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The effects of trichinosis upon certain excretory products, the adrenals, blood, activity and fur of mesocricetus auratus.

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Boston University

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Boston University
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GRADUATE SCHOOL

Dissertation

THE EFFECTS OF TRICHINOSIS UPON CERTAIN
EXCRETORY PRODUCTS, THE ADRENALS, BLOOD,
ACTIVITY AND FUR OF MESOCRIGETUS AURATUS

by

George Raymond Bernard, Jr.
(A. B., Harvard College, 1948;
A. M., Boston University, 1949)

Submitted in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy
1954
Approved

by

First Reader Arthur Hull
Associate Professor of Biology

Second Reader
Instructor of Microbiology
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INTRODUCTION

The research reported below was undertaken to investigate some of the effects of a sublethal infection by a metazoan parasite, *Trichinella spiralis* (Owen) Railliet, upon the golden hamster, *Mesocricetus auratus* (Waterhouse) Nehring. It was hoped that, by the use of suitable histochemical and morphological tests upon the host's tissues, and biochemical tests upon certain of the host's excretory products and the adrenal glands, the course of the disease and the amount of "stress" to which the host was subjected could be ascertained.

The golden hamster has been used as an experimental host for the following parasites: pig ascaris, *A. lumbricoides* var. *sum*, (Larsh and Gravatt, 1948), *Schistosoma mansoni* and *S. japonicum* (Cram and Figgett, 1947), *Endamoeba histolytica* (Reinertson and Thompson, 1951), and *Leishmania donovani* (Adler and Theodor, 1931; Fulton, Joyner, and Chandler, 1950; Leathem and Stauber, 1952; and others).

Previous reports of experimental trichinosis in the golden hamster have been those of Mauss (1940) on the transfer of immunity from infected females to their offspring, the paper of Humes and Akers (1952) describing the vascular changes about *Trichinella* larvae in the cheek pouch, and the abstract of a paper delivered orally by Boyd and Huston (1952) comparing infections with this parasite in the mouse and male hamster. Details of the life cycle of *Trichinella* in the hamster are not completely known.

A knowledge of the life cycle of *Trichinella spiralis* is essential for the understanding of the disease which this parasite produces.
The first phase of the life cycle is the gut phase during which the ingested larvae mature, mate and the ovoviviparous females deposit their larvae in the lymphatic system (first postulated by Opie, 1904 and experimentally proven by Lewis, 1928). This phase lasts for several weeks. Gursch (1949) reported that the ingested worms immediately penetrate the infected rats mucosa but by 24 hours have reappeared in the lumen. Here they mate. After 48 hours, however, all worms repenetrate the mucosa where they cause considerable destruction of the villi. The females then deposit their larvae in the lacteals. The migratory phase begins when the larvae are transported into the blood vascular system via the thoracic duct. The larvae leave the capillaries and drawn by an as yet unknown chemotactic agent (Lewis, 1928) penetrate the sarcolemmata of the muscle fibers thus initiating the muscle phase. The larva then coils, the muscle fibers degenerate and an inflammatory reaction ensues. The locus is the encapsulated by hyaline tissue and finally a calcareous cyst is deposited about the worm. Several weeks after the muscle is invaded, the host becomes adapted to the parasite, the symptoms subside and a convalescent stage ensues. Thus the initial phases of the disease involve a variety of tissues—the digestive tract, the circulatory and lymphatic systems and the organs they service. The later muscle phase involves only the muscle fibers directly. Toxins elaborated by the larvae, however, may affect more distant tissues. Since the host does not become adapted to the parasite for several months after ingestion of the larvae, the infection can be considered a long-term stress composed of a series of waves of destruction involving extensive regions of the body.
It is the purpose of this thesis to demonstrate the extent to which the host is stressed and perhaps chart by indirect evidence the development of accord between host and parasite.

Since Selye (1936) first propounded the rudiments of the theory of "stress" and the relationship of this phenomenon to the adrenocortical tissue, the literature has abounded with reports of "quantitative" research on "stresses" involving either human subjects or animals. Selye (1936 et seq.) and others have reported similar changes in various tissues following different noxious stimuli, e.g., cold, heat, formalin poisoning, surgical trauma, electroshock and many others (see below). These stresses in most cases lasted from several minutes to a few days and cannot be considered "long-term". Some others reported as long-term stresses actually were a series of short-term stresses applied sometimes days apart, e.g., the paper of Stein et al. (1949) on the effect of cold and heat on military men. Some of the main paths for investigation involved urinalysis, blood analysis and, most important, adrenocortical function studied either by morphological, histochemical or biochemical changes.

The tests applied to the hamsters with trichinosis and reported below were: urine volume and pH, urinary uric acid, creatinine and creatine, the diazo test, adrenal weight, adrenal ascorbic acid and non-specific dehydrogenase activity, adrenal lipids, alkaline phosphatase and birefringence. In addition, data concerning the total leucocyte and eosinophil counts, the differential count, the activity of infected hamsters, and the fur changes associated with trichinosis have been reported in the Appendices to the Dissertation.
The blood changes associated with trichinosis or other parasitic or allergic disease involve chiefly an increase in eosinophils (in most animals) (Kirk, 1942) whereas during a period of marked stress, e.g., cold exposure or surgical injury the eosinophils decrease in numbers. Since trichinosis is considered in this dissertation as a stress, a report of the blood picture divorced from the other measurements of the stress upon the animal is necessary.

For the sake of simplicity and brevity the pertinent literature has been summarized in Table I. The meanings of the symbols used in the Table are: "+/" means an increase in that particular substance; "O" means no change; "−/" means a reduction.
<table>
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<td>cold man</td>
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<td>Stein et al. (1949)</td>
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<td>$\neq$</td>
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**URINARY**

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<td></td>
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<tr>
<td></td>
<td>elderly men</td>
<td>$\neq$</td>
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</tr>
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**URINARY**

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<td>Bass and Place (1949)</td>
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<td>Surgery man</td>
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<td>Bruche and Linke (1949)</td>
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<td>man</td>
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<td>Forsham et al. (1948)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lievre (1949)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tawsky et al. (1951)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Conn et al. (1951)</td>
</tr>
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<td></td>
<td>gouty</td>
<td>✗</td>
<td>Lievre (1949)</td>
</tr>
<tr>
<td></td>
<td>men</td>
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<tr>
<td>DOCA</td>
<td>man</td>
<td>0</td>
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<td></td>
<td>rat</td>
<td>0</td>
<td>Friedman et al. (1949)</td>
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</table>

| TRICHINOSIS | cats & dogs | ✗ | Flury and Groll (1913) |
|            | man | ✗ | Wohl (1916) |
|            |     | | Burger (1922) |
|            |     | | Markowicz and Bock (1931) |
|            |     | | Rogers (1941) |
| URINARY    | rat | ✗ | Flury and Groll (1913) |
| CREATININE | rabbit | ✗ | Burger (1922) |
| POLIOENCEPHALITIS | man | ✗ | |

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<td>rat</td>
<td>✗</td>
<td>Clark et al. (1945)</td>
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<tr>
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<td>dog</td>
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**URINARY CREATININE (cont.)**

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**ADRENAL WEIGHT (size of cortex)**

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<tr>
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<td>rat</td>
<td>✓</td>
<td>Long (1947)</td>
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<td>&quot;Work&quot;</td>
<td>rat</td>
<td>✓</td>
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<tr>
<td>Fighting</td>
<td>vole</td>
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<tr>
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| ADRENAL BIREFRINGENCE       | ACTH (small doses) | castrated rat | Weaver and Nelson (1943) |
|                             | ACTH (large doses) | castrated rat | Weaver and Nelson (1943) |

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Perry and Cumming (1952)
In trichinosis, Flury and Groll (1913) found nitrogen retention during the period of development of the larvae in the muscles. As soon as the capsules were formed, an increased nitrogen excretion began which was interpreted as resulting from the elimination of substances originating from destroyed muscle fibers. These studies were amplified by Rogers (1941, 1942) who found that the protein digestion of the infected rats fell to a low point 8-12 days post infectionem (p.i.), perhaps due to secretion of antienzymes by the worms and mechanical damage to the mucosa. The urinary nitrogen and urea output rose immediately after infection, fell off, then rose again by the 13th day. These changes were attributed to a toxic action of the parasites since they occurred before a large scale invasion of the muscles took place. Urinary creatine and creatinine were markedly increased only after 13 days p.i., while the excretion of these compounds was abnormally low during the period 4-12 days p.i.

Forsham et al. (1948) first suggested the use of the urinary uric acid/ creatinine ratio (UA/C) as a measure of adrenocortical function and stress. During stress (the injection of ACTH, cortisone, or other hormones which would lead to hormonal imbalance is considered here to be stressful), the ratio has been found by numerous investigators to be raised due to increased excretion of uric acid. Creatinine output usually remained unaltered or fell slightly. (The number of circulating eosinophils fell simultaneously). Stresses involving severe injury, however, were followed by increased excretion of creatinine and creatine due to increased catabolism especially of muscle tissue. This must be kept in mind when one attempts to measure the UA/C ratio in animals undergoing stresses
such as trichinosis. ACTH and cortisone simulate the effects of stress whereas DOCA was ineffective.

The UA/C ratio studies outlined above were performed on men where uric acid is the chief end-product of purine metabolism. Like most mammals, the hamster has the ability to degrade uric acid to allantoin through the action of the enzyme uricase found in both the liver and kidney (unpublished data). There is no evidence, however, that the increased uric acid excretion encountered following stress is due to an alteration of any enzyme system but rather is due to reduced tubular reabsorption of urate (Friedman et al., 1949; Friedman and Byers, 1950; and Ingbar, 1951). Krizek et al. (1948) found that X-rayed rabbits produced more urinary uric acid whereas their excretion of allantoin was unaffected by that particular stress. Measurement of uric acid in the case of trichinosis in the hamster is, therefore, probably a valid test of stress in this animal.

The diazo reaction, although one of the older non-specific qualitative clinical diagnostic tests, is not fully understood. The only time the test reacts positively is when the animal or person is suffering from febrile disorder such as typhoid fever, measles, tuberculosis, etc. (Hawk et al., 1947). In all cases considerable tissue catabolism is involved and the animal is clearly stressed. The present author has not found any instance of this test applied to cases of overdosage of ACTH, cortisone or other hormones or to stresses not involving extensive tissue catabolism. The test has been applied to human cases of trichinosis by Gould (1945). He obtained a positive reaction in the only case discussed.
Flury and Groll (1913) obtained positive reactions from the urines of heavily infected dogs and cats. The urine from a lightly infected cat gave a positive reaction only during the period immediately following infection. Urine specimens from guinea pigs never reacted positively. Holzhauer (1933), however, always obtained negative results with urine from infected dogs.

Stress or multiple doses of ACTH cause an increase in the adrenal weight either due to hyperplasia or hypertrophy in response to the needs of the organism for additional steroids. DOCA and stilbestrol cause a reduction in weight. Gould (1945) reported in one human case of trichinosis a reduction in size. The weight of the adrenals was not recorded.

The adrenal gland is unique among the organs of the body in having the greatest concentration of ascorbic acid (Long, 1947). The stainable ascorbic acid of the adrenal is located almost exclusively in the cortex (Szent-Gyorgi, 1928; Huszak, 1933; Giroud and Leblond, 1936; and Leblond and Gardner, 1938). Giroud and Santa (1940) found, however, that the medullary cells contribute from 36 to 43 per cent of the total adrenal ascorbic acid (of slaughtered animals) measured by the dichlorophenol technic. Pirani (1952) considered that the vitamin's function in the cortex is probably of a non-specific nature related to cellular respiratory activity and metabolic rate. In the medulla its main function is probably epinephrin stabilization. Sayers et al. (1945) postulated that its role may be that of a prosthetic group attached to Ring D of the steroid molecule which would render the molecule water-soluble.

The ascorbic acid content of the adrenal is immediately depleted after stress and injection of ACTH but soon returns to normal or slightly above normal. Adrenalin is without effect upon the adrenal ascorbic acid.
of the rabbit, whereas in the rat it is decreased. The effects of tularemia on the rat's adrenal ascorbic acid are variable. The fasting male rat's adrenal shows an augmented ascorbic acid content.

The relation of adrenal birefringence to stress has not been extensively studied. Greep and Deane (1949) found that following stress there was usually a decrease in the fine birefringent particles. These are superseded by larger, coarse granules considered by most authors to represent storage.

Adrenal birefringence has been suspected of either revealing the presence of cholesterol crystals not in lipid solution (Bennett, 1940; Yoffey and Baxter, 1947) or marking the presence of steroids (Greep and Deane, 1949; Chan, 1950). The crystals are not sudanophilic (Sarason, 1943; Yoffey and Baxter, l.c.) but are found most abundant in regions of maximal sudanophilia. The sudanophilic substance is according to Sayers and Sayers (1949) the ester cholesterol fraction. Only further research will reveal the true identity of these crystals.

In view of the suspected relationship of cholesterol and birefringence the effects of stress upon cholesterol either stained or biochemically determined should be considered. Various stresses result in an immediate decrease of cholesterol (Goormaghtigh, 1918; Baumann and Holly, 1925; Levin, 1945; North and Nims, 1949; Sayers and Sayers, 1949; and Yoffey and Baxter, 1949). Leatham and Stauber (1952) found that in leishmaniasis of the golden hamster a staining reaction first occurs four weeks p.i. in the usually cholesterol-free adrenal (Alpert, 1950; Mirossy, 1952).
During work on this thesis certain alterations in birefringence paralleling other adrenal changes were found. Hence data on this property of the hamster's adrenal tissue have been included.

