# SOME STUDIES ON *TRYPANOSOMA LEWISI* INFECTIONS IN HYPERGLYCEMIC AND HYPOGLYCEMIC RATS

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ABSTRACT

SOME STUDIES ON TRYPANOSOMA LEWISI IN HYPERGLYCEMIC AND HYPOGLYCEMIC RATS

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Alloxan diabetes was induced in rats to produce long term hyperglycemia, and the administration of insulin was used to study the effect of hypoglycemia on the parasitemia.

Trypanosome population changes were determined and the coefficient of variations for these populations are recorded.

From the results of these studies it is evident that increases in the concentration of blood glucose does not have a significant effect on the population of Trypanosoma lewisi in rats. Likewise, the reduction in the concentration of blood glucose by the administration of insulin does not effect a significant change in trypanosome population.

It has also been shown by calculation that the in vitro rates of glucose metabolism by trypanosomes as determined by previous workers would normally cause hypoglycemic shock in the rat. Subsequent administration of insulin should depress further the blood glucose concentration to a level inadequate for the continued life of the rat.

These studies indicate that increased blood glucose from gluconeogenesis does not have a significant effect on the *T. lewisi* parasitemias in rats.

# SOME STUDIES ON *TRYPANOSOMA LEWISI* INFECTIONS IN HYPERGLYCEMIC AND HYPOGLYCEMIC RATS

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# INTRODUCTION

The infection of rats by the protozoan hemoflagellate, Trypanosoma lewisi (Kent, 1880; Laveran and Mesnil, 1901) normally follows a specific pattern. These organisms initially occur in increasing numbers sometimes reaching several hundred thousand per cubic millimeter. At about the eighth to tenth day there occurs an abrupt decrease in the number of organisms, which is referred to as a number crisis. The parasitemia remains at this low level for varying periods of time when a second number crisis occurs which removes all of the organisms from the host. During the period prior to the first number crisis, trypanosome reproduction is rapid and the organisms vary greatly in size both in length and breadth. The population consisting of small and varying sized organisms increases until the eighth day when reproduction declines to a low rate and the crisis occurs. The few parasites remaining are of large size and with little variation. It has been demonstrated that these population changes are directly correlated with the formation of several antibody-like substances by the host.

Clark and Patton recently demonstrated (personal communication) the absence of trypanosome reproduction-inhibition normally seen in rats by treating the hosts with the corticosteroid dexamethasone. They believed that this effect is due to the inhibition of antibody formation by the corticosteroid, giving support to the idea that the agent responsible for the inhibition of reproduction is antibody in nature. However, dexamethasone and other glucocorticosteroids also stimulate gluconeogenesis; increasing available glucose which could conceivably support increased rates in metabolism and reproduction.

It has been speculated from this information that blood glucose levels may affect parasitemia levels. The purpose of this study is to determine the effect which hyperglycemia or hypoglycemia may have on T. lewisi infections in rats.

## REVIEW OF LITERATURE

Immunological studies of Trypanosoma lewisi infections have been in progress since before 1900 and before the parasitemia had been formally described by Laveran and Mesnil (1901). Taliaferro and Taliaferro (1922) described an uninfluenced infection as one in which the trypanosomes divide rapidly during the first five or six days after inoculation into a previously uninfected rat. During the period between the sixth and tenth day, reproduction ceases and a population containing large organisms of similar size ensues. At approximately ten days after infection there appears a sudden decrease in the number of organisms found in the peripheral circulation. These few remaining organisms may survive for a number of days without reproduction until a second number crisis appears when the balance of organisms disappear from the blood stream.

Concurrent with the investigations of Laveran and Mesnil (1901), Kanthack, Durham and Blandford (1898) observed that recovery from infection by T. lewisi confers immunity to T. lewisi. Since this original observation, details of T. lewisi infections have been studied by numerous investigators.

Rabinowitch and Kempner (1899; as reviewed by Taliaferro, 1932), demonstrated that the serum of a rat recently recovered from an infection of T. lewisi prevented the development of T. lewisi when the two were injected intraperitoneally at approximately the same time into a susceptible rat. However, a normal parasitemia was observed if immune serum was injected one week after infection had been initiated.

Laveran and Mesnil (1901) further concluded from Rabinowitch and Kempner's work and additional studies of their own, that the mechanism involved in both active and passive immunity is phagocytosis. In direct opposition, MacNeal (1904) experimenting with previously immunized guinea pigs could not demonstrate phagocytosis after injection of living T. lewisi organisms, but found "evidence only of the immobilization and gradual solution of the trypanosomes in the peritoneal fluid." Taliaferro (1932) reports that Manteufel (1909) also disagreed with Laveran and Mesnil concluding that the mechanism of phagocytosis is secondary to the part played by lytic mechanisms. As a result of spleen function studies in relation to resistance, Regendanz and Kikuth (1927; as reviewed by Taliaferro,

1932), concluded that the removal of T. lewisi organisms from the peripheral circulation was not due to an immunological response but to an increase in non-specific phagocytosis by the reticuloendothelial system. Their conclusions were based upon the observation that splenectomy was associated with an increase in the length of infection. Taliaferro, Cannon and Goodloe (1931) and Taliaferro (1938) demonstrated that passively transferred immune serum was as effective in inhibiting trypanosome reproduction in splenectomized rats intensively blockaded with India ink as in normal rats.

Taliaferro and Taliaferro (1922) demonstrated by a unique method that certain "resistances" develop in the host against T. lewisi which retard its rate of reproduction. He states: "this resistance is so pronounced that during the last stage of the infection the parasites exist in the blood in the so-called 'adult' condition, without exhibiting any stages of reproduction or growth." The method consists of determining the coefficient of variation of the trypanosome population during the course of infection based on measurement of total length of the organism. The coefficient of variation is greater during

the first five days of infection, when reproduction is occurring at a rapid rate, than during the latter stages of infection when the coefficient of variation is lower. The coefficient of variation is a value from which the state of reproduction in a population of trypanosomes can be evaluated irrespective of the number of trypanosomes destroyed by the host's defenses. Taliaferro and Taliaferro (1922) also noted resistance against T. lewisi after reproduction has nearly ceased and the coefficient of variation is very low. However, this resistance suddenly destroys most of the organisms in the peripheral circulation and after disappearance trypanosomes can not be demonstrated in greater numbers in the tissues. Taliaferro (1924) first refers to the reproduction-inhibiting substance as a reaction product "in view of the fact that it arose as a result of infection and was passively transferred." Later, Taliaferro (1932) refers to this substance as antibody and proposes the name ablastin (Greek ablastos not budding: barren). Coventry (1925) showed that ablastin became evident by the fifth or sixth day and rose in titer until the thirty-fifth day after which it slowly decreased. Also, ablastin is most likely present before the fifth day

as indicated by the decrease in the coefficient of variation on approximately the fourth day.

The presence of other antibody-like substances that exhibit their action by decreasing or removing the T. lewisi organisms from the blood of the host was described by Coventry (1930). Serum collected before the termination of infection but after the first number crisis will, when injected, cause a disappearance of organisms in one to five hours in infected rats which have not yet undergone the first number crisis. Coventry (1930) contends that the removal of organisms indicates a trypanolytic substance probably correlated with the first number crisis. Serum collected after termination of the infection shows similar trypanocidal activity but diminishes in titer in 33 to 96 days as demonstrated by the inability to passively protect rats.

