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THE EFFECT OF HOST ADRENALECTOMY ON THE PHYSIOLOGY OF *TRYPANOSOMA RHODESIENSE*

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Abstract—1. Adrenalectomized rats infected with *T. rhodesiense* succumbed sooner to the infection than nonadrenalectomized infected rats as a result of an increased parasitemia.

2. Trypanosomes isolated from adrenalectomized rats had reduced rates of glucose consumption.

3. Decreases in total liver glycogen prior to infection of the adrenalectomized rats were observed and are related to the earlier death of the infected rat.

4. The data is discussed with respect to the endocrine system as a regulator of trypanosome biochemistry and a possible mechanism in the pathogenesis of trypanosomiasis.

INTRODUCTION

INFECTIONS by *Trypanosoma rhodesiense* in humans and animals have been extensively studied. While much is known about the pathology of the disease and the metabolic pathways of the parasites little is known about the mechanism of pathogenesis and regulation of the parasite's biochemistry and physiology.

Recently, Sanchez & Alderete (1975) have shown that a pronounced hypoglycemia and almost complete exhaustion of hepatic glycogen occur in albino rats infected with *T. rhodesiense*. Furthermore, the onset of the hypoglycemia and hepatic glycogen depletion occur at about 12 hr prior to death of the rat. Sanchez (1973) showed that the hormone, epinephrine, altered the biochemistry and physiology of *Trypanosoma lewisi* by making 4-day post-infection trypanosomes similar to 8-day trypanosomes. These findings are important since a hypoglycemia in this system also occurs prior to the eighth day of infection. Thus, Sanchez (1973) proposed a model for regulation of the parasites by hormones or as a result of hormone action. Selye (1973) has described changes in endocrine and lymphatic structures as a result of stress conditions, and Ashman & Seed (1973) have proposed that physiological changes in *Microtus montanus* infected with *T. gambiense* might be explained by neurological lesions caused by stress from infection or parasite toxic products. The adrenals, therefore, are of interest because of their physiological regulatory functions in the host. It is known that adrenal hypofunction is characterized by decreased blood glucose levels and hepatic glycogen content as well as disturbances in carbohydrate, fat, and protein metabolism.

In general the role of hormones and endocrine systems on host-parasite relationships have been overlooked. The present studies were an attempt to provide additional information, and to lend support to the model proposed by Sanchez (1973) where hormonal action may be partially responsible for the regulation of the parasite's physiology and biochemistry.

MATERIALS AND METHODS

Rat adrenalectomy

Rats used in the experiments were randomly selected and weighed about 180 g. The adrenals were surgically removed by the procedure described by Zarrow *et al.* (1964). The adrenalectomized rats were isolated and allowed to recover for 2 days on a normal diet (Purina Lab Chow) and given physiological saline (0·85% NaCl) *ad lib.*

Preparation of trypanosomes

T. rhodesiense used in these experiments is a strain maintained in our laboratory in female albino rats (Holtzman Co., Madison, Wisc.) by subinoculations of tail-vein blood dilutions in a glucose Krebs–Ringer solution. The experimental animals were routinely inoculated with a dose of 0·2 ml of 10^7 suspension. The parasites were harvested and isolated as previously described by Sanchez & Read (1969) and suspended in Glucose-KRT.

Parasitemia

The growth rate of *T. rhodesiense* in control and adrenalectomized rats was determined by preparing blood smears from tail-vein blood during the course of the infection. The smears were stained with Wright's stain, and the ratio of trypanosomes to erythrocytes was determined by microscopic examination.

Glucose transport

The glucose transport was determined using ^{14}C-labelled glucose (New England Nuclear Corporation) and prepared in Glucose-KRT at a specific activity of 0·1 μCi-m-mole glucose. The procedure is as previously described (Sanchez, 1974).

Hepatic glycogen assay

The liver was removed from the infected rats immediately after death from the infection. Livers from uninfected animals were obtained within the same time range as those obtained from the infected rats. The livers were then placed in a closed Pyrex container and immersed in liquid nitrogen for rapid freezing. Liver glycogen was assayed by the method described by Good *et al.*, (1932) except for the following modifications: Approximately 300 mg of frozen liver was placed in tubes containing 1·0 ml of 15% KOH and placed in a 100°C ethylene glycol bath for 1 hr. Recrystallization was accomplished by the addition of 2·0 ml distilled water, 2·4 ml 95% ethanol, and 0·2 ml 2%

Na_2SO_4. The tubes were then recapped and placed in the 100°C bath until the first boil, allowed to equilibrate to room temperature (approximately 10 min), placed in a crushed ice water bath for 50 min, finally centrifuged for 30 min at 1732 *g*. The supernatant was drained, and the recrystallization repeated twice. After the final centrifugation the supernatant is decanted and the tubes are allowed to drain. One ml of 2N H_2SO_4 is pipetted into each. The tubes are then recapped and placed in the 100°C bath for 4 hr. After hydrolysis, 1·0 ml of 2N NaOH and 1·0 ml pH 7 phosphate buffer is added.