Since Sayers (1950) doubted the value of ascorbic acid or cholesterol determinations in chronic diseases, such as trichinosis in the golden hamster, a new indirect method of assaying the tissue activity by the measurement of the dehydrogenase activity of tissue slices or homogenates was used. This test was applied to the adrenals of infected and control animals.

The dehydrogenases (one of the groups of oxidative enzymes) catalyze the transfer of hydrogen atoms to immediate acceptors other than oxygen and peroxides, although the ultimate acceptor may be oxygen. The principle of their demonstration is the observation of the change in color of suitable hydrogen acceptors when they are reduced by the enzyme. The colorless, water-soluble tetrazolium compounds are reduced by this group of enzymes to the red or purple lipid-soluble formazan compounds.

Zweifach et al. (1951) and Perry and Cumming (1952) applied this test to adrenal tissue. Zweifach et al. found that the oxygen consumption of tissues was directly related to their capacity to reduce tetrazolium salts. These workers emphasized that the tetrazolium reaction is a measure of one or more hydrogen transfer mechanisms and, therefore, cannot be directly compared to oxygen consumption measurements which are a reflection of the total oxidative metabolism of the cell. Furthermore, cells carry out metabolic processes not involving reductase or dehydrogenase activities of the type measured by tetrazolium salts. The formazan production is rather a reflection or a measure of the secretory activity of these cells.
Alkaline phosphatase is involved in the transformation of fructose-6-phosphate to hexosediphosphate (Swanson, 1950), the dephosphorylation of glycerophosphate to glycerol in the production of lipids from carbohydrates (Bullock, 1949), and in the synthesis of protein (Li et al., 1947). The concentration of this enzyme in the adrenal could be considered, therefore, to be an indication of the synthetic activity of this organ. Alkaline phosphatase has not been extensively studied during stress but Leathem and Stauber (1952) reported an elevated phosphatase content in the adrenals of male hamsters lethally infected with *Leishmania donovani*. DOC also causes an increase while hypophysectomy, epinephrin and thyroidec- tomy cause a decrease.

The lipids of the adrenal cortex which are stained by the Sudan stains are usually depleted following stress. If the stress is terminated or is continued but not too severe, the lipid is soon replenished and sections may actually show storage droplets. The male hamster with leishmaniasis was found by Leathem and Stauber (1952) to have increased sudanophilic lipid content. Stilbestrol and thyroxin also caused an increase in sudanophilic lipids. Injections of crude extracts of the anterior pituitary gland into rats also caused an increase (Emery and Atwell, 1933). The results of more modern research are at variance with this earlier work.

Augustine (1936) studied carbohydrate metabolism of trichinized humans and rabbits and found that the changes observed were "slight, transient, and variable". Retention of guanidine caused by extensive lysis of necrotic tissue was considered by Harwood et al. (1937) and Pierce et al. (1939) to be responsible for some of the toxic effects of this
disease. Pierce et al. in their study of human trichinosis found that the guanidine retention led to extreme hypoglycemia whereas the serum cholesterol was normal throughout the period studied. They were not able, however, to study the first month post-infection. Hartman et al. (1940) investigated blood calcium, phosphorus, NPN, sugar, chloride, food intake and blood cholesterol. They found that the blood cholesterol twice rose above normal —2 weeks post-infection and 5 weeks post-infection.

The only biochemical study undertaken on the isolated larvae of Trichinella was that of Stannard et al. (1938) who investigated the respiration of isolated larvae.

Carrick (1944) investigated the effects of trichinosis on the guinea pig's parathyroid glands and serum calcium, inorganic phosphorus and phosphatase. These blood constituents were not significantly altered.

METHODS

In the experiments described below a total of 237 golden hamsters, Mesocricetus auratus, were used. Of these 114 were males. All animals were obtained from one commercial breeder and cannot be considered inbred. Their weights ranged from 85 to 100 grams at time of infection and they were from eight to ten weeks old. Strict adherence to this age and weight range was necessary since other investigators had found that age (Bachmann, 1938; Hendricks, 1948; Matoff, 1943 a and b; Nolf and Zaiman, 1941; and Riedel, 1948) and weight (Hendricks, 1950) directly affected the intensity of infection in experimentally induced trichinosis in other laboratory animals.

The diet of the hamsters consisted of Purina Lab Chow and tap water ad libitum. No dietary supplements were given.
A. Method of Infection: The hamsters (unanaesthetized) were infected by feeding them 225 Trichinella larvae (or more in certain instances noted below) through a copper stomach tube attached to a lcc. tuberculin syringe. This dose was found to cause serious illness in the hamster and was considered as a sublethal dose. A dose of 500 larvae killed 18 of 20 hamsters (during the intestinal phase) whereas 1000 larvae had the same effect as 225 larvae (see "Discussion" below). The larvae were isolated from rat muscle by digestion. The rats had been infected from 35 to 60 days previously by either eating infected hamster muscle or by being forced-fed larvae isolated from hamster meat. Use of young larvae is considered to increase their infectivity (Hendricks, 1949) as is alternation of hosts (Oliver-Gonzales, 1941). A third factor influencing the infectivity of larvae is the length of time of the artificial digestion process (Gursch, 1948; and Larsh and Kent 1949). Longer exposure decreases the infectivity of larvae. The method of isolating larvae devised by Larsh and Kent (l.c.) was in most particulars followed.

The following method was used. An infected rat was killed by decapitation, skinned and the bulk of the muscles removed and ground through a coarse meat grinder. The muscle was then weighed and placed in warm (37°C) digesting fluid (a fresh mixture of 1 per cent hydrochloric acid (Tech.) and 0.7 per cent pepsin NF). Twenty-five cc. of fluid were used for each gram of meat. The large beakers containing the meat-pepsin-HCL were then placed in a water bath at 37°C. The wooden paddles of an agitating device patterned after that described by Newman et al. (1936)
were then lowered into the mixture. The paddles produced a gentle swirling of the fluid. After an hour, the material was allowed to settle for 15 minutes, the supernatant fluid discarded, and the remaining mixture filtered through four layers of cheese-cloth into a modified Baerman funnel (Gould, 1945) consisting of a large funnel connected to a 15-ml centrifuge tube by a 4-inch length of soft rubber tubing. After the larvae had settled into the centrifuge tube (10 minutes), it was disconnected. The material was not centrifuged but the supernatant fluid was discarded. A 0.1-ml. sample of the sediment was then measured out on a glass microscope slide by means of a tuberculin syringe fitted with the copper stomach tube. The drop was covered with a coverslip and the number of larvae within the drop counted under the low power of the microscope. The stock material was then diluted with 0.9 per cent saline solution so that 0.1 ml. would contain approximately 100 larvae. At least three counts were then made on well-mixed samples of the diluted worm suspension, the mean taken, and the volume of the dose determined on the basis of the mean value of the three (or more) counts. The variation in the dose never exceeded 30 larvae. Subsequent examination at autopsy showed that all hamsters became infected.

B. Methods of Urinalysis: Two types of metabolism cages were used in the following experiments. Both cages permitted the animals access to food and water and adequate room for exercise. They were both constructed of quarter inch galvanized wire mesh. Ten drawer type, cylindrical cages (Figure 1) were mounted in a two-tiered rack. Beneath each cage was an aluminum pan (11 x 7 x 1.5 inches) containing a quarter inch deep layer of white mineral (paraffin) oil
components of properties measured) and the filtrate collected in a separatory funnel. The more dense urine was then drawn off into a volumetric cylinder and the volume recorded. An alternative method, utilized
Figure 2: Revolving metabolism cages
when the volume was small, consisted of pipetting the urine directly into a calibrated test tube. The mineral oil was reused until its color became dark. In control experiments the oil soluble substances causing discoloration were found not to influence the results.

The pH of the urine sample was determined by immersing a clean glass rod into the urine sample and transferring a drop to a piece of Nitrazine paper (Squibb) and noting the color change.

If the urine volume was less than 4 ml., it was brought to that volume by the addition of distilled water and the dilution recorded. One-ml. aliquots of the well-mixed urine sample were introduced into each of two 100-ml. volumetric flasks. A third 1-ml. aliquot was introduced into a 125-ml. Erlenmeyer flask.

The first of the volumetric flasks was filled to the mark with distilled water, shaken to insure homogeneity of the mixture and a portion centrifuged for 10 minutes. The centrifugation removed the finely-particulate amorphous material which colors hamster urine (probably phosphates and perhaps mucoproteins). Five ml. of the supernatant was then used for the determination of uric acid according to the method of Bidmead (1951). Exactly five minutes was the time allowed for color development and the color was read in a Klett-Summerson photoelectric colorimeter using a 660 m/Å filter.

The Erlenmeyer flask was used for the "total creatinine" determination by Folin's (1914) autoclave technic for the transformation of creatine to creatinine. Sixteen ml. of a saturated aqueous solution of picric acid AR were added. The flask was then "sealed" with aluminum foil
and autoclaved for 45 minutes at 15 lbs. (120° C.). After cooling, 4 ml. of 10 per cent aqueous sodium hydroxide were added and 20 minutes later the mixture was transferred with rinsings to a 100-ml. volumetric flask. Distilled water was used for the rinsings and to bring the volume to the mark. The color was read in the Klett-Summerson colorimeter with a wavelength of 540 m/μ filter. As in the case of the uric acid, controls were run daily.

The "preformed creatinine" was measured by the classical Folin (1914) technic. To the urine in the second volumetric flask was added 20 ml. of a freshly prepared saturated aqueous picric acid-10 per cent sodium hydroxide (4:1) solution and the color allowed to develop for 20 minutes before dilution to the mark. The color was read as described above for total creatinine. If the color was masked by turbidity this was removed by centrifugation before reading the color.

The excretion of creatine was determined by subtracting the concentration of preformed creatinine from that of total creatinine and multiplying the remainder by 1.16. In control experiments using aqueous creatine solutions it was found that the autoclave technic employed for determining total creatinine gave results that were low and varied with the concentration of creatine (unpublished data). This is in agreement with the findings of Albanese and Wangerin (1944) and at variance with those of Lambert (1945). A suitable correction curve was utilized to give values for creatine that more closely approximated the absolute values.

In consideration of the size of the hamster, the unit selected for expressing the amounts of uric acid, creatinine and creatine excreted was microgram (μg/m.) per gram body weight per day. (This unit equals milli-
gram per kilogram per day.) The hamsters in the metabolism cages were weighed daily.

The diazo test employed was that of Ehrlich as found in Hawk et al. (1947). About 1 ml. of urine was placed in a small test tube. An equal volume of the freshly prepared reagent (sulfanilic acid-sodium nitrite, 50:1) was then added, the mixture shaken and several drops of concentrated ammonium hydroxide added. (Very little ammonia water is needed since hamster urine is neutral or alkaline in reaction.) The formation of a reddish color was considered a positive reaction.

C. Adrenal Methods: The number, sex and age groups of the hamsters whose adrenals were used in this study are summarized in Table II.

Elaborate precautions were taken before the animals were sacrificed. Gloves were not worn and the animals were not handled unduly during weighing, etc. They were killed by neckstroke. In order to eliminate the effects of any diurnal variations in the physiological or morphological status of the adrenal glands all animals were sacrificed between 7:00 and 11:00 P.M.

Immediately after death the body cavity was opened and the kidneys with their attached adrenals removed. With fine scissors and forceps the adrenals were quickly freed of the periadrenal fat. This operation took about 4 minutes. Both adrenals were then weighed on a 500 mg. Roller-Smith Model B Precision Balance. The limits of accuracy of the weights as determined by this torsion balance are plus or minus one fifth of 1 per cent. After weighing, the adrenals were either fixed for histochemical and birefringence studies or were subjected to biochemical determination of their ascorbic acid content or non-specific dehydrogenase activity.
# TABLE II. THE NUMBER, SEX, AND AGE GROUPS OF THE HAMSTERS WHOSE ADRENALS WERE USED FOR THE VARIOUS TESTS OF ADRENAL FUNCTION

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**Note:** The table represents the number of hamsters used for various tests of adrenal function, categorized by sex and age group.
For the study of sudanophilic lipids in adrenal sections the technic of Baker (1944) with certain modifications was used. One of the pair of adrenals was fixed in neutral buffered 10 per cent formalin; the other was fixed in ice-cold acetone for alkaline glycerophosphatase activity (see below). The tissue was kept in the formalin until it was cut (3 days--3 months). The frozen sections, 15 to 20/μ thick, were affixed to the slide with Mayer's egg albumen. At least two slides were prepared from each specimen. One slide was allowed to dry three hours then placed directly into 70 per cent ethyl alcohol saturated with Sudan Black B (Harleco) for 2 minutes. The excess stain was removed by successive short baths in 70 and 50 per cent alcohol. Mayer's glycerin jelly was the mounting medium. The other slide was allowed to dry for 2 hours and then immersed in distilled water to remove the remaining formalin and then coverslipped using Mayer's glycerin jelly as the mounting medium. Slides made in this manner were used for birefringence studies. An American Optical Co. Model P45 Polarizing Microscope equipped with a turntable stage for the measurement of angles of extinction was used. The magnification was 100X and the temperature of the room, 20°C. The temperature appears to be critical since experiments using cooled slides (0°C) showed areas of birefringence (cholesterol?) not observable at 20°C. (Recently, Gomori (1952) has claimed that since it is "insensitive and too dependent on factors such as state of aggregation of the lipid, the nature of the medium, etc., which cannot be controlled.")

