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A Thesis
by
CHARLES ARTHUR DALEY

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A SEQUENTIAL STUDY OF THE PATHOGENESIS OF DISEASE CAUSED BY TRYANOSOMA VIVAX IN EXPERIMENTALLY INFECTED CALVES, UTILIZING CLINICAL, PATHOLOGICAL, HISTOPATHOLOGICAL AND IMMUNOFLOUORESCENT TECHNIQUES

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Approved as to style and content by:

[Signatures and names of committee members]

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ABSTRACT

A Sequential Study of the Pathogenesis of Disease Caused by *Trypanosoma vivax* in Experimentally Infected Calves, Utilizing Clinical, Pathological, Histopathological and Immunofluorescent Techniques. (May 1971)

Charles Arthur Daley, B. S., M. S., Montana State University, D. V. M., Washington State University

Directed by: Dr. Charles H. Bridges

*Trypanosoma vivax* obtained from a clinically sick cow near Neiva, Colombia, was passed in a sheep and a calf and inoculated into the jugular vein of 14 Holstein-Friesian calves. Fever occurred by 24 hours, and recurring parasitemia commenced after 72 hours. It was estimated that practically all of the 14 calves would have died spontaneously within 3 months if none had been euthanitized. Associated with the first and subsequent parasitemias were decreases in hemoglobin, PCV, M:E ratio, serum albumin, A:G ratio and neutropenia.

All calves exhibited gradual weight loss by 2 weeks and later submandibular edema usually became evident. Consistent post mortem lesions seen after 4 weeks were conspicuously hypertrophied, edematous lymph nodes, hypertrophied hemal lymph nodes, emaciation, rounded right heart, palpably firm liver, atrophied thymus and hypertrophied femoral bone marrow.

Associated with *T. vivax* of the infecting inoculum and
succeeding parasitemias were generalized endothelial hypertrophy
and mononuclear cell infiltration along blood and lymph vessels
with proteinuria and bone marrow hyperplasia. Generalized lymphatic
and RE hyperplasia occurred, but was not proven to be due to
the trypanosomiasis. At 3 weeks there were aggregations of
macrophages containing engulfed material distributed along
capillaries in pulmonary interalveolar tissue, and this lesion
in combination with the anemia and apparent cardiac insufficiency
were thought important in the development of anoxia, and pro-
bably contributed to the single fatality observed. Periacinar
congestion and fatty metamorphosis of midzonal hepatocytes were
probably related to the failing heart and indirectly to the pul-
monary lesion. Cystitis and pyelonephritis in the last 2 calves
to be killed probably was a reflection of generalized, chronic
debility.

The detection of trypanosomes in histologic sections, using
a direct fluorescent antibody technique, was impeded by a gen-
eralized fluorescence of tissues of infected calves. It was
suspected that the fluorescence of tissues was related to the
presence of soluble antigens which are known to exist in T. vivax
infections.
ACKNOWLEDGMENTS

The completion of this research was facilitated by the cooperation of many people from several countries.

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CHAPTER I

INTRODUCTION

Bovine trypanosomiasis is caused by a flagellated protozoan belonging to the genus Trypanosoma (29). Kubes, in 1944, presented evidence that Trypanosoma vivax had spread through certain countries and adjacent islands in Central and South America during this century (34). His findings were based on the morphologic similarities of organisms found there with T. vivax isolated during the original African studies.

Wells, et al. in 1969 found evidence that clinically normal cattle of various ages harbored T. vivax in Colombia, South America, although the vector was not discovered (50). The tsetse fly which is the known vector of bovine trypanosomes in Africa has never been found in the New World.

The importance of trypanosomiasis in South American cattle has not been established. The blood of cattle frequently contains T. vivax as well as Anaplasma marginale and/or Babesia spp. A need therefore existed to characterize the clinical, hematologic, biochemical and pathological aspects of trypanosomiasis alone in cattle, and to obtain a basic understanding of the pathogenetic process.