Taliaferro (1932) concluded that both the trypanocidal and reproduction-inhibiting antibodies are involved in the immunity to reinfection by T. lewisi. Augustine (1943) attributed reinfection-immunity to a single trypanocidal antibody which sensitizes reproducing forms rendering them vulnerable to phagocytosis and agglutinates "adults" which are then mechanically removed.

Characterization of antibody or antibody-like components of immune serum was initiated by Taliaferro (1932). He showed that the trypanocidal property of immune serum which demonstrates both curative and protective properties, precipitates with the globulin fraction. The trypanocidal component reveals a marked in vitro affinity for "adult" and reproducing forms and it can be adsorbed from immune serum by the addition of living trypanosomes. Trypanosomes incubated in immune serum are sensitized and when introduced into previously uninfected rats are rapidly removed from the circulation. Taliaferro (1932) has shown that ablastin resides in the globulin fraction, but unlike the trypanocidal component has no in vitro affinity for "adult" or dividing T. lewisi and can not be adsorbed from immune serum. Thillet and Chandler (1957) and Chandler (1958) stated that they could remove the protective value of serum from recovered rats by adsorption with lyophilized metabolic products, but not by lyophilized washed trypanosome bodies. With repeated injections of these same metabolic products, they protected rats against infection by T. lewisi. This study lead Chandler (1958) to propose that inhibition of reproduction is brought about by lower titers of ablastin

than is necessary for agglutination and is due to the neutralization of excreted metabolic products. These metabolic products are stated to be enzymes necessary for obtaining components vital to reproduction and growth of the trypanosome. Thus Chandler (1958), states that ablastin is responsible for the immunity to reinfection and is responsible for at least the first number crisis. D'Alesandro (1962) reports that he could not duplicate the experiments of Thillet and Chandler (1957).

At the present, several antibody theories for immmunity of rats to T. lewisi exist: (1) the one antibody theory of Chandler (1958); (2) the two or possibly three antibody theory of Taliaferro (1938); (3) the two antibody theory of Culbertson (1941) who proposed that there existed in serum one antibody, ablastin, which checks the propogation of the parasite and lysis the young dividing forms causing the first number crisis; and a second antibody which terminates the infection by lysis of the trypanosomes; (4) the three antibody theory of Barnes (1951) with ablastin bringing reproduction to an end and a trypanolytic antibody killing many of the "adults." In addition she stated there was an agglutinating antibody which caused "adult" trypanosomes to cohere in groups.

The Culbertson and Barnes theories are similar and are perhaps combinations of the Chandler and Taliaferro theories. Until further immunochemical and physicochemical characterization of the antibodies present in immune serum was undertaken, the theory of Taliaferro (1938) seemed to have had the greatest support.

D'Alesandro (1959) turned to physicochemical methods of investigating serum components of rats immune to T. lewisi. Using zone electrophoresis and ultracentrifugation he could not demonstrate a qualitative change in the animal's serum after infection. Both ablastin and the first trypanocidal antibody are associated with a small molecular weight globulin with a sedimentation rate of six Svedbergs while the terminal trypanocidal antibody is associated with a large molecular weight globulin with a sedimentation rate of 16 Svedbergs. Electrophoretically, both ablastin and the trypanocidal antibodies migrate between the beta and gamma globulins. These physicochemical studies support the theories proposing the sameness of ablastin and the first trypanocidal antibody; however the trypanocidal antibody can be removed by adsorption while ablastin can not.

In a later publication, D'Alesandro (1962) describes a method in which T. lewisi can be cultured in vitro for approximately 36 hours in the same physiological and morphological form as found in the circulatory system of the rat. He showed that results obtained in vitro agree with those found earlier in vivo in repect to ablastin activity. The in vitro activity of ablastin could not be removed from serum by adsorption with living trypanosomes and the differences in ablastin antibody concentration can be shown by titration. In addition, ablastin is not complement dependent as are the trypanocidal antibodies. Pizzi and Taliaferro (1960) and Taliaferro and Pizzi (1960) using radioisotope methods showed that ablastin supresses the synthesis of proteins and nucleic acids thus giving some indication of its mode of action.

Linton (1929) reported that the blood sugar of rats is not affected by infection with T. lewisi. However when rats are concurrently infected with T. lewisi and Haemobartonella muris a hypoglycemia developes during the terminal stages following splenectomy. Regendanz (1929) was able to cure Haemobartonella muris infections by treating rats with arsenicals without affecting T. lewisi. With this method he found T. lewisi became pathogenic and,

under these conditions, these rats showed a terminal hypoglycemia.

The rate of glucose metabolism has been studied in other trypanosome infections. Trypanosoma equiperdum in rats will produce a hypoglycemia by the consumption of the blood sugar at a rate in excess of that furnished by the rat, (von Fenyvessy, 1926, and Schern, 1928). Savino (1927) has demonstrated the effect of infections with T. equiperdum and T. equinum on the blood glucose in dogs. His studies revealed that the number of trypanosomes varied with the concentration of glucose; being less after the administration of insulin and greater after glucose was given intravenously. He further noted that the spleen affects the level of parasitemia since after splenectomy neither insulin nor glucose affected the number of trypanosomes and a normal parasitemia was observed. Poindexter (1935) produced hyperglycemia in guinea pigs by intraperitoneal injections of glucose, and he demonstrated a shortened prepatent period and an increased rate of reproduction of T. equiperdum. Groups of guinea pigs on various carbo hy drate diets infected with equal numbers of trypanosomes showed that animals on the lower carbohydrate diet lived 20 to 27% longer.

He also states: "that the decrease in blood sugar by insulin injection decreases the rate of multiplication of trypanosomes, as shown by examination of peripheral blood."

Culbertson and Malamut (1938) infected hypophysectomized rats with T. lewisi and found 70% more trypanosomes at the peak of infection than in normal infections.

Moulder (1948) reported that there are changes in the aerobic glucose metabolism of the trypanosomes which are chronologically related to the appearance of ablastin. He states: "that the changes in glucose metabolism are caused by the inhibition by ablastin of the oxidative assimilation of glucose in such a manner as to stop cell division and growth of T. lewisi."

Dunn, Sheehan and McLetchie (1943) and Dunn and McLetchie (1943) reported the successful production of diabetes in rats by the parenteral administration of alloxan (mesoxalylurea), an uric acid derivitive. They described a hyperglycemia greater than 300 mg/100 ml of blood. The diabetogenic action is destroyed within five minutes after an intravenous dose of alloxan is administered, but in this time considerable damage to the islets of Langerhans has occurred (Leech and Bailey, 1945 and Gomori and Goldner, 1945).

According to Lazarow and Palay (1946) the height of the resulting blood sugar after an intraperitoneal injection of alloxan is directly correlated with the severity of pancreatic damage.

The morphological changes which take place in the islets of Langerhans consist chiefly of selective necrosis and disappearance of the beta cells from the islet. During this process the beta cell appears degranulated and shrunken with nuclear pyknosis. After treatment with alloxan the destroyed islet is composed only of its capillaries and normal alpha cells. Lazarow and Palay (1946) have also cited renal and hepatic changes, but these changes could not be correlated with the size of injection of alloxan or the resulting blood sugar level.

A return to normal blood sugar levels after a demonstrated hyperglycemia does occur in some animals several weeks to several months after alloxanization, (Kass and Weisbren, 1945). They also demonstrated that starvation from 48 to 60 hours previous to subcutaneous administration of alloxan increases susceptibility to alloxan hyperglycemia and fewer animals will return to normoglycemia. They could not demonstrate any protection against alloxan damage by the administration of glucose.

