taminated with several viral agents. Therefore, it would be advisable to examine uninoculated cultures for the presence of possible endogenous contaminants.

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Serum Transaminases and Aldolase in Rats Inoculated with *Trypanosoma lewisi.** (30832)

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It is usually assumed that parasitic infections have some detrimental effects on the host organism. In the case of the *Trypanosoma lewisi*-rat relationship such effects are not readily apparent. There is, in fact, evidence that the relationship may be mutually satisfactory in that the parasite contributes substances which enhance the growth of the rat(1,2). Although *T. lewisi* augments the growth rate of the host, it seems likely that it would also produce a stress on the host by utilizing host materials for its own growth, elaborating metabolic by-products which affect the host's metabolism; or more simply, by the production of mechanical damage to the host as a result of high numbers of parasites in the tissues or blood stream. Since a large number of non-specific stresses are known to result in the elevation of serum enzymes(3-6), it was felt that any stress imparted to the rat by *T. lewisi* may be manifested by the elevation of serum enzymes. A study, therefore, was initiated to determine the changes in the levels of several rat serum enzymes during the course of *T. lewisi* population growth and decline. In addition, the relative activities of these enzymes were established in trypanosome cells which were isolated from rat blood.

Materials and methods. Two hundred fifteen immature Sprague-Dawley female rats, each weighing approximately 75 g, were employed. Trypanosome infections were initiated by intraperitoneal inoculation of the "L" isolate of *Trypanosoma lewisi*. Cells for inoculation were harvested and prepared as a pure suspension in physiologic saline according to the method of Lincicome and Watkins (7). The inoculum in one series of experiments contained 5×10^7 cells. In a second experiment two levels were employed: 10^2 and 5×10^7 cells. Estimations of all trypanosome populations in peripheral tail blood and in saline suspensions were made with a hemocytometer, using Toisson's fluid as the diluent. All dilutions were at 1:200.

Blood for serum enzyme analysis was drawn by cardiac puncture of rats under sodium pentobarbital anesthesia. Each rat was sampled only once. Samples to be analyzed

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TABLE I. *Trypanosoma lewisi* Population Changes in Peripheral Tail Blood of Rats (hemocytometer count ± S.E.).

Days after inoculation	Inoculum (numbers of cells)		
	10^2	5×10^7	5×10^7
3			72 ± 10(6)
6	9 ± 2(6)*	153 ± 39(6)	
7			554 ± 70(6)
8	114 ± 23(6)	167 ± 52(6)	
9			457 ± 87(6)
10	73 ± 53(6)	286 ± 121(6)	
11			436 ± 109(5)
14			716 ± 303(6)
15	36 ± 8(6)	118 ± 46(6)	
16			110 ± 26(5)
18			125 ± 39(6)
20	45 ± (6)	97 ± (4)	
21			139 ± 46(4)
23			104 ± 51(4)

* Represents numbers of animals used for the determination.

on the day of collection were stored at +4°C; otherwise they were stored frozen at −4°C. No sample was aged more than 48 hours before enzyme determinations were made. To measure the enzyme activity in trypanosome cells isolated from rat blood, the cells were separated from a standardized saline suspension by centrifugation, resuspended to distilled water and lysed by a single slow freezing in the refrigerator followed by 3 rapid freezings in an acetone-dry ice mixture. The lysed preparation was centrifuged to remove cellular debris and the clear supernatant was assayed for enzyme activity.

Glutamic-oxalacetic acid transaminase (GOT), glutamic-pyruvic transaminase (GPT), and aldolase were measured colorimetrically using commercial test kits.‡ Units of transaminase activity are expressed so that one unit obtained colorimetrically is equal to that quantity of enzyme producing a change in optical density of 0.001/min at 340 mμ and 25°C (equal to one unit in the UV method). One aldolase unit corresponds to 0.045 μmoles of fructose 1,6-diphosphate split per ml of serum per hour at 37°C.

Results. Trypanosome cell populations. The population levels of *T. lewisi* in peripheral tail blood of rats are shown in Table I. Rats inoculated with 5×10^7 cells developed higher parasitemias than those given a smaller inoculum. Based on mean hemocytometer count, the maximum population appears to develop between the 8th to 10th post-inoculation day in rats given either an initial injection of 10^2 or 5×10^7 cells.

