table II). The data indicate a period of larval growth between days 23 and 28 PI by the observed increase in the total protein of worms. Micrograms RNA/mg protein of larvae, and the rate of incorporation of thymidine by larvae decreased between days 23 and 32 PI.

Larvae incorporated larger amounts of thymidine into DNA per gram protein per hour than trichinosed muscle on day 23 PI (Table II); thereafter they incorporated lesser amounts than infected muscle. Table III presents the results of subtracting larval thymidine incorporation and
Figure 22. Changes in the rates of incorporation of $^{3}$H-thymidine by trichinella-infected and uninfected mouse diaphragm. $V =$ picomoles thymidine incorporated into DNA per gram protein per hour. Standard errors are shown for all significantly different points ($P < 0.05$).
Figure 23. Changes in RNA per unit protein of trichinella-infected and control mouse diaphragm. Significantly different points are shown by standard errors (P < 0.05).
Table II. Total μg protein, total μg RNA, μg RNA per mg protein, and picomoles thymidine incorporated into DNA per g protein per hour for larvae on days 23, 25, 26, 28, 32, 35 and 38 are presented. Standard errors for all data are listed in adjacent columns.
<table>
<thead>
<tr>
<th>DAY PI</th>
<th>Total Protein Per Larva (ugms)</th>
<th>S.E.</th>
<th>Total RNA per Larva (ugms)</th>
<th>S.E.</th>
<th>Micrograms RNA per Milligram Protein</th>
<th>S.E.</th>
<th>Picomoles Per Gram Protein Per Hour</th>
<th>S.E.</th>
</tr>
</thead>
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<tr>
<td>23</td>
<td>0.107</td>
<td>.004</td>
<td>0.027</td>
<td>.003</td>
<td>257.7</td>
<td>42.18</td>
<td>15.72</td>
<td>1.21</td>
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<td>25</td>
<td>0.143</td>
<td>.015</td>
<td>0.034</td>
<td>.002</td>
<td>246.9</td>
<td>33.89</td>
<td>9.02</td>
<td>1.07</td>
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<tr>
<td>26</td>
<td>0.213</td>
<td>.016</td>
<td>0.033</td>
<td>.006</td>
<td>153.5</td>
<td>17.99</td>
<td>5.34</td>
<td>0.27</td>
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<tr>
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<td>0.027</td>
<td>.004</td>
<td>102.5</td>
<td>11.28</td>
<td>4.03</td>
<td>0.34</td>
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<tr>
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<td>.027</td>
<td>0.012</td>
<td>.005</td>
<td>75.1</td>
<td>12.12</td>
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<td>.005</td>
<td>0.017</td>
<td>.001</td>
<td>59.1</td>
<td>6.99</td>
<td>2.25</td>
<td>0.25</td>
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<tr>
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<td>.041</td>
<td>0.018</td>
<td>.001</td>
<td>56.2</td>
<td>4.63</td>
<td>2.24</td>
<td>0.38</td>
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Table III. The incorporation of thymidine (in picomoles) into DNA per g protein per hr by trichinosed diaphragms (Column A) is compared with that of trichinosed muscle less the larval protein and thymidine incorporation components (Column B).
<table>
<thead>
<tr>
<th>Day P.i.</th>
<th>A. Infected Diaphragm Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>10.99</td>
</tr>
<tr>
<td>26</td>
<td>9.35</td>
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<tr>
<td>29</td>
<td>6.83</td>
</tr>
<tr>
<td>32</td>
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<tr>
<td>38</td>
<td>5.48</td>
</tr>
<tr>
<td>40</td>
<td>3.82</td>
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</table>

<table>
<thead>
<tr>
<th>Column B</th>
<th>Less Incorp. By Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.79</td>
</tr>
<tr>
<td></td>
<td>9.36</td>
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<tr>
<td></td>
<td>7.00</td>
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<td></td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td>4.06</td>
</tr>
</tbody>
</table>
protein from that of host muscle on days 23, 26, 29, 32, 35, 38, and 40 PI. Control larvae not exposed in vivo to $^3$H-thymidine did not incorporate amounts of label greater than background during exposure to label in the excystment process in pepsin-HCl for 2 hr. In addition it was found that after day 30 PI the DNA per unit protein of larvae was about 3.41 μg DNA/mg protein (S.E. = 0.123).

G. Some aspects of cyst synthesis and changes in protein synthesis in trichinosed muscle.

1. Cyst synthesis

In this experiment changes in the rates of incorporation of $^{14}$C-proline, the total hydroxyproline (as a measure of total collagen), and the total RNA in trichinella-infected versus uninfected mouse diaphragms over a 39-day period of study were determined. Analyses were carried out every 3rd day from day 4 to day 21 PI (samples from day 19 PI were lost), and every other day between days 21 and 39 PI.

In Figure 24 the rate of incorporation of proline by trichinosed diaphragm muscle exceeded that of uninfected by day 13 PI and remained elevated above that of controls throughout the period of study.

The hydroxyproline per unit protein of infected diaphragms (Fig. 25) was higher than that of uninfected by day 26 PI and remained above that of controls for the duration of the experiment.

