Properties of immune sera in Trypanosoma lewisi infection

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PROPERTIES OF IMMUNE SERA IN
TRYPANOSOMA LEWISI INFECTION

by

Michael George Lysenko

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Protozoology

Approved:
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Iowa State College
1950
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INTRODUCTION

There has been described in *Trypanosoma lewisi* infections in rats an immune reaction that operates in two ways: first, an anti-reproductive antibody is formed which inhibits reproduction of the parasites; then, a second antibody is formed which destroys the trypanosomes. Taliaferro (1932a) called the first antibody ablastin and the second the trypanocidal antibody.

The question of the existence, formation, and operation of ablastin has received a great deal of attention by many investigators. Taliaferro (1924, 1925, 1926, 1932a) reported studies on the properties of ablastin: he said that it was a protein in the eglobulin fraction of serum, it was passively transferable, it would not unite with trypanosomes in *vitro*, and that it neither killed the parasites nor affected their motility or vitality. Its only manifestation seemed to be that of *in vivo* suppression of reproduction.

Blood sugar studies by Schern (1928) and Poindexter (1933) indicated an inverse relation between the amount of sugar and number of trypanosomes in pathogenic *trypanosomiasis*. Linton (1929) found that in the non-pathogenic *T. lewisi* there was no change in the blood sugar during the course of the infection.

In the laboratories at Iowa State College, Becker and co-workers (1943, 1947) reported that deficiencies in
Pantothenic acid affected the antireproductive activity of ablastin so that reproduction continued and the infection was pathogenic. Becker and Gallagher (1947) found that treatment of infected rats with sodium salicylate had a similar effect on ablastic activity.

Minoru (1940) reported changes in blood sugar levels in rabbits as a result of sodium salicylate administration. In low amounts there was a hypoglycemia. In greater amounts there was hyperglycemia.

Brown (1915) and Taliaferro (1926) suggested that the difference between pathogenic and non-pathogenic trypanosomes might be based on the absence or presence of an antireproductive factor in the reaction of the host.

The relationships among pathogenicity, antireproductive activity, glucose utilization and glucose levels, pantothenic acid deficiency, and sodium salicylate treatment suggested that further studies be made on immune sera with respect to these various factors.

In this investigation, therefore, are reported studies on immune sera of rats both treated with sodium salicylate and not treated with respect to (1) ablastic activity, (2) changes in protein levels, (3) pantothenate levels and (4) blood glucose levels.
REVIEW OF LITERATURE

Extensive reviews of the literature on immunity of the rat to *T. lewisi* have been made by Taliaferro (1929), Saul (1948) and Barnes (1950). This review therefore, will indicate only the investigations that have a direct bearing on the problem under discussion.

The earliest observation of immunity of the rat to *T. lewisi* was made by Kanhack, Durham, and Blandford (1898). Rabinowitsch and Kempner (1899) studied the immunity further. They showed that the immunity was passively transferable, for when they injected one ml. of immune serum into susceptible rats either 24 hours before or after the infective dose of trypanosomes was given, no infection developed in the animals.

Laveran and Mesnil (1901) determined the degree of reproduction by examining blood smears for variability in size. They believed that the basis for the immunity of the rat to the trypanosome was phagocytosis. They said that they had observed leucocytes engulfing trypanosomes in hanging drop suspensions of guinea pig peritoneal exudate.

MacNeal (1904) brought forward evidence that the disappearance of the trypanosomes was due to lysis rather than to phagocytosis. He repeated the experiments with the peritoneal fluid of guinea pigs, but could not observe the engulfment of trypanosomes. He
observed that the parasites became paler and paler until they finally dissolved. From this he concluded that the basis for the immunity was lysis.

Taliaferro and Taliaferro (1922) made studies of the course of the infection in the rat. They developed the method determining the rate of reproduction by measuring the lengths of the trypanosomes and determining the coefficient of variation. They found that reproduction was high at first, then it slowed down and stopped by the 10th day. With respect to the numbers of trypanosomes, they noted that there was a sharp drop in numbers between the 9th and 13th days. The numbers then remained more or less constant for a period ranging from a few weeks to a few months, at which time there was another number crisis and the trypanosomes disappeared from the peripheral blood completely. From this evidence the authors suggested that there were two effects in the immunity of the rat to the parasite: first, there was inhibition of reproduction; and secondly, there was destruction of the parasites by lysis. In comparing this immune reaction to the non-pathogenic *T. lewisi*, they said that in pathogenic forms there was evidence of neither the inhibition of reproduction nor the destruction of the parasites. This latter type of infection was found in trypanosomes like *T. brucei*, *T. rhodesiense*, and *T. equiperdum* in the rat. A relapsing type of infection was also
described, like *T. equinum* in the rat and *T. equiperdum* in the
guinea pig. The immunity in this type of infection involved the
formation of an antibody which destroyed the parasites, but there
was no inhibition of reproduction. Relapses occurred when not
quite all the trypanosomes were destroyed, leaving the survivors
free to multiply.

These observations of the Taliaferros corroborated some of
the earlier investigations with respect to the difference between
pathogenic and non-pathogenic forms. Brown (1915) found that in a
pathogenic strain of *T. lewisi* reproduction continued and did not
stop as it does in the ordinary strain. Van Saeeghem (1923) also
suggested that pathogenic forms of trypanosomes became harmless
commensals whenever an antireproductive factor was present in the
blood of the host.

Taliaferro (1924) reported further studies on the antire-
productive principle. He found it was passively transferable
and that it thus prevented reproduction for 13 days. He reported
that trypanosomes grown in culture did not form this product, and
that the substance neither killed nor affected the vitality of the
parasites. He suggested therefore that this reaction product was
different from the antibody which destroys the trypanosomes at the
termination of the infection.

Coventry (1925) studied the changes in the titer of the anti-
reproductive factor. She collected serum from rats on various
days of the infection and injected this serum together with a
dose of trypanosomes into experimental rats. The titer was
determined by noting the degree of inhibition of reproduction
in the experimental rats. She was not able to demonstrate the
presence of the product before the fifth day, although she thought
it was present because the rate of reproduction was decreasing.
On the fifth and sixth days there was a rapid rise in titer, with
a further but more gradual increase from the seventh to the 35th
day. The titer then gradually decreased from the 35th day to
the end of the infection.

After further studies, Taliaferro (1925, 1926, 1932a, 1932b,
and 1934) reported additional properties of this antireproduc-
tive product. He found that the substance was found in the
euglobulin fraction of immune serum, that it would not inhibit
reproduction in vitro, and that it was not absorbable as was the
trypanocidal antibody. It was possible to separate the two anti-
bodies on this basis. He thought it was a large molecular
substance because it would not pass through a filter unless
hemoglobin would. It was found in the non-ether-soluble portion
of serum, so it was concluded to be protein in nature. In the
rabbit infected with T. lewisi, there was formed the trypanocidal
antibody but not the reproduction-inhibiting one. Because the
author believed that this reaction product was an antibody, but
different from other antibodies in some ways, he proposed to call
They were found free and active in the thymus, but not in the spleen. They were also found in the liver and bone marrow. There were some abnormal infections, but these were not found in the spleen or thymus. Some were found to be normal in the presence of antibodies, but some were not affected by them. The author suggested that the abnormal infections were due to some unknown factor, and that the normal infections were due to the presence of antibodies. If the abnormal infections were normal for the production of antibodies, then some were not important in the passage of antibodies, but others were essential for the production of antibodies. The author expressed the impression that the antibodies were essential after the experiment, in the last group reproduction, and after passage of antibodies, they were again transferred, when antibodies were injected into normal mice.
entry into the blood stream. He further reported that if adult trypanosomes were injected, division did not occur for two days; while, when dividing ones were injected, division continued uninterrupted. The author reported that ablastin played no role in the immunity to reinfection in the rat. He found that the disposal of the parasites was due to a single trypanocidal antibody. He said that dividing cells appeared in the blood but that they were soon killed off, or immobilized and phagocytized. At sufficiently high titers adults were agglutinated but not killed. The masses of agglutinated cells were then removed mechanically. He suggested the possibility of two antibodies acting in recovery from reinfection: an opsonin, acting specifically on dividing cells; and an agglutinin, acting on both dividing and adult cells.

In reply to Augustine, Taliaferro (1948) said that dividing forms may have appeared in Augustine's immune rats because the ablastic titer may have dropped to a low level. He felt that the presence of dividing forms and a rise in numbers, together with a specific opsonization of division forms was a contradictory situation. As trypanosomes multiply by fission, an adult by its division gives rise to two or more young or division forms. If these division forms were opsonized specifically and removed by phagocytosis, then the total number of parasites should drop and not remain static as Augustine suggested.
In the matter of the controversy as to the existence of ablastin, it might be well to note, that after Dochez and Avery (1916) had described an antireproductive phenomenon in their studies with pneumococcus there was strong criticism. Blake (1917) showed that the phenomena described by Dochez and Avery could be explained on the basis of agglutination. Barber (1919a, 1919b) using his single cell technique to eliminate the question of agglutination, could not find any evidence of inhibition of reproduction.

In the case of the rat and *T. lewisi* there seems to be other evidence, which will be noted later, which corroborates the belief of Taliaferro that there is an antireproductive factor involved in the immune reaction present in this host and parasite relationship.