Serum enzyme levels. Serum GOT was elevated in rats inoculated with *T. lewisi* (Fig. 1). The major increases occurred between the 6th and the 10th days, corresponding roughly to the period of highest parasitemia, and again on the 15th day, corresponding to the period of parasite destruction and population decline. Only during the initial period of the infection (6th day) were the levels of serum GOT higher in rats given 5×10^7 cells than in those inoculated with 10^2 cells. This is attributable to the fact that the parasitemia increases at a faster rate in rats given the higher inoculum.

Serum GPT was also elevated in infected rats (Fig. 2). However, the temporal pattern differed from that seen for GOT in that a progressive elevation of GPT occurred which culminated on the 15th day. This increase in serum GPT activity corresponds to the period of parasite destruction. Only in the early phases of the parasitemia (6th day) was there any significant difference between the serum GPT levels in rats given the two levels of inoculum. It should be noted that in rats given 10^2 cells, the highest elevation of GPT in the serum appeared during the initial rapid growth of parasites.

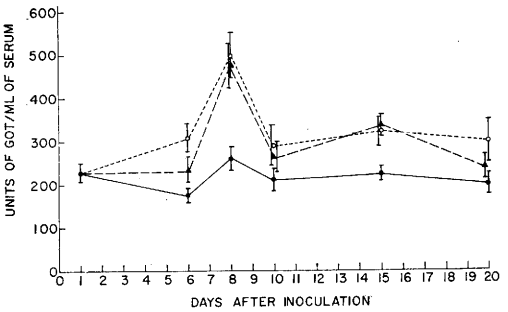

FIG. 1. Effect of *Trypanosoma lewisi* on serum GOT levels in the rat. Each point is expressed as the mean ± S.E., and is calculated from 6-10 rats per point. O- - -O, received a single injection of 5 x 10^7 cells; ▲- - -▲, received a single injection of 10^2 cells; ●——●, are uninoculated controls.

‡ GOT and GPT test kits were Dade Reagents, Inc., products; aldolase test kits were those of D. G. Boehringer and Soehne.

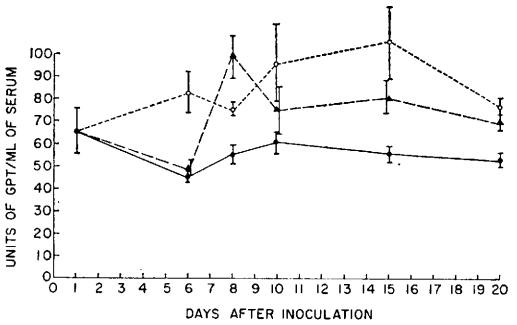

FIG. 2. Effects of *Trypanosoma lewisi* on serum GPT levels in the rat. The legends are the same as in Fig. 1.

Serum aldolase activity in *T. lewisi*-infected rats did not differ significantly from that observed in uninfected rats. Aldolase values for both groups ranged from 45-100 units/ml.

Enzyme levels in trypanosomes. GPT, GOT and aldolase were all present in lysed *T. lewisi* cells (Table II). GPT was present in roughly 10 times greater concentration than GOT. The amount of aldolase activity present in 10^6 trypanosome cells (Table II) was found to be considerably less than that in 1 ml of normal rat serum (45-100 units/ml).

Discussion. The present study shows that serum glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase, but not aldolase, are elevated in rats inoculated with *Trypanosoma lewisi.*

In most instances where stress is applied to an organism, the subsequent elevation of serum enzymes is interpreted as resulting either from pathological lesions and cell necrosis, or from changes in the permeability of the cell membrane(3,5,6). The present study, however, is complicated by the fact that two possible sources of serum enzyme exist: the host tissues and the disintegrating parasite cell. To assess the contribution of enzyme made by the parasite to the host's serum it is necessary to know whether the enzymes are present in the trypanosome cells and, if so, their relative activities. The present findings indicate: 1) that GOT, GPT and aldolase are all present in *T. lewisi,* and 2) that in *T. lewisi,* in contrast to most mammalian tissues (6), there is more GPT than GOT.