Amino acid analysis of hydrolyzed protein samples from the diaphragms of trichinella-infected and uninfected mice killed 24 hr after intraperitoneal injection of $^{14}$C-proline on days 12, 21 and 28 PI,
Figure 24. **In vitro** incorporation of $^{14}\text{C}$-proline into protein of trichinosed and control diaphragms. $V =$ nanomoles proline incorporated into protein per gram protein per hour. Infected mice = ⚫; uninfected mice = ■. Standard errors are shown for all significantly different points ($P < 0.05$).
Figure 25. Changes in the collagen content (μg hydroxyproline per mg protein) of trichinosed (◊) and uninfected (■) diaphragms. Standard errors are shown for those points which differed significantly (P < 0.05).
revealed labeled hydroxyproline only in samples from the infected diaphragms on day 28 PI. In addition, it was found that the total proline of infected muscle rose above that of uninfected about day 21 PI, and on day 28 PI was 3 times the level found in controls.

In Figure 26 the RNA per unit protein of infected diaphragms can be seen to rise above that of controls about day 13 PI, to reach a peak at about 3 times that of uninfected around day 25 PI, and then drop to a level above that of controls. The RNA per unit protein of infected muscle remained higher than that of uninfected up to the time the experiment was terminated.

2. Protein synthesis

Changes in the rates of incorporation of $^{35}$S-methionine by the diaphragms of trichinella-infected versus uninfected animals were determined on days 6 and 10 PI, then on every 3rd day between days 10 and 34 PI, and on day 40 PI. Figure 27 reveals that trichinosed diaphragm muscle began to incorporate greater amounts of methionine than uninfected about day 13 PI. Methionine incorporation remained higher than that of controls throughout the 40-day period of study.

Amino acid analysis of hydrolyzed protein samples from infected and uninfected muscle on days 16, 28 and 40 PI showed that the $^{35}$S label occurred only in methionine sulfone (an hydrolysis product of methionine). On all days of investigation undetectable amounts of label were present in uninfected muscle. Large amounts of labeled methionine sulfone were found on all days in samples from infected diaphragms.
Figure 26. Changes in the RNA per unit protein of trichinella infected versus control diaphragm muscle. Standard errors are shown for all significantly different points ($P < 0.05$).
Figure 27. The in vitro incorporation of $^{35}$S-methionine into protein by trichinosed (●) and control (■) diaphragms. V = nanomoles methionine incorporated into protein per gram protein per hour. Standard errors are shown for all points differing significantly ($P < 0.05$).
In a separate study changes in the rates of incorporation of
$^3$H-tryptophan, -alanine, and -glycine by infected and uninfected muscle
were determined. Mice were infected with 1000 (tryptophan study) or
500 (glycine and alanine studies) larvae and given methyridine on the
same day as above.

The incorporation of tryptophan by trichinella-infected and uninfect-
ted diaphragm muscle was determined on every 3rd day between days 4 and
40 PI and every 5th day between days 40 and 55 PI. Infected muscle
began to incorporate greater amounts of tryptophan than control
diaphragms about day 12 PI and continued to do so out to day 45 PI
(Fig. 28). The pattern and magnitude of change in the RNA per unit
protein of infected muscle (Fig. 29) was similar to that seen in past
studies.

Glycine incorporation by trichinosed and control diaphragm muscle
was examined on every 4th day between days 4 and 32 PI and on day 38 PI.
The protein of infected diaphragms showed incorporation of greater
amounts of glycine than uninfected muscle after day 12 PI (Fig. 30).
The RNA per unit protein from the diaphragms of these same mice revealed
a similar pattern of change seen in past studies (Fig. 31).

The incorporation of alanine by experimental and control diaphragms
was ascertained on every 4th day between days 6 and 34 PI and day 40 PI.
Alanine was incorporated in greater amounts by trichinosed muscle than
uninfected diaphragms after day 12 PI (Fig. 32). The changes in the
total RNA of the diaphragms of these same mice was similar to that seen
in Figure 32 and in previous experiments (Fig. 33).
Figure 28. The in vivo incorporation of $^{3}H$-tryptophan into protein of trichinella-infected (○) and uninfected (■) mouse diaphragms. $V =$ picomoles tryptophan incorporated into protein per g protein per hr. Standard errors are shown for all significantly different points ($P < 0.05$).
Figure 29. Changes in RNA per unit protein of trichinosed (○) versus uninfected (■) diaphragm muscle. Standard errors are shown for all significantly different points (P < 0.05).
Figure 30. The in vivo incorporation of $^3$H-glycine into protein of trichinosed (●) and control (■) diaphragm muscle. $V =$ picomoles glycine incorporated into protein per g protein per hr. Standard errors are shown for all points which differed significantly ($P < 0.05$).
Figure 31. Changes in RNA per unit protein of trichinella-infected (○) versus uninfected (■) mouse diaphragm. Standard errors are shown for all significantly different points.
Figure 32. The in vivo incorporation of $^3$H-alanine into trichinosed (○) and uninfected (■) diaphragm muscle. V = picomoles alanine incorporated into protein per g protein per hr. All significantly different points are shown by standard errors (P < 0.05).
Figure 33. Changes in RNA per unit protein of trichinosed (*) versus control (■) diaphragms. Standard errors are shown for all significantly different points (P < 0.05).
Determination of the final disposition of the $^3$H label and the role played by the larvae in the incorporation of the 3 above amino acids (glycine, alanine and tryptophan) requires further study.
DISCUSSION AND CONCLUSIONS