It has long been observed that diabetic patients frequently are more susceptible to certain infections. Wertman and Henney (1962) reported that the subnormal phagocytizing capacity of the leukocyte was one of the possible causes of the reduced resistance of the diabetic to infection. Their results indicate that leukocytes from rats with a hyperglycemia produced by a diabetogenic dose of alloxan have a reduced capacity for phagocytizing Staphylococcus aureus. Further, leukocytes from animals which are refractory to alloxan, as indicated by a return to normoglycemia, have a normal capacity for phagocytizing Staphyloccoccus aureus.

Krahl (1961) in his publication "The action of insulin on cells," reviews much of the information available on the determination of the mechanism of action of insulin. Although the primary property of interest in this thesis is the ability of insulin to produce hypoglycemia, other actions which may influence the results need to be considered. A deficiency or overabundance of insulin in the mammalian system leads to several changes. Insulin regulates to a certain degree the utilization of glucose in peripheral tissues and storage of glycogen in the liver,

(Krahl, 1961). Nitrogen balance and the action of certain hormones are to a large extent governed by insulin secretion.

Gemmill (1940) and Gemmill and Hamman (1941) have demonstrated that insulin stimulates the uptake of glucose and glycogen synthesis in isolated rat diaphragm. It has been proposed by Beloff-Chain et al. (1955), and a number of later investigators, that insulin specifically facilitates diffusion of glucose through the cell membrane with subsequent glycogen formation. Krahl (1961) indicates that insulin stimulates the incorporation of some natural L-amino acids into muscle protein with the presence of glucose. The metabolic pathway for this can not be accounted for by an increased amino acid transport. An increase in the conversion of glucose to fatty acids is also seen with an increase in insulin.

The pituitary growth hormone and insulin are dependent upon each other. In order that the growth hormone of the adenohypophysis may promote nitrogen retention and subsequent protein synthesis, insulin must be present (Guyton, 1961). While the actions of growth hormone and insulin are mutually supplementary in nitrogen retention, they are mutually antagonistic in terms of carbohydrate

metabolism. Continued injection of pituitary extract produces permanent diabetes mellitus (Young, 1937). Adrenal cortex hormones do not effect insulin action directly but produce hyperglycemia by both inhibition of glucose utilization and increased gluconeogenesis (Long, et al., 1940).

Krahl (1961) points out that although single doses of insulin do not influence the output of glucose by the mammalian liver, perfusion studies show that net glucose output is somewhat decreased. When the animal is hypoglycemic, or after large doses of insulin are administered, glucose output of the liver is increased.

Elliott (1935) showed that insulin had no effect on the growth of the protozoan Colpidium in carbohydrate rich media. Burge and Williams (1927) demonstrated that insulin increased the rate of carbohydrate utilization in Paramecium, but Wichterman and Keltch (1956) as reviewed by Krahl (1961) state that "a heavy suspension of paramecia consumed no glucose either without or with addition of crystalline insulin."

## MATERIALS AND METHODS

Trypanosoma lewisi used in this study was maintained in rats by weekly transfer of blood taken from the tail vein of infected laboratory rats and diluted in 0.85% sodium chloride before intraperitoneal injection into previously uninfected rats. Infections for specific experiments were obtained by transfer of blood from infected stock rats in the sixth to ninth day of infection. Blood was obtained by cardiac puncture using a syringe containing a small amount of heparin dissolved in 0.85% sodium chloride. In Experiments I, II, and IV the inoculum used was a heparinized whole blood suspension diluted with 0.85% sodium chloride or with Hanks' balanced salt solution to the desired number of trypanosomes per $mm^3$. The number of trypanosomes in the peripheral blood of rats infected with T. lewisi was determined by collecting blood from the tail vein of the rat and diluting it one part in 200 parts of 3% acetic acid solution in a Thoma type pipette. The number of parasites in 1 $mm^3$ of whole blood was then determined in a hemocytometer just prior to preparation of the inoculum for experimental infections. The inoculum used in Experiment III contained washed trypanosomes suspended in Hanks'

balanced salt solution. Trypanosome infected heparinized whole blood was centrifuged at 1,000 rpm in an International model UV centrifuge (head no. 240) for ten minutes to concentrate the trypanosomes in the white cloudy layer above the layer of red blood cells. The trypanosomes were drawn off with a Pasteur pipette and mixed with 12 ml of Hanks' balanced salt solution. This mixture was centrifuged as described above and the supernatant discarded. The final inoculum contained the washed trypanosomes suspended in Hanks' balanced salt solution at the desired trypanosome concentration for intraperitoneal injection into experimental rats.

All rats used in these studies weighed between 120 g and 220 g. Rats used in Experiments I, II and III were white rats (CFN strain) obtained from Carworth Farms, Rockland County, New York. Rats used in Experiment IV were white rats (Sprague-Dawley strain) obtained from LuNor Farms, Oshtemo, Michigan. The animals were housed in wire mesh cages in groups not exceeding four animals per cage. Food and water were provided ad libitum. Food provided for Experiments I, II and III was Rockland Complete Rat Diet. This diet was supplemented with Kellogg Basal Diet

in Experiment IV to compensate for possible deterioration of the Rockland diet which had been stored several months.

Alloxan monohydrate manufactured by Nutritional Biochemicals Corporation was used in this study to produce hyperglycemia. Fresh aqueous solutions containing 20 mg/ml were injected subcutaneously after the method of Kass and Weisbren (1945). Permanent hyperglycemia usually resulted after injection of 175 mg alloxan per kg body weight if administered after a 48 to 60 hour fast.

Hypoglycemia was produced by the injection of Protamine Zinc Insulin U.S.P. prepared by Eli Lilly and Co. When uniformly suspended, each ml contains 40 units of insulin, together with approximately 0.50 mg of protamine and 0.80 mg of zinc. Each ml also contains approximately 1.6% glycerin (w/v), approximately 0.25% phenol (w/v) as a preservative, and approximately 0.2% dibasic sodium phosphate (w/v) as a buffer. The desired hypoglycemia was produced by a subcutaneous injection of 0.04 ml (1.6 units) every 12 hours into the loose dorsal tissue at the base of the tail. This procedure was found necessary since the inoculum volume was relatively small and requires accuracy. Accidental injection of excess insulin or intravenous injection resulted in hypoglycemic shock and death of the animal. To avoid these acci-

dents and to enable one person to make the inoculation, the tail of the rat was drawn through a piece of three-eighths inch wire hardware cloth and held firmly while the animal rested on a slippery surface.

Blood sugar determinations were made by the iron reduction micro method of Folin and Malmros (1929). The modifications and the adaptation to the colorimeter by Horvath and Knehr (1941) were followed. Blood samples were collected from the rat's tail vein in a 0.1 ml Folin micro pipette wetted with heparinized 0.85% sodium chloride solution. The optical density of the blood sugar samples was determined with a Bausch and Lomb Spectronic 20 colorimeter with a constant power source at a wave length of 520 mμ.

Since the blood sugar of some alloxanized rats spontaneously returned to normal, it was necessary to remove the pancreas from alloxanized and control rats at the termination of the experiment to determine the relative histological changes. The animals were sacrificed and portions of the pancreas were removed immediately. The tissues were fixed at room temperature in Bouin's fluid for 12 hours, and standard paraffin embedding techniques were used for preparation of slides for staining. Sections were cut at 6 μ and stained with aldehyde fuchsin (Halmi, 1952), and counter

stained with orange G. Using this method, the beta cells of the islets of Langerhans appear light blue with darker blue granules dispersed throughout the cytoplasm. The alpha cells appear light yellow and granular; and the pancreatic acini cells stain yellow-orange. Rats which have been alloxanized and demonstrate a hyperglycemia at the termination of the experiment usually show no evidence of beta cells in the pancreas, but in some cases a very few lightly stained beta cells may be present. Animals which have been alloxanized and have demonstrated a hyperglycemia, but at some time during the experiment returned to a normoglycemia, show large numbers of pancreatic beta cells which appear identical to the stained beta cells of normal animals.