A filtrate is prepared by bringing the volume to 4·0 ml with distilled water adding 1·0 ml 0·3N BaOH plus 1·0 ml 5% $ZnSO_4$. The tubes are allowed to stand 10 min. They are then centrifuged at 770 *g* for 30 min.

RESULTS

Parasitemia

Figure 1 illustrates the parasitemia of control infected rats vs adrenalectomized infected rats. The data is indicative of a greater rate of multiplication by the trypanosomes in adrenalectomized rats as compared to the controls. While initially the number of trypanosomes is not significantly different in adrenalectomized rats as compared to the controls, there is a significant difference (33%) at the terminal stages of the infection. It is important to note, however, that an increased parasitemia is observed in the adrenalectomized rats almost from the beginning.

Glucose transport

The results of the glucose transport studies performed in an attempt to study the effect of adrenalectomy on the respiratory activity of the trypanosomes are shown in Fig. 2. Upon examining the results for the controls it is clear that most of the glucose uptake occurred in the first 30 sec incubation followed by a leveling off. Trypanosomes isolated from the adrenalectomized rats were found to saturate at about 1 min compared to 30 sec for the controls. Furthermore, the data shows that glucose uptake by the controls is 10 times greater than in trypanosomes from adrenalectomized rats in the first 30 sec and approximately $2\frac{1}{2}$ times at the 1 min interval.

Preliminary oxygen uptake studies performed by standard manometric techniques suggest an increase

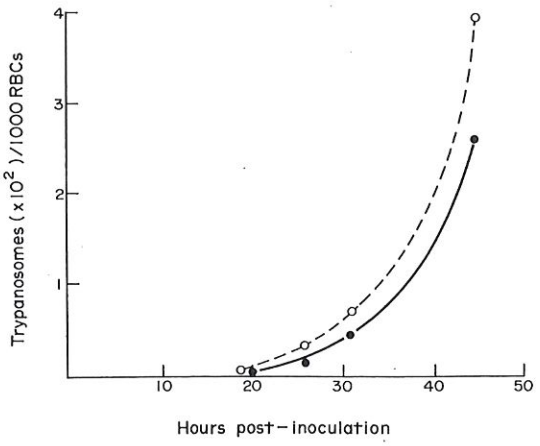

Fig. 1. Parasitemia of *Trypanosoma rhodesiense* in control rats (●) and adrenalectomized rats (○).

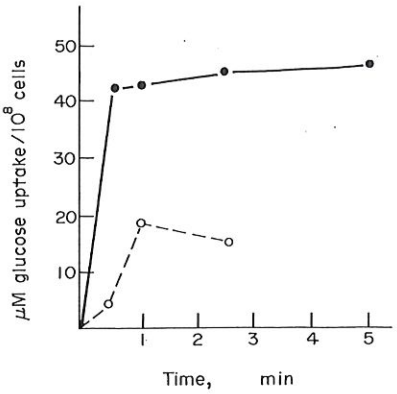

Fig. 2. Glucose transport by *Trypanosoma rhodesiense* isolated from control (●) and adrenalectomized (○) rats.

in the rate of oxygen uptake by trypanosomes isolated from adrenalectomized rats than those from control rats. While the difference (10%) is not considered very significant by this technique, the trend correlated to that of glucose transport. Further studies on this physiological parameter are required by more sensitive methods.

Hepatic glycogen content

Figure 3 shows the difference in liver glycogen levels between infected and uninfected rats as well as the decreased glycogen content, 51% of control, resulting from adrenalectomy. The data strongly indicates liver glycogen mobilization is extensive since more than 95% of the glycogen was utilized, for an almost total depletion.

DISCUSSION

The present study clearly suggests that removal of the adrenals results in an "altered environment" of the host so that trypanosomes isolated from adrenalectomized rats appear to be metabolically different from those isolated from control rats.