Another phase of investigation on ablastin has been in the direction of the mechanism of its action. Becker, Manresa, and Johnson (1943) tested the effect of pantothenic acid deficiency on the immunity of the rat to *T. lewisi*. They obtained higher parasite populations and more reproduction of the parasites in deficient rats. Definite conclusions were not made, however, because it was thought that the increased numbers may have been due to an intercurrent *Bartonella* infection. Later, the work was taken up again. Becker, Taylor, and Fuhrmeister (1947) worked
II.

with deficient rats in which there was no *Bartonella* infection. They concluded that the parasite became a pathogen in pantothenic acid-deficient rats. They reported increased multiplication period, higher populations, anemia, neutrophilia, basophilia, less than normal and more delayed increase in total leucocyte count, either reduced growth rate or loss of weight of the host, and in extreme cases death.

Becker and Gallagher (1947) reported that there was a prolongment of reproduction in rats treated with sodium salicylate. The effect obtained was similar to that obtained with pantothenic acid deficiency. Just as before there was an interference with ablatic activity. These investigations seemed to support the ideas of Taliaferro with respect to the presence in normal infections of an antireproductive principle. The authors suggested that sodium salicylate may have interfered with pantothenate usage. They speculated further that the antireproductive antibody might be an oxidizing type of enzyme in which pantothenate is coupled to a protein as a coenzyme.

Moulder (1948) studied oxygen uptake and glucose metabolism by trypanosomes at various stages in the course of the infection in the rat. He noticed that there were differences in these respects between dividing and adult populations. He found that there was a decrease in the amount of glucose utilized by adult populations as compared with dividing ones. There was also an
increase in the amount of oxygen uptake, and an increase in the respiratory quotient. These changes came on about the fifth day. That is, they coincided with the time at which the inhibition of reproduction by ablastin occurred. The author suggested that ablastin might have acted as a mild inhibitor of the oxidative system of the dividing trypanosomes for glucose utilization. He thought that the role of ablastin might have been similar to that of urethane, azide, and dinitrophenols in yeast cells and in sea urchin eggs as was described by Fisher and Stern (1942) and by Fisher and Henry (1944).

In reviewing this work of Moulder (1948), Taliaferro (1948) suggested that young cells oxidized glucose incompletely because they were assimilating glucose oxidatively, whereas adult non-reproducing trypanosomes oxidized glucose more completely because oxidative assimilation had been inhibited by ablastin. He suggested that it might be associated with a loss of power of carrying the dissimilation of the glucose molecule beyond succinate.

Considerable work has been done on the relationship between carbohydrates and trypanosomiasis. Most of the investigation has been with the pathogenic forms, and the evidence varies. Some studies indicated that there was no noticeable change in blood sugar during the infection (Savino, 1927; Tubangui and Yutuc, 1931). Most evidence indicated that there is a drop in sugar during the infection, or particularly at the termination of the infection.
This was shown in the work of Linton (1930) who reported that the blood sugar dropped very late in a case of trypanosomiasis. Poindexter (1933, 1936) reported that in guinea pigs with *Trypanosoma cruzi*, there was an increase in parasite count after injection of glucose. There was also a shortening of the prepatent period, and an increase in the rate of reproduction. When insulin was given to lower the sugar levels, there was a decrease in the rate of reproduction and treated animals lived longer than those that were not treated with insulin. The author concluded that trypanosomes used glucose just as bacteria growing in a test tube would.

Yorke, Adams, and Murgatroyd (1929) were the first to show that glucose was essential for the life of trypanosomes and that they required large quantities of sugar in *vitro*. Reiner, Smythe, and Pedlow (1936) reported differences in the consumption of sugar between pathogenic trypanosomes and *T. lewisi*. They reported that pathogenic forms consumed 7 - 8 mgm. of glucose per 1000 million trypanosomes per hour at 37°C. Under similar conditions *T. lewisi* consumed 1.5 mgm. The end products of metabolism were different also. In pathogenic forms the end products were pyruvic acid and glycerol. End products reported in *T. lewisi* were succinic, acetic and formic acids, ethyl alcohol and carbon dioxide. Von Brand (1938) thought that the trypanosomes consumed such large quantities of sugar in comparison to man, because they were very
active, were small and so had a relatively large surface, and because they utilized only a fraction of the energy in the carbohydrate molecule as they did not totally oxidize the sugar to carbon dioxide and water.

With respect to sugar levels in rats with *T. lewisi*, Linton (1929) reported that there was no difference in sugar levels between normal rats and those at the peak of the parasite infection. He reported means of 96 and 115 mgm. of glucose per 100 ml. of blood. He also found that in splenectomized rats in which the parasite numbers were greater there was a mean value of 107 mgm. But he thought all these values in the range of experimental error. Regendanz (1929) reported that in a pathogenic strain of *T. lewisi*, which was often fatal, there was always a final hypoglycemia.
EXPERIMENTAL METHOD

This discussion will be in several parts. Some of the general procedures which were used in most of the experiments will be mentioned first. Then the materials and methods particular to the various phases investigated will be discussed separately.

General

The host animal was the laboratory rat, *Mus norvegicus* albinus, of the Wistar A inbred strain. The animals were obtained from the stock colony maintained on a ration that had provided satisfactory growth and reproduction. The colony was louse-free, and there was no evidence of Bartonella infection. Male animals were used as experimental hosts.

The strain of *Trypanosoma lewisi* used was Becker's strain, which is characterized by having a somewhat shorter reproductive period, lower maximum populations, and a shorter duration than other strains. The stages in the rat, however, correspond to those of the usual strains.

Rats were usually infected with the parasite by injecting 2 ml. of inoculum intraperitoneally on the zero day of the infection following the method of Barnes (1950). In experiments in which both trypanosomes and serum were injected into rats, the serum
was injected intraperitoneally, while the trypanosomes were injected intravenously. In small rats this was done by injecting into the tail vein; while in larger ones this was done by exposing and injecting into the common carotid vein.

Sodium salicylate treatment consisted of giving daily doses of 45 mgm. per 100 gm. of weight of rat after Saul (1948).

The rate of reproduction was indicated by determining the percentage of division forms by the method of Becker and Gallagher (1947). In most cases 200 parasites were counted. In some experiments in which immune serum was injected the parasite population was low and smaller numbers such as 25 or 50 were counted.

Plasma or serum was obtained from blood drawn from the postcaval vein just at its entry into the diaphragm by means of a 10 ml. syringe with a 20-gauge needle attached. The rat used was anesthetized in a chloroform jar, pinned to a board, and the vein exposed by incision in the abdominal wall and cutting the falciform ligament. If plasma was desired an anticoagulant like heparin or sodium oxalate was used (Moulder, 1948 - 0.1 volume of 10 per cent solution). The cells were removed by centrifugation and the plasma was pipetted off. If serum was desired, no anticoagulant was used. The collected blood was placed in centrifuge tubes and allowed to stand 20 minutes. The clot was loosened from the side of the tube and either centrifuged immediately or allowed to stand in the
refrigerator for some time before centrifugation. The serum was then pipetted off.

When washed trypanosomes were wanted for intravenous inoculation, the procedure for the collection of plasma was followed. The suspension was then centrifuged slowly (1500 r.p.m. for 10 minutes) and the supernatant with the trypanosomes was pipetted off. This was then centrifuged rapidly (2700 r.p.m. for 15 minutes) and the supernatant plasma poured off. The trypanosomes were washed by adding physiological saline, centrifuged rapidly, and the saline poured off. The trypanosomes were then suspended in the desired volume of saline.

The absorption of immune serum or plasma to remove the trypanocidal antibody was accomplished following the method of Taliaferro (1932a). Both dividing and adult washed trypanosomes were added to the plasma or serum to be absorbed. The mixture was allowed to stand in the refrigerator for an hour or two and then centrifuged strongly to remove the trypanosomes.

Statistical analyses were made following methods found in Snedecor (1946). Probability values were obtained from this source also.
Sodium Salicylate and Ablastic Plasma

In the first experiment in this group plasma from sodium salicylate treated rats infected with *T. lewisi* was tested for the presence of ablastin by passive immunity tests. The plasma used was obtained from rats on the 10th day of the infection. The 10th day was chosen because by then the ablastin titer was fairly high (Coventry, 1925) while the trypanocidal antibody was not of sufficiently high titer to have removed all the trypanosomes. Transfer of passive immunity was determined by observing whether the injected plasma would prevent the reproduction of trypanosomes in susceptible rats.

The experiment was run in three series. In each a group of about 14 rats was infected with *T. lewisi*. About eight of these were put on a regimen of sodium salicylate, while the remainder served to produce normal ablastic immune plasma. This group of rats was called "plasma" rats. Later two groups of rats were infected with trypanosomes to serve for absorption. The dates of infection were such that on the 10th day of the infection of the plasma rats, the absorption rats would be on the fifth and on the eighth day of the infection to provide both dividing and adult trypanosomes. Another group, the "seed" rats was infected to provide dividing trypanosomes for the infection of the "experimental" rats. The plasma rats were sacrificed on the 10th day of the
infection. Their plasma was collected and absorbed. It was then injected into the experimental rats along with a dose of dividing trypanosomes. Some of the experimental rats received the plasma from salicylate-treated infected rats; some received plasma from the untreated infected rats; while the controls received an equivalent injection of saline. Each of the rats received about three ml. of plasma or saline. Blood smears were made immediately on the experimental rats and checked for the percentage of division forms. Smears were then made daily until the percentage of division forms was zero. In the three series of experimental rats, 12 received plasma from salicylate-treated infected rats, six received normal ablasic immune plasma, and six controls received saline.