The method used by Taliaferro and Taliaferro (1922) for determining the coefficient of variation of trypanosome populations was used to determine whether the parasites were reproducing or nonreproducing populations. Smears of blood from the tail vein of rats infected with T. lewisi were stained with Giemsa stain. The total length including the flagellum of 50 randomly chosen trypanosomes was measured by "stepping-off" their lengths with a calibrated dividers on a ground glass plate attached to a bellows

extension on a microscope. The standard deviation of the mean total length was calculated by the use of the formula:

$$\sigma = \sqrt{\frac{\Sigma x^2}{n} - M^2}.$$

The symbol $\sigma$ denotes the standard deviation, x is the magnitude of measurements, n is the number of individuals measured, and M is the mean length. The coefficient of variation was obtained from the above data by means of the formula: $c.v. = \frac{100\ \sigma}{M}$. Coefficients of variation may be as high as 40 for reproducing populations and as low as 2 for "adult" populations.

Experiment I: THE INFECTION OF ALLOXAN-TREATED RATS WITH TRYPANOSOMA LEWISI.

Thirteen rats of mixed sexes weighing 120 g to 170 g were randomly alloted to four groups: (A) seven alloxan-treated and trypanosome-infected rats, (B) three nontreated trypanosome-infected rats, (C) one alloxan-treated noninfected rat and (D) two nontreated and noninfected rats. Seventeen days previous to the infection of rats with T. lewisi, the animals were starved 48 hours and injected intraperitoneally with 175 mg of an aqueous alloxan mono-

hydrate solution per kg body weight. Each animal of groups A and B was inoculated with blood from stock rats in the sixth day of infection which was diluted with 0.85% sodium chloride so that the 0.5 ml of inoculum contained 2.1 X $10^7$ organisms. Values of the parasitemia were determined at one to three day intervals for 21 days and at five day intervals thereafter. Blood sugar values were determined on all animals 1, 3, 7, 10 and 18 days after inoculation with T. lewisi.

Experiment II: A CONTINUATION OF STUDIES OF THE INFECTION OF ALLOXAN-TREATED RATS WITH TRYPANOSOMA LEWISI.

Nineteen male rats weighing 170 g to 220 g were randomly alloted to four groups: (A) ten alloxan-treated and trypanosome-infected rats, (B) five nontreated trypanosome-infected rats, (C) one alloxan-treated noninfected rat and (D) three nontreated and noninfected rats. Eleven days previous to the infection of rats with T. lewisi the animals received alloxan monohydrate as in Experiment I. Each animal of groups A and B was inoculated with blood, from stock rats in the sixth day of infection, diluted with Hanks' balanced salt solution so that the 1.0 ml of inoculum

contained 1.0 X $10^5$ organisms. Values of parasitemia were determined on alternate days for 14 days. Blood sugar values were determined one day before inoculation of trypanosomes and on day seven of the infection.

Experiment III: A CONTINUATION OF STUDIES OF THE INFECTION OF ALLOXAN—TREATED RATS WITH TRYPANOSOMA LEWISI.

Fourteen male rats weighing 160 g to 210 g were randomly alloted to four groups: (A) five alloxan-treated and trypanosome-infected rats, (B) five nontreated trypanosome-infected rats, (C) one alloxan-treated noninfected rat and (D) three normal nontreated and noninfected rats. Rats in this experiment were treated with alloxan twice; 53 and 18 days previous to infection with T. lewisi. Each animal of groups A and B was inoculated with washed trypanosomes suspended in Hanks' balanced salt solution in a concentration of 2.8 X $10^7$ organisms in the 1.0 ml of inoculum. The trypanosomes were obtained from stock rats on the ninth day of infection. Values of parasitemia were determined on alternate days for 14 days. Blood sugar values were determined on all animals four days before infection and on day nine of the infection.

Experiment IV: THE INFECTION OF INSULIN-TREATED RATS WITH TRYPANOSOMA LEWISI.

Fifteen male rats weighing 155 g to 200 g were randomly alloted to four groups: (A) five insulin-treated and trypanosome-infected rats, (B) five nontreated trypanosome-infected rats, (C) three insulin-treated noninfected rats and (D) two nontreated and noninfected rats. The administration of insulin was initiated 48 hours previous to the inoculation of T. lewisi. Each animal of groups A and B was inoculated with 1.0 ml of heparinized whole blood from a stock rat in the seventh day of infection which contained $1.4 \times 10^7$ T. lewisi organisms. Values of parasitemia were determined on alternate days for 11 days. Blood sugar values were determined on all animals on the day of injection with T. lewisi and then on days three, five and nine.

# RESULTS

## Experiment I — THE INFECTION OF ALLOXAN-TREATED RATS WITH *TRYPANOSOMA LEWISI*.

Results of Experiment I are presented in Table I and the group means from these data are graphed in Figure I. Parasitemias in alloxanized rats reached a higher peak approximately two days after the parasitemias in non-alloxanized infected animals. One individual animal (number one of group B) reached a parasitemia level approximately the same as rat number five of group A; the lowest parasitemia level of the alloxanized group of animals. The parasitemias in alloxanized rats attained a higher level and remained at this level approximately six days longer than in conventional animals infected with *T. lewisi*.

The average blood sugar concentration of normal non-infected rats was 119 mg/100 ml of blood. Rats infected with *T. lewisi* had an average blood sugar level of 112 mg/100 ml of blood. Average blood sugar levels of alloxanized rats was 243 mg/100 ml of blood while alloxanized infected rats showed 240 mg/100 ml of blood. Thus the blood sugar concentration of alloxanized rats was increased

TABLE I

RESULTS OF INOCULATING TRYPANOSOMA LEWISI INTO WHITE RATS WHICH HAVE BEEN TREATED WITH ALLOXAN IN EXPERIMENT I

| Rat No. | Sex | $10^4$ trypanosomes in one $mm^3$ of blood on day: | | | | | | | | | | | | Blood sugar in mg/100 ml of blood on day: | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 5 | 6 | 8 | 10 | 13 | 15 | 17 | 19 | 21 | 25 | 31 | 1 | 3 | 7 | 10 | 18 |
| | | Group A (alloxanized and infected) | | | | | | | | | | | | | | | | |
| 1 | F | 9 | 21 | 18 | * | * | * | * | * | * | * | * | * | 249 | 242 | * | * | * |
| 2 | F | 3 | 45 | 28 | 66 | 52 | 54 | 31 | 30 | 29 | 32 | 0 | 0 | 242 | 242 | 236 | 249 | 223 |
| 3 | F | 32 | 53 | 65 | 47 | 47 | 44 | 23 | 38 | 36 | 22 | 4 | 0 | 242 | 242 | 242 | 249 | 229 |
| 4 | F | 13 | 39 | 43 | — | 16 | 10 | 3 | 0 | 0 | 1 | 0 | 0 | 249 | 242 | 236 | 249 | 223 |
| 5 | M | 14 | 37 | 41 | 31 | 23 | 23 | 27 | 18 | 19 | 18 | 11 | 0 | 249 | 242 | 242 | 249 | 229 |
| 6 | F | 32 | 57 | 56 | 41 | 11 | 25 | 22 | 12 | 6 | 1 | 0 | 0 | 223 | 116 | 148 | 113 | 152 |
| 7 | F | 21 | 61 | 75 | 55 | 29 | 37 | 40 | 43 | 19 | 8 | 0 | 0 | 242 | 169 | 165 | 139 | 152 |
| | | Group B (nontreated and infected) | | | | | | | | | | | | | | | | |
| 1 | M | 21 | 38 | 47 | 22 | 18 | 14 | 10 | 0 | 0 | 0 | 2 | 0 | 116 | 119 | 118 | 109 | 124 |
| 2 | M | 19 | 28 | 28 | 24 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 109 | 119 | — | 116 | 113 |
| 3 | M | 16 | 28 | 35 | 34 | 14 | 12 | 7 | 3 | 1 | 2 | 1 | 0 | 120 | 127 | 121 | 131 | 135 |
| | | Group C (alloxanized and noninfected) | | | | | | | | | | | | | | | | |
| 1 | M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — | — | 242 | 242 | 242 | 249 | — |
| | | Group D (nontreated and noninfected) | | | | | | | | | | | | | | | | |
| 1 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 113 | 119 | 114 | 128 | 120 |
| 2 | F | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 116 | 116 | — | — | 124 |

