THE EFFECT OF LYSED TRYPANOSOMA EQUIPERDUM PREPARATIONS

ON BLOOD SUGAR IN THE RAT

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INTRODUCTION AND HISTORICAL REVIEW

Notwithstanding various theories as regard the method by which __Trypanosoma equiperdum__ causes death to the host, it is generally agreed that, at least prior to death, the carbohydrate metabolism of the host is disturbed. This circumstance is manifested in the severe decrease of glucose in the blood, accumulation of lactic acid as an end-product of glucose metabolism, depletion of the glycogen reserves, and reduced ability to synthesize glycogen from sugar. Furthermore, it is agreed that the carbohydrate metabolism of these pathogenic trypanosomes is of an intense nature and proceeds only as far as pyruvic acid.

In view of these characteristics of the parasitic infection, the following hypothesis suggests itself: Trypanosomes contain or elaborate a substance, either enzymatic or insulin-like, which directly or indirectly influence the glucose metabolism of the host.

Chen and Geiling (11) were able to demonstrate glycolysis with an __in vitro__ preparation of lysed __Trypanosoma equiperdum__. The experimental results obtained by Chen (9) indicate glycolysis was due to the presence of hexokinase, 3-phosphoglyceraldehyde dehydrogenase, and adenosine triphosphatase systems in the parasites. It therefore appeared desirable to substantiate such an effect in animals. Accordingly, the purpose of this study has been an attempt

to demonstrate the activity of glycolytic enzymes in lysed preparations of _Trypanosoma equiperdum_ by _in vivo_ methods.

Trypanosomiasis and diseases directly caused by trypanosomes, affecting both man and beast, have been referred to since the beginning of the sixteenth century (40). However, only with the development of slave trade and commercial exploitation of equatorial Africa was there made any effort to investigate these diseases.

In 1880, Evans (14) showed that surra in horses was caused by trypanosomes. Bruce (8), in 1895, proved trypanosomes to be the causal organism of nagana, a disease of domestic cattle and horses. He demonstrated that the infection was transmitted by glossina, tsetse flies, with wild game serving as reservoirs of infection.

The Royal Society Sleeping Sickness Commission (1903 to 1906) proved that sleeping sickness was caused by _Trypanosoma gambiense_ and was transmitted from man to man by the tsetse fly, _Glossina palpalis_ (41). Stephens and Fantham (61) showed that another form of sleeping sickness was caused by _Trypanosoma rhodesiense_ which was transmitted by the tsetse fly, _Glossina morsitans_. The trypanosomes within the tsetse fly, acting as vector, undergo a cyclic development before they are transmitted.

Trypanosma brucei, the organism seen by Bruce (8), may be identical with _Trypanosoma rhodesiense_, but further investigation appears necessary to prove this. _Trypanosoma cruzi_ infects not only man but is fatal for guinea pigs,

rats, and mice. Its insect vector is any one of several species of _Triatoma_ or bedbug. The trypanosome seen by Evans in 1880 and named for him, _Trypanosoma evansi_, is fatal to most laboratory animals as well as horses. Cattle, sheep, and goats, however, usually survive the infection. The infection produced by _Trypanosoma lewisi_ is only midly pathogenic to the rat and occurs only in this species (46).

Sixty years after the first trypanosome was discovered by Valentin of Berne (63) in 1841, the causal organism of dourine in horses was identified and named _Trypanosoma equiperdum_ by Doflein (64). This parasite was variously named by succeeding investigators (_Trypanosoma rougeti_ Laveran and Mesnil, 1901; _Trypanozoon equiperdum_ Luhe, 1906; _Castellanella equiperdum_ Chalmers, 1918), but the original designation of Doflein persists.

Trypanosoma equiperdum infections are endemic in several European countries, North and South America, and North Africa. "Mal de coit," as this malady is also called, occurs in horses and donkeys and is of a chronic character. Edema of the sexual organs manifests itself initially within ten to fourteen days after infection, and patches of peculiar lesions appear on the skin about a month later. These plaque-shaped lesions resemble hard subcutaneous discs and persist from a few hours to several days. Intermittent symptoms, suggesting a relapse phenomenon, occur quite frequently, but progressive weakening and loss of weight, associated with fever and anemia, continue until the animal

succumbs to the infection. Death is preceded by various
nervous symptoms and a general paralysis. Ordinarily,
horses and donkeys survive only two to twelve months after
becoming infected with Trypanosoma equiperdum.

Infections with Trypanosoma equiperdum have been pro-
duced in white rats, white mice, rabbits, hamsters, cotton
rats, guinea pigs, deer mice, dogs, monkeys, sheep, and
goats (19) (65). The infection in cattle is chronic but,
usually, non-fatal. Duration of the disease in inoculated
laboratory animals varies, depending on the virulence of the
strain and the number of parasites injected. Rabbits dis-
play a chronic infection characterized by unusual involve-
ment of the respiratory tract (19) plus the symptoms apparent
in the horse. Recovery from the disease by rabbits has been
noted (64), but death usually occurs in three to four months.
Mice and rats may survive three to five days, guinea pigs
one to three months, and dogs two to three months (65) and
sometimes as long as five months.

Dogs and rabbits, like horses and donkeys, may transmit
the parasites during coitus, i.e., by direct contact of the
mucous membranes. This method appears to be the only one by
which Trypanosoma equiperdum is transmitted in nature since
ostensibly there is no intermediate host.

There are a number of different strains of the causal
organism, in addition to the differences in species, which
vary in their virulence to man and animals and in their sus-
ceptibility to drugs. According to Napier (42), "It seems

possible that many, if not all, the puzzling variations in the pathogenicity of trypanosomes in different animal species, and in drug resistance might be explained on a theory of strain selectivity of vectors, hosts, and drugs."

Trypanosomes belong to the phylum Protozoa, class Mastigophora, subclass Zoomastigina, order Protomonadida, suborder Eumonadea, family Trypanosomidae, and genus Trypanosoma.

These parasites, most highly developed of the hemo-flagellates, are never found within cells but are always free in the plasma. Due to their transparency, study of the structural details of the unstained specimen is unsatisfact-ory. In a blood smear stained by Leishman's or Giemsa's method, trypanosomes are spindle-shaped bodies with pointed ends, the average length of different species varying from 10 to 70 microns. They are flattened and contain in the central part a large, pale-staining, oval-shaped nucleus with a karyosome which occupies about two-thirds of the breadth of the cytoplasm. The cytoplasm stains a light blue and contains dark-blue granules and sometimes vacuoles. In the posterior end are two bodies appearing as one dark-red mass, the parabasal body and blepharoplast, from which an undulating membrane and marginal flagellum arise. The flag-ellum in most species passes forward along the whole length of the body to which it is attached by the undulating mem-brane, and extends for about one-quarter of its length be-yond the anterior end of the body. The stained undulating

membrane is a transparent pale-violet membrane.

Several forms of the same species of trypanosome may be encountered, the thin slender forms usually seen and the broad stumpy ones in which the flagellum ends with the undulating membrane at the anterior end of the body. Many intermediate forms are recognizable. Crithidial forms, in which the blepharoplast is anterior to the nucleus, occur at certain stages in the insect vector, but are not found in man. Reproduction by binary longitudinal fission takes place in the blood stream (20).

Death of the host, infected with Trypanosoma equiperdum, has been attributed at various times to one or another of six general causes: death from specific toxins, from acidosis, from mechanical obstruction, from asphyxiation, from hypoglycemia, and more recently, from potassium poisoning.

Proponents (58) (37) (50) (51) of the toxin theory proposed that trypanosomes elaborate toxins which ultimately cause death to the host. Andrews, Johnson, and Dormal (2) and Kligler, Geiger, and Comaroff (28) refuted this theory, contending that the presence of toxins was alleged only from the effects which a toxin might produce and not from direct experimental evidence. To support their contention these latter workers injected by several routes massive doses of trypanosomes, blood, plasma, and serum obtained from infected animals, and also various trypanosomal proteins into healthy normal rats, and observed no toxic symptoms. Owing to lack of supporting evidence the toxin theory has fallen

into discard.

The acidosis theory has been embraced by numerous investigators (44) (54) (55) (27) (34) (35) (2) (36) (7) (28), none of whom refers to it solely, without qualification, as the cause of death. Depletion of the alkali reserve and apparently lowered oxygen consumption, resulting from excessive lactic acid produced by the metabolism of the trypanosomes, is the cause of death, according to Kligler, Geiger, and Comaroff (28). Several authors (2) (35) (26) (20) claim that acidosis is not the primary cause of death but that it is a part of the syndrome produced by the incomplete combustion of glucose and the insufficiency of glucose for the oxidation of lactic acid.

Andrews, Johnson, and Dormal (2) correlate the acidosis theory with the mechanical obstruction and asphyxiation theories. They reason that by some unexplained phenomenon the trypanosomes agglutinate in the blood of the host, but only "after reaching a certain concentration." This mechanical impediment to the flow of blood, especially in the heart and lungs, produces pulmonary edema as well as stasis. The ensuing insufficiency of oxygen induces an anoxemia which, by restricting oxidation of the metabolic products of host and trypanosomes, induces a "non-volatile, uncompensated acidosis."

Johnson (26), after further investigation, modified the theory promulgated by him and his collaborators. He regards the reduction of oxygen in the blood as the first step in

the syndrome leading to death of the host. In failing to
sustain the oxygen level concomitant with normal metabolic
activity the blood allows an accumulation of lactic acid, a
condition averse to trypanosomal subsistence. The trypano-
somes agglomerate and create the respiratory embarrassment
causing death to the animal.

Opposing views of the mechanical obstruction theory re-
solve themselves into the question of whether trypanosomes
agglutinate and form emboli, and, if emboli are formed,
whether they are capable of completely blocking blood
vessels and asphyxiating the host. Raffel (43) found ap-
parently no relationship between the aggregation of the
trypanosomes and the clotting phenomenon. By administering
glucose, Hoppe (20) was able to prolong the life of rats
infected with Trypanosoma equiperdum until the blood of the
rats contained an average concentration of trypanosomes 2.38
times the normal count at death. Therefore, it would be
difficult to explain the sustenance of life of the rats be-
yond the point where the mechanical obstruction theory would
have the rats dying from clumping trypanosomes and emboli in
the blood vessels, especially since clotted blood and
clumped trypanosomes would probably be little influenced by
an increased blood sugar level.

The most recent theory regarding cause of death in the
host, infected with Trypanosoma equiperdum, is that proposed
by Zwemer and Culbertson (67). These authors attribute
death to an increase in serum potassium to a level fatal to

rats. The increase in serum potassium commences about twenty-four hours prior to death and arises either from an alteration in the differential permeability to electrolytes of the red blood cells or body cells or from injury by the parasites to the "selective secretory mechanism by which the kidneys aid in maintaining a constant blood plasma level." Ikejiani (22) (23) has corroborated these results but concludes they demonstrate only a terminal effect. Later work by Scheff and Thatcher (56) on survival time of potassium tolerant and non-tolerant rats indicates that increased serum potassium is not the cause of death but only a secondary terminal effect, inasmuch as the death rate within the two groups of rats is the same.

Since Schern's (57) hypothesis, in 1925, which states that animals infected with trypanosomes die of sugar depletion, numerous investigators have revealed considerable data concerning this pathologic device. Schern (57) believes that the exhaustion of blood sugar by the parasites is the immediate cause and that the inability of the liver to maintain the blood sugar level is the indirect cause of death of the host. No explanation is given for the breakdown of liver function except the great strain involved in sustaining the blood sugar level. Andrews, Johnson, and Dormal (2) ascribe the degeneration of the liver to an anoxemia resulting from mechanical obstruction of blood vessels in the heart and lungs. This degeneration prevents glycogen storage; consequently, after the sugar in the blood and remain-

ing glycogen in the liver are consumed the host dies from
lack of nourishment. That complete liver glycogen exhaus-
tion occurs before death is disclaimed by the work of
Regendanz and Tropp (50) and Scheff (54). The latter in-
vestigator killed trypanosomes (Trypanosoma equiperdum) in a
rat with Bayer '205' and observed a prompt restoration of
blood sugar to normal. For this observation he offered no
comment. Others (50) (49) (32) succeeded in raising the blood
sugar level by injection of epinephrine, further proof that
some available carbohydrate remains in the body, probably in
the liver or muscle glycogen. Scheff (54), however, was un-
able to note this rise with epinephrine. To account for the
hypoglycemia if liver glycogen is only partially spent,
Andrews, Johnson, and Dormal (2) propose that the damage to
the liver is so great that it "interferes with the withdraw-
al of glycogen." That the rats die of hypolycemia might be
criticized by the results of Krijgsman's (32) experiments
which indicate that the course of infection is not altered
by the administration of insulin to infected animals, and by
Poindexter's (47) work demonstrating that infected animals
live longer when injected with insulin. However, Hoppe
(20) finds that rats infected with Trypanosoma equiperdum
are able to withstand progressively smaller doses of insulin
coincident with age of infection. It was the likeness in
symptoms of death due to trypanosomes and to fatal doses of
insulin which prompted Hoppe and Chapman (21) to investigate
further the hypoglycemia theory of death. By administering

doses of glucose, based on the _in_ _vitro_ rate of consumption determined by Yorke, Adams, and Murgatroyd (66), these investigators were able to prolong the life of infected rats an average of eighteen hours beyond the normal death point. They assume that failure of other investigators to prolong the life of infected animals is due to administration of amounts of glucose insufficient to provide for the metabolism of both trypanosomes and host. Their work likewise contradicts von Brand's (4) claim that "the intensity of the carbohydrate metabolism of the parasites, though surprisingly high, is not high enough to allow assumption of an effective interference with the metabolism of the host." Infected rats dying shortly before they were to receive the next supplement of glucose demonstrated a marked hypoglycemia, whereas rats, too far gone to be aided, died with a hyperglycemia following the last dose of glucose. These authors (21) point out that the rats died, even though they received glucose, due to the fact that throughout the experiment a constant concentration of glucose was used, and this did not take into consideration the needs of the host and the increase in the number of parasites during the progress of the disease. It would be interesting to know the survival time of rats supplied glucose by a continuous intravenous flow method.

After Yorke, Adams, and Murgatroyd (66) showed that sugar was necessary to sustain the life of trypanosomes _in_ _vitro_ impetus was given to research on the carbohydrate

metabolism of the trypanosomes and the sugar requirements of the host.