In order to "semiquantitate" the sudanophilic lipid content of the adrenal sections the following technic was evolved. Since the adrenal cortex of the hamster is considered "lipid poor" (Alpert, 1950; Leathem
and Stauber, 1952) the number of droplets in a small field (ca. 300 \( \mu^2 \)) under oil immersion was counted. The number was then divided by the number of nuclei in the field. Several fields were counted from each morphological region of the same section and the mean used as an indication of the lipid content of that particular region. Photometric measurement of the sudanophilia was considered impractical since the optical density depends on the thickness of the section and this was impossible to control.

Alkaline phosphatase activity was determined by the Gomori-Takamatsu technic (1939) with the modifications suggested by Danielli (1946). The adrenals were fixed in ice-cold acetone (Doyle, 1948; Gomori, 1950), and embedded in tissuemat, with two changes, 15 minutes each. The second tissuemat change was conducted at a vacuum of 15 mm. Hg. Nowry (1949) found that brevity in the infiltration process was necessary to preserve the enzyme. The paraffin blocks were stored at 5°C. until they were cut (not more than 2 months). Doyle (1950) found that storage of uncut blocks up to one year did not influence the activity of the enzyme. The blocks were cut at 6 \( \mu \), the sections affixed to the slides with a minimum amount of albumen and stained. The substrate was sodium glycero-phosphate (28 per cent alpha salt) buffered to pH 9.2 (Wachstein, 1946). Incubation was for a period of two hours at 37°C. The counterstain used was safranin. Control sections were also made. The amount of phosphatase activity and the distribution of the enzyme was ascertained under oil immersion. Sections from normal animals of the same age were compared with those from infected animals.

For the quantitative determination of ascorbic acid the method of Glick
(1949) by microtitration with 2,6-dichlorophenolindophenol was used. After weighing, each of the pair of adrenals was slit sagittally and quickly immersed in 1 ml. of ice-cold 2 per cent aqueous metaphosphoric acid in a small test tube. The tissue was then macerated with the end of a glass rod. The rod and walls of the tube were then rinsed with 1 ml. of distilled water. The material in the tube was then titrated with standardized sodium 2,6-dichlorophenolindophenol (LaMotte reagent) delivered from a microburette. One ml. of this reagent had been standardized by the manufacturer to be reduced by 0.02 mg. of ascorbic acid. This technique measures only the reduced ascorbic acid, but Sayers and Sayers (1948) found in guinea pigs and rats that results obtained by this method were no different from those obtained by methods which measure both reduced and dehydroascorbic acid.

The non-specific dehydrogenase activity of the adrenals of infected and control animals and other tissues was determined quantitatively by the method of Black and Kleiner (1949). This method is based on the effect of dehydrogenase enzymes upon colorless triphenyltetrazolium chloride (TTC) transforming it into the red acetone-soluble formazan. Kun and Abood (1949) showed that the amount of dye measured by the optical density is a linear function of the amount of enzyme present.

The adrenal glands from a recently sacrificed animal were weighed, cut in half and placed in a small test tube containing 3 ml. of 1 per cent TTC. The substrate was buffered at pH 7.2 with 0.1M phosphate (Lillie, 1949). The tube was then placed in a vibrating rack in an incubator at 37°C. After an incubation period of an hour, the substrate
was decanted. The red-colored tissue was then washed with 4 changes of acetone (2 ml., 1 ml., ½ ml., and ¼ ml.). The washings were added to a calibrated Klett colorimeter tube. The volume was brought to 5 ml. with acetone and mixed thoroughly. The color was read in the Klett Summerson Photoelectric Colorimeter at a wavelength of 420 m\u00b4. The tissue was dried for 8 hours at room temperature and weighed on the Roller-Smith Torsion Balance. The amount of enzyme present was estimated by dividing the color reading by the dry weight in milligrams.

RESULTS

A. Appearance of a Typical Hamster with Trichinosis: There is considerable adhesion of the eyelids to the conjunctiva. In lethal cases, several days before death, the eyes close because of the periorcular edema. In one case there was observed loss of the fluid from the eye chamber. The mouth cannot be opened completely and the action of the forepaws is impaired so that the animal cannot handle pellets of food larger than one half inch in diameter. The ears are folded back.

The most common external symptom of the disease is the arching of the back caused by the extension of the hind limbs. The tail is then utilized as an additional support (see Figure 3). The gait of the animal is slow and its activities are lethargic. Signs of unthriftness often appear. There is also a loss of the subdermal fatty tissue (the scruff). This will be considered in more detail later (see Appendix III). The respiratory rate of the animal declines.

Autopsy findings in those animals dying from trichinosis showed that
there were no gross alterations of the uro-genital systems of either males or females. The lungs were not hemorrhagic or otherwise grossly abnormal. The digestive tract appeared empty; no fecal pellets were seen in either the large intestine or the rectum. The cecum was bloated in some cases. The animals with severe cases were often observed to have difficulty in swallowing and there were oftentimes mounds of meal from gnawed food pellets under their cages which implies that infected animals can chew but the activity of those structures involved in deglutition is impaired.

B. The Effects of Trichinosis Upon the Urine

1. The Urine of Normal Hamsters: There was no noticeable increase in urine volume with increasing age. There was, however, considerable variation in the volume from individual to individual. Some hamsters always produced less than 2 ml. whereas others produced over 20 ml. daily. The urine of the latter animals was necessarily more dilute. These animals spent a great deal of time drinking and gnawing the water delivery tube and thereby ingested quantities of water in excess of their needs. In their normal habitat, under desert conditions, it is probable that the hamster's normal urine production is small. The mean urine volume of normal hamsters under the conditions of the experiment was about 8 ml.

Normal hamster urine is slightly alkaline. Under the conditions of the experiment and with a diet of Purina Lab Chow, the pH was found to be between 7.2 and 7.5 (Nitrazine paper determination). No effect of age was noted.

The uric acid/creatinine ratio (UA/C) decreases from birth to maturity. The UA/C of a month old hamster is approximately twice that of
Figure 3: Typical appearance of infected animal.
of the mature hamster (more than 10 weeks old). The decline of the UA/C with increasing age is the result of an increased production of creatinine. The output of uric acid is not affected by age. The concentration of creatine, on the other hand, decreases as the animal matures. This is a common finding in human urinalysis (Hawk et al., 1947). Table III contains a summary of the findings on the influence of age upon the urinary components studied.

2. The Urine of Infected Hamsters: Alterations in the urine volumes of animals infected with *Trichinella* are summarized in Figure 4. Hamsters which subsequently died from their infections showed an oliguria which became extreme after the 16th day p.i. (Each point on the graph represents the mean output of at least 4 animals.) The sublethal cases showed an initial polyuria which developed into oliguria by day 24 p.i. In the hamsters which had been infected with 225 larvae, the oliguria remained constant until day 65 p.i.; in those infected with 1,000 larvae the oliguria was variable but nevertheless present. (Each point on the graph represents the mean output of from 4 to 10 animals.) The urine volume changes of three of four sublethally infected hamsters chosen at random showed similar changes; one had a consistently low output.

In only one group (of four) of animals sublethally infected was there an alteration in the urine pH. In this group the urine became slightly acid during the period 24 to 33 days p.i. As might be expected, hamsters that later died of trichinosis during the latter portion of the intestinal phase produced an acid urine (pH 5.5) beginning on the 3rd day p.i. These animals were either unable to absorb their food or to ingest
### TABLE III. URINARY COMPONENTS OF NORMAL HAMSTERS

<table>
<thead>
<tr>
<th>AGE (in weeks)</th>
<th>NUMBER OF ANIMALS</th>
<th>AVERAGE WEIGHT (g.)</th>
<th>UA/C</th>
<th>RANGE</th>
<th>UA (g/day)</th>
<th>C (g/day)</th>
<th>CREATINE (g/day)</th>
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<td>.61</td>
<td>.50-.75</td>
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<td>.42-.68</td>
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<td>.36-.50</td>
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<td>5</td>
<td>74</td>
<td>.37</td>
<td>.29-.45</td>
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<td>83-142</td>
<td>.27</td>
<td>.13-.41</td>
<td>10</td>
<td>35</td>
<td>12*</td>
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</table>

(* based on 40 control determinations)
Figure 4. Urine volume alterations following infection
URINE VOLUME OF LETHALLY INFECTED HAMSTERS

URINE VOLUME CHANGES IN FOUR SUB-LETHALY INFECTED HAMSTERS CHOSEN AT RANDOM

URINE VOLUME OF SUB-LETHALLY INFECTED HAMSTERS
enough food. The sequelae of malnutrition were evident—loss of weight and ketone bodies in the expired breath (a sign of an acidotic state).

Statistical evaluation of the control uric acid, creatinine, and creatine data was difficult. This was caused by differences in the sizes of samples from the various control animals. Of the 21 control animals, only 12 were subjected to more than one urinalysis. These 12 were tested for variability. It was found that there was variability in both uric acid and creatinine, not only in the output from day to day but also from animal to animal (Snedecor "F" test, 1946). Similar findings were made in the creatine output.

The frequency distribution of control values of both these substances showed skewing to the left. The frequency distribution of the control UA/C ratios was only slightly skewed.

In view of these facts, the establishment of limits of significance at any particular instant was difficult. In order to simplify the evaluation of the experimental data the following technique was adopted. The means and standard deviations of the various constituents were computed. A band representing more than 95 per cent of all normal data was then established by adding or subtracting two standard deviations from the mean. The band thus constructed was found to be almost identical with a hypothetical band whose upper and lower limits were the upper and lower limits of the range of normal values. Any experimental mean value falling either above or below the band portrayed in all graphs was considered significant. Each point on the following graphs of the urine components of sublethally infected animals represents the mean output of from 4 to 10 animals.
It was found that the UA/C ratio was a good indication of the general welfare of the animal. In lethal infections there was a significant peak on day 7 p.i. (see Figure 5). Beginning with day 10 p.i. the values steadily increased. The graphical representation of the data during this time shows a series of peaks. Actually the "valleys" between peaks are artifacts caused by changes in the samples occasioned by the deaths of some of the animals. The vacant metabolism cages were then occupied by animals which were ill but whose symptoms were less severe. As they neared death, however, the UA/C of all hamsters rose to extremely high levels.

The UA/C following sublethal infections seems to be dependent upon the number of larvae ingested; less reaction follows infection with 1,000 larvae than with 225 larvae. The two curves will be considered separately.

The UA/C curve following infection with 225 larvae shows peaks on days 7 and 15 p.i. For two weeks thereafter the mean values for infected animals were but slightly above the normal range. A third peak occurred on day 30 p.i. and was followed by a 30 day period of significantly elevated mean ratios. By day 65, the ratio had returned to normal.

Animals infected with 1,000 larvae also showed a slight (but not significant) peak about a week p.i.. During the following 18 days the UA/C was well within the normal range. After day 26 p.i. the mean ratio became significantly elevated and like the animals infected with 225 worms did not return to normal till day 65 p.i..

The elevation of the UA/C following infection with trichinosis was attributed to two factors: an elevation of the uric acid output and a decreased output of creatinine (see Figures 6 and 7).
Figure 5. Uric acid/creatinine ratio following infection
URINARY URIC ACID-CREATININE RATIO CHANGES OF LETHALLY-INFECTED HAMSTERS

The stippled areas represent the mean of 204 control (normal) ratios plus or minus twice the standard deviation.

URINARY URIC ACID-CREATININE RATIO CHANGES OF SUBLETHALLY INFECTED HAMSTERS

- 225 larvae
- 1000 larvae
Figure 6. Uric acid excretion following infection
URINARY EXCRETION OF URIC ACID

A: in lethally infected hamsters

B: in sub-lethally infected hamsters

The stippled areas represent the mean control urinary uric acid excretion plus or minus twice the standard deviation. Based on 204 control (normal) readings.
Figure 7. Creatinine excretion following infection
URINARY EXCRETION OF CREATININE

A  in lethally infected hamsters

B  in sub-lethally infected hamsters

The stippled areas represent the mean control urinary creatinine excretion plus or minus twice the standard deviation. Based on 204 control (normal) readings.

---  225 larvae

---  1,000 larvae
Immediately following infection (day 2 p.i.), the lethally infected animals showed a decreased output of uric acid. Until day 12 the output was normal, but then rose until the mean output was significantly raised (day 17 p.i.). Most of the lethally-infected hamsters produced prodigious quantities of uric acid during the period from 21 to 24 days p.i.. After day 24 (most hamsters had already died) the output fell and was within the normal range.

The curves representing the animals infected with 225 and 1,000 larvae are essentially similar. On all days following infection to day 65 p.i., the output of uric acid was above normal. The mean urinary uric acid output was elevated on the day following infection with 225 larvae. The curve representing output of these animals showed peaks on day 6, days 12-14, day 49, and day 55 p.i.. The output was normal by day 65 p.i.. The uric acid output of hamsters infected with 1,000 larvae, however, was significantly elevated only once, day 32 p.i. The output after day 65 was subnormal.

Figure 7 is a graphical representation of the alterations of creatinine excretion during infection. The output of the lethally infected animals was low for the first week, then gradually rose to average values, fell precipitously to significant levels on day 18 p.i. and remained low until death. The output of sublethally infected hamsters was normal for the first two weeks, then gradually fell. On days 32-34 the decrease was significant. The output remained slightly subnormal thereafter (no observations were made after day 100 p.i.). No real differences could be ascertained between the mean outputs of animals infected with 225 or 1,000 larvae.
The similarity of the uric acid and creatinine curves after day 65 p.i. should be noted. Although the output of the two substances may have varied from week to week, the variation was similar in both cases and the UA/C ratio remained relatively unaltered.