In Experiment 2, the effect of adding sodium salicylate to ablasic immune plasma in vitro was tested. This was done to see whether sodium salicylate might have acted as a hapten to inhibit the activity of ablasin by combining with the antibody. Coburn and Kapp (1943) reported that in the presence of sodium salicylate there was a decrease in the amount of precipitate formed when horse euglobulin was mixed with rabbit anti-horse serum. In their tests they added the equivalent of 5.8 gm. of sodium salicylate per liter to the solution of antibody. The decrease was observed when the sodium salicylate was allowed to stand for two hours or less before addition of the test antigen. This concentration was rather high, so in this experiment the concentration used was equivalent to five
gm. per liter and the solution was allowed to stand for four to five hours.

The general procedure was much the same as in the first experiment. The plasma rats received no treatment. On the 10th day of the infection the plasma was collected, absorbed with trypanosomes, and divided into two portions. To one portion was added sodium salicylate, as indicated above, and it was allowed to stand in the refrigerator for four to five hours. The other portion was not treated with salicylate but was allowed to stand in the refrigerator for the same time. The plasmas were then injected into experimental rats along with a dose of multiplying trypanosomes to determine presence of ablasic activity by passive transfer tests. Some of the experimental rats received the salicylate-treated plasma, some received the untreated plasma, and some received a similar injection of saline. Smears were made immediately and on succeeding days and examined for division forms until the percentage in all was zero.

The experiment involved three series. Altogether, ten rats received injections of sodium salicylate treated ablasic plasma; eight rats received untreated ablasic plasma; while five controls received saline.

In Experiment 3, the effect of dialyzing the plasma from sodium salicylate-treated infected rats was determined. The purpose
was to see whether sodium salicylate, which had interfered with
the inhibition of reproduction, might have combined with the anti-
body in such a way that dialysis might have removed it to free the
antibody.

Again, a group of plasma rats was inoculated with T. lewisi. One part was put on sodium salicylate treatment whereas the other
part was not. On the 10th day of the infection the plasma was
collected and absorbed with washed trypanosomes to remove the
trypanocidal antibody. The plasmas from both the salicylate-
treated and non-treated rats were tested for the presence of
salicylates using the ferric chloride test as in Simmons and
Gentzkow (1948). Dialysis was carried out by placing each of the
plasmas in Visking casings and dialyzing against cold physiological
saline in beakers in the refrigerator. The saline was changed and
tested at three hour intervals for about 15 hours until the
dialysate showed a negative test for salicylates. The plasmas were
then tested again and injected as usual into experimental rats to
notice whether there was any difference in the passive protection
against the reproduction of the injected dividing trypanosomes.

The experiment was carried out in three series. This involved
ten rats which received the dialyzed salicylate-treated infected
plasma, seven with dialyzed ablatic immune plasma, and four with
saline.
Proteins and Ablastic Serum

In an attempt to learn whether sodium salicylate blocks the action of ablastin or whether it prevents the formation of the antibody, it was thought advisable to make determinations of protein levels of immune sera. These determinations were run in a number of series. In any one series there were four different categories of rats: first, there was the one which received sodium salicylate and was infected with *T. lewisi*; second, there was the one which was infected with trypanosomes and received no treatment; third, there was the control that received sodium salicylate only and was uninfected; and fourth, there was the normal control.

In the first experiment of this group, Experiment 4, protein determinations were made on the various types of rats on the tenth day of the infection. The animals were infected with *T. lewisi* on the zero day of the infection. Blood smears were made on the third, fifth, seventh, and tenth day of the infection to determine whether the infection was proceeding as it should. That is, if the rate of reproduction continued at a high rate in the infected rats receiving sodium salicylate; while, in the rats not receiving the drug, there was a cessation of reproduction between the fifth and seventh days. If the course of the infection followed this plan, then the rats were sacrificed on the tenth day, the serum was collected, and
protein determinations were made.

In the first series of determinations, the protein levels were determined by the copper sulphate-specific gravity method as described in Hawk, Oser, and Summerson (1947). It was originally worked out by Phillips, Van Slyke, Dole, Emerson, Hamilton, and Archibald at the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y. The serum was dropped into bottles of the various concentrations of copper sulphate from a hypodermic needle with the point filed away. The needle was attached to a syringe. Thus drops of approximately equal size were carefully dropped from the same height. Two determinations were made for each sample.

In the second series of determinations, the Micro-Kjeldahl procedure was used. Total nitrogen was determined. From this was subtracted the non-protein-nitrogen to obtain the nitrogen due to protein. The amount of protein was then obtained by multiplying by the factor of 6.25. In determining the total nitrogen, 0.5 ml. of serum was run into a tube with an Ostwald pipette. This was then diluted with 5 ml. of water to give a dilution of 1:11. After shaking, one ml. of this mixture was placed in a 30 ml. Micro-Kjeldahl flask and the standard Micro-Kjeldahl procedure was carried out. The ammonia was collected by steam distillation. The non-protein-nitrogen was determined from protein-free filtrate prepared from the same serum by the method of Folin and Wu (1919). The
procedure was then the same as before. The titration value of the acid for the non-protein-nitrogen was subtracted from that for the total nitrogen, and the value for protein was calculated.

In Experiment 5, a study of the electrophoretic distribution of the proteins in immune sera was made. The experiments with the protein levels had showed there was much higher value of protein in the immune serum than in the serum of infected rats receiving sodium salicylate. It was thought advisable to have an electrophoretic analysis made. The sera were prepared by the author, while the electrophoretic analysis was made in the laboratory of Dr. J. F. Foster, Department of Chemistry, Iowa State College.

Two series were made in this experiment. In one there were eight rats divided into three groups: three rats which were infected with T. lewisi and received sodium salicylate; two rats which were infected with T. lewisi and received no drug; and three rats which were normal controls. The infected rats were examined on alternate days to see whether the infection was proceeding as it should. On the ninth day of the infection the animals were sacrificed, and serum collected. The serum was then taken to Dr. Foster's laboratory where the analysis was carried out on the pooled sample of the three groups of rats. In the second series, the procedure was the same with the exception that twelve rats were used with the pooled serum of four rats in each of the three groups.
The electrophoresis was made on the standard Klett instrument modified with a cylindrical lens optical system. The buffer used consisted of 0.0184 M \( \text{KH}_2\text{PO}_4 \), 0.0020 M \( \text{KH}_2\text{PO}_4 \), and 0.1280 M NaCl, giving a total ionic strength of 0.184 and a pH of 7.6. The samples were dialyzed for 24 hours at 2°C, in Visking sausage casing with agitation against 2 liters of the buffer. The current used was 45 milli-amps, and 145 volts for about four hours. The work was done by Mr. M. R. Dieckmann.

In Experiment 6, the effect of supplying blood proteins to sodium salicylate-treated infected rats was determined. The purpose was to see whether the effect of sodium salicylate might have been in preventing the formation of protein, and whether this effect might be nullified by supplying the rats with blood proteins.

Ten rats were infected with *T. lewisi* and divided into four groups. The first group received sodium salicylate treatment and plasma; the second group sodium salicylate and saline; the third group received plasma only; while the fourth group, the controls, received saline. The blood proteins supplied consisted of mixtures of plasma and serum which was collected from normal rats daily and injected into the experimental rats intraperitoneally. On the first two days of the infection one ml. of plasma was given, while from the third to ninth days two ml. were given. Blood smears were made as before and checked to see whether the salicylate effect was shown in the rats receiving the sodium salicylate treatment. On the tenth
day of the infection the rats were sacrificed and protein determinations were made using the copper sulphate-specific gravity method. Two protein determinations were made on normal rats also, for comparison.

**Tryptophan and Sodium Salicylate Activity**

In Experiment 7, an attempt was made to determine the effect of supplying tryptophan to rats infected with *T. lewisi* and subjected to sodium salicylate treatment. The purpose was to see whether the tryptophan would nullify the effect of the salicylate in preventing ablasic activity. Spizizen and Kenney (1949) had reported that in cases where sodium salicylate had inhibited virus growth, the addition of indole, anthranilic acid, or tryptophan relieved the inhibition.

The experiment was carried out by infecting a number of rats with *T. lewisi* and dividing them into three groups. In one group the rats were treated with sodium salicylate and tryptophan. In the second group the rats were treated with sodium salicylate only. The rats in the third group received no treatment. Three series were made with varying concentrations of sodium salicylate being given. The quantities used were 45, 30, and 25 mgm. of sodium salicylate per 100 gm. weight of rat daily. The quantity of tryptophan administered was 62.5 mg. of DL tryptophan per 100 gm. weight of rat daily. The L tryptophan in this amount was the calculated molar equivalent of the 45 mg. dose of sodium salicylate. Both substances
were administered by rubber catheter to the stomach. The salicylate was given in the morning, while the tryptophan was given in the afternoon.