Various authors (66) (10) (18) (49) (5) (29) (4) demonstrated that many Trypanosoma use large quantities of glucose in their life processes. In vitro studies of the rate of glucose metabolized by Trypanosoma rhodesiense, made by Yorke, Adams, and Murgatroyd (66), indicate that 400 million trypanosomes consume 2.0 to 2.5 milligrams of glucose in one hour and 12.0 to 12.5 milligrams in five hours. They used a 20-fold dilution of mouse blood infected with Trypanosoma rhodesiense in sheep serum, to which glucose had been added, and maintained the suspension at a temperature of $37^{\circ}C$. At the end of an hour, when the preparation no longer turned purple from the reduction of oxyhemoglobin of the mouse red cells an examination showed that practically all of the trypanosomes were dead. The preparation was centrifuged at high speed and the supernatant fluid removed and set aside for sugar estimation. Whether or not the original sheep serum had been likewise set aside before the sugar was estimated and if precautions were taken to prevent glycolysis of the remaining sugars was not made known. It is well known that glucose determinations should be made immediately after taking blood samples as glucose rapidly disappears by glycolysis. Efficient refrigeration only retards, but does not prevent, glycolysis. When the analysis can not be made immediately, the proteins of the blood should be precipitated and the filtrate, to which are added a few drops of toluene,

placed in refrigerator. This filtrate will give accurate sugar determinations for 24 hours (30).

Experiments by Chen and Geiling (10) indicate that 400 million trypanosomes (Trypanosoma equiperdum) would metabolize approximately 8.125 milligrams of glucose in one hour and 11.625 milligrams in two hours. These researchers used a reaction medium consisting of equal volumes of rat plasma, M/15 phosphate buffer of pH 7.4, and Ringer-Locke solution containing 0.75 per cent of glucose. Into 0.6 ml. of this medium were suspended 32 million trypanosomes (Trypanosoma equiperdum), and the reaction was allowed to proceed at 37°C. for one hour. At the end of this time the mobility of the parasites was not noticeably reduced in comparison with the mobility at the beginning of the experiment. The rate of glucose metabolism by trypanosomes in this medium was determined and a curve constructed from data obtained at 15-minute intervals. As indicated by the linear portion of the curve, the rate of glucose metabolism was constant during the first 60 minutes, after which time it slowly decreased.

Fulton and Stevens (17) established that the end-products of glucose metabolism of Trypanosoma rhodesiense were succinic, pyruvic, lactic, acetic, and formic acids, glycerol, ethyl alcohol, and carbon dioxide. Reiner, Smythe, and Pedlow (52) and Chen and Geiling (11) determined the end-product of the glucose metabolism of Trypanosoma equiperdum to be only pyruvic acid. It is quite possible therefore, since there may be different end-products, that

dissimilar metabolic pathways are involved in each of these species of trypanosomes, resulting in unlike rates of glucose consumption. On the contrary, Hoppe (20) calculated that the glucose consumption of _Trypanosoma equiperdum_, _in vivo_, in his experiments was the same as the glucose consumption of _Trypanosoma rhodesiense_, _in vitro_, as determined by Yorke, Adams, and Murgatroyd (66).

Chen and Geiling (11) have studied the manner in which glycolysis occurs in _Trypanosoma equiperdum_ and conclude that glucose is degraded into pyruvic acid through phosphorylative processes in a blood-free preparation of lysed parasites. Chen (9) believes that enzymatic destruction of adenosine triphosphate (ATP) may partially account for the failure of previous workers to show glycolysis with lysed _Trypanosoma equiperdum_ and to prove the phosphorylative mechanism involved in the breakdown of glucose. Mapharsen, stibamine, and tryparsamide were shown to inhibit the activity of hexokinase, adenosine triphosphatase, and 3-phosphoglyceraldehyde dehydrogenase systems involved in the breakdown of glucose by the lysed trypanosomes. Cysteine was found in experiments _in vitro_ to antagonize this inhibitive effect (12). It is believed that the trypanocidal action of arsenicals and antimonials on _Trypanosoma equiperdum_ may be principally in their ability to inhibit the activity of sulfhydryl-containing enzymes in the glucose metabolism of the parasites (9).

Several investigators (21) (25) (24) (33) (15) (20) have

succeeded in prolonging the life of the host animal, after
an usually fatal concentration of trypanosomes in the blood
has been reached, by administering to the animal glucose and
other monosaccharids and disaccharids, e.g., d-fructose,
d-mannose, d-galactose, maltose, sucrose, l-xylose, l-arab-
inose, d-arabinose, raffinose, glycogen, and glycerine, as
well as related compounds, e.g., lactate, succinate, pyruv-
ate, malonate, aconitate, hexose diphosphate, glutathione,
and neo-calglucon. It is of interest that Ivanov and
Umanskaya (24) found trypanosomes "consume pyruvic acid as
energetic material" in view of the fact that Chen and
Geiling (11) identified pyruvic acid as the end-product of
glucose metabolism of Trypanosoma equiperdum, suggesting
that pyruvic acid cannot be metabolized by the parasites.

EXPERIMENTAL

<u>Parasites</u>. The strain of <u>Trypanosoma</u> <u>equiperdum</u> used in these experiments was obtained from the National Institutes of Health, Bethesda, Maryland, through the courtesy of Mr. Thomas F. Probey.

Four albino rats, infected with the parasites, were received. To maintain the strain in our laboratory transfers of the trypanosomes were made into guinea pigs and albino rats. A quantity of heavily infected rat's blood, diluted with glucose-citrate-saline solution (described below), was injected intraperitoneally into the rats and guinea pigs. The number of trypanosomes in this suspension was not accurately determined, since it was not necessary to know the exact number of parasites in the "seed" rats. Guinea pigs, which are able to endure the toxic effects of <u>Trypanosoma</u> <u>equiperdum</u> for much longer periods of time than rats, were used to insure maintenance of the parasites. This strain of <u>Trypanosoma</u> <u>equiperdum</u> was the same as that used by Morrell, Chapman, and Allmark (39) and by Hoppe and Chapman (21).

<u>Animals</u>. Normal and castrated female albino rats were used to culture trypanosomes for further experimentation, but no difference in the course of infection was observed in these two types of hosts. The sex of the rats was disregarded because previous investigations (48) (20) (26) (2) disclosed similar responses to infection by both males and

females. Inasmuch as the rats used for culturing purposes were to be sacrificed, no attention was given to their weights; however, general observation of animals dying before withdrawal of infected blood could be made, indicated that the larger rats showed signs of a more intense infection and died earlier than smaller ones, even though they received identical inoculations. Eight to twelve of these rats were inclosed in large cages and six or seven in smaller cages.

In this work healthy, normal, albino rats were used exclusively for test purposes. Two to four test animals were housed in a single cage before the experiment, then individually during the experiment. Food and water were available to the rats at all times except while the experiment was being conducted. Control animals were maintained under identical conditions.

Method of Infection. Since infection of rats with Trypanosoma equiperdum was found to be the only method by which to culture these organisms and quantity of trypanosomes was the chief concern, it was considered desirable to be able to predict the time at which the concentration of parasites in the blood of the host rats would be most intense and still be non-fatal. To this end, a dose of 2,000,000 trypanosomes was given intraperitoneally to each rat. This dosage was determined on the basis of work of Morrell, Chapman, and Allmark (39) and Hoppe and Chapman (21). If the infection were allowed to run its course,

death of the rats would transpire in 90.6 $\not=$ 11.1 hours according to the former authors, and 90.65 $\not=$ 34 hours according to the latter. Therefore, to be reasonably sure of ample infected rats from which to harvest the parasites, blood was withdrawn from them eighty to eighty-five hours after inoculation with the organisms, at which time the majority of rats appeared moribund and their blood swarmed with trypanosomes.

In all cases, tail blood of infected rats was utilized in determining trypanosome concentrations. This blood, obtained by the technique described under Blood Sugar Determination, was diluted in a medium designed to maintain the viability of the organisms. The infected medium was employed in transferring trypanosomes from infected to non-infected rats. In the preparation of lysed trypanosomes this medium also served as a diluent for infected rat blood during storage and centrifugation.

Trypanosoma equiperdum, an organism cultured _in vitro_ with great difficulty, may be kept alive for at least five hours in a glucose-citrate-saline solution such as that used by Hoppe (20) and containing the following:

Sodium Citrate	3.0 Gm.
Sodium Chloride	3.0 Gm.
Anhydrous Dextrose, C.P.	0.8 Gm.
Distilled Water, q.s.	400.0 ml.

To use, this diluent was mixed with an equal volume of blood.

The prescribed 2,000,000 trypanosome inoculum was counted in an Improved Double Neubauer Ruling counting chamber and measured in a Thoma pipette marked 101. An infected rat was held in a retaining device and prepared for tail blood sampling as described under Blood Sugar Determination. Counting of the trypanosomes was performed in a manner similar to that for erythrocytes.

After discarding the first drop, tail blood was drawn exactly to the 0.5 mark of the Thoma pipette and immediately diluted to the mark 101 with glucose-citrate-saline solution, while rotating the pipette between the thumb and forefinger. While holding it in a horizontal position, the pipette was rotated for about a minute, then shaken sidewise for two minutes; the fluid from the stem of the pipette was expelled and a drop of the remaining fluid delivered to the counting chamber. Before counting, five minutes were allowed for the trypanosomes to settle.

Trypanosomes were counted within five of the twenty-five double-ruled squares--specifically, within the four corner squares and the center one. To determine the count within the chamber formed between the central square millimeter on the ruled surface and the subtended area on the cover slip 0.1 mm. above it, the number of trypanosomes within the five double-ruled squares was multiplied by five to "correct for area." This value represented the number of trypanosomes per 0.1 c.mm. of diluted blood. Multiplying this quantity by 10, to "correct for depth of chamber," gave

the number per 1 c.mm. of diluted blood, and again by 200, the dilution factor, gave the number of trypanosomes per 1 c.mm. of undiluted blood. Therefore, routinely, the number of trypanosomes in the aforementioned five squares was counted and four ciphers were added (5 x 10 x 200 = 10,000).

Dilutions of infected rat blood, such that each milliliter of diluted blood would contain 2,000,000 trypanosomes, were made as follows: Heart blood was obtained from an unanesthetized, infected rat whose trypanosome count had been determined. Each foot of the rat was fastened by a spring clamp attached to a string, so that, with proper tension on each string, the rat was held securely, back downward, to a flat operating board. The point of maximum heart pulsation was determined and the needle, attached to a 1 ml. Tuberculin syringe, entered into the heart. The appropriate amount of blood was obtained by gentle suction--either arterial or venous blood being considered suitable. The aspirated blood was immediately added to the calculated quantity of glucose-citrate-saline diluent and shaken for three minutes. Then the 2,000,000 trypanosome dose was immediately injected intraperitoneally into each "culture" rat. Uniform dispersion of the parasites was effected by frequent agitation of the preparation.

Increase in numbers of trypanosomes during the time necessary for determination of the count and the withdrawal of heart blood was disregarded for the purposes of this

procedure, since it was negligible.

Method for Obtaining Lysed Trypanosome Preparations.
The average survival time of white rats infected with two
million trypanosomes is approximately 90 hours (21) (39). A
fresh suspension of Trypanosoma equiperdum was prepared from
the blood of albino rats infected eighty to eighty-five
hours previously.

By means of a 10 ml. hypodermic syringe, fitted with a
19 gauge, 2 inch needle and containing sufficient 1% Heparin
Sodium solution to wet the sides, as much blood as possible
was withdrawn from the heart of the rat without the aid of
anesthesia. Immediately the blood was added to an equal
volume of glucose-citrate-saline solution and refrigerated
at $3^{o}C$. The diluted blood of 16 to 20 rats was thus obtain-
ed and pooled. Refrigeration was necessary to prevent en-
zymatic or other destruction of constituents present in or
elaborated by the trypanosomes which might effect the glu-
cose metabolism of normal rats. The glucose present in the
diluting solution provided the trypanosomes nutrient and
permitted them to continue growing. The citrate prevented
the coagulation of the blood, and the saline, in the correct
proportion, maintained an isotonic medium which prevented
crenation or lysis of the blood cells and trypanosomes. The
diluting solution in addition facilitated the separation of
parasites from blood elements.

Within an hour after collection the mixed, diluted,
chilled blood was centrifuged in cold centrifuge tubes for

ten minutes at 2400 revolutions per minute. The plasma which was turbid and contained the trypanosomes and leucocytes together with some hemolyzed red blood cells was separated from the red blood cells. This turbid layer was then transferred to a cold, narrow centrifuge tube by suction and again centrifuged for ten minutes at the same speed as before. The trypanosome deposit so obtained formed a rather compact layer but usually contained red blood cells enough to color the solution and deposit. Often two to four centrifugations of the trypanosome layer and an equal volume of cold isotonic saline were necessary in order to remove the pink color due to the hemoglobin. The isolated trypanosome layer was then diluted with an equal volume of distilled water and placed in the ice-cube compartment of a refrigerator for a minimum of twenty-four hours. The subfreezing temperature served two purposes: It (1) forestalled the enzymatic destruction of the cell constituents and (2) lysed the trypanosomes by freezing the cell contents, thereby causing the cell membranes to rupture. Just before use the lysed trypanosome preparation was centrifuged for five minutes to separate the lysed trypanosome solution from the lysed trypanosome residue.

About 75 ml. of infected blood, the average amount withdrawn from 16 to 20 rats, yielded approximately 6.2 ml. of sedimented trypanosomes. At the time of withdrawal, 80 to 85 hours after inoculation with 2,000,000 trypanosomes, rat blood contains approximately 1.25×10^6 trypanosomes per

cubic millimeter (20). Therefore, if all of the trypanosomes in the blood had been sedimented by the procedure described above, the average deposit obtained after centrifugation contained about 15×10^9 trypanosomes per ml.

Only about 25 per cent of the trypanosome deposit, diluted with distilled water and placed in the refrigerator to lyse, was recovered in a solid phase as disintegrated particles; the remainder appeared in solution. On this basis, each ml. of lysed trypanosome solution represented the substance of approximately 8.6×10^9 trypanosomes.

Neither live nor motile trypanosomes were observed in either phase.

Administration of Lysed Trypanosome Preparations. As described under Method of Infection, a normal, adult, unanesthetized, albino rat was secured to a flat operating board, in preparation for the administration of the supernatant lysed trypanosome solution, the suspension of lysed trypanosome residue, or of distilled water. Tension on the strings, to which the feet of the rat were fastened, was adjusted to permit the hind legs to be lifted easily.