Observations upon creatine excretion among lethally infected hamsters were limited to three animals. The results were variable. The one constant finding was that all excreted more creatine than normal. One animal's excretion varied daily, rising significantly one day and being normal the next (see Figure 8).

As was found in the study of creatinine, there was a marked similarity between the output of creatine of those animals infected with 225 larvae and those with 1,000 larvae (see Figure 8). The mean creatine values were significantly higher than control values during the period 19–23 days p.i. Before this time it is notable that the level fluctuated—first high, then falling, again rising even higher until it was significantly raised. After day 25 the level was well within the normal range with no such fluctuations.

In summary, the creatinine and creatine excretions of sublethally infected hamsters showed essentially similar alterations. Unlike creatinine which gradually decreased for the first two weeks, the creatine output was variable during this period. Both showed peaks at about the 18th day, followed by a diminished output until the 5th week, then a gradual rise to "normalcy".

Since these findings were at variance with other reports of elevated creatinine excretion during trichinosis, it was suspected that
Figure 8. Creatine excretion following infection
URINARY EXCRETION OF CREATINE

A in three lethally infected hamsters

B in sub-lethally

the stippled areas represent the mean control urinary creatine excretion plus or minus twice the standard deviation. Based on forty control (normal) readings.

--- 225 larvae

-.- 1,000 larvae
Perhaps the lowered excretion of this substance was brought about by the inactivity of infected animals (see Appendix II).

In order to test the effects of forced activity on the creatinine and creatine excretion of infected hamsters the following experiment was performed. Five revolving cages equipped to collect urine were used (see Methods and Figure 2). In these cages were placed 1 normal and 4 infected hamsters. These animals were forced to walk 250 meters (a considerable distance for a hamster) at the beginning of the 24 hour collection period. Another normal hamster was kept in a stationary cage (see Figure 1). The results of urinalyses of these animals are summarized in Figure 9. In this figure each point representing the results from experimental animals is actually the mean output of from 4 to 8 hamsters. The urine of the control non-exercised animal was "normal" throughout the experiment. Its results were, therefore, not tabulated.

It was found that exercise did not affect the creatinine and creatine excretion of normal animals, nor did exercise cause any noticeable increase of these substances in sublethally infected hamsters when compared with non-exercised infected animals (compare Figures 7, 8 and 9). The only striking difference was that the peak of creatine excretion of the exercised infected animals occurred about day 28 ± 2 while in non-exercised infected animals it occurred 10 days earlier. This may have been caused by some alteration in the life history or infectivity of the parasite, since this experiment was conducted several months after the experiment on excretion without forced exercise. (The parasite was two generations older, i.e., had passed through additional hamster and rat hosts.)
Figure 9. Creatinine and Creatine excretion after exercise
URINARY EXCRETION OF CREATININE AND CREATINE
AFTER EXERCISE

CREATININE

CREATINE

Days post infection
3. The Diazo Reaction: Seventy-eight urine samples from four sub-lethally infected and two control animals from the 6th through the 70th day p.i. were tested for the diazo reaction. All but five gave a negative reaction. This group of five (3 from day 7, one each from days 12 and 13 p.i.) all gave different reactions, i.e., the fluid was orange red. A true positive reaction according to Hawk et al. (1947) is supposed to be a deep red. The five reactions, therefore, have been considered questionable and it is concluded that the diazo test is not diagnostic of trichinosis in the golden hamster.

C. The Effects of Trichinosis upon the Adrenal Glands:

1. The Weight of the Adrenals: The adrenals of all golden hamsters studied were weighed on a torsion balance at autopsy. Unlike most other mammalian species but like the cottontail rabbit (Christian, 1953), the adrenal glands of male golden hamsters were heavier than those of the females of the same body weight (also described by Peczenik, 1944; Keyes, 1949; and Wexler, 1952) and generally the left adrenal was heavier than the right.

The method of expressing the adrenal weight was as the wet weight of both adrenals in milligrams divided by the body weight of the animal in grams. Plotting of the means of the control weights expressed in this manner against age of the animal yielded a nearly horizontal line in the case of the males whereas the female control data yielded a curve with a slight peak 3 months p.i. (when the animals were 5 months old). The females were virgin animals and this peak and subsequent decline may be related to their capability of reproduction. This, however, is only conjecture. A straight line correlation between weight and age has been
described in both male and female albino rats by Hatai (1913); and Cole
and Harned (1942).

Figure 10 summarizes the data on the adrenal weights of male and
female animals after infection. The Student's "t" test (Fisher, 1946)
was applied to all data for determining the significance of the differ-
ence of the control and experimental means. P equaling 0.05 was con-
sidered significant.

The adrenals of infected males show an initial decrease in weight
(not significant) followed by an increase over those of the control groups
and finally a gradual decline with the average weights slightly subnormal.
During the period 4 to 8 weeks p.i. the weights were significantly raised
above normal. Two weeks later, however, the weights were normal.

The adrenals of infected female hamsters do not present the same
drastic alterations. There was an immediate increase with a return to
normal by the 7th week. Five weeks p.i. the mean weight was significantly
elevated. It was significantly decreased 3 months p.i. Correlation of
the latter finding with the life cycle of the parasite is difficult since
the symptoms of the disease have abated 3 months p.i.

It should be noted that the body weight after infection is also
decreased.

2. Ascorbic Acid of the Adrenal: Evaluation of the data on quanti-
tative determinations of ascorbic acid was limited because of the paucity
of control data (18 males and 7 females). The control data (and the later
experimental data which are probably normal) seem to show that with increas-
ing age the amounts of ascorbic acid within the glands of female hamsters
Figure 10: Adrenal weight following infection.
Broken line represents mean weights of control animals.
ADRENAL WEIGHT
(MEAN)

WET WT.
BODY WT.

MALES

FEMALES

DAYS POST INFECTION
increases. The concentration of the vitamin is significantly less in males and is not altered with age.

The data are summarized in Table IV. It would seem that there was real alteration of the adrenal ascorbic acid only at one point following infection. Six weeks p.i. the concentration in one male and one female animal was significantly increased.

3. Adrenal non-specific Dehydrogenase Activity: Figures 11 and 12 summarize the observations on non-specific dehydrogenase activity in the adrenals of male and female hamsters. Again, the method selected to show the normal range of control values was to determine the mean and standard deviations and construct a band with a width equal to four times the standard deviation. In the course of statistical analysis it was found that the mean control for males (13 observations) was significantly less than that for the females (13 observations). The concentration of the enzyme, unlike the concentration of ascorbic acid, is unaffected by increased age of the gland (within the period of life studied).

The concentration of these enzymes in the adrenals of male hamsters was found to be altered only once following infection. Twenty-one days p.i. the adrenals of the two animals studied showed activities nearly double those of normal animals. Further experimentation is required to determine whether the increased activity was due to an increase in one, several, or all of the enzymes which possess dehydrogenase activity.

Most measurements of dehydrogenase activity of the adrenals of female hamsters also were well within the normal limits. The exceptions were single observations 21 and 28 days p.i. and the observations on
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Figure 11. Dehydrogenase activity of male adrenals

Figure 12. Dehydrogenase activity of female adrenals
ENZYME ACTIVITY (MALES)

ENZYME ACTIVITY (FEMALES)

DAYS POST INFECTION
adrenals 8 months p.i.. At these three times the results were significantly greater than normal. No explanation could be found for the elevated enzyme activity 8 months p.i. since controls run simultaneously exhibited "normal" activity. The causes of augmented enzyme concentration 3--4 weeks following infection will be considered in the Discussion.

For comparison Table V contains the results of observations upon the non-specific dehydrogenase activities of other organs in normal hamsters plus certain observations on mean activities of normal mouse organs measured by the same technique as reported by Black and Kleiner (1949).

4. Sudanophilic Lipids in the Adrenal: The adrenal glands of all hamsters studied presented the usual cortical zonations: an outer connective tissue capsule, a narrow zona glomerulosa, the cords of cells forming the zona fasciculata which merge almost imperceptibly with the cells of the narrow zona reticularis. Sinusoids were common in both the fasciculata and reticularis. The lipophbic zone (first described by Reiss et al., 1936 in the rat's adrenal and by Wexler, 1952, in the hamster's adrenal) between the glomerulosa and fasciculata was observed in adrenals of both sexes, infected and control of all ages (see Figure 13).

Formalin-fixed, frozen sections of normal hamster adrenals appeared to be stained moderately with Sudan Black B when observed grossly. Under oil, however, the cells of the glomerulosa and reticularis were seen to contain many small sudanophilic granules (10µ) (perhaps the lipid-rich mitochondria postulated by Alpert (1950). In addition, there was a distinct background "wash" suggesting ultra-microscopic sudanophilic substances. Sections from the adrenals of older control animals (10 to 12 months old) showed fine droplets and "spheroid complexes" (first described in adrenals
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(*data from Black and Kleiner, 1949)
of rats by Cain and Harrison, 1951) in the glomerulosa (see Figure 14) and larger droplets in the inner fasciculata and reticularis. These larger droplets approached the size of the nuclei in one 12 months old female and perhaps were a sign of fatty degeneration.

The adrenal cortices of infected hamsters showed the following cytological variations from the normal sections. Instead of fine granules, the reticularis zones of two females, five and seven weeks p.i., showed many large droplets. Two males, eight and nine weeks p.i., showed large droplets in the fasciculata. Six months p.i., there were large droplets in the glomerulosa and very large droplets in the reticularis of one female (T-1, see below). As was observed in control sections from older animals, the glomerulosa of older infected hamsters contained numerous granules and spheroids, and larger droplets in the fasciculata and reticularis.

An attempt was made to quantitate roughly the lipid content of the cells from the different zones at various stages p.i. (see under Methods). Sections from control animals were also studied in this manner. The results are summarized graphically in Figures 15 and 16. The results obtained from sections of both control and experimental glands were so widely divergent that it was impossible to apply any statistical tests to the data. The following points can be noted: of the three regions, the glomerulosa had the most dense concentration of sudanophilic material; the fasciculata (with the exception of one female, T-1), the least. The concentrations of sudanophilic lipids in fasciculata and reticularis remained constant with perhaps a slight increase in the older animals studied. With increasing age, however, the zona glomerulosa of both infected and control animals
showed a steady increase in the concentration of sudanophilic lipid (indicated by the trend lines, the equations of which were derived by the method of least squares). The glomerulosa of male adrenals contained a slightly greater concentration than that of the females of the same age.

The author agrees with Rogers (1942) that the sole reliable indication of the status of an infected animal is its weight at time of sacrifice as compared with its weight at time of infection. The more weight lost, the greater the intensity of the disease. No correlation could be found between the amount of lipid and the severity of infection measured by weight changes. A study of the data from animals considered severely infected shows that in most cases the lipid was slightly more concentrated than in control or in infected animals, with but slight or no weight loss. For example, the high lipid concentrations in the glomerulosa of individual female animals weeks five and six could not have been caused solely by severe infections, since only the female hamster from week five (animal #5-5) was severely affected by the infection. (The female from week six exhibited mild symptoms.) The concentration of lipid in the fasciculata of animal #5-5, however, was much greater than that of any other young infected or control animal. One female three weeks infected was very ill, yet showed an average lipid content in all zones. The only severely infected male, whose adrenal lipid content was observed six weeks, showed a greater than average lipid content. A male, four months, however, showed a still greater concentration, yet had apparently completely recovered. At no time following infection was there observed a decrease in the concentration of sudanophilic lipids.
Figure 15. Sudanophilic lipid content of adrenals from infected male hamsters

Figure 16. Sudanophilic lipid content of adrenals from infected female hamsters
5. Alkaline Phosphatase: The most conspicuous difference between adrenal sections was the striking concentration of alkaline glycerophosphatase in adrenal sections from male animals of all ages, both control and experimental. Females demonstrated only very slight activity.

The normal male hamster adrenal showed activity in the fibrous connective tissue cells of the capsule. The nuclei of the subcapsular cells were positive. There was slight cytoplasmic staining in addition to nuclear staining in the glomerulosa proper. The cytoplasm and nuclei of the fasciculata were stained very intensely, the cells of the inner fasciculata having the greatest concentration of the enzyme. The nuclei of cells of the zona reticularis also were stained. Older animals also showed cytoplasmic staining in this zone. The staining reaction of the medulla was variable, with the nuclei sometimes showing enzyme activity. The nuclear activity here was confined chiefly to the nuclear membrane and perhaps is an artifact caused by the diffusion of the enzyme before fixation or of the calcium salts during the staining procedure (Martin and Jacoby, 1949; and Feigin, Wolf and Kabat, 1950).

In most cases the activity of the normal female adrenal was confined to a slight reaction of the capsular connective tissue and the nuclei of the glomerulosa and fasciculata.

Infection with *Trichinella* did not markedly influence the concentration or distribution of alkaline glycerophosphatase. The adrenal sections from infected male hamsters were similar to control sections except for the following: One animal sacrificed one week p.i. had a greater concentration of cytoplasmic alkaline glycerophosphatase in the fasciculata cells than did any control animal. Another male, 8 weeks p.i., showed cytoplasmic staining of the reticularis.

The sections from infected females also resembled those from control
females. One female infected two weeks previously showed augmented activity in the glomerulosa. In only one case, that of a four month infection, was there any enzyme activity in the cytoplasm, consisting of slight activity in the reticularis.

6. Birefringence: Formalin-fixed, unstained frozen sections mounted in glycerine jelly were studied for birefringence activity. Birefringence was observed primarily in the cells of the inner fasciculata except in older animals, especially old females, where the nature of the birefringence changed from finely-particulate, faintly-birefringent crystals in the inner fasciculata to large birefringent crystals distributed throughout the fasciculata and the reticularis (see Figure 17).