Blood smears were made on the third day and on alternate days subsequently. The smears were examined for the percentage of division forms.

Pantothenic Acid and Ablastic Serum

In Experiment 8 pantothenic acid determinations were made on rat sera in order to ascertain whether there was any difference in the pantothenic levels of ablatic immune serum and sodium salicylate-treated infected rat serum. The pantothenic acid assays were run on the same groups of rats on which protein levels were determined. That is there were four groups: one, which was infected with *T. lewisi* and received sodium salicylate treatment; two, which was infected with *T. lewisi*; three, which received sodium salicylate; and four, which was the normal control.

Two series of determinations were made. One series was made in the summer of 1949 using the method of Coryell et al (1945) with myalase P enzyme as the liberator of any bound pantothenic acid. The myalase P was made up in solution with acetate-acetic acid buffer. The acetic acid solution (12 ml. glacial acetic acid per liter) and the acetate solution (27.2 gms. sodium acetate per liter) were kept separate. They were mixed before using and the
equivalent of 225 mg. myalase P per liter was weighed out and added. The bacterium used was *Lactobacillus arabinosus* 17-6, obtainable from the American Type Collection, Georgetown Washington University Medical School, at Washington, D. C.

In preparing the sample, 0.5 ml. of serum was transferred to a 50 ml. Erlenmeyer flask by means of an Ostwald pipette. To this was added 10 ml. of myalase P solution and the procedure carried out as indicated in Coryell *et al* (1945). The basal medium for growth of the bacteria in the assays was that of Landy and Dicken (1942). The amount of growth was determined by titrating with 0.1 N NaOH for the amount of lactic acid produced.

The work of Novelli, Kaplan, and Lipmann (1949) showed that in material containing coenzyme A it was necessary to use a mixture of intestinal phosphatase and fresh pigeon liver extract to liberate the pantothenic acid before it could be used by the organism in microbiological assay. In the same paper these authors reported no coenzyme A in human plasma. It was thought wise to try their method in the hope that the enzyme system they suggested might have liberated any pantothenate that might have been bound in the antireproductive antibody as suggested by Becker and Gallagher (1947). In the second series of pantothenic acid determinations, therefore, the method of the above authors was tried on five samples of serum.
The method was much the same as that used in the previous series with the exception that, instead of using myalase P as the liberator of the vitamin, the mixture of enzymes of intestinal phosphatase and liver extract was used. The intestinal phosphatase was purchased from Armour and Company, Chicago, Ill. The liver extract was prepared according to the method of Kaplan and Lipmann (1948). Again, 0.5 ml. samples of serum were used, incubated with the enzymes as described and finally diluted to 50 ml. before assaying. Assays were carried out in duplicate using two, three, and four ml. aliquots. The amount of growth was determined turbidimetrically after 24 hours using a Klett-Summerson Photoelectric Colorimeter. Otherwise the method was that of Novelli, Kaplan, and Lipmann (1948).

At the same time assays were made on the same five samples without the enzyme mixture. One-half ml. of serum was diluted with 10 ml. of distilled water and autoclaved as in the enzyme procedure. Then the volume was made up to 50 ml. and the procedure followed as for the one of Novelli, Kaplan, and Lipmann (1948) from then on. The remaining samples were assayed by this method as values were as high or higher than in the enzyme treated group and recoveries of 90.1 per cent were obtained.

In both series, the assays were made on serum collected from the various types of rats on the 10th day of the infection. The
Rats were those used for the protein assays in the first series, and those used in the blood sugar experiment in the second series.

Glucose Levels and Ablastic Blood

In Experiment 9, an attempt was made to determine whether there was any relation among the blood glucose levels, rate of reproduction, and the numbers of trypanosomes, in sodium salicylate treated and in untreated rats infected with T. lewisi. Four rats were used in each series. One of these was infected and received sodium salicylate; another was infected only; another received the salicylate but was not infected; and the last was a normal rat. Twelve such series were made.

On the zero day of the infection a blood sugar determination was made on four rats after about 18 hours fasting. Then the rats were divided into the four above mentioned categories and the appropriate treatment was started. Additional blood sugar determinations were made on the third, fifth, seventh, and tenth days of the infection. At the same time smears were made and the percentage of division forms was estimated. Also, the number of trypanosomes was estimated using the standard hemocytometer method used for counting erythrocytes. Blood was collected from the tail, diluted with Hayem's solution, and the number of trypanosomes in 20 center squares was counted. If the number was low 40 squares were counted.
The method of Horvath and Knahr (1941) was used for the determination of the blood sugar values. The instrument employed was the Klett-Summerson Photoelectric Colorimeter with a No. 54 (green) filter. Tail blood was used and as it was found to coagulate very rapidly an anticoagulant was used. Following Hawk, Oser and Summerson (1947) two mg. of sodium oxalate was used per ml. of blood. A solution of the oxalate was made up to contain 0.2 mg. per 0.1 ml., and 0.1 ml. of this solution was placed in a depression of a spot plate and dried in the oven. In collecting the blood then, the tip of the tail was cut and the blood allowed to collect in one of these depressions in the spot plate. When sufficient blood was collected 0.1 ml. was removed with a "contam" pipette and added to 10 ml. of dilute tungstic acid solution. Then the procedure was followed as described by Horvath and Knahr (1941).
RESULTS AND DISCUSSION

The results of the experiments and their discussion are placed in one section for convenience of reference. The material will be presented and discussed under the same divisions as in the section, Experimental Method.

Sodium Salicylate and Ablastic Plasma

Becker and Gallagher (1947) found that sodium salicylate interfered with the antireproductive phenomenon in rats infected with T. lewisi. Instead of the reproduction of the parasites stopping between the fifth to seventh days, as normally, reproduction continued at a high rate. Barnes (1950) showed further that this effect was due to the salicylate radical. Two possibilities presented themselves with respect to the effect of salicylate. It prevented either the formation of the antibody or the action of the antibody.

In an attempt to learn which possibility was in operation, the first experiment was set up to determine whether the antibody could be demonstrated in plasma of salicylate treated rats by passive immunity tests. If the antibody were present combined with the salicylate, it might be released by dilution when injected into another rat. Taliaferro (1924) had shown that the antireproductive factor is passively transferable.
The results of the passive immunity tests of Experiment 1 are shown in Table 1. When susceptible rats were injected with immune plasma along with a dose of multiplying trypanosomes, the rate of reproduction dropped immediately. In the controls, which received a saline injection, the reproduction continued at a high rate until it was brought to a stop by the rat producing its own ablastin. In the rats which received plasma from the sodium salicylate-treated rats, in which the trypanosomes had still been in the reproductive phase, the rate of reproduction continued as in the controls. Salicylate treated rats were those that received 45 mgm. sodium salicylate per 100 gm. of weight of rat daily.

This evidence indicated that either the antibody was not present in the plasma rats which had received sodium salicylate; or that if the salicylate were in combination with the antibody, it was not released by dilution when injected into another rat.

If the salicylate acted by combining with the antibody and thus prevented it from uniting with the antigen to stop the reproduction, it was thought possible to have this combination take place in vitro. It has been mentioned before that Coburn and Kapp (1943) had observed an inhibition of the precipitation of antigen with antibody. The treatment followed in Experiment 2 was similar to that used by these two authors.
Table 1. Percentage of division forms in rats injected with (1) sodium salicylate infected rat plasma, (2) immune plasma, and (3) saline.

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* Day of infection on which blood smears were made.

** Rats received 45 mgm. sodium salicylate per 100 gm. weight daily.
The results of this experiment, which are shown in Table 2, indicated that the ablasic immune plasma was not affected by the treatment with sodium salicylate. When the treated plasma was injected into susceptible rats along with an inoculation of dividing trypanosomes, the division forms disappeared as they did in the untreated plasma injected group. Similar doses of trypanosomes continued to multiply as usual in the control animals.

These data indicated that under the conditions of this experiment, the salicylate would not combine with the antireproductive antibody in vitro. This does not mean of course, that the combination might not take place in vivo in the animal receiving sodium salicylate during the course of the infection. There is the possibility too, that it is not the salicylate that combines with the antibody. It could be that a metabolic derivative formed in the host unites with the antibody.

Previous work has presented evidence of an in vivo reaction between sodium salicylate and ablisin. Becker and Lysenko (1948) were able to reinfect recovered or near-recovered rats with T. lewisi when both donor and receptor rats received sodium salicylate and the reinfecting dose of dividing trypanosomes was very large. In recovered rats that did not receive sodium salicylate, no reinfections were obtained. Yet when the recovered receptor rats were treated with sodium salicylate and reinjected with dividing trypanosomes, there was a recurrence of reproduction in a few but
Table 2. Percentage of division forms in rats injected with
(1) immune plasma treated with sodium salicylate*,
(2) immune plasma, and (3) saline.

<table>
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* Immune plasma with equivalent of 5 gm. sodium salicylate added and allowed to stand 4 hours.