After shaving the hair over the skin area covering the femoral vein of the right leg, an incision about one-half of an inch long was made one-quarter of an inch to the right of and parallel to the vein. The skin was then rolled over to the left, bringing the vessel into view. The vessel was then dilated by compression above the site of injection. Depending on the amount of fluid to be injected, either a

2 or 3 ml. all-glass hypodermic syringe, fitted with a
No. 26 gauge needle, five-eighths of an inch in length, was
employed. The needle was passed through the fascia and
upper surfaces of the muscles, about one-eighth of an inch
to the right of the vein and almost parallel to it. The
vein was entered from the side by advancing the needle
slightly and changing its direction toward the vein. As
much as 3 ml. of lysed trypanosome preparations were thus
administered at a rate of about 1 ml. per minute. After the
injection was made, the skin which had been pulled back to
expose the vein was released; thus, this skin flap and the
muscles through which the needle had been advanced acted as
mechanical checks to hemorrhage.

Blood Glucose Determination. Single determinations
were made of the glucose concentration in blood of normal,
adult albino rats. Multiple determinations were made
initially, then at intervals $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, $3\frac{1}{2}$, 4, 5,
and 6 hours after injection in similar rats injected with
lysed trypanosome solutions, with suspensions of lysed
trypanosome residue, and with distilled water. Determina-
tions were also made of glucose in in vitro preparations
initially and at intervals of $\frac{1}{2}$, 1, 2, and 4 hours after
start of incubation at 37°C. In addition, duplicate analys-
es of glucose in the injected preparations were run.

It is common knowledge that there are reducing sub-
stances other than glucose in blood. If present in a high
concentration, these so-called saccharoids, mainly gluta-

thione and glucuronic acid (16), give the apparent glucose estimation an inflated value. For this reason, the Nelson-Somogyi colorimetric method (43) (59) for the blood glucose determination was adopted in this work. Somogyi's (60) method of blood deproteinization employs zinc sulfate and barium hydroxide as the precipitating agents. A filtrate is obtained containing practically no reducing substances other than glucose. If fluoride or oxalate is used as an anti-coagulant it too is precipitated along with the proteins by these reagents. This colorimetric method is probably not the most rapid procedure for blood sugar determination but is one of the most sensitive. Reoxidation of the reduced copper is avoided by saturation of the reagent solutions with sodium sulfate. Nelson's arsenomolybdate reagent extends the useful range of the determination from five gamma to three milligrams of glucose and thus presents an excellent method for the estimation of glucose in the lower range of values. Another advantage gained by the use of this method is the stability of the color produced on addition of the arsenomolybdate reagent to the reduced copper solution. The density of the blanks as well as of the test preparations remains unchanged and allows reading at convenience.

An AC Model Fisher Electrophotometer and Filter 525-B, transmitting a spectral band at approximately 525 milli-microns, were employed. A glucose calibration curve was constructed for the method and instrument used by carrying through several series of standards and blanks. The stan-

dard solutions ranged in value from 5 to 250 gamma of glucose per ml., corresponding to 1 ml. of a 1:20 dilution of blood containing 10 to 500 mgm. of glucose per 100 ml. respectively. The transmission values obtained from the Logarithmic Scale were plotted against representative blood glucose values on linear graph paper. The resultant calibration graph (Figure 1) was a straight line between the values representing 5 to 350 mgm. per cent of blood sugar and a line curved upward from 350 to 500 mgm. per cent. The data for the straight-line portion of the curve then are in conformity with Beer's Law, and the concentration of the sugar is directly proportional to the scale reading. Consequently, a calibration factor may be used. However, all blood sugar values in this investigation were obtained directly from the calibration curve.

Tail blood was used for the determination of normal blood sugar and blood sugar of test rats. A rubber tube (black pure gum; 3/16 in. bore; 3/64 in. wall) for aspiration was attached to a 0.2 ml. pipette, calibrated in 0.001 ml. divisions. The pipette was fastened in a clamp with the tube-end slightly elevated to prevent the blood from flowing back out of it, and 0.005 ml. of 1% Heparin Sodium solution was drawn up into its tip. The rat was then placed in a retaining device to contain the animal while blood was being drawn from its tail. This piece of apparatus was fashioned from an eight and one-half inch long, thick-glass drug percolator with an inside diameter of three and one-quarter

inches. A clamp, attached to a ringstand, held the percolator by the small end, which also served as an air inlet. A notched cork was placed in the large opening after introducing the rat into the percolator and drawing its tail through the notch in the cork. The retainer was placed at an angle of forty-five degrees above the clamped pipette so that the tip of the rat's tail was easily accessible to the tip of the pipette. About 3 to 5 mm. of the tip of the tail were snipped off; the first drop of blood was discarded; and blood was drawn into the pipette to the 0.105 ml. mark and immediately mixed with 1.5 ml. of distilled water.

Reagents used in the deproteinization procedure were: (1) 0.3 N barium hydroxide and (2) 5.0% solution of $ZnSO_4.7 H_2O$. It is necessary that the alkali neutralize exactly the zinc sulfate solution, volume for volume, using phenolphthalein as indicator. On the basis of this titration, appropriate dilution of the more concentrated is made so that the two reagents are correctly balanced.

Three additional reagents were required for the colorimetric determination of glucose: (1) Copper Reagent A, made by dissolving 25 Gm. of anhydrous sodium carbonate, 25 Gm. of Rochelle salt, 20 Gm. of sodium bicarbonate, and 200 Gm. of anhydrous sodium sulfate in about 800 ml. of water and diluting to one liter; (2) Copper Reagent B, containing 15% $CuSO_4.5 H_2O$ and twenty drops of concentrated sulfuric acid per liter; and (3) Arsenomolybdate Color Reagent, made by dissolving 25 Gm. of ammonium molybdate in 450 ml. of dis-

tilled water, adding 21 ml. of concentrated sulfuric acid, mixing, adding 3 Gm. of dibasic sodium arsenate ($Na_2HAsO_4 \cdot 7 H_2O$) dissolved in 25 ml. of water, mixing, and placing in an incubator at 37°C. for 48 hours.

All the reagents were contained in glass-stoppered, clear-glass bottles except the Arsenomolybdate Color Reagent, stored in a glass-stoppered brown bottle and the barium hydroxide solution, stored in an aspirator bottle connected to a 5 ml. burette graduated to 0.01 ml. and protected by soda lime from the carbon dioxide in the air.

For deproteinization, 0.2 ml. of barium hydroxide solution was added to the laked blood and mixed. After the solution had turned brown 0.2 ml. of zinc sulfate solution was added and the mixture shaken, then allowed to set for a few minutes before centrifuging. Centrifugation for four or five minutes separated the mixture sufficiently to permit withdrawal of 1 ml. of protein-free filtrate into a 1 ml. measuring pipette tipped with washed cotton. The filtrate was drained into a Folin-Wu tube to which was added and mixed 1 ml. of a mixture of 25 parts of Copper Reagent A and 1 part of Copper Reagent B prepared the day of use. Blanks were set up in the same way except that distilled water was substituted for blood. The solutions were mixed and heated for twenty minutes in a boiling water bath, at the end of which time they were cooled in a pan of cold water. One milliliter of Arsenomolybdate Color Reagent was added to each tube by means of a 1 ml. measuring pipette; upon

mixing, the reaction evolved carbon dioxide, and the color developed quickly. The solutions were then diluted to 25 ml. with distilled water, mixed, and read in the electro-photometer at 525 millimicrons. Before readings were taken, the electrophotometer was adjusted to read zero on the Logarithmic Scale with a blank in the chamber.

In Vitro Preparations. To ascertain their in vitro effects on glucose concentration, lysed trypanosome solutions and suspensions of lysed trypanosome residue were incubated in specific media at 37°C. for four hours.

The reaction medium was of the type employed by Chen and Geiling (10) to study in vitro effects of antimonials on live trypanosomes. It consisted of equal volumes of rat plasma, M/15 phosphate buffer of pH 7.4, and Ringer-Locke solution containing 0.75% of glucose.

The phosphate buffer solution was prepared by the following procedure (31): 0.9078 Gm. of pure, crystalline KH_2PO_4 was dissolved in 100 ml. of freshly distilled, ammonia-free water, and 1.1876 Gm. of pure, crystallized $Na_2HPO_4.12H_2O$, which had lost 10 molecules of water by exposure to the air for 14 days, were also dissolved in 100 ml. of freshly distilled, ammonia-free water. The M/15 phosphate buffer solution of pH 7.4 was obtained by mixing 19 ml. of the former solution and 81 ml. of the latter.

Ringer-Locke solution was prepared which contained the following:

Solution A.

Sodium Chloride	9.00 Gm.
Potassium Chloride	0.42 Gm.
Calcium Chloride	0.24 Gm.
Distilled Water, q.s.	500.00 ml.

Solution B.

Sodium Bicarbonate	0.30 Gm.
Anhydrous Dextrose, C.P.	7.50 Gm.
Distilled Water, q.s.	500.00 ml.

To use, one part of Solution A was mixed with one part of Solution B, the mixture being prepared the day of use.

The glucose concentrations of three types of in vitro preparations--medium plus lysed trypanosome solution, medium plus suspension of lysed trypanosome residue, and medium alone--were determined initially and at intervals of ½, 1, 2, and 4 hours after start of incubation at 37°C. The amount of trypanosome preparations added to the medium varied from 6.25% to 25%. A fourth in vitro preparation, consisting of the medium minus rat plasma, was tested for glucose concentration initially and 4 hours after start of incubation at 37°C.

RESULTS

Statistical Manipulations. The mean, standard devia-
tion, per cent standard deviation, standard error, and per
cent standard error were determined for all series of abso-
lute glucose values. These quantities appear with the tab-
ulated glucose concentrations.

Standard deviation was derived by means of the formula:

$$s = \sqrt{(Sd^2) / (n-1)}$$

where s is the standard deviation, d is the deviation of
each observation from the mean, n is the number of observa-
tions, and S signifies summation. The mean plus or minus
two standard deviations is an index of the average observa-
tion and 95.45 per cent of the values obtained by repeated
trials.

Standard error was computed as follows:

$$s_{\bar{x}} = s / \sqrt{n}$$

where $s_{\bar{x}}$ is the standard error and s and n have the same
meaning as above. This measurement is an estimation of the
standard deviation of the means. Twice this value, measured
on each side of the true mean, would include all except five
per cent of the means of similar samplings.

Means of two types were derived for the experimental
data in each of the various studies undertaken--the mean per
cent change in glucose concentrations for all rats or in
vitro preparations at each time interval and the mean per

cent change in glucose concentrations for each rat or *in vitro* preparation during the entire period of the test. For example, (1) the mean per cent change in blood glucose, at 1 hour, in all rats receiving supernatant lysed trypanosome solution was determined, and (2) the mean per cent change in blood glucose during the 6 hour test period, for each rat receiving this preparation, was likewise determined. It then became necessary to know whether the average per cent change in glucose differed significantly in normal rats from time interval to time interval following injection with a lysed trypanosome preparation and whether the average per cent change in glucose in individual rats during the entire test period differed significantly from rat to rat. If all the means related to one variable are from the same population there should be no significant difference among them. Conversely, if there is no significant difference among the means of a variable it can be expected that, at least 95 per cent of the time, any change which may occur in the means during the test period is no more than what might be expected among individual observations. The variation of the observations is caused not only by the basic experimental errors which are always present but also by differences which might be due to a difference in the time interval necessary for a change to occur (first variable) or a difference inherent among the rats (second variable). The following null hypothesis was then tested: The observed variance among (1) the "time interval" means and (2) the

"animal" means is equal to the variance calculated from the population of individual per cent changes. The variance ratio of F ratio test was applied to the observations recorded as per cent change in glucose concentrations because of the decided variation in absolute values among rats.

The per cent changes at each time interval for all the rats were tabulated in a column and the individual per cent changes for each rat were tabulated in a row. The total sum of squares for each experiment was then computed. This quantity is equal to the sum of squares of deviations from the over-all mean, which is the mean of all the individual observations. In the make-up of the total sum of squares are included the sums of squares for each of the three variables present in the experiment. These values provide a measure of variation among time intervals and among rats or in vitro preparations and provide a measure of discrepancy (error) among the glucose changes observed in the different rats at the different time intervals. Corresponding to the three sums of squares into which the total has been partitioned, the mean squares (mean variances) may be calculated by dividing each sum by the corresponding number of degrees of freedom. The row and column components are independent of each other, neither component appearing in the other mean square. The test of the hypothesis that there is no change in mean glucose concentration with time following injection is

$$F = \frac{\text{mean square for time intervals}}{\text{mean square for error}}.$$

It may be learned, also, if the difference occurring in rats is attributable to sampling from a uniform population, in which case

$$F = \frac{\text{mean square for animals}}{\text{mean square for error}}.$$

Tables of F, found in most statistics books, give those values of F which will be equalled or exceeded in the proportion of cases indicated by the table for the respective degrees of freedom involved in computing the two variances concerned. A 5 per cent level of probability has been chosen in this work to test the various hypotheses. If two sets of observations show a discrepancy between mean squares, signified by a value of F that would occur in less than 5 per cent of pairs of sets taken from the same series, then it is considered unlikely that the two sets came from the same series, and they are called significantly different.

Normal Blood Glucose Concentration. The mean blood glucose concentration of 59 normal, adult albino rats was established at 91.04 ± 15.15 milligrams per cent (Table 2). Hoppe (20), using the Folin-Wu method for blood glucose determination, reported a mean of 145.6 ± 21.8 milligrams per cent in the normal, adult albino rat.

In Vivo Effect of Distilled Water on Blood Glucose. Six normal control rats, injected intravenously with distilled water, yielded blood sugar levels at half-hour intervals which fluctuated over a rather wide range in a period of four hours following injection (Tables 3, 4). However, the

means of the absolute values (Table 3, Figure 2) and of the
per cent changes (Table 4) at these intervals were quite
consistent. The mean blood glucose concentrations at the
time intervals following injection of the distilled water
are:

$$97.18 \pm 13.46 \text{ mgm.\% before injection}$$
$$99.25 \pm 16.00 \text{ mgm.\% at 0.5 hour}$$
$$97.29 \pm 22.61 \text{ mgm.\% at 1.0 hour}$$
$$97.79 \pm 15.84 \text{ mgm.\% at 1.5 hours}$$
$$102.58 \pm 14.61 \text{ mgm.\% at 2.0 hours}$$
$$102.88 \pm 24.08 \text{ mgm.\% at 2.5 hours}$$
$$101.75 \pm 16.94 \text{ mgm.\% at 3.0 hours}$$
$$98.25 \pm 14.63 \text{ mgm.\% at 3.5 hours}$$
$$99.04 \pm 17.09 \text{ mgm.\% at 4.0 hours}$$

From an analysis of variance of data in Table 4 the hypoth-
esis, that the time intervals have equal means, is accepted
since the observed F, 0.27, is less than the theoretical
5 per cent value, 2.29 (Table 5). In other words, the aver-
age changes in the blood sugar concentrations of the 6 rats
at the specified time intervals following intravenous admin-
istration of water may be attributable to chance (with a
high degree of significance) and need not be the result of
the water administration. This analysis also indicates a
marked variation from rat to rat in their blood glucose
levels at the half-hour intervals--derived F, 16.33, greater
than theoretical 5 per cent probability value, 2.49. Ad-
ditional test animals would probably reduce this variation.