In view of the supposed relationship between lipids and birefringence (see Introduction) the data collected from both the birefringence material and the Sudan-stained material have been summarized in Table VI. Since birefringence activity was confined to the fasciculata, only the sudanophilic activity of the fasciculata was tabulated.

This table shows the overall lack of correlation of the birefringence reaction and the sudanophilia of sister sections from the same glands. Statistical analysis of this data by the chi-square test also shows no correlation.

D. Recapitulation: Because adrenal studies were confined to animals sublethally infected, only the results from sublethal infection upon the urine of hamsters will be summarized.

The urine of infected animals showed an initial polyuria followed by oliguria (by day 24 p.i.). The volume returned to "normal" by day 65.
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COMPARISON OF BIREFRINGENCE AND LIPID ACTIVITY
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The pH of the urine in most cases was unaffected by the infection. The UA/C showed peaks 7, 15, and 23 days p.i. It returned to "normal" by day 65 p.i.

The uric acid output was increased following infection. The creatinine output fell simultaneously. After an initial increase, the output of creatine fell. All three urinary components were "normal" by day 65.

The excretion of creatinine and creatine by infected animals was not elevated by forced exercise.

The diazo reaction was found to be an unreliable diagnostic test for trichinosis in the hamster.

The following were normal values obtained from hamster adrenals:

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Weight (wt/wt./ body wt.)</td>
<td>0.22</td>
<td>0.15-0.27</td>
</tr>
<tr>
<td>Ascorbic Acid (mg./gm. wet wt.)</td>
<td>1.19</td>
<td>0.80-1.54</td>
</tr>
<tr>
<td>Dehydrogenase Activity (colorimeter reading/dry wt. (mg.))</td>
<td>103</td>
<td>70-159</td>
</tr>
</tbody>
</table>

The weights of the adrenal glands of males and females were altered by infection but not similarly.

Ascorbic acid concentration was significantly raised 42 days p.i. In both sexes the concentration of this vitamin in the infected adrenal was elevated over the normal values.
Figure 17: Birefringence activity in old hamster adrenal
Two and three weeks p.i., the dehydrogenase activity of the adrenal glands was significantly raised.

The concentration of Sudan stained adrenal lipid droplets was slightly greater in the more severely affected cases than in control or less severely infected animals.

In two cases, 1 and 2 weeks p.i., the alkaline phosphatase reaction was slightly more intense.

Birefringence activity was unaltered by the infection.

With increasing age the adrenal glands from normal hamsters showed the following changes. In females, the ascorbic acid concentration was increased and the birefringence activity became more striking; in males the birefringence activity was similarly altered. Sections of adrenal glands from older animals also showed that the quantity and nature of the sudanophilic substance(s) was altered as a result of the ageing process.

DISCUSSION

In order to appreciate the time sequence of the metabolic and anatomical alterations induced by trichinosis, the life cycle of the worm in the golden hamster should be considered. Details of the life cycle are not completely known. Boyd and Huston (1952) reported upon the survival of adults in the intestine following infection with 100 and 500 larvae. In the former case all adults had been eliminated by the 15th day p.i., whereas those from the heavier infection survived longer. These investigators reported, however, a rapid loss of adults (83-93 percent lost)
by the sixth day p.i.. Humes and Akers (1952) working with an inoculum of approximately 1,000 larvae found that after the larvae are ingested, they quickly mature and the adults can be found in the small intestine and cecum of the hamster from the first day to the 34th day p.i. The larvae were recovered from the blood beginning on the third day and ending on the 22nd day after infection. The larvae were first observed invading the muscle fibers of the cheek pouch on the 16th day and were coiled by the 19th day.

The first report of urine volume alterations in experimental trichinosis was that of Flury and Groll (1913). They observed in experimentally infected dogs, cats and rabbits that the initial diuresis was followed by oliguria during the stage of muscle invasion. Human trichinosis presents a different picture. In man, Gould (1945), reported oliguria associated with edema of the muscles and sweating during the intestinal and muscular phases. Following the febrile stage there is a marked diuresis. The experimental animals above and the hamster, however, unlike man, cannot lose water through sweating. The hamster produced excessive amounts of urine up to day 24 p.i., i.e., about a week following the initial invasion of the muscle. The onset of oliguria may have been due to decreased water intake of the infected animals but other experiments (see Appendix II) show that infected hamsters become lethargic 6 days after the onset of oliguria (day 30 p.i.). No data are available on water consumption but it seems probable that the water consumption would be diminished only after
day 30. The oliguria before this time may have resulted from hydration of the invaded muscle tissue as Rogers (1942) found in trichinosis in the rat.

The only report of any alteration in urine pH of experimentally infected animals was that of Rogers (1942) who found an increase in the pH in two rats. He neglected, however, to include the data. The finding of lowered pH in certain of the hamsters (attributed to an acidotic state accompanying the altered assimilation of food) is at variance with Roger's report.

The UA/C ratio of lethally infected hamsters was significantly increased on day 7 p.i. probably because of two factors—the damage to the intestine and cecum caused by the adults and the initial wave of larvae in the blood stream (or the toxins, if any, liberated by the larvae or the damaged tissues). After day 10 the UA/C ratio steadily increased as the animals became sicker.

The alterations of the UA/C ratio of sublethally infected hamsters seemed to be dependent upon the number of larvae ingested—more reaction followed infection with 225 than 1,000 larvae. The elevation of the UA/C ratio was found to be directly proportional to the stress undergone by the animal since the animals infected with the lesser number of worms lost more weight and generally appeared more ill. The paradoxical situation of a greater infection resulting from a smaller inoculum of Trichinella larvae has also been reported by McCoy (1940) in the rat. He considered that the situation was due to fewer larvae maturing in the intestine probably because the greater irritation to the intestinal mucosa following
heavy infections adversely affects the ability of the larvae to become established. Nolf and Edney (1937) studied larval production in rats given graduated numbers of larvae. If the hamster's gut were similar to that of the rat as an environment for Trichinella, infection with 1,000 larvae would lead to a population of larvae in the muscles amounting to approximately 115,000 worms. Infection with 225 would produce 16,000 larvae. This obviously is not the case.

An intermediate dose of 500 larvae was a lethal dose in 90 per cent of the cases studied. (Beahm and Downs (1939) found that the lethal dose for rats was 900 larvae, and Rogers (1942) found 2,100 larvae to be a lethal dose although he thought his worms had a low viability.) Boyd and Huston (1952) used 500 larvae as one of the standard dosages in their study of hamster trichinosis. They, however, fed larvae encapsulated in diaphragm tissue, whereas in the present study larvae digested from the muscle were used. It may be that the herbivorous hamster cannot completely digest meat, and the larvae were not freed from the capsules and were, therefore, quickly eliminated. This would explain their finding that 83 to 93 per cent of the adults were lost by day 6 p.i. Another alternative is that the worms grown in this laboratory were more virulent (see under Methods).

In summary, 225 larvae can be considered a good stressing dose, 500 larvae a lethal dose, and 1,000 larvae, because of the attendant intestinal mucosa damage and development of fewer adults, less stressful than a dose of 225 larvae.
Following sublethal infection the curves of the daily UA/C ratios were elevated at one week p.i. and for an extended period beginning four weeks p.i. The first peak was probably attributable to the effects of the larvae in the blood stream and adult damage to the intestinal mucosa; the second to the lysis of the muscular tissue. Infection with 225 larvae was followed by an additional peak in the UA/C ratio at about 15 days p.i. The cause(s) of this elevation is not clear but may have been due to the effects of muscle fiber penetration by the larvae.

Of interest (but probably of little significance) are the facts that the two antibodies, the antiadult and antilarval antibodies, produced by the host in response to the parasite appeared in the rat's blood, according to Oliver-Gonzalez (1941) at about the same times as the peaks in the hamster's urinary UA/C ratio. The antiadult antibody was found in rats blood beginning on the 15th day and its titre was highest from 25 to 35 days p.i. It disappeared by day 50. The antilarval antibody first appeared on the 30th day (also several weeks after the introduction of the noxious agents into the blood stream) and its titre was highest 45-60 days p.i. Immune reactions then are probably occurring throughout the period when the UA/C ratio is grossly altered.

The return of the UA/C ratio to normal values about 65 days p.i. indicated the beginning of the recuperative or convalescent stage. Other corroborative evidence that day 65 marks the onset of the convalescent stage is that infected animals began gaining weight at this time, most of the differential counts returned to "normal" at this time (see Appendix I), the activity of infected hamsters also returned to the pre-infection level,
and various skin changes occurred (see Appendix III).

The alterations of the UA/C were caused by elevation of the uric acid output and decrease in the output of creatinine. As metabolic products these two substances are probably independent of each other, the former being a product of purine catabolism, the latter resulting by an as yet unknown process but certainly not from purines. The composition of the diet was constant. Any changes in excretion of these two substances, therefore, would be due either to endogenous alterations or to starvation.

Elevation of the urinary uric acid level following trichinosis could have been due either to extensive cellular destruction or to the effects upon tubular reabsorption of urate brought about by the liberation of excess ACTH in response to the stress. Evidence is presented in Appendix I of excessive blood lymphocyte destruction, a rich source of nuclear (purine rich) material. Furthermore, the first indications of augmented uric acid excretion coincide with the initial phases of the muscle invasion. The question is then raised as to why the uric acid output remains high while the creatine output is subnormal after day 25 P.I.? If the increased uric acid output were brought about exclusively by muscle and lymphocyte destruction, then it would be expected that creatine (and its end-product, creatinine) would also be excreted in excessive amounts. Creatinine and creatine are not elevated along with the uric acid. The augmented excretion of uric acid then must be attributed mostly to the increased secretion of ACTH.

Increased creatinine excretion has been reported in human trichinosis (Wohl, 1916; Burger, 1922; Markowicz and Bock, 1931) and in experimental trichinosis in dogs and cats (Flury and Groll, 1913), and in rats...
(Rogers, 1941).

Decreased output of this substance was reported by Flury and Groll (l.c.) in experimental trichinosis of the rabbit. Both sublethally and lethally infected hamsters also have a diminished output although excessive muscular catabolism must be occurring. This catabolism would contribute to an increased output. Perhaps the diminished absorption of exogenous creatinine and its precursors negated the increases due to catabolism. If the hamsters were suffering of malnutrition, however, the creatine output would probably be elevated (Hawk et al., 1947) provided its metabolic pathways were similar to man’s. An infected man’s diet cannot be rigidly controlled and the augmented excretion of creatinine in human trichinosis may be due partly to an increased exogenous creatinine supply. Exercise seems to have no effect upon the excretion of either creatinine or creatine by the infected (or control) hamsters. There seems to be no way of reconciling the findings by other authors in experimental and clinical trichinosis with those in hamster (and rabbit) trichinosis. The answer may be one of species difference.

The creatine excretion curve of sublethally infected animals, following a significant peak on day 19, quickly fell to normal. The increased production of creatine coincides with the penetration of the muscle fibers by the larvae. This penetration, however, is followed by an extended period of muscle fiber destruction (Gould, 1945), yet the creatine excretion curve does not remain elevated as would be expected. The entire explanation of these alterations in creatine and creatinine excretion following infection requires further investigation.
The following factors must be taken into account in an analysis of the effects of any stress upon the adrenal glands: (1) the intensity of the pituitary stimulation, (2) the duration of this stimulation, and (3) the time during or after pituitary stimulation at which the adrenals are analyzed. Infection with 225 larvae is followed by a series of destructive waves which probably result in a moderately severe continuous stimulation, with the greatest stimulation (if one accepts the U6/C ratio as a valid measure of ACTH secretion) during the period from 15 to 50 days p.i. Theoretically, an adrenal gland extirpated from an infected animal during this period should present evidence of moderate stimulation, e.g., hypertrophy, depleted cholesterol (birefringence?), and depleted sudanophilic lipids because of the continuous demand for the cortical hormone(s) (Sayers et al., 1944 et seq.).

The alterations in the cortical components listed above would last only until adrenal adaptation occurred. Such a type of stress would bring about specific adaptations since the stress, though constant and continuously applied, would induce less and less change in the internal environment. The requirement for cortical hormone(s) would be reduced and the gland would return to a pre-stress state of functional activity; the chemical constituents restored to normal concentration or even stored in excess.

Hypertrophy of the adrenal cells is an indication of increased secretion (Zwemer, 1936). Pinchot, Close and Long (1949) studied the adrenals of rats infected with tularemia (which they considered a long-term stress). They found the adrenal weight to be increased to 2 1/2 times that of normal. In the present investigation it was found that the adrenal weights of both male and female hamsters were increased following infection. The mean weights of males remained higher than the mean of controls for 100 days, i.e., until long after the stress had been ter-
minated. The means were significantly higher than the means of control weights during the period 4 to 8 weeks po.i. (the period of greatest stress) and terminating convalescence. The weights of adrenals from females were greater than control weights for only 42 days. Thereafter the means of experimental weights were less than control weights and at one time (90 days po.i.) their mean was actually significantly less than the mean of the control weights. This difference in the response of the males and females is probably correlated with their ability to withstand stress. Female hamsters according to Wexler (1952) react more quickly to a stress, and are more hardy. In the present work the mortality rate was higher and the weight losses more drastic in males, and, following lethal doses of worms, the females lived longer. McNaught, Beard and DeEds (1939) found strong evidence of a sex variation in the susceptibility of rats. Females were found to have a higher resistance as indicated by the number of encysted larvae found after feeding standard doses of larvae.