** Day of infection on which blood smears were made.

/ In these controls counts were not made, but the presence of division forms were noted.
not all cases. The question arises as to what the effect of the salicylate was on ablastin in the recovered rats in order to allow reproduction to start again. A number of possibilities present themselves. 1. The salicylate, or perhaps a metabolic derivative in some way destroyed the ablastin that was present in the recovered rats. 2. The ablastin might have been dissipated naturally in the organism and the salicylate prevented its continued formation. 3. The ablastin present at the time of reinjection might have been used up by uniting with the large numbers of trypanosomes which were injected, and the salicylate treatment prevented any further production of the antibody. Thus reproduction was reinitiated in recovered rats because the ablastin present was removed in one of the three ways suggested above, and salicylate treatment allowed the reproduction to continue by destroying or preventing the formation of any further antibody.

In the third experiment in this group, another attempt was made to see whether a possible antibody-salicylate combination might be reversed. The method used was the common method of dialysis which has been used to separate substances of small molecular size from those of large molecular size. Ablastin immune plasma from untreated rats was also tested to see whether dialysis would affect it.

Table 3 shows the results for the passive immunity tests for the two types of dialysed plasma. Dialysis was shown to have no
Table 5. Percentage of division forms in rats injected with dialyzed plasma from sodium salicylate treated and non-treated infected rats.

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* Day of infection on which blood smears were made.
effect on either. In the case of the dialyzed plasma from salicylate-treated immune rats, there was no effect on the rate of reproduction. This was the same as in Experiment 1 where similar plasma, which was not dialyzed, was used. In the case of the ablastic immune dialyzed plasma, the reproduction was arrested just as it was in the rats injected with non-dialyzed immune plasma in Experiment 1. Similar T. lewisi inoculations into control rats showed that the trypanosomes injected were dividing and that they continued to divide for five to seven days.

The evidence from this experiment showed that dialysis would not separate any possible combination of the antireproductive factor and the salicylate. This might mean that some other procedure would have to be used to effect such a possible separation. The fact that dialysis had no effect on ablustin from untreated rats indicated that if ablustin is an oxidative enzyme-like complex with pantothenic acid as coenzyme and protein as apoenzyme as suggested, hypothetically, by Becker and Gallagher (1947), this complex did not separate under the dialysis technique used. Again, some other procedure might be necessary to effect such a separation of the possible pantothenic acid-protein complex.

From these three experiments, it would appear then, that the effect of sodium salicylate treatment in preventing the antireproductive phenomenon in T. lewisi infections in the rat is other than by
union with the antibody. Any possible union between salicylate and antibody was not separated by dilution, or dialysis. Nor was it possible to effect a union of the salicylate and antibody in vitro under the conditions investigated. The work of Becker and Lysenko (1948), however, did indicate an in vivo reaction between sodium salicylate and ablastin.

Proteins and Ablastic Serum

The other suggested possible way in which sodium salicylate might interfere with the antireproductive phenomenon in T. lewisi infections in rats, was that of preventing the formation of the antibody. Cannon (1942) suggested a possible relation between protein deficiency and the ability to form antibodies. It was possible that salicylate caused a hypoproteinemias and so interfered with antibody production. Or perhaps the salicylate interfered specifically with the formation of this particular antibody, ablastin.

Caldwell and György (1947) reported that rats, which were suffering from anemia and inanition due to a low protein toxic linoleic diet, had an infection that was no different from that in rats fed normal diets. Saul and Becker (1949) reported that ablastin was affected in rats receiving sodium salicylate, but that the trypanocidal antibody was not. That is, reproduction continued as long as sodium salicylate was given, but the parasite population fell off eventually. This would indicate that the salicylate did
not interfere with the formation of the trypanolyzin.

In terms of these observations it might be possible to set up the hypothesis that the salicylate interfered with the formation of the antireproductive antibody. To test this hypothesis, it was decided first to make determinations of total serum protein levels on treated and untreated infected rats.

Table 4 summarizes the statistics of the serum protein levels that were made in Experiment 4. Determinations were made on four types of rats and the number in each category varied from 14 to 17. The mean of both the normal and the salicylate treated uninfected group was 53.8 gm. of total protein per liter of serum. The mean of the salicylate treated infected group was 49.5 gm., while that of the trypanosome infected untreated group was 65.4 gm. An analysis of variance showed an F value of 6.69 (P < 0.01) which is indicative of a significant difference among the means. That is, if the experiment was repeated the results would be the same unless less than one in 100 chance had taken place. In comparing the means statistically it may be seen from the table that there were significant differences among: the infected group and the salicylate-treated infected group, the infected group and the normals, the salicylate-treated infected group and the normals. There was no difference between the means of the salicylate-treated uninfected group and the normals.
Table 4. Summary of statistics comparing serum protein levels among (1) T. lewisi infected rats, (2) sodium salicylate treated infected rats, (3) sodium salicylate treated, and (4) normal rats.

<table>
<thead>
<tr>
<th></th>
<th>Tryps. only</th>
<th>Tryps. only vs. Tryps. and salicylate</th>
<th>Tryps. only vs. Normals</th>
<th>Tryps. and salicylate vs. Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Means*</td>
<td>65.4</td>
<td>65.4</td>
<td>49.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.5</td>
<td>55.8</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>Mean diff.</td>
<td>15.9</td>
<td>11.6</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>S - x</td>
<td>1.6</td>
<td>1.7</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Degrees of Freedom</td>
<td>31</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>9.93</td>
<td>6.82</td>
<td>2.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.02)</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>62</td>
<td>9211.31</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>2357.78</td>
<td>779.2</td>
</tr>
<tr>
<td>Within treatments</td>
<td>59</td>
<td>6873.53</td>
<td>116.5</td>
</tr>
</tbody>
</table>

Sodium salicylate rats: n 16, mean 55.8.

* Means expressed in gm. per liter of serum.
This indicated that sodium salicylate treatment alone had no effect on the protein levels at the tenth day of the infection. That is, there was no evidence that salicylate treatment interfered with the protein supply of the animals as shown in the blood serum. There was evidence to indicate that in salicylate-treated infected animals the protein values, as measured in this investigation, were lower than in the normal animals. The levels of the infected group were higher than those of the normals under the same conditions. Further, there is evidence that the protein levels in the salicylate-treated infected rats were lower than in the infected untreated animals.

The data suggested the possibility that in the infected animals the serum proteins were higher than in normal ones because of the presence of ablastin. The lower levels in the sodium salicylate-treated infected animals might have been due to the absence of ablastin.

Experiment 5 was set up in order to examine this difference in protein levels further and to determine in what fractions the differences in the sera of the various types of animals used in Experiment 4 might be found. Electrophoretic patterns were made therefore on the sera of (1) sodium salicylate-treated rats with T. lewisi, (2) untreated rats with T. lewisi, and (3) normal rats. As the analyses were made through the kindness of Dr. J. F. Foster a minimum number of samples were used. For this reason no runs were
made on sodium salicylate-treated uninfected rats, because the means of these rats had been found to be the same as those of the normal rats in Experiment 4. Pooled samples were used, as indicated in Table 5, in order to have as few runs as possible.

In Figure 1 are shown the electrophoretic patterns of pooled sera of the second series. At the top are the patterns of the ascending and descending columns of the sera of normal rats. These patterns were obtained by tracing on paper from the negatives of the distribution of the boundaries of the various components. Photographs of the boundaries were made after the sera had been subjected to the effect of an electric field of 45 m. amps. at 145 volts in phosphate-chloride buffer of 0.184 ionic strength and pH of 7.6. The tallest peaks represent the albumen fraction. The subsequent three peaks represent the three common globulin fractions (α, β, γ). The remaining two boundaries (ε, δ) are those of the buffer salts. The middle and bottom patterns are similar ones for the other two groups of rats.

An inspection of these patterns shows a larger peak for the gamma-globulin fraction in the T. lewisi infected group of rats than in the other two groups. The differences in the various fractions are more clearly seen in Table 5, in which are shown the distributions of the various components in grams per 100 ml. of serum. These components were calculated by Mr. M. R. Dieckmann from the areas under the peaks in the patterns as shown in Figure 1.
Figure 1

Electrophoretic patterns of pooled sera of normal rats (top), rats with T. lewisi (middle), and rats with T. lewisi and sodium salicylate treatment (bottom).
In Table 5 it may be seen that increase in the protein level in the infected rats over the normal and the salicylate-treated infected rats was in part due to higher albumen and in part to higher gamma-globulin. In the case of the latter the difference in the infected rats was about twice that of the normals in each series. The gamma-globulin fractions in the *T. lewisi* rats were approximately two and 3.6 times larger than those of the *T. lewisi* and sodium salicylate-treated group as shown in Series 1 and 2.

It may be interesting to note that in making an electrophoretic analysis of two cases of Kala-azar, a disease caused by *Leishmania donovani* in the family *Trypanosomatidae*, Cooper, Rein, and Beard (1946) reported a similar high gamma-globulin content.

The increase in the protein levels in the immune sera was not entirely due to the increase in the gamma-globulin fraction, however. There was an indication that the albumen fraction was higher in the immune rats than in the other two groups. This fluctuation in the albumen levels in hypoproteinsemia and hyperproteinsemia is a common phenomenon (Greenberg, 1948). The increase in the albumens may be associated with a general increase in the rate of protein synthesis, when the production of antibodies is stimulated by an antigen. In the case of these experiments, the presence of trypanosomes in the blood of the host stimulated the formation of ablastin. This stimulation may then have become general with an increased production of all fractions of the serum.
Table 5. Electrophoretic analysis of pooled samples of sera from (1) salicylate treated infected rats, (2) T. lewisi infected rats, and (3) normal rats.