<u>In Vivo Effect of Supernatant Lysed Trypanosome Solution on Blood Sugar</u>. Analyses showed that on an average only 0.7 mgm. of glucose was present in the material injected. The results of sugar determinations obtained at half-hour intervals for the first four hours following injection of the lysed trypanosome solution, then at the fifth and sixth hours, gave a range of values from 67.80 mgm. per cent to 170.75 mgm. per cent in 7 normal, adult rats (Table 6). The means for these results are as follows:

96.89 ± 17.76 mgm.% before injection

99.81 ± 18.29 mgm.% at 0.5 hour

101.55 ± 33.84 mgm.% at 1.0 hour

95.08 ± 17.68 mgm.% at 1.5 hours

99.11 ± 19.63 mgm.% at 2.0 hours

100.11 ± 24.08 mgm.% at 2.5 hours

98.04 ± 35.60 mgm.% at 3.0 hours

97.96 ± 30.98 mgm.% at 3.5 hours

89.84 ± 25.91 mgm.% at 4.0 hours

95.67 ± 14.89 mgm.% at 5.0 hours

85.42 ± 7.67 mgm.% at 6.0 hours

The means remained fairly constant for the initial four hours, then dropped slightly and irregularly in the last two hours, modified by the fact that only three results were obtained at each of these intervals (Tables 6,7; Figure 3). By the F ratio test all of the average interval changes were shown to be members of a uniform population (Table 8); the F value, 0.80, in the present comparison indicates no

significant difference in the variation when compared to the tabular F value for the 5 per cent point, 2.10. It may then be inferred that administration of the supernatant lysed trypanosome solution has no effect on blood glucose of rats within a period of six hours and that results of repeated experiments will be no different than the ones obtained, with a probability of 95 per cent. A significant difference in the variation between animals is evidenced by the larger F value, 6.48, when compared to the F table value for the 5 per cent point, 2.32 (Table 8).

In Vivo Effect of Lysed Trypanosome Residue on Blood Sugar. All suspensions of lysed trypanosome residue which were injected into the rats were found to be glucose-free. A range of concentrations from 60.25 mgm. per cent to 151.75 mgm. per cent was obtained from glucose determinations run at half-hour intervals for the first four hours, then at the fifth and sixth hours, after injection of suspensions of lysed trypanosome residue into 9 normal, adult rats (Table 9). The blood glucose means at the various time intervals fluctuated, but no deviation was greater than 7.47 mgm. per cent (Figure 4); the largest mean per cent change was -10.49 per cent at the end of the sixth hour (Table 10). The time interval means of the individual observations are as follows:

96.22 ± 14.97 mgm.% before injection

101.67 ± 20.10 mgm.% at 0.5 hour

96.53 ± 11.06 mgm.% at 1.0 hour

91.72 ± 15.55 mgm.% at 1.5 hours

97.76 ± 12.93 mgm.% at 2.0 hours

95.13 ± 11.89 mgm.% at 2.5 hours

95.17 ± 20.32 mgm.% at 3.0 hours

97.69 ± 25.42 mgm.% at 3.5 hours

95.56 ± 14.22 mgm.% at 4.0 hours

92.30 ± 12.15 mgm.% at 5.0 hours

88.75 ± 16.86 mgm.% at 6.0 hours

The F value, 1.07, for time interval means, calculated from
data in Table 10, is less than the theoretical 5 per cent
value, 2.03, based on 9 and 64 degrees of freedom (Table 11).
Therefore, the hypothesis, that the time intervals have
equal means, is again accepted. It may then be stated that
intravenous administration of lysed trypanosome residue into
normal rats produces no change in blood glucose within six
hours after the injection. The results of parallel sets of
determinations would be no different and would be what might
be expected from random sampling, with a probability of
95 per cent. As with distilled water and lysed trypanosome
solution, the blood glucose values obtained in this experi-
ment showed a definite variation from rat to rat, the ob-
served F value, 14.52, being significantly larger than the
expected 5 per cent point, 2.09 (Table 11).

Incubation and Glucose Content of Suspending Medium.

Four samples of media showed glucose concentrations ranging from 271.5 mgm. per cent to 325.5 mgm. per cent during a four hour incubation period (Table 12). These values were obtained before incubation and after incubation for 0.5, 1.0, 2.0, and 4.0 hours. The mean glucose values at these times are as follows:

$$293.0 \pm 17.2 \text{ mgm.\% before incubation}$$
$$300.9 \pm 19.2 \text{ mgm.\% at 0.5 hour}$$
$$310.0 \pm 4.4 \text{ mgm.\% at 1.0 hour}$$
$$300.4 \pm 12.8 \text{ mgm.\% at 2.0 hours}$$
$$304.0 \pm 31.8 \text{ mgm.\% at 4.0 hours}$$

An inconsistent variation among the means of the absolute glucose values (Table 12, Figure 5) and of the per cent changes (Table 13) is apparent. However, an analysis of variance of the per cent changes reveals that the incubation time intervals have equal means, since the computed F value, 0.23, is much less than the expected value for 5 per cent probability, 3.86. That is to say, the variations in glucose concentrations of the four media at the stated incubation periods may be ascribable to chance and need not be the result of incubation. In ninety-five times out of a hundred replications of this experiment would yield glucose concentrations whose variance ratio would also not exceed the tabulated F value. The observed F value, 2.67, for samples of media, when compared to the 5 per cent probability figure, 3.86, indicates that these four samples are members of a

normal population possessing the same mean and that the var-
iation among the samples may also be ascribed to chance.

Incubation and Glucose Content of Suspending Medium
Minus Rat Plasma. Three samples of suspending media minus
rat plasma furnished estimates of their glucose concentra-
tions. These quantities, determined before and after a
four-hour incubation period, ranged from 348.5 mgm. per cent
to 396.0 mgm. per cent (Table 15, Figure 6). The mean glu-
cose concentration before incubation was 388.7 mgm. per cent
and after incubation 366.2 mgm. per cent. Comparison of the
time interval means by the F ratio test (calculated F for
time intervals, 4.06, against theoretical F value at the
5 per cent point, 7.71) indicates all were obtained from one
population, with a probability of 95 per cent (Table 16).
It can be rightfully postulated then that incubation has no
effect upon the glucose concentration of the suspending med-
ium minus rat plasma.

In Vitro Effect of Supernatant Lysed Trypanosome Solu-
tion on Glucose Content. Since two of the four 15 ml.
samples were diluted with 1 ml. of supernatant lysed trypan-
osome solution and the remaining two with 5 ml., the in-
dividual glucose concentrations were not comparable. How-
ever, the time interval means of absolute glucose values
(Table 17, Figure 7) and the per cent changes in glucose
(Table 18) could be correlated. The mean glucose concentra-
tions of these preparations before incubation and at the in-
dicated times during incubation are as follows:

$$259.0 \pm 29.0 \text{ mgm.\% before incubation}$$

$$249.9 \pm 14.1 \text{ mgm.\% at } 0.5 \text{ hour}$$

$$254.4 \pm 29.2 \text{ mgm.\% at } 1.0 \text{ hour}$$

$$265.6 \pm 13.3 \text{ mgm.\% at } 2.0 \text{ hours}$$

$$265.5 \pm 16.4 \text{ mgm.\% at } 4.0 \text{ hours}$$

The means at the various time intervals were shown, by the F ratio test, to be members of normal populations having equal means, with a probability of 95 per cent. The observed F value for time interval means, 2.35, was less than the tabulated F value at the 5 per cent point, 3.86 (Table 19). It may then be assumed that supernatant lysed trypanosome solution has no effect upon glucose in suspending media incubated at $37^{\circ}C$. for 4 hours, and variation in glucose content during that time may be due to inherent experimental errors. An obviously significant variation among preparations is substantiated by the analysis of variance of data in Table 18. The F value for preparation means, 13.86, is larger than the expected F value at the 5 per cent level of significance, 3.86 (Table 19). The inference is that these preparations were not drawn from a normal population, and consequently, similar random samplings of four preparations would also yield unlike means. One would be wrong in this assumption in only 5 per cent of the cases.

In Vitro Effect of Lysed Trypanosome Residue on Glucose Content. Two of the four 15 ml. samples of media were diluted with 1 ml. of lysed trypanosome residue and the remaining two with 2.5 ml. For this reason the individual

glucose concentrations were not comparable. If there were
no change in glucose content during the incubation period
any variations before incubation would be carried along;
consequently, the means at the time intervals would be com-
parable (Table 20, Figure 8). The mean glucose concentra-
tions at the various time intervals are as follows:

$$276.6 \pm 10.5 \text{ mgm.\% before incubation}$$
$$288.5 \pm 8.8 \text{ mgm.\% at 0.5 hour}$$
$$274.0 \pm 15.2 \text{ mgm.\% at 1.0 hour}$$
$$288.4 \pm 7.6 \text{ mgm.\% at 2.0 hours}$$
$$285.5 \pm 12.4 \text{ mgm.\% at 4.0 hours}$$

Since per cent changes in glucose content are also compar-
able, an analysis of variance was run on data in Table 21 to
test the hypothesis of no difference in time interval means
at the 5 per cent level of significance (Table 22). The F
value calculated for time interval means, 1.02, was found to
be less than the theoretical value, 4.07. The hypothesis of
no difference in means is acceptable, and the assumption can
be made that addition of lysed trypanosome residue to the
medium causes no change in glucose concentration when the
preparation is incubated for 4 hours.

Immunity. Although immunity studies were not a part of
the original design of this work, the result noted may be of
some worth. Fourteen rats, inoculated 33 to 101 days pre-
viously with either lysed trypanosome solution or residue,
received a dose of 2,000,000 live trypanosomes. Their sur-
vival time was no different than that of normal rats.

DISCUSSION

While it is widely held (2) (10) (20) (21) (46) (49)
(50) (54) (57) that an hypoglycemia inevitably accompanies
Trypanosoma equiperdum infections in the rat, a theory is
yet to be found which can explain satisfactorily and com-
pletely the mechanism by which the hypoglycemia is produced.
Andrews, Johnson, and Dormal (2) tried to explain it on the
basis that the liver was so seriously damaged by anoxemia
that it was incapable of mobilizing sufficient glycogen to
maintain the sugar needs of the host. Hoppe and Chapman
(21) advocated the theory that the consumption of glucose by
the trypanosomes and host was so extravagant that a fatal
hypoglycemia was effected. Proponents (37) (50) (51) (58)
of the toxin theory offered no suggestion for a correlation
between probable trypanosome toxins and sugar metabolism of
the host. It remained for Chen and Geiling (11), in 1946,
to reveal the metabolic pathway by which Trypanosoma
equiperdum degrades glucose to pyruvic acid. Through their
(10) efforts and those of Yorke, Adams, and Murgatroyd (66)
a measure of the trypanosomes' rate of glucose consumption
was ascertained. This rate was as high as 8.125 milligrams
of glucose in one hour according to the former authors.
Superficially, at least, it appears that Hoppe and Chapman
(21) were correct in assuming that death of the host was due
to a deficiency of glucose resulting from the tremendous

"appetite" of the parasites. However, to negate the possibility that the trypanosomes contain or release a substance, either enzymatic or insulin-like, which effects the production or metabolism of glucose by the host, a series of _in vivo_ tests on the rat were conducted.

If the substance were present in trypanosomes, lysis of a fresh batch of the parasites should release the material. That being the case, the change in blood glucose concentration exhibited in a normal rat, to which has been administered one of these lysed trypanosome preparations, can then be employed as an index of the metabolic effects, and consequently, of the presence of a trypanosomal "factor," i. e., an increase in blood sugar might indicate the presence of a glycogenolytic agent, and a decrease might indicate a glycogenetic or insulin-like substance.

The Nelson-Somogyi colorimetric method (68) (70) was adopted for the determination of "true" glucose values. For determining the density of the individual oxidized glucose solutions, the logarithmic scale of the electrophotometer was used in preference to the scale indicating per cent transmission because of the greater accuracy possible in reading it. However, the per cent transmission values recorded in Table 1 were used as a check. When compared to the average normal blood glucose concentration as determined by the Folin-Wu procedure the average obtained herein is evidence of the discrimination shown by the Nelson-Somogyi method. The average obtained in this work on 59 normal, adult rats

was 91.04 ± 15.15 mgm. per cent; that of Hoppe (20), employing the Folin-Wu procedure on 100 rats, was 145.6 ± 21.8 mgm. per cent.

When a mass of trypanosomes, obtained from and concentrated by centrifugation of infected rats' blood, was allowed to lyse in distilled water for 24 hours at sub-freezing temperatures two phases were produced--a clear, supernatant solution and a residue. Presumably, the contents of the parasites were given up to the solution, since microscopic examination of the residue revealed only fragments of ruptured cells. This solution was injected into 7 normal, adult albino rats through the femoral vein. Glucose analyses of the rats' blood were made at specific intervals during the course of 6 hours, even though it was deemed unlikely that the effect of the sought-after material would fail to present itself within or remain longer than a 4-hour period, especially since the solution was administered intravenously. Deviations from the normal blood glucose concentration did appear from time interval to time interval in each rat, but these were irregular and resulted in a mean deviation of only -0.29 mgm. per cent from the average normal value over the entire 6-hour period (Table 7). By a statistical analysis of variance of the data, no significance could be attached to these changes. Therefore, by these results it was assumed that lysed trypanosome solution produces no effect whatsoever on the blood glucose picture of the rat, and the presence of a specific factor in Trypanosoma equiperdum which effects the glucose metabolism of the host is ruled out.