If adrenal weight changes per se. are indicative of the intensity of stress and adaptation to the stress, then male hamsters are stressed more and require a longer period to adapt to the parasite than do females. Yet there was no difference observed in the UA/C alteration following stress in males and females.

Pinchot et al. (1949) and Sayers and Sayers (1949) warn that the determination of ascorbic acid in a prolonged stress is not a reliable index of the functional state of the adrenal. Pinchot et al. (l.c.) found that the ascorbic acid of the adrenal of rats following infection with _P. tularenses_ was variable in concentration. Merten and Smith (1936)
found a decrease in adrenal ascorbic acid in children dying of childhood diseases. Their results, however, may have been due in part to post mortem changes or may be related to the inability of man to synthesize this vitamin. The rat and the hamster according to Cooperman et al. (1943) do not have to rely upon exogenous ascorbic acid.

The ascorbic acid concentrations of infected glands, instead of being reduced following infection, were in most cases elevated, significantly so in two cases (one male and one female), 42 days p.i. Alpert (1950), working only with male hamsters, found that ACTH injection (an artificial short-term stress) caused a reduction, then rise, then reduction in ascorbic acid. The reduction, however, was not marked and the male hamster's adrenal was not easily stimulated by ACTH. This sequence was not observed in hamsters with trichinosis.

The values for adrenal ascorbic acid obtained for normal male hamsters were found to correspond closely with those reported by Alpert (1950) and Wexler (1952). The values for normal females reported by Wexler (l.c.) were much lower than those reported in Table IV.

Zweifach et al. (1951) reported that stress caused a decreased formazan production in the fasciculata and reticularis of the rat. All observations upon adrenals from infected hamsters with the exception of four observations of increased activity, 21-28 days p.i., were normal. The adrenals of the four hamsters with the augmented dehydrogenase activity were probably secreting hormones more actively, as would be expected at this stage of the stress. The reduction in enzyme activity, i.e., the return of hormone production, to normal following day 28 may be correlated
with the development of adaptation on the part of the adrenal to the stress (see below).

When compared with that of the rat, the hamster's adrenal was found to be "lipid poor", a term applied to this tissue by Koneff et al. (1946), Alpert (1950) and Wexler (1951). Leathem and Stauber (1952) reported an occasional "tinge" of sudanophilic lipid in the adrenals of their control hamsters (8?) which they attributed either to individual variation or to the seasonal alteration reported by Kayser and Aron (1950). Since their experiments lasted for a period of two months and involved only male animals, the latter explanation can probably be eliminated. Sections from normal adrenals from both male and female hamsters (also collected during a two month period) were found by the present author consistently to contain an appreciable quantity of lipid material. However, unlike the mouse (Whitehead, 1933 and Dalton, 1944) where the lipid concentration decreases with ageing, the hamster's adrenal lipid was found to increase with age. This was most evident in the glomerulosa but also seen in the other two zones. Koneff et al. (1946) found Sudan Orange stainable material only rarely in the zona reticularis of the hamster.

Alpert (1950) claimed that the male hamster's adrenals do not contain typical sudanophilic lipid. The fine sudanophilic granules observed he attributed to lipid-rich mitochondria. He, however, worked with relatively young animals. Sections from adrenals of year-old hamsters would be difficult indeed to distinguish from normal rat adrenal tissue, in that they contain larger droplets, spheroids, etc., characteristic of "typical sudanophilic lipid".
No real difference was observed between the sexes with respect to the amount of sudanophilic lipid in the adrenal cortex. The adrenals of males may have had slightly more lipid material in the glomerulosa. In contrast, both Whitehead (1933) and Dalton (1944) found that the adrenals of female mice contained more lipid than those of males.

The suprarenal glands of children who died following infections of long duration were depleted of lipid. The kind of organism causing the infection, its virulence, and its numbers were unimportant (Menten and Smith, 1936). If the hamster's adrenal responded similarly to that of the young human being and rat (Sayers, 1950), an intense continuous stress such as trichinosis would probably lead also to depletion of the sudanophilic substance. Zwemer (1936) postulated that the amount of lipid within a cell was inversely proportional to its secretory activity. Leathem and Stauber (1952) noted a "tinge of lipid" in male hamster adrenals 2 weeks p.i. with Leishmania donovani and the presence of fine or large lipid droplets (stainable with Sudan IV and Sudan Black B) in the reticularis and fasciculata six weeks p.i. Heavily infected hamsters that survived eight weeks exhibited an enhancement of the lipid throughout the reticularis and fasciculata. No mention was made of the glomerulosa lipid.

In adrenals from trichinized hamsters no correlation was found between the amount of lipid and the severity of infection as measured by weight changes. Data from animals considered severely infected showed that in most cases the lipid was slightly more concentrated than in control or infected animals with but slight or no weight loss. Neither in-
creased nor decreased were noted in sudanophilic lipids during the initial and recuperative stages of the disease. During the period five to seven weeks p.i. the reticularis and fasciculata zones of two females showed large lipid droplets. Eight and nine weeks p.i., two males showed large droplets in the fasciculata. These alterations, it must be noted, came after the onset of stress as determined by the UA/C ratio. No gross changes were noted in the lipids of the glomerulosa as a result of infection.

The finding of increased sudanophilia in the fasciculata and reticularis of trichinized hamsters only after a considerable period of delay is similar to the findings of Leatham and Stauber (1952) in Leishmania-infected hamsters. These authors thought the sudanophilia may have resulted from prolonged ACTH release. Knouff et al. (1941) warned that the interpretation of changes in the sudanophilic droplets was difficult in that differential lipid changes in various lipid fractions may occur without altering the total lipid content. During the stage when the UA/C ratio is elevated such changes may occur in certain fractions of the lipids (the hormones or their precursors) which are coalesced to form the fine sudanophilic droplets of the hamster's adrenal. There would result no gross change in the droplets. As specific adaptations are made, the stress would induce less secretion of ACTH and the adrenal would return to a pre-stress state of functional activity. A restoration of the chemical constituents to normal concentrations would follow and there might even follow a stage of excess storage of these substances (Greep and Deane, 1949; Sayers, 1950). This perhaps is the explanation for the appearance of the larger sudanophilic droplets and the increased ascorbic acid con-
centration long after the stress had been initiated.

Since the exact chemical nature of birefringent material is unknown, but may be cholesterol, it would be pertinent to consider other reports of this substance in the hamster's adrenal. Alpert (1950) could not demonstrate cholesterol histochemically in the hamster adrenal except in one old male. The birefringent material in both trichinized and control hamsters was exceptionally faint if the adrenals were from younger animals but the granules became coarser and more dispersed with age. Coarser granules are considered to be characteristic of inactivity (Deane and Greep, 1947). The presence of faintly birefringent material in the adrenal before "senility" probably represents stored material and is probably not concentrated enough to give a positive cholesterol reaction (if it could). Leathem and Stauber (1952) reported increased hamster adrenal cholesterol 4 to 8 weeks following infection with Leishmania. The reason for this increase is probably the same as that offered for the increase in sudanophilia. Trichinosis did not alter the birefringent material consistently. The study of hamster adrenal birefringence following infection was found to be of little value.

In the present study no evidence of consistent alteration of adrenal alkaline glycerophosphatase in either male or female adrenal cortices was found following infection with Trichinella. Alpert (1950) first described the distribution of this enzyme in the male hamster adrenal. It is located in the cytoplasm and nuclear material of the outer half or two thirds of the fasciculata. The nuclei of the other regions are faintly stained.
The present study corroborates these observations. The finding of a greater enzyme activity in glands of older normal male hamsters is similar to the finding of Kochakian and Fox (1944) in older male mice. In addition, the adrenal cortices of females were also subjected to the histochemical procedure for alkaline phosphatase. It was found that the adrenal glands of female hamsters, like those of Swiss mice (Elftman, 1947) and Sprague-Dawley rats (Dempsey et al., 1949), contain only small quantities of the enzyme.

The increased enzyme concentration observed in a pair of infected animals during the first two weeks p.i. might indicate an increased synthetic activity in the adrenal. The lack of any consistent alteration in adrenal alkaline phosphatase following trichinosis was unlike the findings of Leathem and Stauber (1952) who found increased concentration of this enzyme in adrenals from male hamsters infected with leishmaniasis beginning with the fourth week p.i.. They found in the few hamsters studied that only those with augmented sudanophilic lipids showed this increased phosphatase reaction.

The mobilization of cortical alkaline phosphatase may be mediated by the hypophysis (Elftman, 1947). Alpert (1950) injected hamsters with ACTH for 12 days but could not produce any change in the adrenal alkaline phosphatase. Leathem and Stauber (1952) expressed the idea that had Alpert continued his injections of the hormone he might have induced changes. Since the present investigation was similar to that of Leathem and Stauber except for the parasites involved and no consistent change could be induced by trichinosis, the present author tends to agree with Alpert that the
hamster's adrenal is not easily stimulated (by visual standards) by ACTH release and that the changes observed by Leathem and Stauber were probably not caused by increased adrenocortical secretion but rather by a staining artifact caused by long incubation of sections from a very small number of animals or to a specific reaction of this adrenal enzyme to leishmania bodies or their metabolic products.
SUMMARY AND CONCLUSIONS

1. The golden hamster, *Mesocricetus auratus*, was found to be easily infected with *Trichinella spiralis*. The strain of worm developed in this laboratory was found to be 100 per cent infective for the hamster. The number of larvae capable of producing death was 500, whereas 225 and 1,000 larvae were found to produce a sublethal infection. The reasons for this apparent paradox are discussed. Lethally infected hamsters died during the 4th week post infectionem (p.i.).

2. Details of the construction and operation of two metabolism cages of novel design, one stationary and the other an "exercising wheel" type are given.

3. Techniques are described for the handling of small volumes of an opaque urine such as is produced by the hamster.

4. Arching of the back was the most common external symptom of trichinosis in the hamster.

5. Emaciation of the host usually accompanies infection. It is postulated that the emaciation was caused by the inability of infected hamsters to swallow the Lab Chow.

6. Under the conditions of the experiment, the following were found to be normal values (range in parentheses) for hamster urine:

   Volume: 8 ml. (0.5 - 25.0) 
   pH: 7.25 
   Uric Acid: 10/g. / day / g. (4 - 15) 
   Creatinine: 35/g. / day / g. (17 - 54) 
   Creatine: 12/g. / day / g. (0 - 30) 
   Uric Acid / Creatinine Ratio: .27 (.13 - .41)

7. The diazo reaction was found to be ineffective as a diagnostic test
for trichinosis in the golden hamster.

8. Following infection, there is an initial diuresis lasting the first three weeks, followed by oliguria which lasts until about day 65 p.i. The urine volume then returns to normal.

9. The uric acid/creatinine (UA/C) ratio was found to be a good indicator of the general health of the animal. An elevation of the value of the ratio was directly correlated with the "stress" undergone by the animal. Various stages in the life cycle of the parasite in the host caused striking alterations in the UA/C ratio.

10. The elevation of the UA/C was found to be caused by an increase in the output of the uric acid and a concomitant decrease in the creatinine output. The increased uric acid production was theoretically caused principally by an increased secretion of adrenocorticotrophic hormone in response to the stress. The entire explanation for the decrease in output of creatinine and creatine requires further investigation.

11. Forced exercise (a "brisk" walk of 250 meters) did not increase creatinine and creatine output of normal or sub-lethally infected hamsters.

12. The average normal male hamster adrenal was significantly heavier, contained significantly less ascorbic acid (per mg. wet weight), significantly less non-specific dehydrogenase activity (per mg. dry weight), perhaps a little more sudanophilic substance (chiefly in the glomerulosa) and a much greater alkaline phosphatase reaction than the average normal female hamster adrenal.

13. With increasing age, there was a noticeable increase in the adrenal sudanophilic lipids, birefringence and a slight increase in ascorbic acid.
No effect of age was noted in the weights of adrenals from male animals. The adrenals of females were heaviest when the animals were 5 months old.

14. Trichinosis caused an increase in adrenal weight, increased ascorbic acid, and initial increases in alkaline phosphatase and dehydrogenase activity.

15. It was found that the UA/C ratio was a more exact measure of stress than any of the histochemical or biochemical changes of the adrenal which were studied. Certain correlations, however, were noted. The immediate increase of alkaline phosphatase and dehydrogenase enzyme systems plus the hypertrophy of adrenal glands following infection were indicative of increased secretory activity. The increase of ascorbic acid and lipid a considerable period after the onset of the stress is probably correlated with excess storage of these substances indicating adaptation on the part of the host to the presence of the parasite.

16. The histophysiology of the hamster adrenal was found to be slightly different from that of other animals. The lipid droplets evidently are not easily depleted (at least by this type of stress) nor is the ascorbic acid concentration immediately affected by trichinosis. The normally faint birefringence was not affected nor was the weight of the adrenal drastically affected. Two enzyme systems, however, were found to be immediately affected, alkaline phosphatase and non-specific dehydrogenases. The idea is advanced that perhaps measurement of the activity of these two "substances" following stress might be fruitful in the determination of the general metabolic and secretory rates of the adrenal.
APPENDIX I

Hematological Changes in Trichinized Golden Hamsters

Introduction

Since the report of Brown (1898) describing the elevation in the number of eosinophil cells of human blood following infection with *Trichinella*, many authors have reported a similar eosinophilia following experimental trichinosis of laboratory animals. Elevated eosinophil cell percentages were found in the guinea pig (Opie, 1904; and Hamann, 1943), the dog (Bittner, 1913; and Beahm and Jorgensen, 1941), the pig (Bachman and Rodriguez-Molina, 1933; and Maass, 1933), and the rabbit (Wantland, 1937). Rats experimentally infected, however, show at most a very slight eosinophilia (Van Someren, 1938; Beahm and Downs, 1939; Hamann, 1943; and Larsh and Nichols, 1949). There have been conflicting reports concerning the blood picture of trichinized mice. Larsh and Nichols (1949) failed to demonstrate an eosinophilia, whereas Stein (1949) reported eosinophilia and used the depletion of these cells following injections of adrenocortical hormones and adrenalin as a bioassay of adrenocortical activity. The experiment reported below was undertaken to study the effects, if any, of trichinosis upon the blood picture of the golden hamster.