* tail gangrenous, no sample taken — no sample taken

Figure I: Average T. lewisi parasitemias in alloxan treated rats (group A) and nontreated rats (group B) and average blood sugar concentrations of rats in these groups.

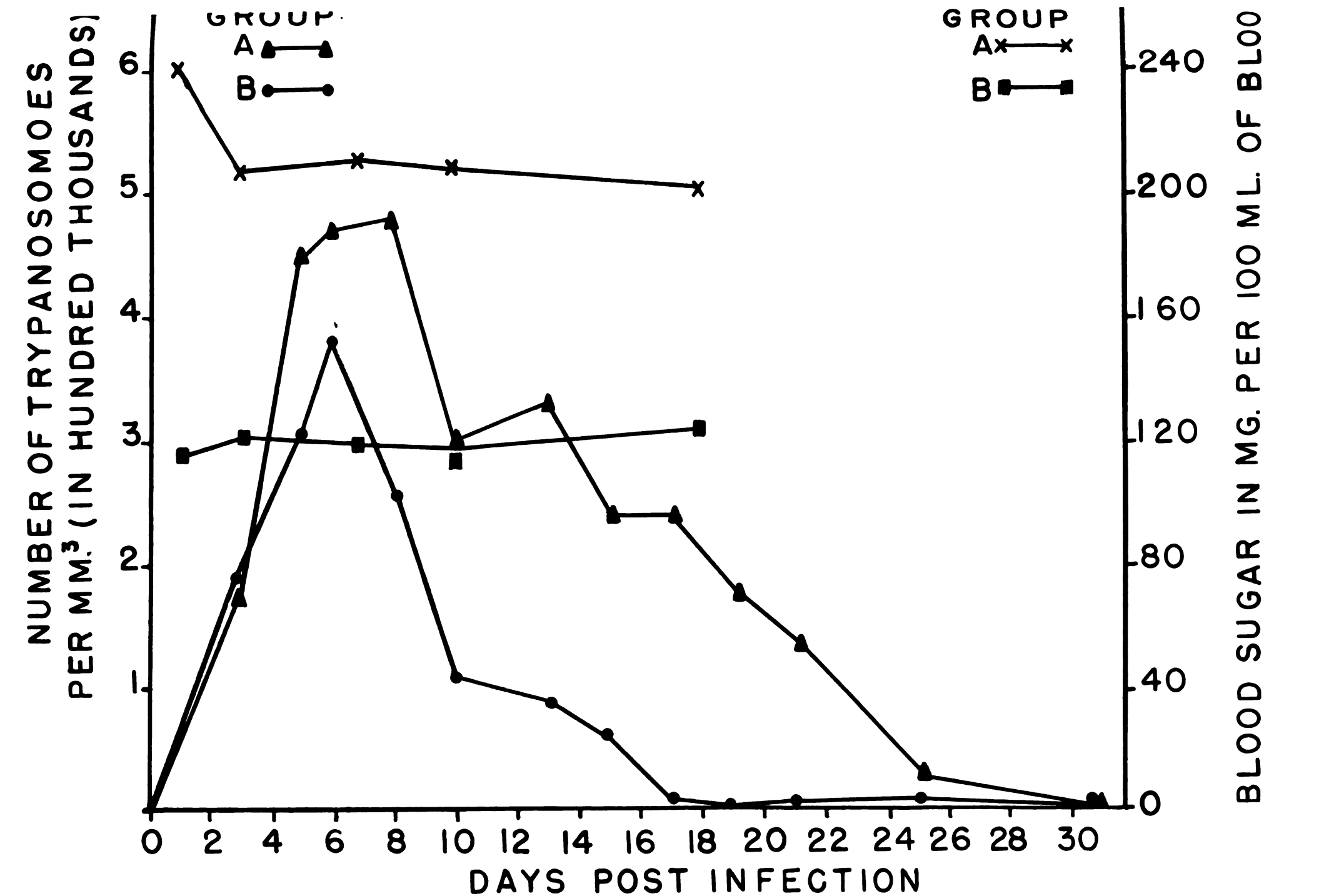

GROUP
A
B
GROUP
A
B
NUMBER OF TRYPANOSOMOES PER MM.³ (IN HUNDRED THOUSANDS)
BLOOD SUGAR IN MG. PER 100 ML. OF BLOO
0
1
2
3
4
5
6
0
40
80
120
160
200
240
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
DAYS POST INFECTION