Lest the unknown "factor," if there be one, had not been given up to the solution during the lysis procedure and had remained in the cellular substance of the trypanosomes instead, a similar test was applied to 9 rats using the lysed trypanosome residue in place of the solution. As with the lysed trypanosome solution irregular deviations from the normal glucose concentration occurred during the test period. These resulted in possessing a mean deviation of +0.31 milligram per cent (Table 10).

A statistical analysis of variance of these data indicate there was no significant difference in these changes, and the hypothesis was validated experimentally that lysed trypanosome residue does not contain a factor effecting blood glucose in the host animal.

To avoid misinterpretation of these results control rats were subjected to the same test procedures as were the test rats except that they received distilled water intravenously in place of either lysed trypanosome preparation. The resulting mean deviation in this group of rats was +3.26 milligrams per cent. Analysis of variance again indicated no difference between the mean per cent changes at the time intervals which could not be attributed to chance.

The large individual deviations from normal blood glucose concentration noted in some of the rats are probably explainable by two lines of reasoning. If the changes were in only one direction, i. e., plus or minus, and were of approximately the same magnitude throughout (e. g., Rat No. 47,

Table 4) apparently the normal glucose concentration was an erroneous observation. A truer determination of the normal would be one equal to the algebraic sum of the observed reading and the mean deviation for the particular rat. Dr. R. W. Bates (3) has brought the author's attention to the fact that deviations such as those observed in Rat No. 38, Table 4, wherein the blood glucose concentrations at the various time intervals fluctuate considerably, may be conditioned by several factors. Bates has observed similar irregularities of adrenal ascorbic acid content in transfering rats from one room to another or in handling untrained rats. The stress of handling untrained rats will lower the adrenal ascorbic acid as much as 40 per cent. It is well known that an increase in blood epinephrine results in a corresponding increase in blood glucose. The increase in blood glucose is used as an indication of the amount of adrenal ascorbic acid change, since adrenal ascorbic acid content is reduced by epinephrine called forth during a state of stress, and since the amount of epinephrine required to produce a fall in adrenal ascorbic acid exceeds that required to produce an increase in blood glucose. Because the rats on which tests were conducted in this work were neither trained nor sheltered in the same room during the testing as before, the same reasoning may be used to explain the irregularities noted in this research. Various degrees of stress to which the rats were subjected during the experiment and their individual sensitivities undoubtedly summon varying amounts of epinephrine and, consequently, of glucose.

In vitro tests were run to check on possible action of the "factor" in lysed trypanosome solution and residue outside the animal organism. Preparations of medium alone, medium minus rat plasma, medium plus lysed trypanosome solution, and medium plus lysed trypanosome residue were incubated at body temperature (37°C.) for 4 hours. The changes in glucose concentration of these preparations were noted at ½, 1, 2, and 4 hours, except of the preparation containing medium minus rat plasma which was observed at 4 hours. A statistical analysis of variance of these data indicate no changes occurred in any of the preparations which could not be attributable to chance. Therefore, it has been shown by in vitro methods, under conditions of this experiment, that no factor affecting glucose is present in lysed trypanosome solution or residue.

That glycolysis was not demonstrated in this work, either by in vivo or in vitro methods, does not preclude that such is the case, since only to a limited extent was efficient refrigeration of the trypanosomal preparations possible during centrifugation and separation procedures. And, as aforementioned, Chen (47) believes failure of such a demonstration may be partially due to enzymatic destruction of adenosine triphosphate (ATP). However, under the conditions of this experiment, no trypanosomal "factor" affecting blood glucose was apparent.

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TABLE 2

BLOOD GLUCOSE CONCENTRATIONS IN
NORMAL, ADULT ALBINO RATS

Rat No.	Blood Glucose Concentration (mgm./100ml.)	Rat No.	Blood Glucose Concentration (mgm./100ml.)
2	83.75	22	90.00
3	91.25	23	76.75
4	69.75	24	100.50
5	90.75	25	93.50
6	85.75	26	89.50
7	93.25	27	85.75
8	85.75	28	78.00
9	78.50	29	86.25
10	98.25	30	89.50
11	91.50	31	107.75
12	86.50	32	77.00
13	81.50	33	63.25
14	82.25	34	88.00
15	78.00	35	91.75
16	69.00	36	74.50
17	64.50	37	113.50
18	97.50	38	94.75
19	97.00	39	92.50
20	70.50	40	125.25
21	99.75	41	132.25

TABLE 2--Continued

Rat No.	Blood Glucose Concentration (mgm./100ml.)	Rat No.	Blood Glucose Concentration (mgm./100ml.)
42	118.75	52	105.50
43	121.00	53	80.75
44	72.00	54	89.50
45	97.00	55	112.00
46	93.25	56	102.00
47	78.50	57	85.75
48	81.50	58	85.75
49	105.50	59	117.25
50	80.00	60	116.50
51	83.75		

Number of rats......................... 59

Mean blood glucose concentration....... 91.04 mgm.%

Standard deviation..................... 15.15 mgm.%

Standard deviation (%)................. 16.64

Standard error......................... 1.97 mgm.%

Standard error (%)..................... 2.16

TABLE

BLOOD GLUCOSE CONCENTRATIONS IN NORMAL, ADULT
INTRAVENOUS INJECTION

Rat No.	Rat Weight (Gm.)	Water Injected (ml.)	Blood Glucose		
				Time	
			Normal	0.5	1.0
38	302	3.0	93.63	113.00	83.00
42	295	2.3	119.88	110.00	116.50
44	240	1.0	100.80	105.50	128.25
45	290	1.0	97.00	72.75	65.50
46	262	1.0	93.25	86.50	93.50
47	214	1.0	78.50	107.75	97.00
Total..................			583.06	595.50	583.75
Mean..................			97.18	99.25	97.29
Standard deviation.....			13.46	16.00	22.61
Standard deviation (%).			13.85	16.12	23.24
Standard error.........			5.49	6.53	9.23
Standard error (%).....			5.65	6.58	9.49

3

ALBINO RATS AT VARIOUS INTERVALS FOLLOWING
OF DISTILLED WATER

Concentration (mgm./100 ml.)
Following Injection (hr.)

1.5	2.0	2.5	3.0	3.5	4.0
89.00	89.50	93.00	96.00	99.00	93.25
119.50	129.75	140.75	135.00	122.50	131.25
112.00	107.75	104.00	103.50	101.00	105.00
77.75	94.00	67.00	91.75	83.75	86.50
88.00	97.50	111.50	90.25	82.25	88.75
100.50	97.00	101.00	94.00	101.00	89.50
586.75	615.50	617.25	610.50	589.50	594.25
97.79	102.58	102.88	101.75	98.25	99.04
15.84	14.61	24.08	16.94	14.63	17.09
16.20	14.24	23.41	16.65	14.89	17.26
6.47	5.96	9.83	6.91	5.97	6.98
6.62	5.81	9.55	6.79	6.08	7.05

TABLE

PER CENT CHANGE IN BLOOD GLUCOSE CONCENTRATIONS
FOLLOWING INTRAVENOUS

Rat No.	Water Injected (ml.)	Normal Glucose (mgm.%)	Per cent Change in Time		
			0.5	1.0	1.5
38	3.0	93.63	+20.69	-11.35	- 4.94
42	2.3	119.88	- 8.24	- 2.82	- 0.32
44	1.0	100.80	+ 4.66	+27.13	+11.11
45	1.0	97.00	-25.00	-32.47	-19.85
46	1.0	93.25	- 7.24	+ 0.27	- 5.63
47	1.0	78.50	+37.26	+23.57	+28.03
Mean...........		97.18	+ 3.69	+ 0.72	+ 1.40

* Per cent change from normal.

TABLE

ANALYSIS OF VARIANCE

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Animals..............	5	7,789.92	1,557.98
Time intervals.....	7	192.42	27.49
Residual (error)...	35	3,338.48	95.39
Total.............	47	11,320.82	

4

OF NORMAL, ADULT ALBINO RATS AT VARIOUS INTERVALS
INJECTION OF DISTILLED WATER

Blood Glucose Concentration*

Following Injection (hr.)					Mean
2.0	2.5	3.0	3.5	4.0	
- 4.41	- 0.67	+ 2.53	+ 5.74	- 0.41	+ 0.90
+ 8.23	+17.41	+12.61	+ 2.19	+ 9.48	+ 4.82
+ 6.89	+ 3.17	+ 2.68	+ 0.20	+ 4.17	+ 7.50
- 3.09	-30.93	- 5.41	-13.66	-10.82	-17.65
+ 4.56	+19.57	- 3.22	-11.80	- 4.83	- 1.04
+23.57	+28.66	+19.75	+28.66	+14.01	+25.44
+ 5.96	+ 6.20	+ 4.82	+ 1.89	+ 1.93	+ 3.33

5

OF DATA IN TABLE 4

F Ratio

$F = 1,557.98/95.39 = 16.33$, $F_{.95} (5,35) = 2.49$

$F = 25.49/95.39 = 0.27$, $F_{.95} (7,35) = 2.29$

TABLE

BLOOD GLUCOSE CONCENTRATIONS IN NORMAL, ADULT
OF SUPERNATANT LYSED

Rat No.	Rat Weight (Gm.)	Supernatant Injected (ml.)	Blood Glucose			
						Time
			Normal	0.5	1.0*	1.5
32	240	2.40	70.25	72.75	84.50	91.45
34	144	1.44	90.00	76.15	67.80	77.85
36	266	3.0	94.00	108.50	.....	77.00
40	258	2.0	124.25	119.50	118.75	124.00
55	212	2.0	112.00	100.50	159.50	101.00
56	156	2.0	102.00	107.75	102.50	110.50
57	172	2.0	85.75	113.50	76.25	83.75
Total......................			678.25	698.65	609.30	665.55
Mean.......................			96.89	99.81	101.55	95.08
Standard deviation.........			17.76	18.29	33.84	17.68
Standard deviation (%)....			18.33	18.32	33.32	18.59
Standard error............			6.71	6.91	13.82	6.68
Standard error (%)........			6.93	6.92	13.61	7.03

* One sample lost.

6

ALBINO RATS FOLLOWING INTRAVENOUS INJECTION TRYPANOSOME SOLUTION

Concentration (mgm./100 ml.) Following Injection (hr.)						
2.0	2.5	3.0	3.5	4.0	5.0**	6.0**
88.80	82.60	76.25	78.60	80.75	......	......
93.00	82.20	71.50	70.40	75.65	......	......
71.25	88.75	72.25	72.75	58.25	......	......
122.50	145.00	170.75	147.50	137.00	......	......
117.25	103.50	111.50	118.00	90.25	103.50	83.00
116.50	118.00	102.50	123.00	109.25	105.00	94.00
84.50	80.75	81.50	75.50	77.75	78.50	79.25
693.80	700.80	686.25	685.75	628.90	287.00	256.25
99.11	100.11	98.04	97.96	89.84	95.67	85.42
19.63	24.08	35.60	30.98	25.91	14.89	7.67
19.81	24.05	36.31	31.63	28.84	15.56	8.98
7.42	9.10	13.46	11.71	9.79	8.60	4.43
7.49	9.09	13.73	11.95	10.90	8.99	5.19

** Only three samples run.

PER CENT CHANGE IN BLOOD GLUCOSE CONCENTRATIONS
FOLLOWING INTRAVENOUS INJECTION OF

Rat No.	Supernatant Injected (ml.)	Normal Glucose (mgm.%)	Per cent Change in Time			
			0.5	1.0*	1.5	2.0
32	2.40	70.25	+ 3.56	+20.28	+30.18	+26.41
34	1.44	90.00	-15.39	-24.67	-13.50	+ 3.33
36	3.0	94.00	+15.43	.....	-18.09	-24.20
40	2.0	124.25	- 3.82	- 4.43	- 0.25	- 1.41
55	2.0	112.00	-10.27	+42.41	- 9.82	+ 4.69
56	2.0	102.00	+ 5.64	+ 0.49	+ 8.33	+14.22
57	2.0	83.75	+32.36	-11.08	- 2.09	- 1.46
Mean.............		96.89	+ 3.93	+ 3.83	- 0.75	+ 3.08

* One sample lost.

ANALYSIS OF VARIANCE

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Animals.............	6	7,166.06	1,194.34
Time intervals.....	9	1,322.28	146.92
Residual (error)...	45	8,298.97	184.42
Total............	60	16,787.31	

7

OF NORMAL, ADULT ALBINO RATS AT VARIOUS INTERVALS
SUPERNATANT LYSED TRYPANOSOME SOLUTION

Blood Glucose Concentration***

Following Injection (hr.)						Mean
2.5	3.0	3.5	4.0	5.0**	6.0**	
+17.58	+ 8.54	+11.89	+14.95	.....	.....	+16.67
- 8.67	-20.56	-21.78	-15.94	.....	.....	-14.65
- 5.59	-23.14	-22.61	-38.03	.....	.....	-16.60
+16.70	+37.42	+18.71	+10.26	.....	.....	+ 9.15
- 7.59	- 0.47	+ 5.36	-19.42	- 7.59	-25.89	- 2.86
+15.69	+ 0.49	+20.59	+ 7.11	+ 2.94	- 7.84	+ 6.77
- 5.83	- 4.96	-11.95	- 9.33	- 8.45	- 7.58	- 3.04
+ 3.18	- 0.38	+ 0.03	- 7.20	- 4.37	-13.77	- 0.30

** Only three samples run.
*** Per cent change from normal.

8

OF DATA IN TABLE 7

F Ratio

$F = 1,194.34/184.42 = 6.48$, $F_{.95} (6,45) = 2.32$

$F = 146.92/184.42 = 0.80$, $F_{.95} (9,45) = 2.10$

TABLE

BLOOD GLUCOSE CONCENTRATIONS IN NORMAL, ADULT
INTRAVENOUS INJECTION OF SUSPENSION

Rat No.	Rat Weight (Gm.)	Suspension Injected (ml.)	Blood Glucose			
						Time
			Normal	0.5	1.0	1.5
48	292	3.0	81.50	86.75	90.75	78.50
49	240	1.0	105.50	86.25	97.00	85.75
51	228	1.0	83.75	110.00	95.50	103.50
52	232	1.0	105.50	107.75	102.00	103.50
53	202	1.0	80.75	77.75	75.00	60.25
54	252	1.0	89.50	85.75	89.50	86.25
58	164	1.5	85.75	121.75	102.00	107.75
59	220	2.0	117.25	140.00	115.00	96.00
60	164	1.0	116.50	97.00	102.00	104.00
Total.....................			866.00	915.00	868.75	825.50
Mean......................			96.22	101.67	96.53	91.72
Standard deviation......			14.97	20.10	11.06	15.55
Standard deviation (%)..			15.56	19.77	11.46	16.95
Standard error..........			4.99	6.70	3.69	5.18
Standard error (%)......			5.19	6.59	3.82	5.65

* One sample lost.