<table>
<thead>
<tr>
<th></th>
<th>Sodium salicylate plus</th>
<th>T. lewisi only</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series</td>
<td>1 2</td>
<td>1 2</td>
<td>1 2</td>
</tr>
<tr>
<td>No. rats</td>
<td>3 4</td>
<td>2 4</td>
<td>3 4</td>
</tr>
<tr>
<td>Albumen</td>
<td>35.8* 59.2</td>
<td>39.0 40.5</td>
<td>54.7 65.5</td>
</tr>
<tr>
<td>α-globulin</td>
<td>3.2 3.3</td>
<td>5.1 2.1</td>
<td>3.0 2.9</td>
</tr>
<tr>
<td>β-globulin</td>
<td>9.2 10.1</td>
<td>7.9 10.0</td>
<td>7.2 9.7</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>3.4 2.8</td>
<td>7.0 9.1</td>
<td>3.4 4.7</td>
</tr>
<tr>
<td>Totals</td>
<td>49.6 54.2</td>
<td>59.0 61.6</td>
<td>48.0 73.9</td>
</tr>
</tbody>
</table>

* Grams per liter of serum.

There was an exceptionally high value for the albumen fraction in the second series on normal rats. No explanation was available for this.

The evidence from the protein studies indicates that in the rats which received sodium salicylate, the protein and gamma-globulin were lower than in infected rats which did not receive the drug.
These lower levels were associated with the continued reproduction of the parasites in the former and a cessation of reproduction in the latter. On the basis of this evidence, the salicylate effect of Becker and Gallagher (1947) may be due to the inhibition of the synthesis of the antibody.

The fact that high gamma-globulin and ablasic activity were associated in the T. lewisi rats, while low gamma-globulin was associated with no ablasic activity in the salicylate-treated rats, supported Taliaferro's idea of the presence of ablasic in rats. It supported further his observations that ablasic is a protein. There is also agreement with Taliaferro's finding that ablasic was in the euglobulin fraction. The evidence here, although indirect, seemed to indicate its presence in the gamma-globulin fraction. This is not contradictory because after studies on the comparison of electrophoretic and precipitation fractions, Svensson (1941) considered that "euglobulin" contained all the electrophoretic components of total globulins. The evidence from this experiment would limit the location of ablasic further by placing it in the gamma-globulin fraction.

In the next experiment, Experiment 6, an attempt was made to see whether the injection of plasma proteins from normal rats would influence the salicylate effect on the antireproductive phenomenon in T. lewisi infections in the rat. It was thought that if the salicylate affected the synthesis of serum proteins, it might be
possible to offset this salicylate effect on the reproduction by supplying the treated and untreated rats with plasma proteins. Then, if the other fractions of the blood were higher, there might be sufficient raw materials with which to build the antibody.

From Table 6 it may be seen that the rate of reproduction of the trypanosomes in the rats with salicylate and plasma continued at about the same rate as in those with salicylate and saline. With

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Salicylate and plasma</th>
<th>Salicylate only</th>
<th>Plasma only</th>
<th>Saline only</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30 32 42 40 -- 30 20 24 26 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10 30 60 35 12 30 36 40 36 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>48 42 58 48 40 32 46 50 45 48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>52 48 51 52 42 52 2 1 3 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>45 44 49 34 33 39 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10 Protein levels in gm. per liter of serum

<table>
<thead>
<tr>
<th>Protein levels in gm. per liter of serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.3 42.3 49.5 44.1 54.9 -- 66.6 66.6 58.5 70.2 51.3 54.9</td>
</tr>
</tbody>
</table>

* Day of the infection
the same infection, the rate of reproduction stopped as usual in
the controls with plasma alone, or saline alone. This would in-
dicate that the injection of plasma into the salicylate treated
animals did not affect the salicylate interference with ablasic
activity.

An examination of the protein levels in the same table
indicates that they corresponded with those found in Experiment 4.
The levels in the salicylate-treated group were lower than those
in the normals, those with saline and trypanosomes, and those with
trypanosomes and plasma. Although there are too few samples to
form any conclusions, there seems to be an indication that the in-
jection of the plasma proteins had very little effect in raising
the protein levels in the serum of the rats which received sodium
salicylate.

Tryptophan and Sodium Salicylate Activity

Experiment 7 was another experiment in which an attempt was
made to neutralize the effect of sodium salicylate in inhibiting
the formation of the antireproductive antibody. This time,
tryptophan was given to the experimental animals. Spizizen and
Kenney (1949) had reported that in their studies on the inhibition
of virus growth by sodium salicylate, the addition of indole,
antranilic acid or tryptophan, completely relieved the inhibition.
In the salicylate effect on inhibiting the formation of ablastin, it
was thought that the sodium salicylate might have been interfering with the synthesis of tryptophan by replacing competitively one of the precursors of tryptophan, anthranilic acid. This might be so because the structures of salicylate and anthranilic acid are similar. When there was an excess of tryptophan supplied, then the salicylate interference with the synthesis of tryptophan would be nullified. If this were true, then the presence of the antibody should have been demonstrable.

In the experiment the rate of reproduction was estimated by examining blood smears for division forms of trypanosomes. The results are shown in Table 7. It may be seen that the rates of reproduction of trypanosomes in the tryptophan plus salicylate group were no different from those in the group not receiving tryptophan. In the controls, which did not receive salicylate, the rate of reproduction was at zero by the fifth day. In the rats which received a daily dose of 25 mgm. of sodium salicylate, the rate of reproduction did not continue as in those receiving 30 or 45 mgm. daily. The 25 mgm. dose must be near the minimum level of sodium salicylate to bring about the prolonging of the reproductive phase of the parasites. In preliminary tests before the experiment, levels of 25 mgm. had shown the salicylate effect. The shortening of the reproductive phase in this 25 mgm. group could not have been due to the tryptophan, because it was
Table 7. Percentage of division forms of trypanosomes in rats receiving (1) tryptophan plus sodium salicylate, (2) sodium salicylate only, and (3) untreated controls.

<table>
<thead>
<tr>
<th>Mg. of Na sal.</th>
<th>Tryptophan* plus sodium salicylate</th>
<th>Sodium salicylate only</th>
<th>Untreated controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat No.</td>
<td>127 128 137 138 150 151 152 129 130 139 140 153 154 141 155 156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3**</td>
<td>40 50 60 52 34 25 32</td>
<td>30 50 64 30 44</td>
<td>46 26 29</td>
</tr>
<tr>
<td>5</td>
<td>41 29 3 34 25 35 30</td>
<td>20 55 53 44 43 37</td>
<td>0 0 0</td>
</tr>
<tr>
<td>7</td>
<td>7 14 1 1 36 44 42</td>
<td>24 51 1 0 43 37</td>
<td>0 0 0</td>
</tr>
<tr>
<td>9</td>
<td>20 12</td>
<td>21 34 39 29 27</td>
<td>45 27</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>34 24</td>
<td></td>
</tr>
</tbody>
</table>

* 61.5 mgm. DL tryptophan per 100 gm. weight of rat daily.
** Day of infection on which smears were made.
present in the group not receiving tryptophan also.

From these data it may be said that there is no evidence that additional tryptophan would offset the salicylate effect of prolonging the reproduction of *T. lewisi* in the rat. That is, no evidence was obtained that the salicylate effect might involve the replacement of anthranilic acid, a precursor, in the synthesis of the tryptophan molecule.

Pantothenic Acid and Ablastic Serum

Pantothenic acid assays were made in an attempt to learn whether there might be some relation between pantothenic acid levels in the blood and the antireproductive antibody against *T. lewisi* in the rat. Becker and co-workers (1942, 1947) had shown that rats which were deficient in pantothenic acid did not show the phenomenon of inhibiting the reproduction of the parasites. This suggested that the vitamin is associated with the formation or the functioning of the antibody, ablastin. When Becker and Gallagher (1947) found that the administration of sodium salicylate had the same effect as the deficiency of pantothenic acid on the inhibition of reproduction, it was suggested, hypothetically, that the antireproductive substance was an oxidative enzyme. It was further speculated that the enzyme consisted of a protein apoenzyme, and of pantothenic acid as a coenzyme. If this were true, then in pantothenic acid
deficiency, there would be no coenzyme and so the antibody could not be present in a complete form. The two authors thought also that the salicylate interfered with pantothenic acid usage, and thus brought about the same effect on the reproduction of the parasite.

If pantothenic acid were a part of the antireproductive antibody, then it was possible that it would be present in large quantities in immune ablasic sera. An attempt was made in Experiment 8 to see whether any differences could be detected in sera of rats undergoing different treatments. The same groups were used that were involved in the experiment on protein levels in Series 1. In Series 2, the same groups were used that were studied for blood glucose levels. These groups included (1) salicylate-treated infected rats, (2) T. l. l. infected rats, (3) rats receiving sodium salicylate only, and (4) normal rats.