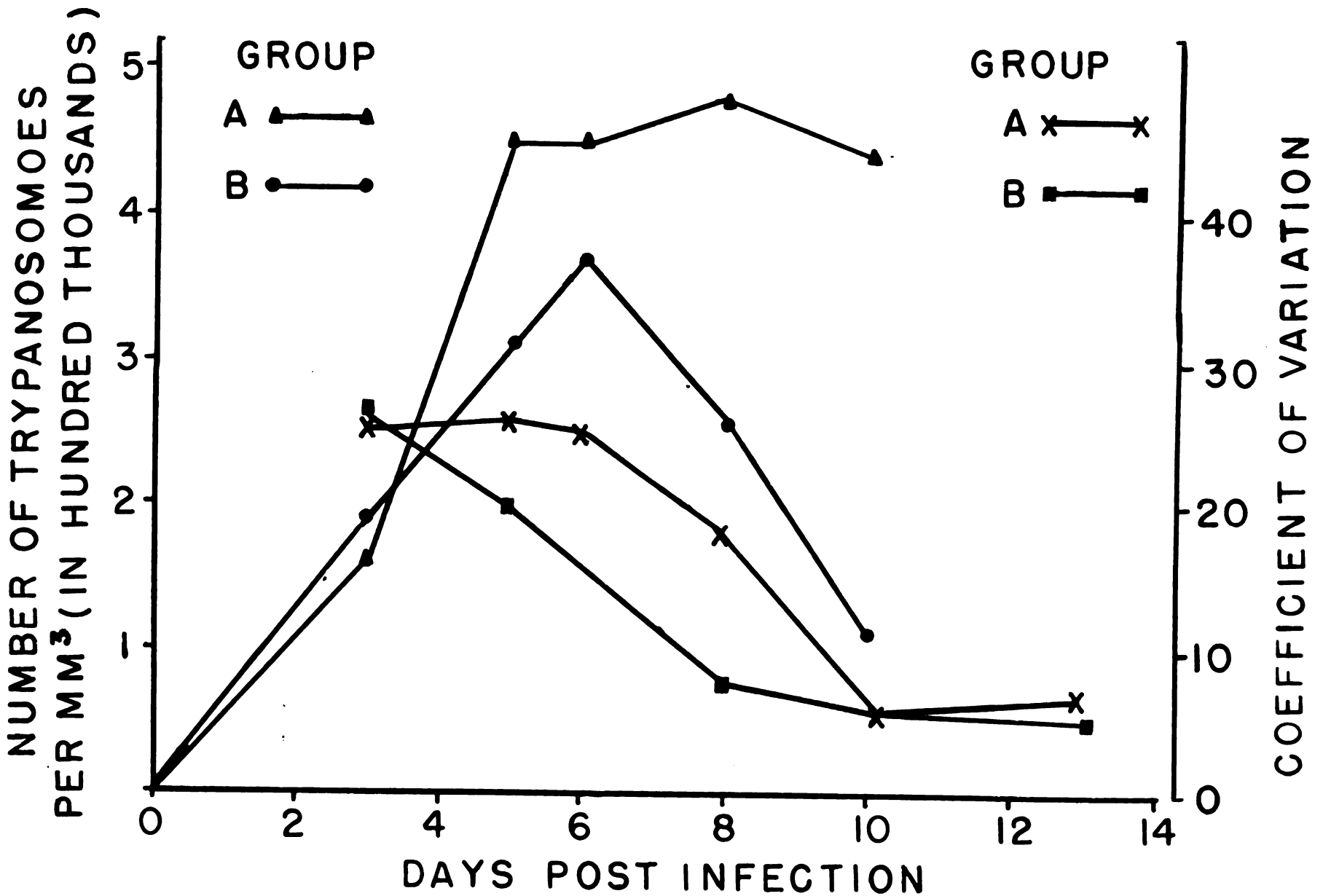
TABLE II

RESULTS OF DETERMINATIONS OF COEFFICIENTS OF VARIATION OF RAT PARASITEMIAS IN EXPERIMENT I

| Rat number | Coefficient of variation on day: 3 | 5 | 6 | 8 | 10 | 13 |
|---|---|---|---|---|---|---|
| | | | Group A (alloxanized and infected) | | | |
| 2 | 25.8 | 34.9 | 30.9 | 22.7 | 5.3 | 5.4 |
| 3 | 27.6 | 26.4 | 25.1 | 9.8 | 5.4 | 9.5 |
| 5 | 22.9 | 17.4 | 18.7 | 22.4 | 6.1 | 5.7 |
| | | | Group B (nontreated and infected) | | | |
| 1 | 26.0 | 14.9 | 9.4 | 5.8 | 5.1 | 3.8 |
| 2 | 27.2 | 15.0 | 12.5 | 11.2 | — | — |
| 3 | 24.4 | 28.9 | 25.2 | 7.7 | 6.7 | 5.5 |

— no sample taken

Figure II: Average T. lewisi parasitemias in three alloxanized rats (group A) and three nontreated rats (group B), and the average coefficients of variation of these groups.


NUMBER OF TRYPANOSOMOES PER MM³ (IN HUNDRED THOUSANDS)
GROUP
A
B
GROUP
A
B
COEFFICIENT OF VARIATION
0
1
2
3
4
5
0
10
20
30
40
0
2
4
6
8
10
12
14
DAYS POST INFECTION

approximately 113% more than the normal value in conventional animals. Special note of the resulting parasitemias and blood sugar concentrations of rats six and seven of group A should be made. Both of these animals demonstrated relatively high parasitemias but the blood sugar levels began to return to normal values shortly after the start of the infection. Tissue sections of the pancreases of these two animals, stained with aldehyde fuchsin, demonstrated that there were normal appearing beta cells present in a far greater number than in the hyperglycemic, alloxanized rats of group A.

There is no predetermined point at which the coefficient of variation indicates a change from a reproducing to a nonreproducing population, but values below ten correspond to parasitemias which show very few reproducing forms in stained blood smears with respect to division of cell organelles or cytoplasm. Examination of the coefficient of variation of parasite populations in several animals from groups A and B indicate that the trypanosome reproduction continues at a higher level for approximately seven days longer in alloxanized rats than in conventional rats (see Table II and Figure II). These differences are not significant at the 1% level as determined by a "t" test.

TABLE III

RESULTS OF INOCULATING TRYPANOSOMA LEWISI INTO MALE WHITE RATS WHICH HAVE BEEN TREATED WITH ALLOXAN IN EXPERIMENT II

| Rat No. | $10^4$ trypanosomes in one $mm^3$ of blood on day: | | | | | | | Blood sugar in mg/100 ml of blood on day: | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 0 | 7 | 13 |
| | | | | Group A (alloxanized and infected) | | | | | | |
| 1 | 0.5 | 1.5 | 15.5 | 29.5 | 32.5 | 27.5 | 26.0 | 256 | 249 | 143 |
| 2 | 2.0 | 1.5 | 41.0 | 27.0 | 21.0 | 16.0 | 15.0 | 256 | 263 | 262 |
| 3 | 1.5 | 1.0 | 9.0 | 13.0 | 21.0 | 0.0 | 0.0 | 256 | 263 | 254 |
| 4 | 1.0 | 2.0 | 0.0 | 20.0 | 111.0 | * | | 248 | — | * |
| 5 | 4.5 | 6.5 | 16.5 | 45.5 | 127.0 | 104.5 | 60.0 | 232 | — | 254 |
| 6 | 3.5 | 1.0 | 27.0 | 34.0 | 35.0 | 33.0 | 32.0 | 232 | 242 | 127 |
| 7 | 0.0 | 2.0 | 13.5 | 33.5 | * | | | 232 | — | * |
| 8 | 0.0 | 0.5 | 0.0 | 0.5 | 4.0 | 3.0 | 0.0 | 263 | 263 | 262 |
| 9 | 1.0 | 4.5 | 37.0 | * | | | | 248 | * | |
| 10 | 1.0 | 1.5 | 3.5 | 2.5 | 1.5 | 4.0 | 2.0 | 263 | 263 | 254 |
| | | | | Group B (nontreated and infected) | | | | | | |
| 1 | 1.5 | 1.0 | 29.0 | 35.5 | 43.0 | 26.5 | 31.5 | — | 119 | 111 |
| 2 | 0.5 | 3.5 | 13.5 | 11.0 | 1.0 | 0.0 | 0.0 | — | 123 | 119 |
| 3 | 1.5 | 1.5 | 20.0 | 20.0 | 18.5 | 0.0 | 0.0 | — | 127 | 130 |
| 4 | 2.5 | 3.0 | 4.0 | 9.5 | 3.5 | 0.0 | 0.0 | — | 131 | 123 |
| 5 | 0.5 | 0.5 | 3.0 | 4.0 | 5.5 | 1.5 | 0.5 | — | 108 | 127 |
| | | | | Group C (alloxanized and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 263 | 263 | 135 |
| | | | | Group D (nontreated and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 139 | 116 | 87 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 127 | 135 | 131 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 143 | 108 | 97 |

* died — no sample taken

Statistical evaluation of the differences in peak parasitemias and the parasitemia values on the thirteenth day of infection in groups A and B show that groups A and B are not significantly different at the one percent level as determined by a "t" test.

Experiment II – A CONTINUATION OF STUDIES OF THE INFECTION OF ALLOXAN–TREATED RATS WITH TRYPANOSOMA LEWISI.