9

ALBINO RATS AT VARIOUS INTERVALS FOLLOWING
OF LYSED TRYPANOSOME RESIDUE

Concentration (mgm./100 ml.)
Following Injection (hr.)

2.0	2.5*	3.0	3.5	4.0*	5.0**	6.0**
86.25	102.50	84.50	93.50	107.75	......	......
85.75	82.25	77.75	85.00	92.50	......	......
90.75	85.00	93.25	97.50	118.00	......	......
110.55	81.50	88.00	82.25	85.00	89.75	80.00
88.75	......	68.25	67.25	72.75	97.80	67.00
90.75	95.50	105.50	97.80	90.25	82.00	85.00
124.00	115.75	132.25	151.75	......	85.75	84.50
101.00	96.00	102.50	97.50	93.25	85.00	115.00
102.00	102.50	97.50	123.00	105.00	114.50	101.00
879.80	761.00	856.50	879.25	764.50	553.80	532.50
97.76	95.13	95.17	97.69	95.56	92.30	88.75
12.93	11.89	20.32	25.42	14.22	12.15	16.86
13.25	12.50	21.35	26.02	14.88	13.16	19.00
4.31	4.20	6.78	8.47	5.03	4.96	6.88
4.41	4.42	7.12	8.67	5.26	5.37	7.75

** Only six samples run.

TABLE

PER CENT CHANGE IN BLOOD GLUCOSE CONCENTRATIONS
FOLLOWING INTRAVENOUS INJECTION OF

Rat No.	Suspension Injected (ml.)	Normal Glucose (mgm.%)	Per cent Change in Time			
			0.5	1.0	1.5	2.0
48	3.0	81.50	+ 8.90	+11.35	- 3.68	+ 5.83
49	1.0	105.50	-18.25	- 8.06	-18.72	-18.72
51	1.0	83.75	+31.34	+14.03	+23.58	+ 8.36
52	1.0	105.50	+ 2.13	- 3.32	- 1.90	+ 4.79
53	1.0	80.75	- 3.72	- 7.12	-25.39	+ 9.91
54	1.0	89.50	- 4.19	0	- 3.63	+ 1.40
58	1.5	85.75	+41.98	+18.95	+25.66	+44.61
59	2.0	117.25	+19.40	- 1.92	-18.12	-13.86
60	1.0	116.50	-16.74	-12.45	-10.73	-12.45
Mean.............		96.22	+ 6.76	+ 1.27	- 3.66	+ 3.32

* One sample lost.

TABLE

ANALYSIS OF VARIANCE

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Animals.............	8	20,945.70	2,618.21
Time intervals....	9	1,739.17	193.24
Residual (error)..	64	11,540.27	180.32
Total............	81	34,225.14	

10

OF NORMAL, ADULT ALBINO RATS AT VARIOUS INTERVALS
SUSPENSION OF LYSED TRYPANOSOME RESIDUE

Blood Glucose Concentration***

Following Injection (hr.)						
2.5*	3.0	3.5	4.0*	5.0**	6.0**	Mean
+25.77	+ 3.68	+14.72	+32.21	.....	.....	+12.35
-22.04	-26.30	-19.43	-12.32	.....	.....	-17.98
+ 1.49	+11.34	+16.42	+40.90	.....	.....	+18.43
-22.75	-16.59	-22.04	-19.43	-15.88	-24.17	-11.92
.....	-15.48	-16.72	- 9.91	+21.11	-17.03	- 7.15
+ 6.70	+17.88	+ 9.27	+ 0.84	- 8.38	- 5.03	+ 1.49
+34.99	+62.39	+76.97	.....	0	- 1.46	+33.79
-18.12	-12.58	-16.84	-20.47	-27.51	- 1.92	-11.19
-12.02	-16.31	+ 5.58	- 9.87	- 1.72	-13.30	-10.00
- 0.66	+ 0.89	+ 5.33	+ 0.22	- 3.60	-10.49	+ 0.32

** Only six samples run.
*** Per cent change from normal.

11

OF DATA IN TABLE 10

F Ratio

$F = 2,618.21/180.32 = 14.52$, $F_{.95}$ (8,64) $= 2.09$

$F = 193.24/180.32 = 1.07$, $F_{.95}$ (9,64) $= 2.03$

TABLE 12

GLUCOSE CONCENTRATIONS OF SAMPLES OF
SUSPENDING MEDIUM INCUBATED AT 37°C.

Sample No.	Quantity of Medium (ml.)	Glucose Concentration (mgm./100 ml.)				
		Normal	Time Incubated (hr.)			
			0.5	1.0	2.0	4.0
1	15	304.5	282.0	306.5	316.0	276.0
2	15	271.5	290.0	314.5	304.0	279.0
3	15	287.0	306.0	306.0	286.0	320.0
4	15	309.0	325.5	313.0	295.5	341.0
Total...............		1172.0	1203.5	1240.0	1201.5	1216.0
Mean...............		293.0	300.9	310.0	300.4	304.0
Standard deviation..		17.2	19.2	4.4	12.8	31.8
Standard dev. (%)...		5.9	6.4	1.4	4.2	10.5
Standard error......		8.6	9.6	2.2	6.4	15.9
Standard error (%)..		2.9	3.2	0.7	2.1	5.2

TABLE 13

PER CENT CHANGE IN GLUCOSE CONCENTRATIONS OF SAMPLES
OF SUSPENDING MEDIUM INCUBATED AT 37°C.

Sample No.**	Initial Glucose (mg.%)	Per cent Change in Glucose Concentration*				
		Time Incubated (hr.)				Mean
		0.5	1.0	2.0	4.0	
1	304.5	- 7.39	+ 0.66	+ 3.78	- 9.36	- 3.08
2	271.5	+ 6.81	+15.84	+11.97	+ 2.76	+ 9.35
3	287.0	+ 6.62	+ 6.62	- 0.35	+11.50	+ 2.79
4	309.0	+ 5.34	+ 1.29	- 4.37	+10.36	+ 3.13
Mean...	293.0	+ 2.85	+ 6.10	+ 2.76	+ 3.82	+ 3.88

* Per cent change from normal.
** Each sample contained 15 ml. of medium.

TABLE 14

ANALYSIS OF VARIANCE OF DATA IN TABLE 13

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Samples...........	3	334.88	111.63
Time intervals....	3	29.11	9.70
Residual (error)..	9	376.89	41.88
Total...........	15	740.88	

$F_{smpl.} = 111.63/41.88 = 2.67$, $F_{.95} (3,9) = 3.86$

$F_{time} = 9.70/41.88 = 0.23$, $F_{.95} (3,9) = 3.86$

TABLE 15

GLUCOSE CONCENTRATIONS OF SAMPLES OF SUSPENDING MEDIUM
MINUS RAT PLASMA INCUBATED AT 37°C.

Sample No.	Quantity of Preparation (ml.)	Glucose Concentration (mgm./100 ml.)	
		Normal	Incubated 4 hr.
1	10	385.0	385.0
2	10	385.0	348.5
3	10	396.0	365.0
Total...............		1166.0	1098.5
Mean...............		388.7	366.2
Standard deviation.		6.3	18.3
Standard dev. (%)..		1.6	5.0
Standard error.....		3.7	10.6
Standard error (%).		1.0	2.9

TABLE 16

ANALYSIS OF VARIANCE OF DATA IN TABLE 15

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Samples............	4	748.84	187.21
Time intervals....	1	759.38	759.38

F_{time} = 759.38/187.21 = 4.06 $F_{.95}(1,4)$ = 7.71

TABLE 17

GLUCOSE CONCENTRATIONS OF IN VITRO PREPARATIONS CONTAINING
SUPERNATANT LYSED TRYPANOSOME SOLUTION INCUBATED AT 37°C.

Prepn. No.	Supernatant Added* (ml.)	Glucose Concentration (mgm./100 ml.)				
			Time Incubated (hr.)			
		Normal	0.5	1.0	2.0	4.0
1	1.0	283.0	262.5	279.5	273.0	279.5
2	1.0	283.0	256.5	276.5	276.0	279.5
3	5.0	245.5	230.0	218.0	259.5	248.0
4	5.0	224.5	250.5	243.5	249.0	255.0
Total..............		1036.0	999.5	1017.5	1062.5	1062.0
Mean..............		259.0	249.9	254.4	265.6	265.5
Standard deviation.		29.0	14.1	29.2	13.8	16.4
Standard dev. (%)..		11.2	5.7	11.5	5.2	6.2
Standard error.....		14.4	7.1	14.6	6.9	8.2
Standard error (%).		5.6	2.8	5.7	2.6	3.1

* Supernatant added to 15 ml. of medium.

TABLE 18

PER CENT CHANGE IN GLUCOSE CONCENTRATIONS OF IN VITRO
PREPARATIONS CONTAINING SUPERNATANT LYSED
TRYPANOSOME SOLUTION INCUBATED AT 37°C.

Prepn. No.**	Initial Glucose (mg.%)	Per cent Change in Glucose Concentration*				
		Time Incubated (hr.)				Mean
		0.5	1.0	1.5	2.0	
1	283.0	− 7.24	− 1.24	− 1.77	− 1.24	− 2.87
2	283.0	− 7.24	− 2.30	− 2.47	− 1.24	− 3.31
3	245.5	− 6.31	−11.20	+ 5.70	+ 1.02	− 2.70
4	224.5	+11.58	+ 8.46	+10.91	+13.59	+11.14
Mean...	259.0	− 2.30	− 1.57	+ 3.09	+ 3.03	+ 0.56

* Per cent change from initial.
** 1.0 ml. Supernatant contained in No. 1 and No. 2.
** 5.0 ml. Supernatant contained in No. 3 and No. 4.

TABLE 19

ANALYSIS OF VARIANCE OF DATA IN TABLE 18

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Preparations......	3	596.89	198.96
Time intervals....	3	101.05	33.68
Residual (error)..	9	129.14	14.35
Total..........	15	827.08	

$F_{prepn.} = 198.96/14.35 = 13.86$, $F_{.95}(3,9) = 3.86$

$F_{time} = 33.68/14.35 = 2.35$, $F_{.95}(3,9) = 3.86$

TABLE 20

GLUCOSE CONCENTRATIONS OF IN VITRO PREPARATIONS CONTAINING
SUSPENSION OF LYSED TRYPANOSOME RESIDUE INCUBATED AT 37°C.

Prepn. No.	Suspension Added* (ml.)	Glucose Concentration (mgm./100 ml.)				
			Time Incubated (hr.)			
		Normal	0.5**	1.0	2.0	4.0
1	1.0	289.0	297.0	289.0	295.5	298.0
2	1.0	287.0	289.0	285.0	294.0	291.0
3	2.5	276.0	279.5	259.5	280.0	269.0
4	2.5	266.5	.....	262.5	284.0	284.0
Total...............		1118.5	865.5	1096.0	1153.5	1142.0
Mean...............		279.6	288.5	274.0	288.4	285.5
Standard deviation.		10.5	8.8	15.2	7.6	12.4
Standard dev. (%)..		3.7	3.0	5.5	2.6	4.3
Standard error.....		5.2	5.1	7.6	3.8	6.2
Standard error (%).		1.9	1.8	2.8	1.3	2.2

* Suspension added to 15 ml. of medium.
** One sample lost.

TABLE 21

PER CENT CHANGE IN GLUCOSE CONCENTRATIONS OF IN VITRO
PREPARATIONS CONTAINING SUSPENSION OF LYSED
TRYPANOSOME RESIDUE INCUBATED AT 37°C.

Prepn. No.***	Initial Glucose (mg.%)	Per cent Change in Glucose Concentration*				
		Time Incubated (hr.)				Mean
		0.5**	1.0	2.0	4.0	
1	289.0	+ 2.72	0	+ 2.25	+ 3.11	+ 2.03
2	287.0	+ 0.70	- 0.70	+ 2.44	+ 1.39	+ 0.96
3	276.0	+ 1.27	- 5.98	+ 1.45	- 2.54	- 1.45
4	266.5	.....	- 1.50	+ 6.57	+ 6.57	+ 3.88
Mean...	279.6	+ 1.58	- 2.05	+ 3.18	+ 2.13	+ 1.19

* Per cent change from initial.
** One sample lost.
*** 1.0 ml. Suspension contained in No. 1 and No. 2.
*** 2.5 ml. Suspension contained in No. 3 and No. 4.