Shortly after infection by the migratory larvae of *Trichinella spiralis* host muscle fibers undergo several marked morphological changes including: a) an increase in the sarcoplasmic matrix; b) an increase in the size and number of nuclei with an accompanying increase in the size of nucleoli; c) loss of the structural integrity of the sarcomeres, with subsequent degeneration and loss of myofilamentous elements; d) positional changes of the triads followed by intense proliferation of elements of the T system and an increase in the number of free-ribosomes and ribosome-studded membranes of the endoplasmic reticulum; c) an increase in the size and number of mitochondria; and f) an increase in the number of Golgi complexes (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968; Despommier, personal communication). Zarzycki (1963) demonstrated a marked increase in the succinic acid dehydrogenase activity in infected muscle, and Maier and Zaiman (1966) showed, by histochemical methods, an increase in the RNA and in the activity of the Golgi of infected muscle. Gabryel and Gustowska (1967) were able to correlate an increase in RNA with the augmented basophilia of infected muscle, and autoradiographically demonstrated the incorporation of $^3$H-thymidine by infected muscle nuclei. It might be anticipated that morphological and chemical changes of this magnitude would be accompanied by profound alterations in trichinosed muscle biochemistry.

*Trichinella* migratory larvae invade host muscle fibers between days 6 and 24 PI (Shanta and Meerovitch, 1967). This continuing recruitment
over at least 3 weeks would be expected to confound study of the order of biochemical events occurring in infected muscle.

In agreement with the work of Shanta and Meerovitch (1967), migratory larvae were found in greatest numbers in the blood and peritoneal washings of mice harboring adult worms between days 9 and 14 PI. Infection of experimental mice with between 500 and 1000 larvae, followed by injection of animals with 500 mg/kilo (Denham, 1965) of the anthelminthic methyridine on day 11 or 12 PI resulted in heavy infection of mouse muscle, and restriction of the age difference between larvae to 5 or 6 days, respectively.

It was reasoned that the chemical pathology in trichinosis must involve highly modified patterns of protein synthesis and that changes in RNA metabolism should occur.

The RNA content of trichinella-infected diaphragms (Fig. 2) rose above that of uninfected about day 13 PI, and continued to rise to about day 27 PI at which time it was about 3 times that of uninfected diaphragms. Thereafter there was some decrease in the RNA per unit protein of trichinosed muscle, although by day 72 PI the RNA content of infected muscle remained significantly higher than that of uninfected controls.

Changes in RNA per unit protein of the diaphragms of mice infected with varying numbers of trichinella larvae were investigated (Fig. 3), and the results indicated that a large number of infected fibers was required for detection of the above mentioned changes in RNA per unit protein in trichinosed diaphragm muscle. It has been demonstrated that
an increase in the number of muscle larvae, over that found in the
diaphragms of animals from group I (Fig. 3), did not produce an earlier
increase or greater magnitude of change in infected diaphragm RNA per
unit protein.

Khamboonruang (1971) demonstrated that the number of larvae produced
by an individual adult female trichinella was inversely proportional to
the number of worms in the inoculum. His conclusions were supported by
the present biochemical evidence when the micrograms RNA per unit
protein of diaphragm muscle from the 4 infected groups of mice were
compared (Fig. 3) on day 25 PI. The RNA per unit protein of group II
mouse diaphragm was approximately equal to 73% of the difference between
that of group I and that of controls; group III mouse diaphragm RNA per
unit protein was about 35% of the difference between that of group II
and that of control animals; and the RNA per unit protein of group IV
mouse diaphragm was equal to 50% of the difference between that of group
III muscle and that of uninfected animals.

From work in this laboratory (Fig. 1) and from the findings of Shanta
and Meerovitch (1967) it is known that the greatest number of migratory
larvae are found in the blood and peritoneal washings of infected
animals between days 9 and 14 PI. The lives of adult trichinella in the
intestines of host animals was abbreviated with the anthelminthic
methyridine on days 11 (Group A), 9 (Group B), and 8 PI (Group C) (Fig.
4). There was an increase in the number of muscle larvae in the diaphragms
of experimental animals as methyridine was given later during the infec-
tion with adults (days 11, 9, or 8 PI). On day 24 PI the RNA per unit
protein of diaphragm muscle from group B mice was approximately equal to
40% of the difference between that of group A and that of controls; and the RNA per unit protein of diaphragms from group C mice was about 43% of the difference between that of group B diaphragm and that of control diaphragm muscle. These results, and those shown in Figure 3, seem to indicate that reduction of larvae produced by the administration of methyridine before day 11 PI, or the infection of experimental animals with less than 500 larvae (methyridine given on day 11 PI), results in a decrease in the magnitude of the biochemical alterations observed in infected host diaphragm muscle.