This experiment was designed to repeat Experiment I, but due to some unknown cause, rats used in Experiments II and III developed what appeared to be a nervous system disorder which subsequently resulted in death of several animals. Animals showing the nervous symptoms were submitted to the Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University for diagnosis, but no conclusive diagnosis was made. A nutritional deficiency is a possibility.

Parasitemia values of this experiment are presented in Table III, and it can be seen that parasitemias were erratic not only in the alloxanized rats but in nontreated infected animals. Although alloxanized rats four and five of group A developed extremely high parasitemias, there were also animals which were alloxanized but developed only low parasitemias (i.e. rats three, eight and ten of group A).

TABLE IV

RESULTS OF INOCULATING *TRYPANOSOMA LEWISI* INTO MALE WHITE RATS WHICH HAVE BEEN TREATED WITH ALLOXAN IN EXPERIMENT III

| Rat no. | $10^4$ trypanosomes in one mm3 of blood on day: | | | | | | | | | Blood sugar in mg/100 ml of blood on day: | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 0 | 9 |
| | | | | | Group A (alloxanized and infected) | | | | | | |
| 1 | 0.0 | 0.0 | 1.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 263 | 254 |
| 2 | 0.0 | 0.0 | 3.5 | 15.5 | 10.0 | 4.0 | 6.5 | 2.0 | 0.0 | 271 | 254 |
| 3 | 0.0 | 0.0 | 1.0 | 4.5 | 0.0 | 0.5 | 0.0 | 0.0 | 0.0 | 156 | 189 |
| 4 | 0.0 | 4.0 | 5.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 152 | 148 |
| 5 | 0.0 | 0.0 | 12.5 | 116.0 | 138.0 | 155.0 | 209.0 | * | | 139 | 117 |
| | | | | | Group B (nontreated and infected) | | | | | | |
| 1 | 0.0 | 7.0 | 39.5 | 71.0 | 47.0 | 55.5 | 48.5 | ** | | 124 | 96 |
| 2 | 0.0 | 0.5 | 3.5 | 17.5 | 7.5 | 8.5 | 4.0 | 4.5 | 0.0 | 113 | 139 |
| 3 | 0.0 | 0.0 | 2.0 | 13.5 | 7.0 | 7.0 | 3.0 | 0.0 | 0.0 | 124 | 84 |
| 4 | 0.0 | 0.0 | 5.5 | 48.0 | 55.5 | 73.5 | 53.5 | 53.0 | 21.5 | 128 | 103 |
| 5 | 0.0 | 10.0 | 35.5 | 26.0 | 23.0 | 21.0 | 23.5 | 0.0 | 0.0 | 113 | 99 |
| | | | | | Group C (alloxanized and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 236 | 170 |
| | | | | | Group D (nontreated and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 124 | 80 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 131 | 120 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 120 | 128 |

* died ** sacrificed for pathological examination

In this experiment, variations in blood sugar levels were observed which were not present in Experiment I. The alloxanized control rat, number one of group C, demonstrated a spontaneous reduction in blood sugar level. Two of the rats in group D (normal rats) showed a progressive hypoglycemia when compared to normal blood sugar levels of 119 mg/100 ml of blood recorded in Experiment I.

## Experiment III — A CONTINUATION OF STUDIES OF THE INFECTION OF ALLOXAN-TREATED RATS WITH TRYPANOSOMA LEWISI.

The rats used in Experiment III were free of symptoms suggesting nervous system pathology until the onset of the trypanosome infection. At the termination of the experiment, six of the rats behaved abnormally. The results of Experiment III are similar to those of Experiment II and are given in Table IV. Some parasitemias reached extremely high levels while others never equaled the normally observed parasitemia of T. lewisi. Rats one, three and four of group A appear to have been refractory to infection by T. lewisi. The period of prepatency was lengthened by approximately two days in animals one, two, three and five of group A and rats three and four of group B.

Blood sugar levels fluctuated in all four groups with the one alloxan treated noninfected rat returning to a

lower blood sugar level. The nontreated and noninfected rats showed slightly higher blood sugar concentrations than normal.

Experiment IV — THE INFECTION OF INSULIN-TREATED RATS WITH TRYPANOSOMA LEWISI.

In order to avoid the diseased conditions of the animals in Experiments II and III, all materials which might come in contact with the experimental rats were sterilized and a new stock of rats was obtained from a different source. The modified diet stated under "Materials and Methods" was fed to these animals. Throughout the experiment no animal abnormalities were observed except those produced by T. lewisi and insulin.

Trypanosome parasitemias were nearly identical in both groups A and B. (See Table V and Figure III.) Rats in group B demonstrated a slightly higher parasitemia on about the fifth day. After the ninth day of infection the parasitemias of groups A and B coincided.

Statistical evaluation of peak trypanosome population differences show that groups A and B are not significantly different at the one percent level as determined by a "t" test.

TABLE V

RESULTS OF INOCULATING *TRYPANOSOMA LEWISI* INTO MALE WHITE RATS WHICH HAVE BEEN TREATED WITH INSULIN IN EXPERIMENT IV

| Rat no. | $10^4$ trypanosomes in one $mm^3$ of blood on day: | | | | | Blood sugar in mg/100 ml of blood on day: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | 5 | 7 | 9 | 11 | 1+ | 3++ | 5++ | 9++ |
| | | | Group A (insulinized and infected) | | | | | | |
| 1 | 8.5 | 31.5 | 45.5 | 37.0 | 4.5 | 152 | 37 | 66 | 37 |
| 2 | 18.5 | 47.0 | 42.0 | 46.0 | 36.5 | 152 | 37 | 35 | 54 |
| 3 | 15.0 | 30.0 | 29.0 | 21.5 | 21.0 | 70 | 51 | 35 | 32 |
| 4 | 9.0 | 45.5 | 46.5 | 34.0 | 31.0 | 139 | 54 | 54 | 32 |
| 5 | 6.0 | 22.5 | * | | | 70 | 63 | 57 | * |
| | | | Group B (nontreated and infected) | | | | | | |
| 1 | 14.0 | 26.0 | 26.0 | 13.0 | 9.5 | 139 | 111 | 114 | 100 |
| 2 | 13.5 | 48.0 | 33.5 | 19.5 | 14.0 | 134 | 111 | 107 | 122 |
| 3 | 12.0 | 50.5 | 92.5 | 86.0 | 55.5 | 126 | 96 | 96 | 103 |
| 4 | 14.0 | 47.5 | 31.0 | 22.0 | 3.5 | 113 | 103 | 89 | 89 |
| 5 | 12.5 | 84.0 | 55.0 | 53.0 | 28.5 | 134 | 100 | 93 | 111 |
| | | | Group C (insulinized and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 111 | 57 | 51 | 37 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 111 | 45 | 45 | 43 |
| 3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 51 | 57 | 82 | 51 |
| | | | Group D (nontreated and noninfected) | | | | | | |
| 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 130 | 111 | 113 | 114 |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 111 | 100 | 100 | 100 |

* animal died from an overdose of insulin
\+ eight hours after administration of insulin
++ six hours after administration of insulin

Figure III: Average T. lewisi parasitemias in insulin treated rats (group A) and nontreated rats (group B), and average blood sugar concentration of rats in these groups.