TABLE 22

ANALYSIS OF VARIANCE OF DATA IN TABLE 19

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Squares
Preparations......	3	52.65	17.55
Time intervals....	3	25.37	8.46
Residual (error)..	8	66.65	8.33
Total...........	14	144.67	

$$F_{prepn.} = 17.55/8.33 = 2.10, \quad F_{.95} (3,8) = 4.07$$

$$F_{time} = 8.46/8.33 = 1.02, \quad F_{.95} (3,8) = 4.07$$

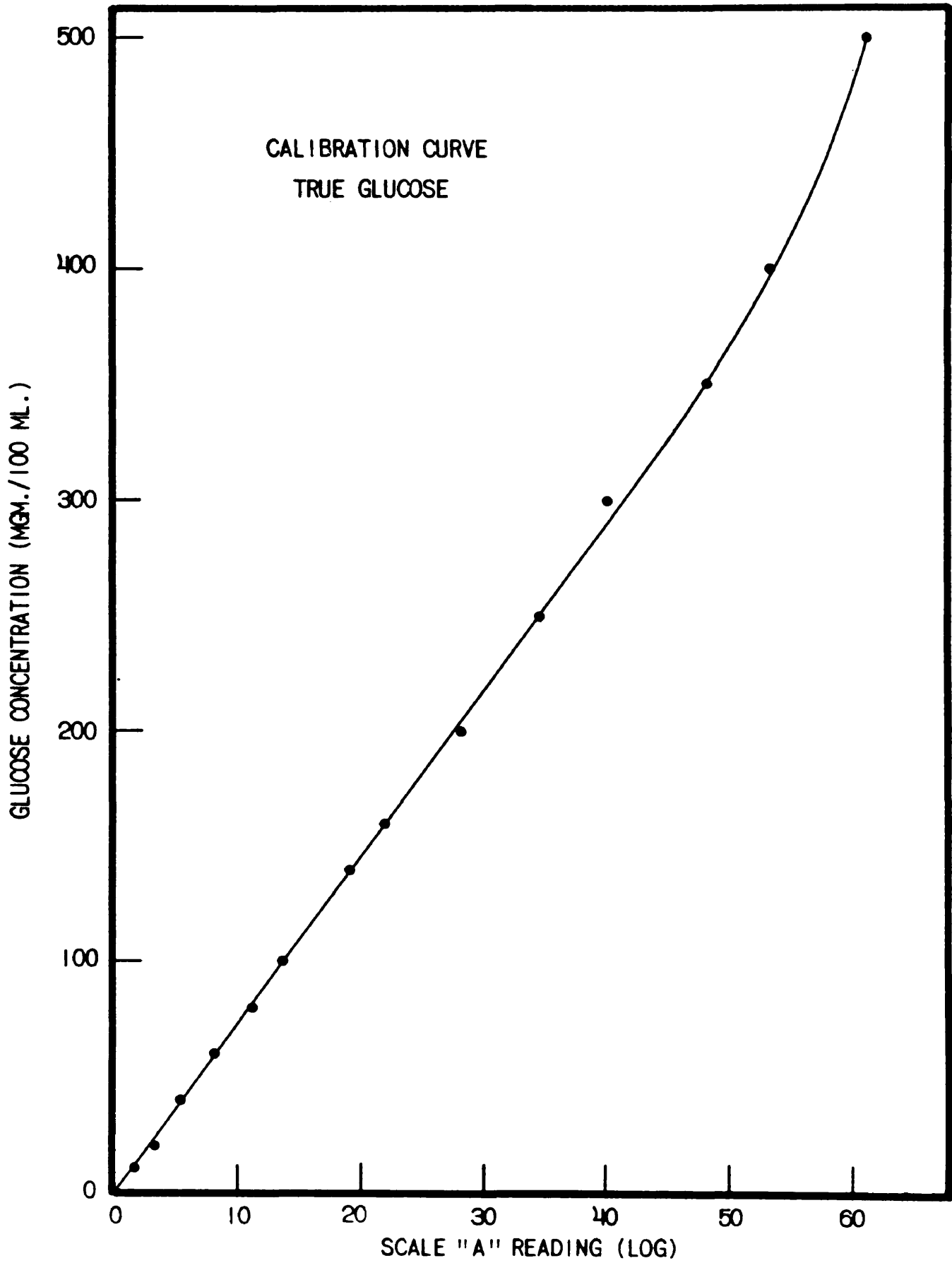FIGURE I

MEAN BLOOD GLUCOSE CONCENTRATION AFTER
DISTILLED WATER INJECTED IN RATS
(CONTROLS)

●————● MEAN GLUCOSE CONCENTRATION
○- - - -○ ONE STANDARD DEVIATION
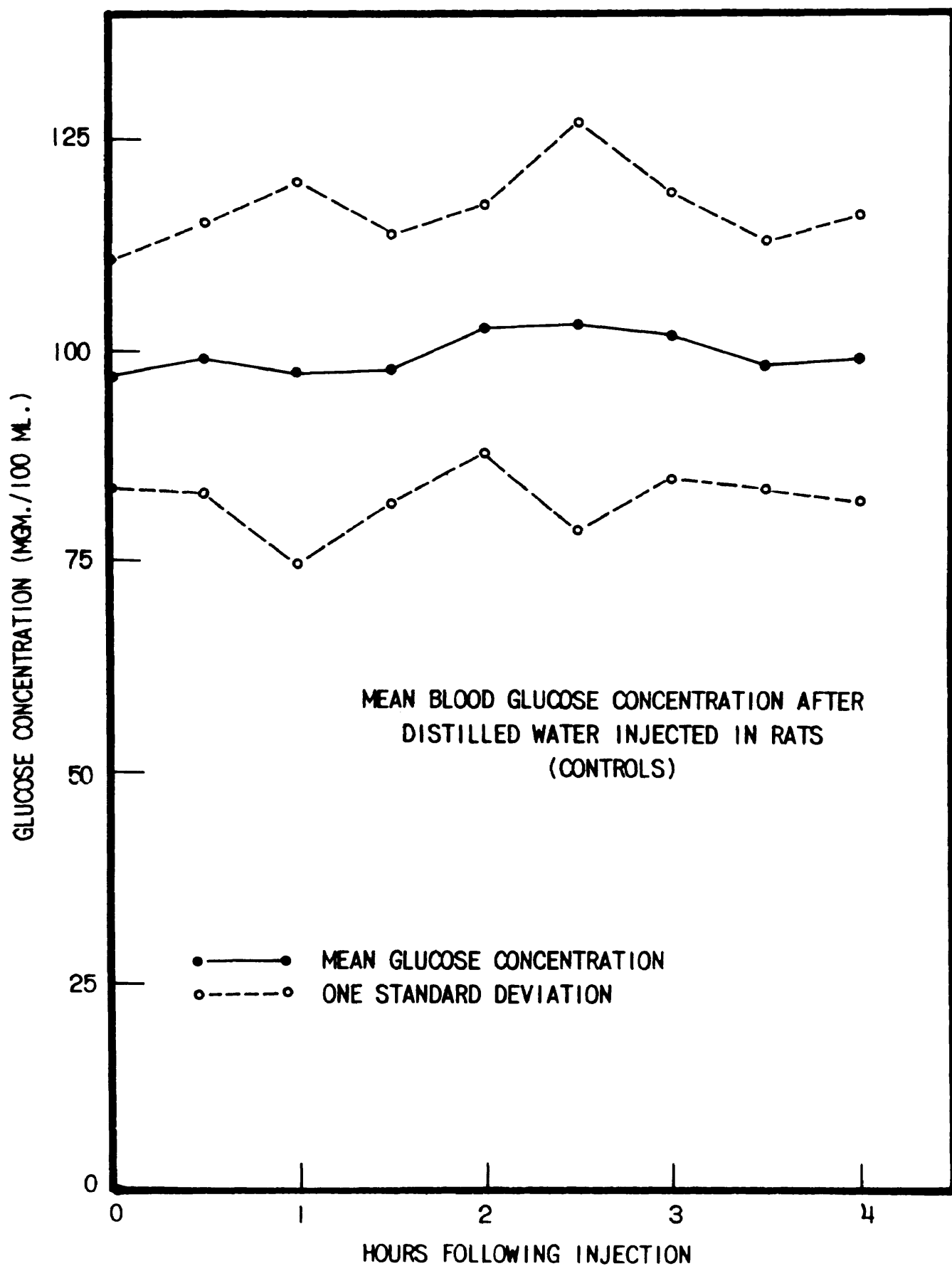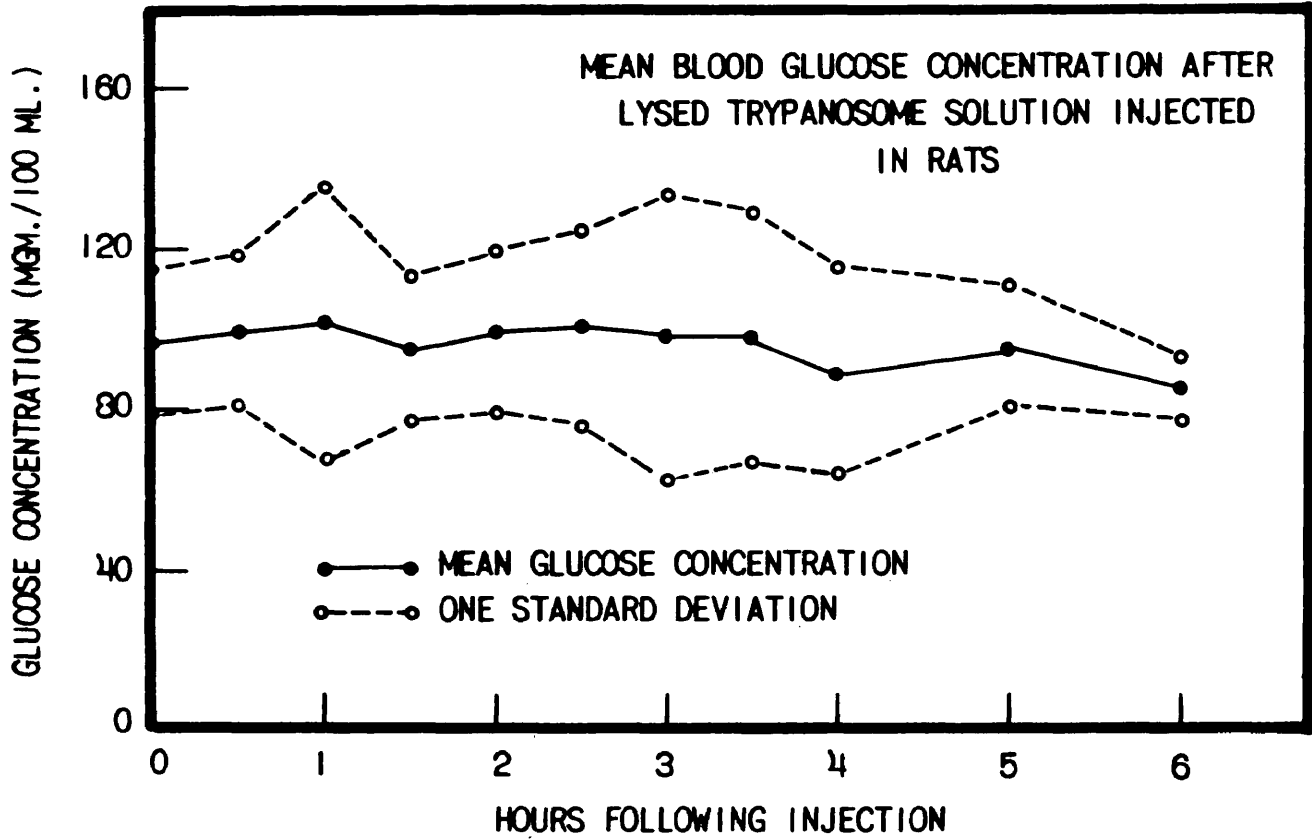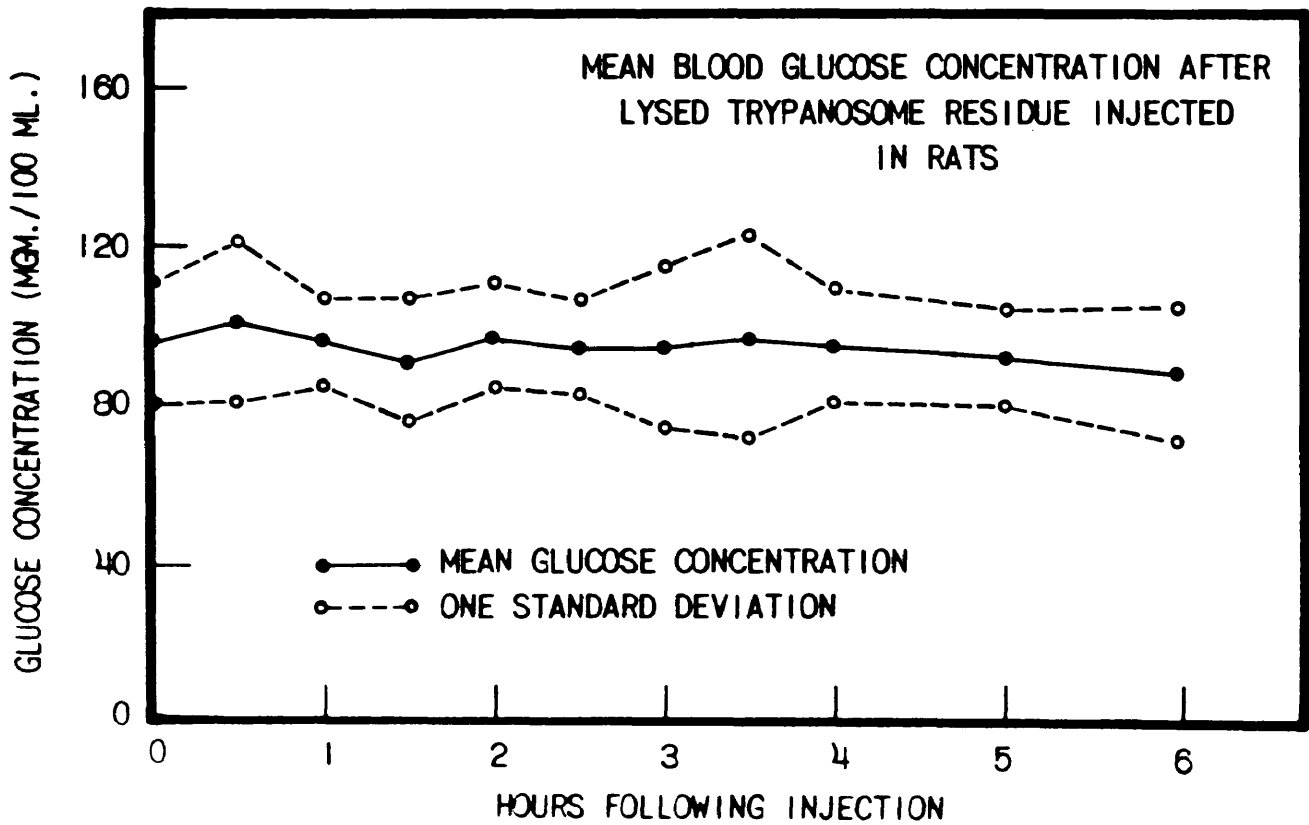
GLUCOSE CONCENTRATION (MGM./100 ML.)
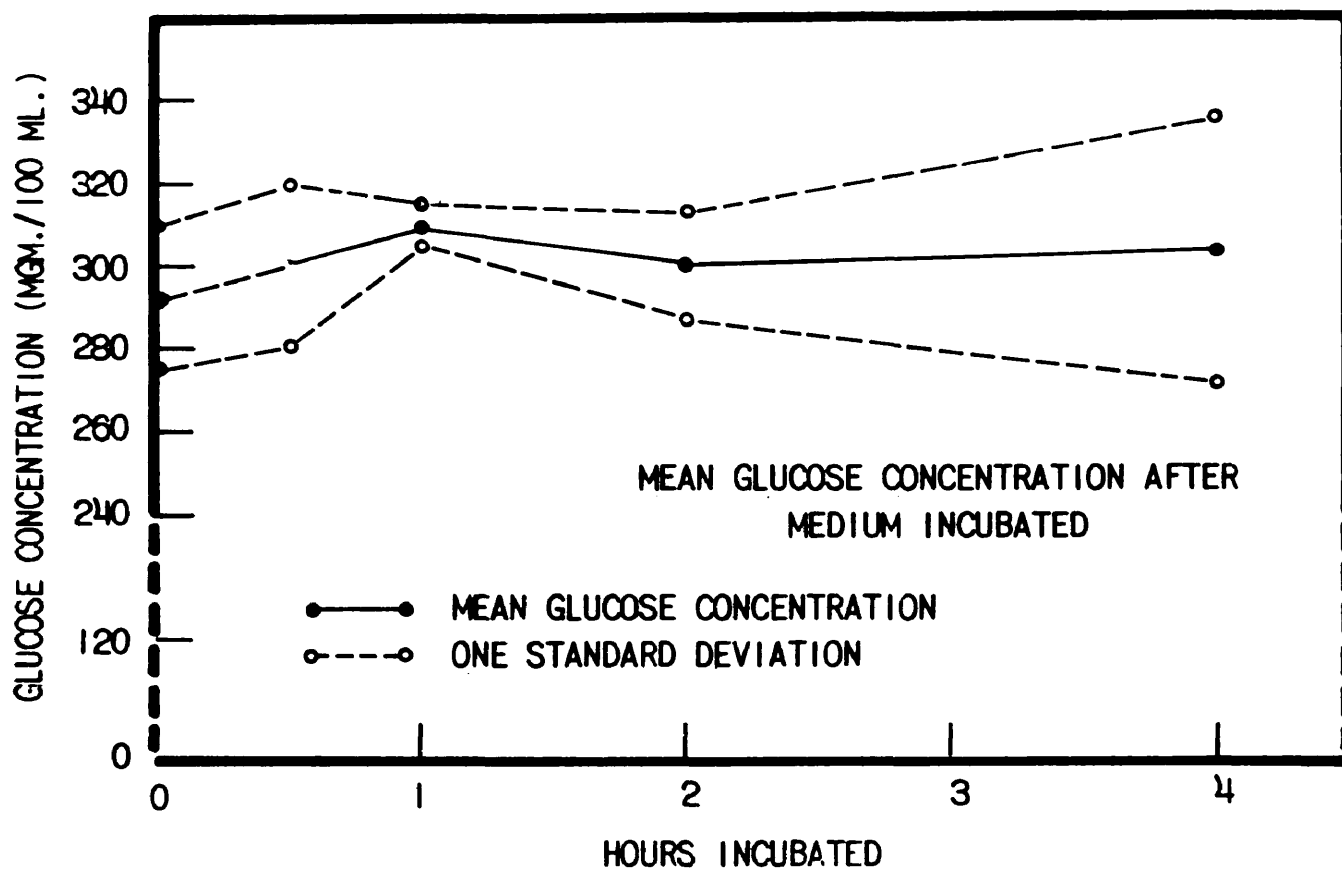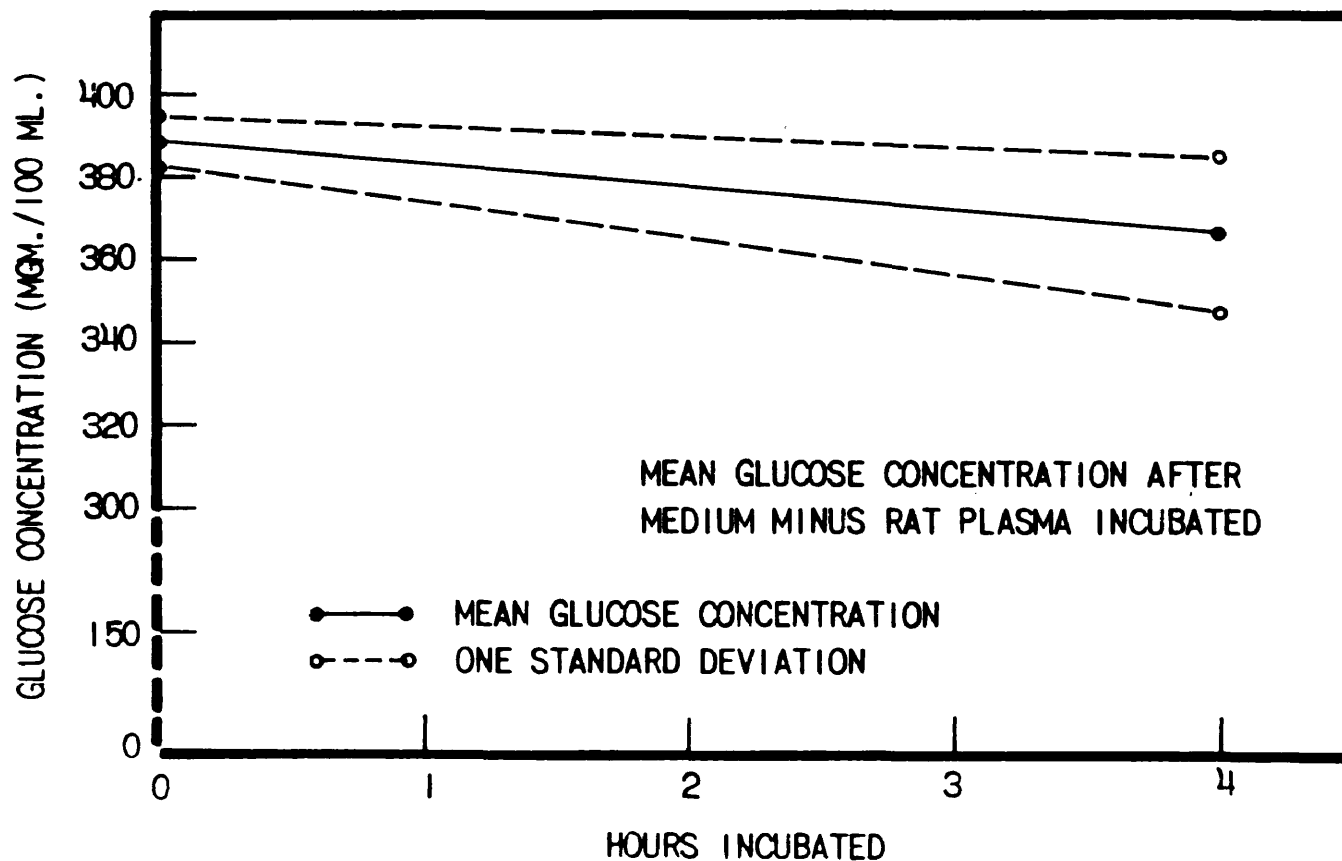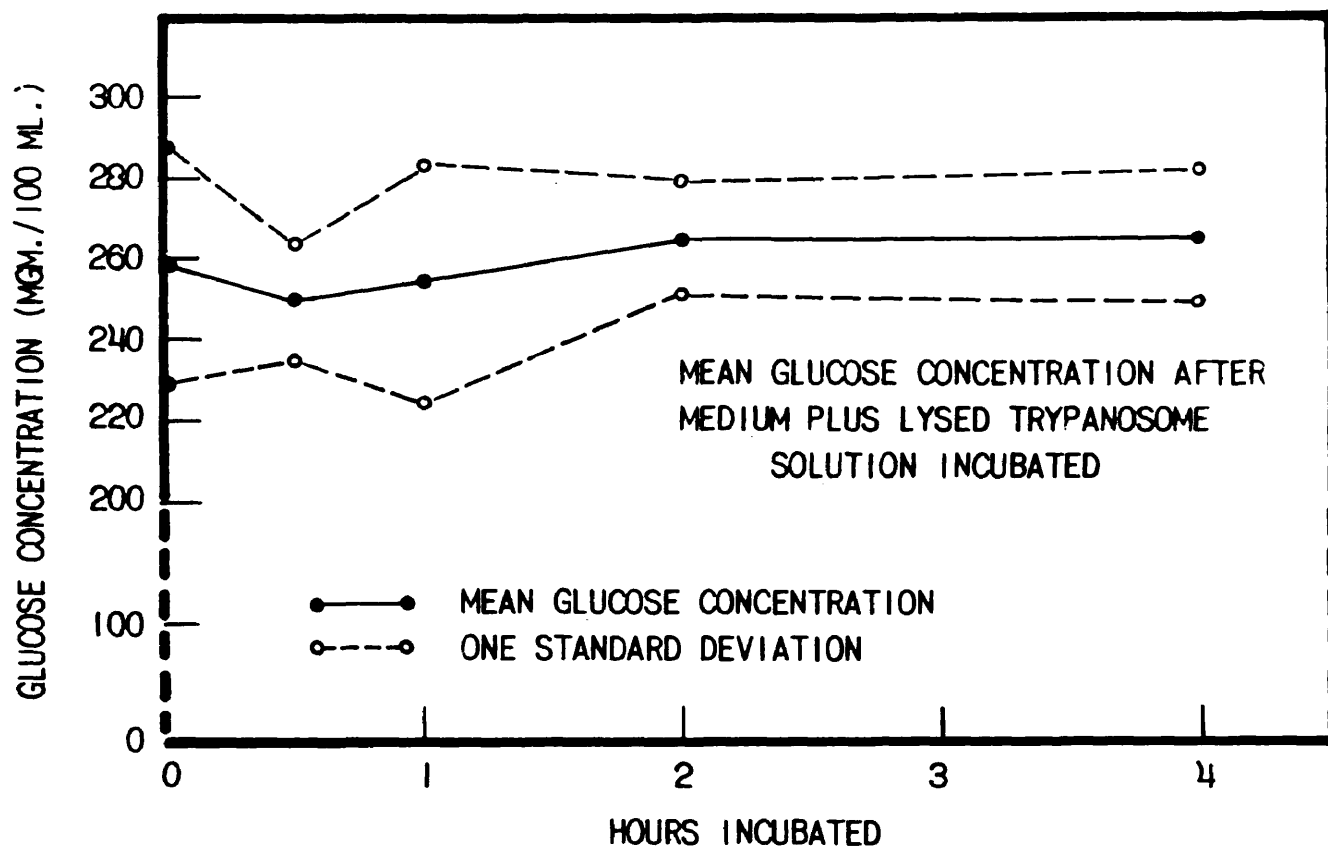
HOURS FOLLOWING INJECTION