To determine that the observed changes in the RNA per unit protein of muscle infected with trichinella are not due to a decrease in the total protein, total RNA was examined in terms of diaphragm wet weight (Fig. 6) and diaphragm dry weight (Fig. 7). The results presented in Figures 14 and 15 reveal that the wet and dry weights, respectively, of infected diaphragms remained equal to those of controls to day 28 PI, at which time the weights of trichinosed diaphragms rose and remained above those of uninfected mouse diaphragm muscle to day 40 PI. It was found that the magnitude and pattern of change of diaphragm total RNA related to diaphragm tissue wet weight (Fig. 6) and diaphragm dry weight (Fig. 7) was similar to that obtained when total RNA was examined in terms of diaphragm protein (Fig. 5).

Cells characteristic of inflammatory response are sometimes found associated with infected fibers. In order to determine if the RNA component of the histiocytes, macrophages and eosinophils associated with some of the infected fibers of trichinosed muscle contributed to the
enhanced total RNA of infected diaphragms, cortisone was given to one
group of infected animals and the changes in the RNA per unit protein
was compared with that of diaphragms from trichinosed mice not given
cortisone. In Figure 8 the RNA per unit protein of muscle from the 2
groups of infected mice are seen not to differ from each other but to
demonstrate the same magnitude of variation from that of control
diaphragms as seen in past experiments (e.g., Figs. 2, 5, 6 and 7).

To assure that methyridine played no role in the enhanced RNA of
infected muscle, the RNA per unit protein of 2 groups of uninfected
mice were compared (one group was given methyridine and the other was
given no anthelminthic). It was revealed that methyridine does not
effect the RNA per unit protein of mouse diaphragm muscle over a period
of 40 days (Fig. 9).

In the experiment on the incorporation of $^{14}$C-uridine into RNA
during a 60-min period in vitro, the incorporation by uninfected
diaphragms remained relatively stable. A slight tendency to decline
may be attributed to a declining rate of mouse growth between 5 weeks
(day 0) and 10 1/2 weeks (day 40) in mouse age. On the other hand, by
day 10 PI, trichinosed diaphragms showed a clearly elevated incorpora-
tion of $^{14}$C-uridine.

An investigation of changes in RNA per unit protein of infected
versus control diaphragm muscle in greater chronological detail (Fig.
11) revealed a similar magnitude and pattern of change as that observed
in Figure 2.

Although there was no increase in the RNA per unit protein on day
10 PI, the increase in uridine incorporation by infected muscle was
highly significant and, when calculated in terms of specific activity of RNA (nanomoles uridine incorporated into RNA per μg RNA per hr), maximum specific activity was obtained on day 10 PI (Fig. 12). This may imply that considerable RNA turnover occurred in this early period between days 8 and 14 PI, a period just preceding a dramatic rise in the rate of incorporation of amino acids by infected muscle (Figs. 24, 27, 28, 30 and 32). Since RNA accumulation continues to increase to about day 24 PI (Fig. 11), and since the elevated uridine incorporation is more or less constant after day 16 PI (Fig. 10), it follows that specific activity would be expected to decline. After day 10 PI the specific activity of RNA indeed does decline and is actually significantly less than that in uninfected muscle by day 18 PI. Thus, breakdown in RNA sharply declines after day 10 PI and this is accompanied by a marked increase in RNA synthesis.

It had been previously reported that there is an apparent increase in the RNA of trichinella after it enters the muscle fiber (Zarzycki, 1962) and an apparent increase in the histochemically demonstrable RNA of the infected fiber itself (Maier and Zaiman, 1956; Gabreyel and Gustowska, 1967). It therefore seemed desirable to determine the extent to which the enhanced RNA per unit protein of trichinosed diaphragms reflected the RNA content of trichinella and that of the infected muscle fibers. The isolation of larvae on several days after day 32 PI showed the RNA content of trichinella to be relatively stable. Clearly, both host and parasite make substantial contributions to the elevated RNA of infected muscle during this period. Unfortunately, it has not proven
feasible thus far to examine the parasite and the host as sources of elevated RNA in infected muscle during the earlier stages of infection. There are serious technical difficulties in separating them for chemical analysis. The trichinella RNA per unit protein accounted for about 30% of the difference between trichinella-infected and control diaphragm RNA per unit protein (Fig. 13).

Other changes in the chemical composition of trichinosed diaphragms occur during the first 40 days of infection. An increase in the percent water in trichinosed over that of control diaphragm was observed between days 10 and 40 PI (Fig. 16); and an increase in the wet (Fig. 14) and dry (Fig. 15) weights of infected diaphragms was found to occur between days 28 and 40 PI.