Methods

The technic employed for the collection of blood was as follows: a hamster was lightly anaesthetized with Veterinary Nembutal Abbott (dosage 7.2 mg. per 100 g. body weight), the hairs of the tail and base of the tail
clipped close to the skin with fine scissors, and a transverse cut made in the median dorsal portion of the tail with a sharp, heavy razor blade. This usually produced a free-flowing wound which would deliver at least 3 drops of blood.

For the study of the alterations of the differential count in trichinosis, eight animals (4 males and 4 females) were infected with the sub-lethal dosage of larvae (225 worms). Two animals, one male and one female, were used as controls. The days post infection (p.i.) upon which blood was drawn were: day 0 (just prior to infection), 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 30, 33, 39, 42, 45, 48, 52, 56, 60, 65, 70, 75, 85, 100.

The smears were stained with freshly prepared Wright's stain and differentiated with McJunkin-Haden solution. At least 200 cells were counted (under oil) from each smear.

Total eosinophil and leucocyte counts were made from blood samples from similarly infected animals which were to be sacrificed for the study of their adrenal tissue (see above).

Two days prior to sacrifice all animals were lightly anaesthetized, tails cleaned and blood drawn. The age groups studied and the number of animals in each group are summarized in Table VII.

In order to eliminate the effects of any variations in the physiological or morphological status of the blood elements brought about by activity, etc. (Carnicero, 1944; Holberg and Visscher, 1950; and Tatai and Ogawa, 1951), all animals were bled between 7:00 and 11:00 P. M.,
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Leucocytes</th>
<th></th>
<th>Eosinophils</th>
<th></th>
<th>% Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>range</td>
<td>mean</td>
<td>range</td>
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<tr>
<td>1 week control</td>
<td>10</td>
<td>4425</td>
<td>1550-7500</td>
<td>40</td>
<td>15-125</td>
<td>0.9</td>
</tr>
<tr>
<td>1 week</td>
<td>9</td>
<td>5260</td>
<td>3100-7600</td>
<td>40</td>
<td>0-85</td>
<td>0.8</td>
</tr>
<tr>
<td>2 weeks</td>
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<td>3700-6650</td>
<td>148</td>
<td>65-205</td>
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<td>3 weeks</td>
<td>9</td>
<td>6540</td>
<td>2300-14200</td>
<td>85</td>
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the most active period in the hamster's day (see Appendix II).

The eosinophil counts were made according to the technique of Speirs as found in the directions supplied with the Speirs-Levy Eosinophil Counting Chamber (Clay-Adams Co.). The diluent was composed of the following substances: 10 drops 1 per cent aqueous phloxine, 5 drops diethylene glycol, 22 ml. distilled water, 2 drops 1 percent alconox (wetting agent) and 4 ml. acetone (added just before use). The blood and diluent were mixed in a white-cell pipette for a period of 10-15 seconds. The first 4 discharge drops from the pipette were discarded and then the fluid was admitted into two chambers of a Speirs-Levy Eosinophil Counting Chamber.

The absolute leucocyte counts were made by the usual technique—the diluent being Turk's solution. In all cases two chambers were counted.

Results

Alterations of the differential counts of the infected hamsters are summarized in Figures 18 and 19. Cells bearing eosinophilic granules were affected by the presence of Trichinella in the host. Beginning about day 13 p.i., the numbers of these cells increased, reaching a peak from 16 to 19 days p.i., and returning to normal about a week later. The intensity of eosinophilia was not correlated with the intensity of the infection as measured by body weight losses.

In the normal hamster, the lymphocytes outnumber the neutrophils approximately 2 to 1. Four to 12 days p.i. this relationship becomes altered so that the neutrophils become dominant. A second marked decrease in the lymphocyte number was observed about 40 days p.i. The neutrophils remain The neutrophils remain dominant for varying periods returning to within the normal range as early as day 20 or as late as day 70 p.i. No correlation between the intensity of the infection (body weight loss) and the dis-
Figure 18. Alterations in the blood differential percentages in four infected hamsters
Figure 19. Alterations in the blood differential in four infected and two control hamsters.
turbance of the lymphocyte-neutrophil relationship was observed.

A slight leucocytosis was observed in several animals during the second week p.i. Animals considered heavily infected, however, during the critical stage of the disease, i.e., during the 4th to 6th week, showed a slight leukopenia. Data on the absolute leucocyte and eosinophil counts of all animals are summarized in Table VII.

The absolute number of eosinophils was raised during the 2nd week and the numbers remained elevated until the 4th month. Division of the absolute eosinophil number by the absolute leucocyte count ought to produce a quotient equal to the differential eosinophil percentage. Treatment of the data in this manner was attempted. The highest percentage of eosinophilia, however, was 5.2 per cent which was actually only one fourth of the highest percentage found in stained smears. The highest value obtained from control data was 2.7 per cent and all quotients derived from control data were "normal" by differential count standards.

Discussion

The hamster like other mammals has only a limited number of blood cell types with which to defend itself against an infinite variety of noxious agents in the environment. In trichinosis, the eosinophil is the cell type that characterized the response of the hamster to this etiological agent. The function of the increased numbers of eosinophils has been postulated by Bachman (1938) to be one of resorption of disintegrated worm products while the neutrophils combat invasion by active phagocytosis and digestion of the foreign bodies. This explanation falls down when one considers
that the first signs of eosinophilia in the peripheral blood appear almost coincidentally with the appearance of larvae in the blood (Humes and Akers, 1952). It is difficult to conceive of the presence of disintegrated worm products at this early time. Another possible explanation would be that the histamine liberated from tissue destroyed by the migrating larvae and the adults in the gut raises the blood histamine level and the eosinophils act to remove this material from circulation. Hamann (1943) found increased titres of blood histamine in trichinized rats and guinea pigs. Kirk (1942) reported that there is a high concentration of histamine in the eosinophil granules. Whether the histamine has been phagocytized by the blood eosinophils or is an indigenous elaboration product of the cell requires further investigation. The exception to this hypothesis is the finding by Hamann (1943) that there was no consistent correlation between the eosinophil counts and blood histamine levels.

The alterations of the differential eosinophil percentage found in hamster trichinosis were similar to those found by Opie (1904) in the guinea pig, by Wantland (1937) in the rabbit, and by Maass (1933) in the pig, i.e., the numbers were elevated beginning the tenth day and reached a maximum during the latter part of the 3rd week p.i. The eosinophilia then decreased and the second peak of eosinophilia (and leucocytosis) observed in man by Brown (1897) and by Della Vida and Dyke (1941) was not observed in the hamster.

The percentage of eosinophils in the normal hamsters was found to be 0 to 4 per cent. This was in agreement with values reported by Stewart et al. (1944), Rose et al. (1946), and Trincao et al. (1949). The one
exception was the report by Byrd (1942) of the absence of circulating eosinophils in hamsters he "identified" as *Cricetus cricetus* but apparently were *Mesocricetus auratus*.

The mean total leucocyte counts of control animals was 4,200 cells per cmm., with a range of 1,550–7,500. These values are low if compared with the data from normal hamsters reported by Byrd (1942), Stewart et al. (1944), Rose et al. (1946 and Sherman (1953). (Stewart et al. and Sherman counted blood obtained by cardiac puncture.) Crabb and Kelsall (1951), however, reported a mean leucocyte count of 3,900 with a range of 2,700–6,050 cells per cmm.

Since there was no appreciable change in the absolute leucocyte count following infection, the decrease in the percentage of lymphocytes in the differential count indicates that there was an absolute lymphocytopenia and neutrophilia following infection. Lymphocytopenia has been reported in rat trichinosis by Van Someren (1938) whereas Beahm and Downs (1939) found an increase in this type of cell in the rat following trichinosis. The response of dogs to trichinosis does not involve any alteration in the lymphocyte number (Beahm and Jorgensen, 1941). Administration of ACTH or adrenocortical hormones was found by Dougherty and White (1944) and Reinhardt and Li (1945) to cause an absolute lymphopenia and a rise in serum gamma globulin (Dougherty and White, 1947). Trowell (1947) found that in immune reactions the lymphocytes manufacture antibodies. Adrenocortical hormones or ACTH release following a stress such as hemorrhage, X-rays, toxins, etc., stimulate the breakdown of lymphocytes and the release of their stored gamma globulin proteins. Since trichinosis is a
bona fide stress in the hamster (see above) a lymphocytopenia could be expected. Wright and Oliver-Gonzales (1943) found that the anti-larval and anti-adult antibodies could be found in the gamma globulin fraction of infected rabbit serum. The biphasic depression in the number of lymphocytes during the second week and around day 35 p.i. may be correlated with the production of the anti-adult and anti-larval antibodies respectively.

The absolute eosinophil/leucocyte ratio was found to give an eosinophil percentage much lower than that found in differential counts from similar animals. An explanation for this anomaly may reside in increased eosinophil cell membrane fragility following infection. This is mere conjecture but such a condition would lead to the lower counts.

Summary

1. Trichinosis was found to cause a moderate eosinophilia in the blood of infected hamsters. The eosinophilia reached a peak from 16 to 19 days post infectionem (p.i.), and returned to normal about a week later.

2. There was a lymphopenia during the second and fifth weeks p.i.. This lymphopenia was probably associated with the production of antibodies.

3. Methods for the determination of absolute eosinophil numbers in the peripheral blood were found not to be as sensitive to changes as the differential count, at least when the technique was applied to blood from trichinized animals.
APPENDIX II

The Activity of Golden Hamsters Infected with Trichinosis

One of the sequelae of infection with Trichinella in the golden hamster is a marked decrease in activity. (This, of course, is not unusual for an animal undergoing extensive muscular trauma.) In order to measure the amount of activity of non-infected and infected animals during the course of the disease the following experiment was devised.

The recording apparatus was designed after the "activity analyzer" of Harned et al. (1952). It consisted of two cylindrical cages similar to the metabolism cages described previously. The cages were suspended individually by springs and attached to light counterbalanced heart levers which magnified alterations in the floor level of the cages four times. The supporting springs were so constructed that only the climbing activity of the hamsters would register on the smoked drum. A few food pellets were put in the cages and a small water reservoir (25 ml.) was attached to the cage. Kymograph records were made over a ten hour period at night. The room housing the cages was darkened and no one entered, in order to minimize the influence of external stimuli on the activity of the hamsters.

Before commencing, the normal activity of six 10 week old normal male hamsters was recorded. Four of these animals later were infected with 225 larvae. The hamsters were paired, one experimental and one control, at the beginning of each experiment and all future recordings were made with the same animals in the same cages.
Figures 20 and 21 are the kymographic records of 3 sublethally infected hamsters and one lethally infected hamster, with a simultaneous recording of the control animal at various times p.i. The individual daily records represent a two hour period (8:40-10:40 P.M.) which had previously been found to be the most active period of this nocturnal animal. The activity of all animals pre-infection was "normal".

One of the four infected hamsters died on the 26th day p.i. (during the latter portion of the gut phase). Immediately following infection the animal ceased to be active and seldom stirred in the cage. The moribund condition of this animal was due probably to the severity of the intestinal symptoms.

The activity of sublethally infected hamsters can be summarized as follows: five days to a week p.i. there was a significant decrease of activity lasting several days. This period would coincide with the period of destruction of the intestinal tissue by the adults. Infected hamsters during the following two weeks showed normal activity or were slightly hyperactive. About the 30th day their activity practically ceased and did not return to normal until the 10th week p.i.

It has been established by Humes and Akers (1952) that Trichinella larvae are in the peripheral blood stream of the golden hamster during the period from 3 to 22 days p.i. Presumably during this time the larvae are penetrating the muscle fibers, although these investigators did not find larvae in the cheek pouch muscle fibers until the 16th day. The point to be considered is that most of the larvae have penetrated into
Figure 20: Two Hour Activity Records of Lethally (top row) and Sublethally Infected Hamsters
Figure 22: Appearance of infected hamster losing its hair
seen beneath the falling fur. The animals were never naked.

Methods

In order to study the microscopic changes involved in fur loss, a large group of animals of both sexes was infected with 225 larvae of *Trichinella spiralis* (see above). The animals were isolated and their diet consisted of Purina Lab Chow and tap water *ad libitum*. Daily weight records of these animals were kept and those losing the greatest number of grams were used for the necropsy material. Fur loss was more common in those animals which had previously lost a great deal of weight than in those which had not lost weight. It was supposed that animals that lost weight would have lost their fur had they lived and that the appearance of their skin and its appendages would give a good indication of the sequence of events preceding hair loss. Animals were sacrificed on the following days p.i.: 29, 42, 50, 54, 60, 65, 71, 85, and 128. Uninfected controls were sacrificed on days 29 and 128.