The parasitemia of adrenalectomized infected rats is significantly greater than that of the control infected rats. This increased rate of growth is reflected throughout the infection. Sanchez & Knight (1975) have observed an increased parasitemia in *T. rhodesiense* infected rats treated with exogenous epineph-

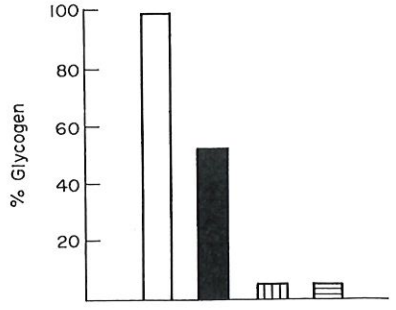

Fig. 3. Rat liver glycogen content from uninfected controls □, uninfected adrenalectomized ■ and *Trypanosoma rhodesiense* infected controls ▥ and infected adrenalectomized ▤

rine. Similarly, Sanchez (1973) showed that an increase in the rate of multiplication is also mediated in *T. lewisi* by exogenous epinephrine. We have recently shown that the hormone, glucagon, produces even more pronounced increases in the rate of multiplication (Sanchez & Alderete, 1975). Therefore, in light of current work on glucagon, an alternate suggestion is that while the role of epinephrine is important, it appears that glucagon plays a greater role in regulating the rate of parasite multiplication. Sanchez & Alderete (1975) have found that rats infected with *T. rhodesiense* have pronounced hypoglycemias approximately 12 hr before the rat dies, and it is well known that glucagon is the primary hormone involved in the homeostasis of blood glucose (Foa, 1973). The parasitemia shows that increases in the numbers of trypanosomes become pronounced at about 35 hr post-infection, and since the rats generally succumb to the infection at 48 hr post infection, the presence of glucagon indicates possible hormonal regulation.

The data on glucose uptake is very suggestive of different metabolic processes between trypanosomes isolated from adrenalectomized rats to those isolated from controls. Two possible explanations exist. Grant & Fulton (1956) have found that "abnormally high blood pyruvate levels are correlated with the degree of infection and are directly caused by the metabolism of the parasite", a similar observation was made by Coleman & VonBrand (1957). Sanchez & Knight (1975) have also shown the same type of phenomena for pyruvate as well as lactate. The increased number of trypanosomes in the adrenalectomized rats could very well produce a high pyruvate-lactate environment resulting in a negative feedback of the glycolytic scheme. Increases in glycerol concentrations would result in increased oxygen uptake due to oxidation of α-glycerophosphate back to dehydroxyacetonephosphate, the mechanism of oxygen reduction first reported by Ryley (1956). Current unpublished work in our laboratory on trypanosome enzyme activity appears to support this shift in metabolism. As indicated earlier in this report, trends toward increased oxygen consumption by the parasites using manometric techniques have been observed in adrenalectomized *T. rhodesiense* infected rats. A second alternative is that decreased glucose transport by *T. rhodesiense* from adrenalectomized rats might also be directly related to the absence of cortisol. It is known that cortisol defiency causes inbalances of carbohydrate metabolism resulting in the decrease or absence of gluconeogenesis. The minimal glucose available in the adrenalectomized rat is suggestive that the absence of cortisol induces more efficient changes in the energy producing metabolism of the parasites. This might indicate that some of Selye's (1973) observations on the changes of endocrine and lymphatic structures as a result of stress are important in host-parasite interactions since atrophy of the adrenals caused by trypanosomiasis could result in the direct or indirect metabolic regulation of the parasites through the absence of a hormone.

VonBrand (1938) also reported depletion of liver glycogen due to *T. rhodesiense* infection. The absence of epinephrine due to adrenalectomy and the continued mobilization of liver glycogen during infection give support to the hypothesis that glucagon is the primary hormone in glycogenolysis (Foa, 1973). Total liver glycogen depletion in this stage also lends support to the concept that excess secretion of glucagon may have regulatory properties on *T. rhodesiense* and contribute to pathogenesis.

Sanchez (1973) forwarded a hypothesis of host factor-regulated metabolism of *T. lewisi* with epinephrine. He proposed that increased hormonal activity induced by the stress of the infection triggers a series of events which ultimately results in the metabolic regulation of the parasite. Sanchez & Alderete (1975) in support of this model strongly suggest the specific role of a hormone (i.e. glucagon) and/or its mode of action as a regulatory mechanism of trypanosomal physiology and biochemistry. The hypothesis proposed by Sanchez, therefore, becomes even more attractive in explaining the regulation of the parasite.

This study again illustrates the importance of the role of hormones and hormone mode of action in host-parasite interactions. This aspect of research has largely been overlooked and requires additional attention.

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Key Word Index—Trypanosoma rhodesiense; host adrenalectomy; physiology; host parasite interactions; glucose transport; liver glycogen; African trypanosomes; trypanosomiasis.