The data of Figure 17 demonstrated an apparent decrease in the percent protein of infected diaphragm dry weight below that of control muscle shortly after day 10 PI. The magnitude of this difference remained at about 4% (S.E. = 0.7) until day 60 PI, after which time the percent protein of infected diaphragms and that of uninfected muscle were equal. In light of the reported breakdown of myofilamentous elements (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968; Despommier, personal communication) in muscle fibers infected with trichinella, this decrease in infected muscle percent protein is not surprising. It is suggested that this is a reflection of the process of dedifferentiation exhibited by trichinosed muscle fibers, and that this is followed by a period of redifferentiation of the infected fiber into a functionally and structurally new cell-type. It is further suggested
that this new cell-type is metabolically and morphologically distinct from the original muscle fiber and may require new structural and enzymatic proteins. The enhanced incorporation of amino acids by infected over control muscle (Figs. 24, 27, 28, 30 and 32) may indicate synthetic activity directed towards the fulfillment of these new protein requirements, as well as the production of cyst collagen.

When RNA per unit protein and rates of incorporation of $^{14}$C-uridine by liver tissue from infected versus uninfected mice was investigated, no differences between the RNA per unit protein of infected and uninfected mouse liver tissue were demonstrated (Fig. 18). Although the rates of incorporation of uridine by liver tissue from infected animals were above those of uninfected on days 24 and 36 PI (Fig. 19), it is felt that these differences may not be significant biochemical changes associated with trichinosis.

Although it is generally accepted that trichinella larvae are incapable of completing their development in cardiac muscle, several workers have found unencysted larvae within myocardial fibers (see review, Ribas-Mujal, 1971). In addition, an increase in the number and size of nuclei, and intense fatty degeneration of infected myocardial fibers has been observed (Gould, 1945). When possible changes in RNA per unit protein of heart muscle during infection with trichinella were investigated, there were no differences in cardiac muscle from infected and uninfected animals.

Although Fasske and Themann (1961) and Ribas-Mujal and Rivera-Pomar (1968) reported an increase in the number and size of myofiber nuclei
during the early stages of infection with trichinella, Despommier (personal communication) has found only an increase in the size of existing nuclei. None of these workers have reported nuclear divisions. The increase in the incorporation rate of thymidine by infected muscle (Fig. 22) and subsequent rise in the total DNA (Fig. 21) of trichinosed diaphragms over that of controls occur on days 10 and 13 PI, respectively, whereas the reported increase in the size of infected muscle nuclei has occurred by day 8 PI. However, as will be seen later, adjustment of the time base of the electron microscopic and biochemical studies will reveal a close chronological correlation between these findings and an increase in the size and number of infected-fiber mitochondria and nuclei (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomer, 1968; Despommier, personal communication). That mitochondria contain DNA is well established, and this marked increase in the size and number of infected-muscle mitochondria may contribute to the observed alterations in DNA metabolism in trichinosed muscle.

Despommier (personal communication) controls the age of larvae by injecting migratory forms, which have been born within the same 20 hr period, directly into mouse muscle. Using this technique he has been able to determine that a period of rapid growth of the larvae occurs between days 10 and 15 PI (days 15 to 26 PI in the present study). This larval growth is demonstrated by an increase in the total protein of larvae between days 23 and 28 PI (Table II). In Table II a decrease (between days 23 and 28 PI) and stabilization (day 28 to 38 PI) of the larval total RNA, total DNA (3.41 µg DNA/mg protein) and incorporation of thymidine imply a cessation of growth and the maintenance of a lowered metabolic state by the mature, encysted worm.
Adjustment of the RNA per unit protein of infected muscle (Fig. 13) and the rate of incorporation of thymidine by trichinosed diaphragm (Table III) for the larval component implies that mouse muscle infected with trichinella is metabolically more active than uninfected muscle.

In Figure 24 the rate of incorporation of proline by infected diaphragms rose above that of uninfected immediately following the period of rapid RNA turnover shown for trichinosed muscle in Figure 12. In Figure 27 the incorporation of methionine (an amino acid known to be present in very small quantities in collagen) by infected diaphragms showed a pattern of change very similar to that observed for the incorporation of proline. From this, and because there was very little proline hydroxylated to hydroxyproline it is suggested that the increased incorporation of proline by infected over uninfected muscle is primarily a reflection of alterations in the total protein synthetic activity of infected diaphragms rather than a simple indication of changes due to the synthesis of cyst proteins alone.

Although the data from Figure 25 give little information about the initiation of encystment, they do imply that most muscle larvae have encysted by day 27 PI. Since, under the conditions of this experiment, the last larvae to invade muscle fibers enter on day 11 PI it is suggested that the encystment process is completed about 16 days after entry into the fiber. The first larvae to enter host muscle fibers do so on day 6 PI (Phillipson and Kershaw, 1961) and would therefore be encysted by day 22 PI. That this may indeed be the case is implied by the work of Shanta and Meervitch (1967) in which they found that larvae began to form cysts on day 21 PI.
Hanks and Stoner (1958) found that, in vivo, infected muscle incorporated greater amounts of $^{14}C$-tryptophan than uninfected, while Stoner and Hanks (1955) showed trichinosed muscle to incorporate less labeled glycine and alanine in vivo than did uninfected. However, these workers infected their experimental animals with a small number of larvae (450 larvae/mouse); did nothing to control the age difference between larvae; sacrificed mice on days 14 and 56 PI only; did not determine the disposition of label in any of the 3 amino acids studied; and determined the radioactivity of some of the most lightly infected muscles in the mouse body (they used hind leg muscles only).