NUMBER OF TRYPANOSOMES
PER MM³ (IN HUNDRED THOUSANDS)
5
4
3
2
1
0
GROUP
A
B
GROUP
A
B
0
2
4
6
8
10
12
DAYS POST INFECTION
200
160
120
80
40
0
BLOOD SUGAR IN MG. PER 100 ML. OF BLOOD

This experiment was designed to keep the blood sugar level as low as possible without jeopardizing the lives of the animals. One rat died showing typical hypoglycemic shock symptoms due to an accidental injection of insulin intravenously. Rats with recorded blood sugar levels of 32 to 35 mg/100 ml of blood did not show any abnormal symptoms with the exception of slightly lowered physical activity. Average blood sugar levels six hours after administration of insulin in group A and C were 46 and 52 mg/100 ml of blood respectively. Values for group B, the infected nontreated rats, and group D, the normal animals, were 109 and 110 mg reducing sugar per 100 ml of blood respectively. Thus the blood sugar concentration of insulin treated infected rats was approximately one-half the concentration of nontreated infected rats.

## DISCUSSION AND CONCLUSIONS

The hyperglycemias induced by alloxan in the rats of Experiments I, II, and III are comparable to the hyperglycemias reported by previous workers (Dunn and McLetchie 1943). The administration of alloxan intraperitoneally produced a blood sugar value which stabilized at a level considered high enough for investigating the effects of hyperglycemia on Trypanosoma lewisi. It was believed unnecessary to use intravenous administration of alloxan which would produce higher blood sugar levels but would also produce excessive pathology.

The results indicate that the parasitemias obtained in untreated controls are comparable in respect to numbers of organisms and duration to those parasitemias reported by previous workers (Taliaferro and Taliaferro, 1922; and Coventry, 1925, 1930). The coefficients of variation of the trypanosome populations were similar to those reported by Coventry (1925).

Statistical analysis of the data indicates that there is no significant difference between parasitemias of T. lewisi observed in normal and alloxan hyperglycemic rats.

Statistical evaluation of the differences in the coefficients of variation of T. lewisi in infected alloxanized rats and infected nontreated rats show that the differences are not significant at the 1% level. Thus, an increase in available blood sugar as produced by alloxan will not alter the parasitemia in T. lewisi infection.

Although additional quantities of glucose are available to the trypanosomes for increasing their metabolism and reproduction, the trypanosomes apparently do not modify their cycle in the rat. This observation indicates that either glucose is not the limiting factor in T. lewisi metabolism; or other changes which result from alloxanization of rats, such as decreases of insulin, increases of acetone bodies, unesterified fats, triglycerides, cholesterol, phospholipids, urates and amino acids prevent T. lewisi from utilizing the increased amount of glucose. Houssay (1955) states that since fasted diabetic animals continue to secrete glucose, gluconeogenesis appears to proceed in the absence of insulin. It is probable that the principal source of glucose here is the glycogenic amino acids of proteins that are catabolized. Thus, the diabetic animal with disturbances in normal glucose oxidation also looses protein which may affect the production

of immune globulins and subsequently influence the infection.

Augustine (1943) concluded that phagocytosis was an important factor in the animals' defense against T. lewisi. Wertman and Henny (1962) showed that alloxan has an affect on infection by Staphylococcus aureus due to the impairment of phagocytic activity. From the data of these workers one might expect these same effects to occur in the present study, however these results are contrary to Wertman's and Henny's.

The results of Experiment IV, in which hypoglycemia was induced, give additional evidence that the blood sugar concentration in the ranges studied has little effect on T. lewisi infections. The indications are that trypanosome growth is not significantly decreased even though the blood sugar concentrations are lowered to 58% of normal values. According to Somogyi (1927) blood sugars in man determined by reduction type methods include approximately 27 ± 4 mg/100 ml of blood of nonglucose reducing substances. Therefore the "true"blood glucose level six hours after administration of insulin in these experiments would be 5 mg/100 ml of blood.

Moulder (1948) determined that 3 X $10^8$ intact T. lewisi organisms in a phosphate-saline medium utilized 0.2 mg of glucose per hour in the case of "adult" organisms and 0.4 mg in the reproducing forms. If an average parasitemia of T. lewisi attains a peak of 3 X $10^5$ organisms per $mm^3$ or 3 X $10^8$ organisms per ml of blood, and the total blood volume of a rat is approximately 15 ml, then the total population of the reproducing T. lewisi would be 4.5 X $10^9$, and this population would utilize 18 mg of glucose per hour using Moulder's 0.4 mg value. The mean blood sugar of the noninfected and nontreated rats in this study was 119 mg/100 ml of blood, but considering Somogyi's (1927) work this would actually be 92 mg/100 ml of blood. This 92 mg/100 ml of blood value provides a total blood glucose concentration of 13.8 mg per rat or 4.2 mg less than the projected trypanosome population could utilize. Hoppe and Chapman (1947), using the Folin Wu copper reduction method for determining blood sugar concentrations, state that 32.8 mg of blood sugar or 5.8 mg of glucose per 100 ml of blood is "below the physiological limit necessary to the life of the rat." By treating the rat with insulin the "true blood glucose" is reduced at six hours after injection to

5 mg/100 ml of blood or 0.75 mg of glucose in the total blood volume of the rat. These calculations indicate that the true blood glucose is below the physiological limit necessary for life of the animal and hypoglycemic shock would ensue. It appears that more investigation is needed to determine the amount of glucose needed for the maintenance of life in the rat, the normal glucose turnover rate in the rat, and the essentiality of glucose to T. lewisi.

Experiments II and III can not be used to establish an conclusions since normal parasitemias were not uniformly present in group B. Although it was not experimentally determined, the reasons for failure were probably due to the nervous system disorder described in the "Results" or to the decreased viability of the trypanosomes by the washing procedure of Experiment II.

Suramin Sodium, an effective trypanocidal agent and alloxan monohydrate are related chemically to the same parent compound urea. Any direct action that alloxan would have on the trypanosome organism per se is remote as Leech and Bailey (1945) have demonstrated that the toxic effects due to alloxan are limited to a few minutes immediately

following an intravenous injection. Since the rats in this study were treated with alloxan at least 11 days prior to inoculation with trypanosomes, any trace of the drug would presumably have disappeared and would not be contributing to the results directly.

The present study does not show a significant increase in the number of trypanosomes in rats with a hypoglycemia produced by insulin. This is not in agreement with Culbertson and Malamut (1938) who reported an increase in parasitemia levels of T. lewisi in hypophysectomized rats. The difference in results suggests that the concentration of blood sugar is not the direct cause of the increased parasitemia obtained by hypophysectomized rats, or by treating rats with insulin.

The relative parasitemias seen in this study compared to the parasitemias reported in the unpublished results of Clark and Patton suggest that increased gluconeogenesis induced by the administration of dexamethasone is probably not the major contributing factor to the extremely high parasitemias observed.

## SUMMARY

It has been demonstrated that the hyperglycemia in rats resulting after the destruction of the beta cells of the islets of Langerhans with alloxan does not significantly affect the peak attained by a parasitemia of T. lewisi. Likewise, the height of the parasitemia after the number crisis on the thirteenth day and the duration of the infection are not significantly different from that attained in normal hosts.

The coefficients of variation of the parasite populations in infected alloxanized rats are not significantly different from infected nontreated rats.

Physiological changes other than hyperglycemia produced by the administration of alloxan which might have an effect on the parasitemia are listed. No experimental evidence is presented which discounts the effects of these changes.

Interpretation of the results indicates that T. lewisi does not utilize glucose in vivo in quantities as great as previously determined in vitro. Results indicate that it is doubtful that rats would survive infections with T.

*lewisi* and hypoglycemia produced by insulin if as much glucose is required by *T. lewisi* as in vitro studies indicate.

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