Combined results on serum and trypanosome cell enzyme levels suggest that in rats infected with *T. lewisi* both the host tissues and parasite serve as a source of serum enzymes. The present finding that during the preclimactic phase (8th day) of the parasitemia, serum GOT increased slightly more than serum GPT, coupled with the finding that GPT is 9-fold more concentrated than GOT in lysed trypanosome preparations, suggests that the elevation of serum GOT may be due principally to the release of this enzyme from rat tissue. Release of enzyme from disintegrating trypanosome cells should result in a differential elevation of serum GPT.

The postclimactic phase of the infection, on the other hand, which is characteristically one of declining populations and destruction of trypanosomes, is here characterized by greater increases in serum GPT. These elevated levels of serum GPT are therefore interpreted as coming, chiefly, from disintegrating trypanosomes. Calculations show that adequate amounts of GPT are present in the trypanosomes in the blood to account for this observed elevation in rat serum GPT. One cannot, however, exclude the possibility that some of the enzyme is of host tissue origin. The excess serum GPT observed earlier in the infection (6th day) may be of rat tissue origin since the trypanosomes are

TABLE II. Enzyme Levels in "Lysed" *Trypanosoma lewisi* Cells.

	Enzyme units/10^6 cells*			Enzyme ratios		
Exp No.	GPT	GOT	Ald	GPT/GOT	GPT/Ald	GOT/Ald
1	108	13.6	1.74	7.9	62.0	7.8
2	62	9.4	2.20	6.6	28.1	4.3
3	128	14.2	2.98	9.0	43.0	4.8
4	137	10.4	3.07	13.2	44.6	3.4
Mean	109	11.9	2.49	9.2	44.4	5.1
± S.E.	20	1.3	.38	1.5	8.1	1.1

* Enzyme units are defined in the text.

still in a positive rather than a negative growth phase at this time, and the amount of enzyme released by disintegrating parasites should be relatively small.

The lack of change in serum aldolase indicates that *T. lewisi* has little or no effect on rat skeletal muscle which is a primary source of the enzyme(6). In view of the low levels of aldolase in *T. lewisi* cells, it is not surprising that this enzyme was not elevated in the serum during the period of population decline.

In view of these data it seems that *T. lewisi* has more stressful effects on its host than previously suspected. The fact that some enzymes appear to be released from rat tissues during the parasitemia suggests that *T. lewisi,* either by chemical or mechanical means, may alter the cellular membrane. This effect, however, is only temporary since the serum enzyme levels return to normal when the parasitemia disappears. Although some pathogenic trypanosomes produce elevated serum enzymes in their hosts(8,9), the mechanism responsible is probably different from that of *T. lewisi* since pathogens produce very definite tissue lesions(8). Further studies elucidating the mechanisms underlying the alterations described here should prove valuable in advancing our basic knowledge of the host-parasite relationship.

Summary. Glutamic-oxalacetic transaminase (GOT), and glutamic-pyruvate transaminase (GPT), but not aldolase, were elevated in the serum of rats infected with *Trypanosoma lewisi.* Increased serum transaminase activity is believed to come from the host's tissues, which presumably are damaged by the parasite, as well as from the disintegrating parasite cells. All 3 enzymes were also present in lysed parasite cells. Here, transaminase activity was high, but aldolase was present in only minimal amounts. GPT was 9-fold more concentrated than GOT.

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Strain Differences in Susceptibility of the Rat to Dietary Cirrhosis.* (30833)

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In the course of experiments with dietary cirrhosis in rats certain strains appeared to be more susceptible to the development of the disease than others. However, there have been few reports in which a study of strain differences has been recorded. Engel(1) and Copeland(2) indicated that genetic factors may be involved in the susceptibility of rats to renal injury produced by acute choline deficiency. These authors compared 2 strains which differed in their minimal requirement for choline. These differences persisted on inbreeding for several generations and seemed to have no relation to rate of growth. Although their studies do not deal with cirrhosis they are pertinent since dietary cirrhosis can

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