A one inch square of skin (which had been clipped) from the middorsum directly over the scapular region was removed. (Of advantage is the fact that the skin in this location is particularly thick.) The skin was stretched and tied over the open end of a test tube containing Bouin's fixative and the tube with its hamster skin "drumhead" was immersed in a bottle of Bouin's fluid. The tissues were fixed for 48 hours. A 1 cm. disk was cut from the center of the drumhead, dehydrated and embedded. Such treatment insured that the tissue sections would consist of a single
flat layer of skin. The sections were cut at 12 micra and stained with haematoxylin-eosin-phloxine or with Van Gieson's connective tissue stain.

Results

Normal hamster skin is composed of the following layers: the epidermis consisting of the stratum corneum and stratum germinativum, the dermis or corium, and the subcutis consisting of the panniculus adiposus and panniculus carnosus. As in other mammals, the stratum corneum consists of kertinized emucleate cells. This layer increases slightly in thickness with age. In normal young hamster skin (7 weeks old) there are indications of a stratum granulosum. New born hamsters have a distinct granulosum. The germinativum is several layers thick in newborn and maturing hamsters but very thin in older animals. Nuclear activity here also diminishes with age. No basement membrane was observed between epidermis and dermis. The dermis lacks papillae and consists of a felt-work of dense fibrous connective tissue through which pass the hair follicles. The bases of the follicles are in the panniculus adiposus. This layer is made up of fat cells, blood vessels and a few elastic fibers. The panniculus carnosus in the shoulder region consists of two layers of muscle fibers.

The hair shafts of this area are lubricated by small sebaceous glands. These glands are composed of at most 20 enlarged holocrine type gland cells (see Figure 24 D #8 and Figure 25 C). Skin removed from the lumbosacral region of normal hamsters presents a different histological appearance. The most obvious anatomical feature of this region is the
great concentration of sebaceous glands (see Figure 23). No definite
orifice from these glands was observed. The other structures are essen-
tially similar to those found in the shoulder region. It is postulated
that these glands in some way are related to sexual activity serving to
produce an odor attractive to the other sex. Hamilton (1939) noted the
presence of hip glands in field mice, especially in old males. Shrews
possess cutaneous side glands which actively secrete during the breeding
season.

At birth the hamster is naked and remains so for about a week.
The hair follicles develop rapidly during this week, as evidenced by
the active cells making up the follicle (see Figure 25 B and C). The
follicles arise individually as ingrowths of the stratum germinativum.
As the animal ages, however, the follicles become grouped together and
by ten weeks the characteristic arrangement is three or more follicles
grouped together with the hair shafts protruding through a common open-
ing in the skin.

Infection with Trichinella 29 days previously caused these hist-
ological alterations in the skin. There was a marked thickening of the
corneum with a thinning of the germinativum to a single layer (Figure 24 B).
As might be expected in an animal which had lost weight the panniculus
adiposus was absent. This loss resulted in a shift of the bases of the
follicles towards the skin surface. Stasis in the blood vessels servicing
the follicles could ensue.

Forty two days p.i. (Figure 24 C) the surface of the skin was rough
and the corneum was very thick. Sections through larvae at this time
Figure 23: Sebaceous glands from the lumbosacral region of the golden hamster
Figure 24: Histological Alterations in the Skin of Hamsters Following Infection. Sections cut at 12 micra, H. and E. stained and photographed at X200.

A. Normal skin, 7 weeks old; B. Skin 21 days p.i.;
C. Skin 42 days p.i.; D. Skin 71 days p.i.;
E. Skin 85 days p.i.; F. Skin 128 days p.i.;
G. Normal skin of the same age as F.

(1) epidermis; (2) dermis; (3) hair follicle, directly above is follicle in tangential section; (4) subcutis or panniculus adiposus; (5) muscular layer or panniculus carnosus; (6) stratum corneum; (7) stratum germinativum; (8) sebaceous gland; (9) smooth muscle (arrector pili m.); (10) transverse section through root of hair.
Figure 25: Comparison of Young Rat Skin (A) and Day-Old Hamster Skin (B and C) with Skin from a Hamster Infected 71 days previously with *Trichinella*. 12 μm sections, H. and E stains, 200X.
showed that the worms were heavily encapsulated and surrounded by numerous large macrophages with small, heavily stained nuclei. The most obvious histological change was the infiltration of the dermis and the muscle layer by numerous cells containing eosinophilic granules. These cells were pleomorphic, but mainly cylindrical or fusiform in shape. The average dimensions of the cytoplasm were 15 by 4 micra while the nuclear dimensions were 6 by 3 micra. The cytoplasm was occupied by a many medium-sized eosinophilic granules. The nucleus presented a "cartwheel" appearance because of peripheral chromatin clumps. On the basis of their morphology and appearance during a pathological condition (trichinosis) these cells have been tentatively identified as Russell body cells (Kindred, 1932).

At 50 days p.i., the corneum had increased in thickness. Sections of skin 60 days p.i. showed that the thickened corneum had become detached from the epidermis indicating that it was being sloughed off.

Figure 24 D shows the appearance of hamster skin 71 days p.i. This particular animal had lost its fur two weeks previously and a new coat had immediately appeared. The section revealed an active stratum germinativum as evidenced by nuclear divisions and other manifestations of nuclear activity. The fatty layer had reappeared. Follicles in sections from this animal were similar to the follicles of young animals. Figure 25 compares the appearances of hamster "pup" skin and day-old rat skin with the skin of this infected animal. The similarity of the stratum germinativum cells and the cells lining the follicle is obvious. The cellular components of
the dermis were also similar. Note the dense epiderchium layer (outer corneum) of the young rat (this disappears as the animal acquires fur) and the differences in the appearance of the stratum germinativum.

Hamster skin 85 days p.i. (this animal had lost its fur 3 weeks previously) shows the following histological alterations. The germinativum had thinned and there was less cellular activity in the dermis. The follicles had grown down into the fatty layer (see Figure 24 E).

The normal appearance had been regained by day 129 (Figure 24 F) although there seemed to be fewer follicles than in normal animals of the same age (Figure 24 G).

Discussion

The significance of the hair loss reported here is unknown. One explanation may be that the mechanical obstruction of the vascular supply brought about by the loss of fatty tissue beneath the dermis may lead to death of the follicles and a considerable portion of epidermis.

The damage caused in the skin as a result of trichinosis can be compared to a second degree burn, i.e., loss of the epidermal tissue in the affected area, according to the classification of Dupuytren (Pack and Davis, 1930).

Summary

1. Hair loss occurred in 30 of 73 hamsters (41 per cent) infected with T. spiralis about 65 days previously. No sex differences were noted.

2. Evidence is presented which demonstrated that the fur of the dorsum is lost in toto and is replaced by new fur developing from new folli-
cles which closely resemble embryonic follicles.

3. During the muscular invasion phase of the disease conditions in the skin arise which predispose the animal to hair loss. The fatty tissue disappears, the dermis becomes very dense and numerous eosinophilic irritation cells, so-called Russell body cells, are found in the dermis and muscular layers. The layer which is affected chiefly, however, is the stratum germinativum. The cells of this layer undergo degenerative alterations which cause an abnormal corneal thickening and sloughing. Evidences of follicular death are seen. The old hairs are entrapped in this dead matrix and fall.

4. Four months p.i. the skin of the infected animal has returned to normal, new fur having developed from follicles proliferated from the stratum germinativum.
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Abstract

The aims of this research were to measure the "stress" produced in the golden hamster (Mesocricetus auratus) by an experimental infection with Trichinella spiralis, to chart the gradual development of accord between host and parasite, and thirdly, to note any correlations between the urinary and adrenal changes caused by the "stress".

The tests used for measuring the stress were: the urinary uric acid/creatinine ratio, the adrenal weight, adrenal ascorbic acid, adrenal non-specific dehydrogenase activity, adrenal sudanophilic lipids, alkaline phosphatase, and birefringence. The urine volume, pH, creatine excretion, and the diazo reaction were also studied.

More than 230 hamsters, half of which were males, were used. They were from 8 to 10 weeks old and weighed from 85 to 100 grams at time of infection. The hamsters were infected by being forced-fed a sublethal dose of 225 Trichinella larvae isolated by a special digestion technique from rat muscle. The urinalyses and adrenal analyses were made by the usual standard techniques, in certain cases slightly modified. Urine was collected by the use of two new types of metabolism cage, a stationary cage and an "exercising-wheel" cage, both of which permitted the collection of uncontaminated and undiluted urine.

The most common visible symptom of trichinosis in the hamster was an extension of the hind limbs resulting in an arching of the back. The emaciation which followed infection was found to be caused by impairment of the activity of those structures involved in deglutition rather than by inability to chew.
The mean urine volume of normal hamsters under the conditions of the experiment was about 8 ml. per day. The pH varied between 7.2 and 7.5. Sublethally infected hamsters showed an initial polyuria followed by oliguria. The daily output returned to normal by day 65 post infectionem (P.I.). Lethally infected hamsters showed oliguria. The pH of the urine was unaffected by sublethal infection. Lethally infected hamsters in the terminal state produced an acid urine.

The diazo reaction was found to be an ineffective diagnostic test for trichinosis in the hamster.

The urinary uric acid/creatinine ratio decreased from birth to maturity of the hamster because of increased creatinine production. The urinary uric acid output was unaffected by age. Creatine excretion decreased with age.

The uric acid/creatinine ratio was applied to hamster urine as a convenient measure of stress. It was found that the elevation of the value of the ratio was directly correlated with the "stress" undergone by the animal. Various stages in the life cycle of the parasite caused striking elevations in the uric acid/creatinine ratio of the host. The ratio showed peaks, for example, on the 7th, 15th, and 28th days P.I., caused respectively by the reinvasion of the gut by the adult worms, the waves of larvae in the circulation and invasion of the muscle tissue. The elevations of the ratio during stress were caused by increased output of uric acid and concomitant decreases in the creatinine output. The increased uric acid production was attributed to an increased secretion of adrenocorticotropic hormone in response to the stress. No explanation for the decreased output of
creatinine and creatine following infection with *Trichinella* could be offered. All the urinary components studied returned to normal by day 65 p.i.

Although infected animals were less active (see below), forced exercise did not increase either the creatine or creatinine output of trichinized or normal hamsters.

The average normal male hamster adrenal was significantly heavier, contained significantly less ascorbic acid, showed significantly less nonspecific dehydrogenase activity, slightly more sudanophilic substance and a much greater alkaline phosphatase activity than the average normal female hamster adrenal. With increasing age, there was a noticeable increase both in the adrenal sudanophilic lipids and birefringence, and a slight increase in ascorbic acid. No effect of age was noted in the weights of adrenals from males. The adrenals of females were heaviest when the animals were 5 months old. Following infection there was an increase in adrenal weight from 28 to 63 days p.i., an increase in ascorbic acid 42 days p.i., and initial increases in alkaline phosphatase and dehydrogenase activity.

It was found that none of the physiological tests applied to the hamster's adrenal following infection was as exact or as sensitive a measure of the stress as the uric acid/creatinine ratio. Certain correlations between the urinary and adrenal findings were noted. The immediate increase of alkaline phosphatase and dehydrogenase enzyme systems, plus the hypertrophy of the glands following infection, were indicative of increased secretory activity. The urinary uric acid excretion during this period
was probably caused by increased adrenocorticotrophic hormone secretion. The increase in the concentration of ascorbic acid and sudanophilic lipid which occurred a considerable period after the onset of this stress, was probably correlated with excess storage caused by decreased demand for these substances. This would indicate adaptation on the part of the host to the presence of the parasite. Simultaneously with these adrenal changes the uric acid/creatinine ratio decreased, supporting the theory that the stressful conditions were abating.

The physiology of the hamster's adrenal appeared markedly different from that of other animals in that neither the lipid droplets nor the ascorbic acid concentration were depleted (at least by this stress). The normally faint birefringence was not affected. The activity of two enzymes, alkaline phosphatase and non-specific dehydrogenase, was found to be increased immediately. Measurement of these two substances following stress might be fruitful in the determination of the general metabolic and secretory rates of the hamster adrenal.

Trichinosis caused a moderate eosinophilia which reached a peak from 16 to 19 days p.i. and returned to normal about a week later. There was a lymphopenia during the second and fifth weeks p.i. This lymphopenia was probably associated with the production of the two antibodies to the parasite, the antiadult and the antilarval antibodies. No leucocytosis was observed.

Methods for the determination of absolute eosinophil numbers in the peripheral blood, when applied to blood from trichinized animals, were
found not to be as sensitive to changes in the numbers of circulating eosinophils as the differential count. Increased membrane fragility of the eosinophilic cells from infected animals to the diluent used may have been responsible.

The activity of hamsters sublethally infected with *Trichinella* was altered. A sequence of activity changes was observed. During the reinvasion of the gut mucosa by the adult worms there occurred a reduction of activity, followed by a period of hyperactivity probably caused by hypersensitivity of the invaded muscles. The activity was curtailed during the inflammatory and necrotic reactions in the parasitized muscles. Normal activity was resumed when these reactions subsided. A lethally infected hamster became inactive immediately following infection.

In 30 of 73 hamsters (41 per cent) sublethally infected about 65 days previously there occurred a marked loss of the fur of the dorsum. This fur was lost in toto and was replaced by new fur developing from newly proliferated follicles which closely resembled embryonic follicles. A series of histological changes preceded the shedding of the fur. The fatty tissue disappeared. The dermis became very dense and numerous eosinophilic irritation cells, so-called Russell Body cells, were found in the dermis and muscular layers. The layer which was affected chiefly, however, was the stratum germinativum. The cells of this layer underwent degenerative alterations which caused an abnormal corneal thickening and sloughing. There were evidences of follicular death. The old hairs became entrapped in the dead matrix and fell, to be replaced immediately by new hairs which developed from new follicles.
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