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

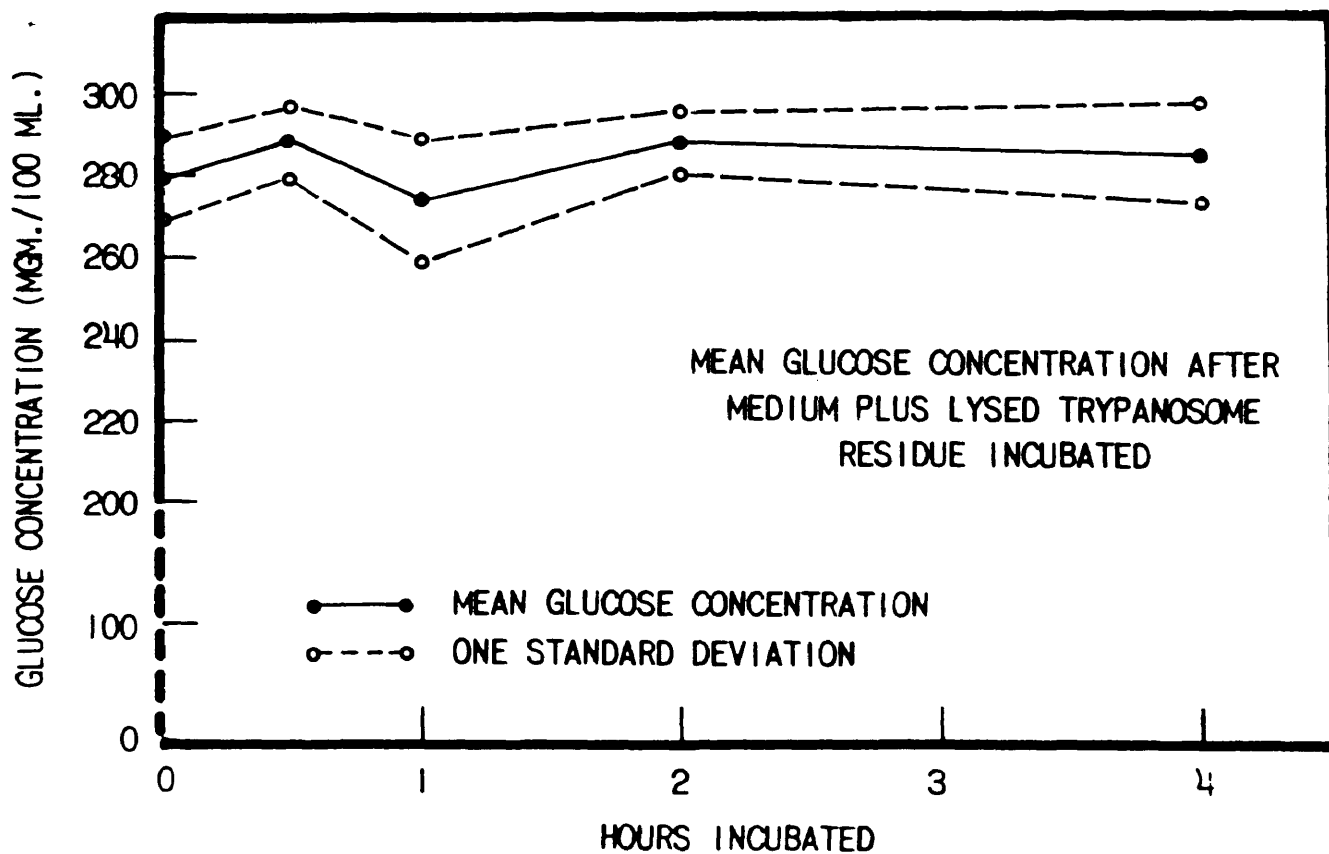FIGURE 6

FIGURE 7

FIGURE 8

MEAN PER CENT CHANGES IN BLOOD GLUCOSE
AFTER ADMINISTRATION OF:

●———● DISTILLED WATER (CONTROLS)
■———■ LYSED TRYPANOSOME SOLUTION
▲———▲ LYSED TRYPANOSOME RESIDUE

HOURS FOLLOWING INJECTION

FIGURE 9

MEAN PER CENT CHANGES IN GLUCOSE CONCENTRATION
AFTER INCUBATION OF MEDIUM;

●--- --● MINUS RAT PLASMA
▲———▲ PLUS LYSED TRYPANOSOME SOLUTION
■———■ PLUS LYSED TRYPANOSOME RESIDUE
●———● 100 PER CENT

HOURS INCUBATED

FIGURE 10