There was a small difference in the assay procedure in the two series. The pantothenic acid values were somewhat different in the two series due to the fact that a different method was used in the second. The method was changed because in the interval between the time when the two series were made, there was evidence that myalase P would not liberate all bound pantothenic acid. Another method was tried therefore, in the hope that if there was pantothenic acid bound in the ablasic antibody, the vitamin would be liberated and so made available to the assay organism.
The results of the assays are summarized in Table 8. The first series seemed to be promising with the highest values in the T. lewisi infected group. When the values were analyzed statistically no evidence was found that there was a difference among the various means. The differences came within the error of the procedure. The usual error in microbiological assays is about 10 per cent. When recovery tests were made, the average recovery was 90.1 per cent.

The values in the second series were higher than in the first, but they were uniformly so. Again, an analysis of variance indicated that there was no evidence of significant difference among the various means.

These data would indicate that the pantothenate is involved in the ablasic immune reaction in a manner other than being a part of the actual antibody. That is, this would be so if the methods used in the assays made all of the pantothenate available in a usable form for the assay organism. It may be that the pantothenate was bound in such a way that it was not released. The full answer to this question must await improved methods for the determination of pantothenate.

Glucose Levels and Ablastic Blood

Experiment 9 on blood glucose levels was carried out in order to ascertain whether there were any relationships among
Table 8. Summary of statistics on pantothenic acid assays of sera of rats with (1) T. lewisi and salicylate, (2) T. lewisi, (3) salicylate, and (4) of normal rats.

| Source of variation | Series 1 | | Series 2 | | |
|---------------------|----------|---|----------|---|
|                     | D.F.     | S.S. (M.S.) | D.F. | S.S. (M.S.) |
| Totals              | 22       | 3475 | 27       | 6643 |
| Treatments          | 3        | 599 | 1153     | 334.3 |
| Within treatments   | 20       | 2876 | 161.0    | 5490 | 228.8 |
| F                   |          | 1.32* |          | 1.68* |

Actual means

|                     | Series 1 | | Series 2 | | |
|---------------------|----------|---|----------|---|
|                     | Means | Numbers | Means | Numbers |
| T. lewisi and       | 92.5**  | 6 | 149     | 8 |
| salicylate only     | 102 | 6 | 147     | 8 |
| Salicylate only     | 97 | 6 | 146     | 6 |
| Normals             | 88.2   | 5 | 132     | 6 |

* Not significant statistically.
** Micrograms of pantothenic acid per 100 ml. serum.
the blood sugar level, the rate of reproduction, the numbers of trypanosomes, and sodium salicylate treatment.

As was mentioned in the review of the literature, Linton (1929) reported that in the non-pathogenic T. lewisi in the rat, there was no difference in the glucose levels between normal rats and infected rats. He made his determinations at the height of the infection; that is, on the sixth to ninth days. He also reported that in his studies of the blood glucose levels of splenectomized and infected rats, in which the parasite numbers were higher, there was also no significant difference in the blood sugar levels from normal values. Regendanz (1929) reported, on the other hand, that there was always a final hypoglycemia in a pathogenic strain of T. lewisi with which he experimented.

Becker and Gallagher (1947) reported that T. lewisi became pathogenic in rats that received sodium salicylate daily in doses of about 45 mgm. per 100 gm. weight of rat. Barbour and Herman (1920) and Minoru (1940) had reported that, at certain levels, sodium salicylate caused an increase in blood sugar in dogs and rabbits respectively.

Yorke, Adams, and Murgatroyd (1929) reported that trypanosomes required glucose for life in vitro. Reiner, Smythe, and Fedlow (1936) reported that pathogenic trypanosomes consumed 7-8 mgm. of glucose per 1000 million organisms per hour at 37\°C. Under the
same conditions, the non-pathogenic *T. lewisi* consumed 1.5 mg. of glucose. Moulder (1948) found that dividing populations of *T. lewisi* consumed larger quantities of glucose than did adults.

From this evidence reported in the literature, it was thought that the prolonging of the reproductive cycle of *T. lewisi* in the rat by sodium salicylate administration might be associated with higher glucose levels. That is, it might be that the salicylate mobilized greater glucose levels in the blood and so made the medium more favorable for the multiplicative stage of the trypanosomes to continue.

Experiment 10 was set up in order to test this hypothesis. Blood sugar levels were determined just before infection and treatment, and then on the third, fifth, seventh and tenth days. The results of the experiment are shown in Tables 9 and 10.

In Table 9 there are listed the means for the various observations that were made on the rats in the experiment. It may be seen that in the *T. lewisi* plus salicylate group, the rate of reproduction continued at a high rate from the third to the tenth days. By comparison, in the untreated *T. lewisi* group, the rate of reproduction was down to zero by the seventh day. As far as numbers of trypanosomes were concerned, in the treated group the numbers rose gradually from the third to tenth days. In the untreated group, the numbers rose at first and then were lower by the seventh and tenth days. These data are similar to those found by
Table 9. Summary of means in Experiment 9.

<table>
<thead>
<tr>
<th>Day of infection</th>
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<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. lewisi Salicylate</td>
<td>87.5</td>
<td>116</td>
<td>103.1</td>
<td>116</td>
<td>125</td>
</tr>
<tr>
<td>T. lewisi</td>
<td>96</td>
<td>103</td>
<td>103.5</td>
<td>109</td>
<td>106</td>
</tr>
<tr>
<td>Salicylate</td>
<td>90</td>
<td>103</td>
<td>104.6</td>
<td>106</td>
<td>111</td>
</tr>
<tr>
<td>Normal</td>
<td>96.1</td>
<td>101.8</td>
<td>93.5</td>
<td>96</td>
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<table>
<thead>
<tr>
<th><strong>Trypanosome numbers</strong></th>
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<tbody>
<tr>
<td>T. lewisi Salicylate</td>
</tr>
<tr>
<td>T. lewisi</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Percentage of division forms</strong></th>
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</thead>
<tbody>
<tr>
<td>T. lewisi Salicylate</td>
</tr>
<tr>
<td>T. lewisi</td>
</tr>
</tbody>
</table>

*Mgms. of glucose per 100 ml. blood.

**Thousands of trypanosomes per cubic millimeter of blood.
<table>
<thead>
<tr>
<th></th>
<th>T. lewisi salicylate</th>
<th>T. lewisi only</th>
<th>Salicylate only</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>11</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td>125</td>
<td>106</td>
<td>111</td>
<td>106</td>
</tr>
<tr>
<td><strong>Mean diff.</strong></td>
<td>37.5</td>
<td>10</td>
<td>21</td>
<td>10.9</td>
</tr>
<tr>
<td><strong>S^-x</strong></td>
<td>5.8</td>
<td>4.84</td>
<td>5.64</td>
<td>5.9</td>
</tr>
<tr>
<td><strong>D.F.</strong></td>
<td>19</td>
<td>20</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td><strong>t</strong></td>
<td>7.38</td>
<td>2.07</td>
<td>3.72</td>
<td>1.85</td>
</tr>
<tr>
<td><strong>(P &lt; 0.01)</strong></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Salicylate only versus Normals</th>
<th>T. lewisi only versus Salicylate</th>
<th>T. lewisi only versus Salicylate</th>
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</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><strong>Means</strong></td>
<td>111</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td><strong>Mean diff.</strong></td>
<td>5</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td><strong>S^-x</strong></td>
<td>6.3</td>
<td>6.4</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>D.F.</strong></td>
<td>15</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td><strong>t</strong></td>
<td>0.79</td>
<td>2.97</td>
<td>3.65</td>
</tr>
<tr>
<td><strong>(P &lt; 0.01)</strong></td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
<td>(P &lt; 0.01)</td>
</tr>
</tbody>
</table>
previous investigators (Becker and Gallagher, 1947; Becker and Lysenko, 1948; Saul and Becker, 1949; and Barnes, 1950).

The means for the sugar determinations are also indicated in Table 9. In the case of the normal rats the means varied from 96.1 on zero day to 106 on the tenth day. When these two means were analyzed statistically by the t-test, it was found that there was no evidence for a significant difference between them. There was an upward trend in the values for the normals from zero to the tenth day. This might be explained by the fact that after the first experience the rats were more frightened and there might have been an adrenalin effect to cause an increase in the blood glucose levels. Similar increases were noted in the other groups as well.

In the case of the T. lewisi infected group the range of values was much the same as in the normal group. Again, the levels on the tenth day were somewhat higher than on the zero day. As before there was found to be no significance in the difference. In comparing the values of the T. lewisi group and the normals for the tenth day it was found that the means were the same. This corroborated the results of Linton (1929) who had reported that there was no increase or decrease in the blood sugar levels in T. lewisi infections.

In the case of the group that received salicylate but in which the rats were not infected, the means were 90 and 111 on the zero and tenth day. The t-test indicated that there was evidence that the difference between these two means was significant. When
the mean for the tenth day of the salicylate group was tested against the mean for the tenth day for the normals, it was found that the difference was not significant. These findings might be interpreted by saying that at the concentration of salicylate used, there was no evidence for an increase in the sugar levels, but that perhaps there was a trend in that direction.