In the present study incorporation of these 3 amino acids is shown in Figures 28, 30 and 32, respectively. These data indicate that, with all 3 amino acids, greater amounts of label are incorporated in vitro into the protein fractions of infected than uninfected diaphragm muscle. Determination of the disposition of label and the role played by the larvae in incorporation of tryptophan, glycine and alanine is presently underway.

In Figures 29, 31 and 33 are shown changes in the RNA per unit protein of the same mouse diaphragms used in the determinations of incorporation of tryptophan, glycine, and alanine, respectively. They all display a pattern and magnitude of change seen in the previous experiments.

Fasske and Themann (1961), and Ribas-Mujal and Rivera-Pomar (1968) have conducted electron microscopic studies of trichinosed muscle fibers.
Although these authors have provided important information on the morphological changes occurring in trichinella-infected muscle, they failed to determine the chronological sequence of ultrastructural alterations. However, Despommier (personal communication) has devised a method for direct injection of trichinella migratory larvae of approximately the same age (all larvae were born within a 20-hr period) into mouse skeletal muscle. This procedure has enabled delineation of the exact chronology of ultrastructural changes in trichinella-infected muscle. In order to chronologically correlate the biochemical changes shown in the present study with the morphological alterations shown by Despommier, the time scale upon which the biochemical studies were based must be adjusted. From work in this laboratory, and from studies by Shanta and Meerovitch and others, it has been shown that very small numbers of trichinella larvae enter host muscle fibers between days 6 and 8 PI. Furthermore, when methyridine was given on days 8 or 9 PI (Fig. 4) the first day upon which infected muscle total RNA became significantly different from that of uninfected moved from day 14 PI (methyridine given on day 11 PI) to day 19 PI (methyridine given on day 9 PI) or day 24 PI (methyridine given on day 8 PI). These findings imply that the initial rise in the total RNA of trichinosed over that of control diaphragm is due to the larvae entering on day 10 PI (about the same numbers of larvae enter muscle fibers on day 10 and 11 PI). If larvae entering muscle fibers on day 6 PI were equal in number to those entering on day 10 PI, the initial rise in total DNA and RNA of, and incorporation of thymidine, uridine and amino acids by infected over control diaphragm
muscle would occur the same number of days after day 6 PI that they have been shown to occur after day 10 PI. The day upon which larvae were injected into mouse muscle in the electron microscopic study by Despommier will be called day 6 PI (the day upon which migratory larvae first enter muscle fibers in an oral infection, such as that used in the present study). In Table IV it is shown that the initial rise in free ribosomes and ribosome-studded membranes of the sarcoplasmic reticulum occurs concomitantly with an increase in the rate of incorporation of $^{14}$C-uridine (Fig. 10) by infected over control muscle. Despommier showed these same organelles to remain in high concentrations in infected muscle up to day 34 PI, a period during which I observed enhancement of the total RNA (Fig. 11) and rate of incorporation of uridine by infected muscle. Despommier reported a period of rapid growth of the larvae and infected fibers between days 16 and 26 PI, an event which is preceded by a period of rapid RNA turnover (Fig. 12) in infected muscle, and accompanied by a marked increase in the incorporation rates of 5 amino acids by infected diaphragms (Figs. 24, 27, 28, 30 and 32). In addition, the total protein of worms increases between days 19 and 24 PI, while the total RNA, μg RNA/mg protein and incorporation of thymidine decreases (Table II). An increase in the number and size of nuclei (day 6-8 PI) and mitochondria (day 12-20 PI) is accompanied by an increase in the rate of incorporation of thymidine (day 6-40 PI; Fig. 22) and in the total DNA (day 9-40 PI; Fig. 21) in trichinosed muscle.