In the case of the rats that were infected with *T. lewisi* and received sodium salicylate, the means on the zero and tenth days were 87.5 and 125. From Table 10 it may be seen that this difference was found to be highly significant. When the tenth day mean of this group was compared with the normal group and the *T. lewisi* infected group, it was found that the differences were highly significant. At first glance this seemed to be contradictory to the usual findings as had been reported in the literature. In most cases it had been found as indicated in the review of the literature, that there was a hypoglycemia associated with pathogenic trypanosomiases. This hypoglycemia was found, however, a very short time before death, as indicated in the work of Tubangui and Yutue (1931). The interest here was in the relation between the sugar levels in the blood and the continued reproduction of the parasites in rats undergoing salicylate treatment and not in the pathological picture.

In summing up the data in the blood sugar experiment, it has been found that in *T. lewisi* infections there was no effect on the
blood glucose levels. Salicylate administration alone showed no effect or perhaps a slight trend to raise the blood glucose levels at the concentration of 45 mgm. sodium salicylate per 100 gm. weight of rat. In the rats with *T. lewisi* in a state of continued reproduction under the influence of sodium salicylate treatment, the blood sugar levels at the tenth day of the infection were higher than in the other three groups. That is, neither the trypanosomes alone, nor the salicylate alone had a significant effect on the blood sugar of the rats. In rats with both trypanosomes and salicylate there was an elevation of the blood sugar. These higher values were associated with continued reproduction of the parasites.

These data would support the hypothesis that in an environment of large quantities of glucose in the blood, the trypanosomes continue in the reproductive phase in which it has been shown by Moulder (1948) that larger quantities of glucose are utilized. It may be that as long as large amounts of carbohydrate are available, the trypanosomes continue to utilize the same metabolic scheme and so reproduction continues. One may speculate that perhaps pantothenic acid deficiency might act in a similar way. Wright (1942) had found that when rabbits were fed glucose by stomach tube, the level of pantothenic acid in the blood dropped. This indicated that pantothenic acid was involved in glucose metabolism. It might be then, that in pantothenic acid deficient rats there is an increase in the blood sugar to such an extent that again, the reproductive
phase of the trypanosomes is favored to continue. There is no evidence for this assumption, however, and the problem would have to be investigated with respect to sugar levels and pantothenic acid deficiency.

Although the hypothesis sounds attractive, it is difficult to tell which is cause and which effect. It may be that the higher sugar levels in the rats with both trypanosomes and salicylate were due to a combined stimulatory effect of the salicylate and the presence of the trypanosomes to cause a greater mobilization of blood glucose. Another problem then would be to study the changes in liver glycogen under the same conditions. Even though the level in the *T. lewisi*-only rats was lower than in the *T. lewisi* plus salicylate rats, it was actually not below normal and there was still ample glucose supply in the blood. It is not likely therefore, that reproduction stopped because of a lack of glucose.

In view of the fact that the experiments on protein studies indicated that there was no evidence of the presence of antibody in the salicylate-treated and infected rats, this stimulation explanation of higher sugar levels is probably nearer the truth. That is, the salicylate treatment prevents the formation of ablustin. In the absence of antireproductive activity the trypanosomes continue to multiply. The reproductive phase is favored by the presence of adequate carbohydrate in the environment. And the larger amount
of blood glucose is caused by the combined stimulatory effect of trypanosomes and salicylate treatment.
SUMMARY AND CONCLUSIONS

1. Passive immunity tests on plasma from *T. lewisi*-infected rats that had been treated with sodium salicylate indicated that either the antireproductive antibody, ablastin, was not present in the plasma, or that a possible salicylate-antibody complex was not separated by dilution when the plasma was injected into susceptible experimental rats.

2. No evidence was obtained that sodium salicylate would combine with ablastin *in vitro* and so prevent its demonstration by passive immunity tests.

3. No evidence was obtained that dialysis would cause a separation of a possible salicylate-ablastin complex.

4. No evidence was obtained that dialysis would cause a separation of a coenzyme from a possible oxidative enzyme-like ablastin.

5. Evidence from the above experiments on salicylate and ablastic plasma suggested that the effect of salicylate in preventing the antireproductive phenomenon in *T. lewisi* infections in the rat is other than by union with the antibody to form a salicylate-ablastin complex. Previous work by Becker and Lysenko (1940) however, indicated the presence of an *in vivo* reaction between sodium salicylate and ablastin.
6. Total serum protein levels were determined on four groups of rats. These were (1) rats with *T. lewisi* and sodium salicylate, (2) rats with *T. lewisi* only, (3) rats with sodium salicylate only, and (4) rats that were normal controls.

7. From the data on serum protein levels of the above mentioned groups of rats, the following conclusions were formed:

   a) Sodium salicylate in the dosage used had no effect on the serum protein levels of the rats so treated.

   b) Serum protein levels in rats with *T. lewisi* only were higher on the tenth day of the infection than those of the other three groups studied.

   c) Serum protein levels in rats with *T. lewisi* and sodium salicylate on the tenth day of the infection were lower than those of the other three groups studied.

   d) This evidence suggested that the higher serum protein levels in the *T. lewisi* group were associated with the presence of ablastin, while the lower serum protein levels of the *T. lewisi* plus sodium salicylate group were associated with absence of ablastin, due to the salicylate treatment.

8. Electrophoretic analyses were made on pooled serum samples of three categories of rats. These were (1) rats with *T. lewisi* and sodium salicylate, (2) rats with *T. lewisi* only, and (3) rats that were normal controls.
9. From the data on electrophoretic analysis of the above mentioned rats, the following conclusions were formed:

a) The values obtained for total serum proteins corroborated in general the values obtained by Micro-Kjeldahl and specific gravity methods in Experiment 4.

b) The higher serum protein levels in the *T. lewisi* group of rats seemed to be due to an increase in albumen and gamma-globulin fractions.

c) The gamma-globulin fractions in the *T. lewisi*-only group were about 2 and 3.6 times greater than those in the *T. lewisi* and salicylate group.

d) The higher and lower values of gamma-globulin mentioned above were associated with the presence and absence of ablatic activity against *T. lewisi* in the host rats.

e) The evidence on serum protein studies supported the possibility that sodium salicylate interfered with the appearance of ablatic activity by preventing the formation of the antibody.

f) The evidence on serum protein studies supported Taliaferro's idea of the presence of ablastin in rats against *T. lewisi*.

g) There was support also of Taliaferro's idea that ablastin is a protein.
h) There was support too of Taliaferro's findings that ablastin was in the euglobulin fraction of serum.

i) The evidence would further limit the location of ablastin to the gamma-globulin fraction of serum.

10. From the experiment on injecting normal plasma proteins into rats with T. lewisi and sodium salicylate, the following were noted:

   a) The added plasma proteins had no influence on the interference with ablastic activity by sodium salicylate.

   b) The added plasma proteins had no influence on the total blood protein levels of rats that were receiving sodium salicylate.

   c) The total serum protein levels were similar to those in Experiment 4.

11. From the experiment on administration of tryptophan by gavage, the following were noted:

   a) There was no evidence that the additional tryptophan influenced the salicylate interference with ablastic activity against T. lewisi in the rat.

   b) This indicated that salicylate acted other than by substituting competitively for anthranilic acid in the synthesis of tryptophan.

12. Pantothenic acid assays were made on sera of four groups of rats. These were (1) rats with T. lewisi and sodium salicylate,
(2) rats with *T. lewisi* only, (3) rats with sodium salicylate only, and (4) rats that were normal controls.

15. From the data on pantothenic acid determinations the following were noted:

a) No evidence was obtained of any differences in the pantothenic acid levels in the four groups of rats mentioned above.

b) Either pantothenic acid is involved in the immune reaction in a manner other than being a part of the antibody itself, or the present methods of assay did not show possible differences in the pantothenate content of ablastic and non-ablastic sera.

14. Blood glucose determinations were made on various days of the infection of four groups of rats. These were (1) rats with *T. lewisi* and sodium salicylate, (2) rats with *T. lewisi* only, (3) rats with sodium salicylate only, and (4) rats that were normal controls.

15. From the data of the blood glucose determinations, the following were noted:

a) The findings corroborated the reports of Linton (1929) that there was no effect on blood glucose levels in rats by *T. lewisi* infections.

b) At the concentration used, sodium salicylate showed no effect, or perhaps a trend to raise the blood glucose
levels in rats.

c) The combined effect of *T. lewisi* and sodium salicylate treatment caused an increase in blood glucose.

d) These higher blood glucose levels were associated with continued high rates of reproduction of *T. lewisi* in the rat due to sodium salicylate treatment.

16. The evidence from the blood glucose experiment offered two possibilities with respect to the association of continued reproduction in the salicylate-treated infected rats and the higher blood glucose levels:

a) The prolonging of the reproductive phase was due to higher glucose levels in the environment in which the trypanosomes were growing.

b) The higher blood glucose levels were caused by a combined stimulatory effect of the trypanosomes and the salicylate on the mobilization of glucose.

17. In view of the evidence from the studies on the relation of proteins and ablasic serum, it would seem that the latter possibility mentioned above is nearer the truth, and that the prolongment of the reproductive phase was due to interference of the salicylate in the formation of ablastin.
LITERATURE CITED


Coventry, P. A. 1925. The reaction product which inhibits reproduction of the trypanosomes in infections with Trypanosoma lewisi, with special reference to its change in titer throughout the course of infection. Amer. Jour. Hyg. 5:127-144.


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