The encystment of larvae entering on day 6 PI was observed electron microscopically to occur between days 18 and 22 PI (Bruce, 1970). By
Table IV. Correlation of ultrastructural alterations (Column B) by Despommier (personal communication) with changes in trichinosed muscle biochemistry shown in the present study (Column C). The day upon which Despommier injected his larvae intramuscularly is designated day 6 PI. The time base for biochemical studies has been adjusted as indicated in context.
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event Occurring in trichinosed muscle</td>
<td>Day PI Ultrastructural Study</td>
<td>Day PI Biochemical Study</td>
</tr>
<tr>
<td>Elevation in number of free ribosomes, polyribosomes, and ribosome-studded S.R.</td>
<td>Day 6-34 PI</td>
<td>-</td>
</tr>
<tr>
<td>Elevation in rate of uridine incorporation.</td>
<td>-</td>
<td>Day 6-40 PI (Fig. 10)</td>
</tr>
<tr>
<td>Elevation in total RNA.</td>
<td>-</td>
<td>Day 9-40 PI (Fig. 11)</td>
</tr>
<tr>
<td>Period during which larvae and fiber undergo greatest growth.</td>
<td>Day 16-26 PI</td>
<td>-</td>
</tr>
<tr>
<td>Period of increase in total protein of larvae, and decrease in total RNA and incorporation of thymidine into DNA.</td>
<td>-</td>
<td>Day 19-24 PI (Table III)</td>
</tr>
<tr>
<td>Period of greatest RNA turnover.</td>
<td>-</td>
<td>Day 6-10 PI (Fig. 12)</td>
</tr>
<tr>
<td>Period of enhanced incorporation of proline, methionine, tryptophan, glycine and alanine.</td>
<td>-</td>
<td>Day 9-40 PI (Figs. 24, 27, 28, 30 and 32)</td>
</tr>
<tr>
<td>Increase in the number and size of fiber nuclei.</td>
<td>Day 6-8 PI</td>
<td>-</td>
</tr>
<tr>
<td>Increase in the number and size of fiber mitochondria.</td>
<td>Day 12-20 PI</td>
<td>-</td>
</tr>
<tr>
<td>Period of enhanced thymidine incorporation.</td>
<td>-</td>
<td>Day 6-40 PI (Fig. 22)</td>
</tr>
<tr>
<td>Period of elevated total DNA.</td>
<td>-</td>
<td>Day 9-40 PI (Fig. 21)</td>
</tr>
<tr>
<td>Microscopic observations on completion of cyst formation for day 6 PI larvae.</td>
<td>Day 18-22 PI</td>
<td>-</td>
</tr>
<tr>
<td>Chemical determination of completion of cyst formation for Day 6 PI larvae.</td>
<td>-</td>
<td>Day 22 PI (Fig. 25)</td>
</tr>
</tbody>
</table>
chemical methods the changes in total collagen (total hydroxyproline; Fig. 25) revealed that the encystment of larvae entering on day 6 PI is completed by day 22 PI.

In electron microscopic studies of trichinella-infected fibers detailed descriptions in changes in T tubule structure have been presented (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968). The T tubules undergo several stages of dramatic development in which they proliferate throughout the infected fiber. However, no explanation has been offered for these changes in T tubule structure. The infected fiber shows a markedly enhanced metabolic state, and all nutrients required by the larva must pass through the infected fiber to reach the worm. This creates a greatly increased burden on the absorptive surface of the muscle fiber. The extensive growth of the tubules of the T system might be explained on the basis of the dramatically increased absorptive obligation placed on the infected fiber by the high level of trichinella-infected muscle metabolism, and by the presence of a worm within the fiber which must undergo a 10-fold increase in size.

It is interesting to note that many of the ultrastructural and biochemical changes occurring in trichinosed striated muscle fibers are not unique to this parasitic myopathy. A discussion of the marked similarities existing between the ultrastructural and biochemical changes which occur in trichinosed fibers and those taking place in muscle fibers under diverse conditions of damage and disease is appropriate at this time.
Adams (1958), Mastaglia and Kakulas (1969), Susheela et al. (1969), and Engel and MacDonald (1970) have observed that similar ultrastructural changes occur in diverse myopathies, and that these alterations "can be resolved into reaction patterns involving fiber organelles which, in turn, appear in different sequences and combinations in various muscle diseases" (Engel and MacDonald, 1970). This statement refers to ultrastructural changes accompanying degeneration in various myopathies. However it has been shown that ultrastructural alterations similar to those seen in regeneration occur in varying numbers of fibers either before, during or after the characteristic degenerative changes associated with: a) myonecrosis induced by rattlesnake venom (Stringer, et al., 1972); b) vitamin E deficiency in the rabbit and "Rottnest quokka" (Zalkin et al., 1962; Weinstock et al., 1965; Van Vleet, 1967; Mastaglia et al., 1970); c) myotonic dystrophy (Schorer, 1970; Tomonaga and Tanabe, 1972); d) toxomyopathies induced by botulimum toxin (Duchen, 1971), chloroquine (MacDonald and Engel, 1970), colchicine (Markland and D'Agostino, 1971) and emetine (Fewings et al., 1972); e) polymyositis (Walton and Adams, 1956; Mastaglia and Kakulas, 1970); f) denervation (Walton and Adams, 1956; Cazzato, 1970); g) mouse dystrophy (Weinstock et al., 1958; Susheela et al., 1969); h) Wohlfart-Kugelberg-Welander syndrome (Adachi et al., 1971); and Duchenne muscular dystrophy (Walton and Adams, 1956; Pearson, 1962; Pellegrino and Franzini, 1963; Pearce and Walton, 1963; Hudson et al., 1967; Mastaglia and Kakulas, 1967; Schafiq et al., 1967; Mastaglia and Kakulas, 1969; Slug and Moser, 1969; Susheela et al., 1969; Mastaglia et al., 1970;
Engel and MacDonald, 1970). Those morphological changes characteristic of regeneration in normal striated muscle fibers are accompanied by:

a) an increase in DNA and RNA content; b) increases in the rates of incorporation of amino acids into protein; and c) increases in the rates of incorporation of thymidine into DNA and of uridine into RNA (Walton and Adams, 1956; Adams et al., 1962; Susheela et al., 1969; Monkton and Nihei, 1972). In several myopathies these biochemical changes have been observed to accompany the period of ultrastructural change resembling regeneration. In denervated muscle, increases in the DNA content (Manchester and Harris, 1967) and RNA content (Walton and Adams, 1956; Manchester and Harris, 1967) of regenerating fibers, and changes in the rates of incorporation of amino acids into regenerating fiber protein (Buse et al., 1965; Holt et al., 1972) have been shown. In Duchenne muscular dystrophy fibers undergoing regeneration-like changes have displayed an increase in total DNA (Weinstock, 1969), total RNA (Walton and Adams, 1956; Pearson, 1962; Hodgson et al., 1967; McArdle, 1967; Ross and Jans, 1968; Mastaglia, 1969) and rates of incorporation of amino acids (Ionasescu et al., 1971). Fibers under the influence of murine dystrophy and undergoing ultrastructural changes similar to regeneration have been reported to undergo an increase in total RNA (Pearce and Walton, 1963; Susheela et al., 1969) and rates of incorporation of amino acids (Simon et al., 1962). Finally, in polymyositis, fibers undergoing regenerative-like alterations have been reported to exhibit an enhanced RNA content (Mastaglia and Kakulas, 1970).

In Table V the ultrastructural (Fasske and Themann, 1961; Ribas-Mujal and Rivera-Pomar, 1968; Despommier, personal communication) and
Table V. Correlation of reported ultrastructural changes known to occur in trichinosed muscle (Column B) with those which have been shown to occur in regenerating myofibers (Column C).
<table>
<thead>
<tr>
<th>Event</th>
<th>B Trichinella infected muscle</th>
<th>C Regenerating muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. An increase in the amount of sarcoplasmic matrix.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2. An increase in the size and number of nuclei with migration of nuclei to a central location in the fiber</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3. An increase in the number of free-ribosomes and polysomes, and intense proliferation of rough sarcoplasmic reticulum.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4. Intense proliferation of T tubules.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5. An increase in the size of fibers.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6. An increase in the size and/or number of mitochondria.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>7. An increase in the number of golgi.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>8. Increase in DNA and RNA content.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>9. An increase in the rate of incorporation of thymidine into DNA and of uridine into RNA.</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10. A dramatic increase in the incorporation of amino acids into protein.</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
biochemical (present study) changes known to occur in trichinosed muscle fibers are compared with those observed during regeneration after massive damage to normal myofibers (Walton and Adams, 1956; Adams et al., 1962; Susheela et al., 1969). This comparison suggests that the biochemical and morphological changes occurring in trichinosed fibers resemble those which occur in association with regeneration in normal striated myofibers which have undergone extensive damage.

It has been observed that, among other myopathies, in toxic myopathies (MacDonald and Engel, 1970; Duchen, 1971; Stringer et al., 1972), polymyositis (Mataglia and Kakulas, 1970), and vitamin E deficiency in the "Rottnest quokka" and rabbit (Van Vleet et al., 1967) regeneration of many of the diseased muscle fibers may go to completion. However, under the myopathic conditions of murine dystrophy (Susheela, et al., 1969), Duchenne muscular dystrophy (Walton and Adams, 1956; Pearson, 1962; Hudgson et al., 1967; Mataglia et al., 1970), denervation (Miledi and Slater, 1968), and Wohlfart-Kugelberg-Welander syndrome (Adachi et al., 1971), among others, the ultrastructural and biochemical alterations resembling those occurring during regeneration have been called "abortive regeneration" (Kakulas, 1970) since the end result of such changes is degeneration of myofibers. Trichinosed muscle fibers do not appear to undergo either degeneration or complete regeneration during the time period over which they have been observed (15 months PI, Fasske and Themann, 1961). On day 60 PI (as at 15 months) trichinosed fibers contain numerous golgi and mitochondria; enlarged, central nuclei with enlarged nucleoli; vast amounts of tubules of the smooth sarcoplasmic
reticulum and t-system; numerous free-ribosomes and polysomes and some rough sarcoplasmic reticulum (Despommier, personal communication).

Based on the above considerations, an hypothesis of events occurring after entrance of trichinella larvae into host muscle fibers is presented. The mechanical damage done to a host fiber by entrance of a larva may trigger redifferentiation resulting in regenerative-like changes in the host myofiber. Some information from the larva early during its stay in the host myofiber may cause the infected fiber to adopt its regenerative-like ultrastructural and biochemical status as important constituents of its new level of metabolism. Having established a new metabolic level, the infected fiber may now assume its role as "nurse cell" (Despommier, personal communication) to the larva. This nurse cell does not undergo complete regeneration or degeneration, but rather synthesizes enzymes and structural proteins necessary for it to maintain its metabolically hyperactive state, and serve to establish a biochemically and structurally suitable environment for the development and continued existence of its parasite. In addition, a portion of the enhanced protein synthesis occurring in the nurse cell may be directed to the production of cyst protein (collagen).
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