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The citations on the following pages follow the style of American Journal of Veterinary Research.
CHAPTER II

LITERATURE REVIEW

The pathogenicity of the disease caused by *T. vivax* in African cattle is generally regarded as of major importance (35). Fiennes stated that *T. vivax* often caused either a chronic or benign disease with a high recovery rate, and only exceptionally caused peracute or acute disease (15). Hudson reported that *T. vivax* caused fever initially, and subsequently hypoglycemia and hemorrhages in the tongue, adrenals and kidney (30). Death occurred in the subacute stage from severe anemia rather than hypoglycemia. In another study, Fiennes found multiple thromboses of the smaller blood vessels in the spleen, liver, lymph nodes, lung and adrenal glands of cattle with *Trypanosoma congoense* (14). The tunica adventitia of many of the vessels of the organs studied became hyperplastic with loss of elasticity, and the endothelial cells reportedly underwent "metaplasia" and passed into the general circulation.

Foci of chronic infection by both *T. congoense* and *T. vivax* were found in bovine cardiac tissue (17). The cattle had been exposed to infected tsetse flies and then treated with trypanosomicidal drugs. Most of the organisms were degenerated or lysed, but some were normal, and were always found in capillaries. The occurrence of myocardial necrosis varied, but often was severe. Lodged parasitic emboli were suspected to have caused local
ischemia.

Most clinical pathological data from cattle infected with *T. congolense* or *T. vivax* included decreased hemoglobin values and erythrocyte counts (15, 30, 34, 39), eosinopenia (16), neutrophilia (39) or neutropenia (13, 16), lymphocytosis (13, 39), periodic monocytosis associated with a reduction in numbers of circulating trypanosomes (16), elevated serum glutamic-oxalacetic transaminase (SGOT) levels (26) and no change in bilirubin levels (13, 16). Serum albumin levels decreased in sheep with *T. vivax* (6, 40), in rats with *Trypanosoma brucei* (31) and in rabbits with *Trypanosoma evansi* (47). Gamma globulins increased (6, 31, 40). Blood glucose levels remained normal in cattle with *T. congolense* (13), but decreased in rats with *T. brucei* (48).

Fiennes summarized certain theories (15) as follows: Direct intervention by the parasites can not be substantiated, because parasites can not be demonstrated by detailed examinations. Intracellular developmental forms have been proven to exist only with *Trypanosoma cruzi*. Histologic lesions caused by intoxication induced by dead trypanosomes has not been proven. An antigen-antibody reaction causing injury to the somatic cells has been thought to occur.

Because of the lack of information on the pathogenesis of disease caused by *T. vivax*, data on other trypanosomal diseases has been reviewed. Results of experimental disease caused by *T. brucei* in mice indicated that infectivity and pathogenicity
appeared to be related (37). Both varied according to the antigenic type involved. Gordon et al. (24) exposed rabbits to *Trypanosoma rhodesiense* from carrier insects and observed local multiplication of the parasites, "chancre" formation and invasion of the blood stream.

Nigerian swine infected with *Trypanosoma simiae* developed fever and died acutely without anemia (32). Thrombi were present in hepatic arterioles, pulmonary arterioles, interlobular veins of the renal cortex, arterioles at the hilus and trabeculae of lymph nodes, small vessels of the brain, medium sized vessels of the adrenals and submucosal vessels of the intestine. The thrombi contained fibrin, mononuclear cells and in some cases whole or fragmented trypanosomes. Endothelial cells of several thrombosed capillaries were hypertrophic.

That trypanosomes are influenced by body temperature was suggested by a report from India in which the parasites were found chiefly in the skin capillaries of cattle infected with *T. evansi* (1). Fiennes found *T. congolense* infections in the skin of cattle and mentioned the rapid removal of the parasites once they entered the main circulation (14). He stated that serum antibody titers and body temperatures above 38 C were probably prime factors in the defense mechanism. Markinelle studied highly pathogenic *T. cruzi* infections in mice and found that a high (36 C) environmental temperature may protect mice for months (36).

Rabbits infected with *Trypanosoma gambiense* developed skin
lesions containing high concentrations of trypanosomes before death (45). A vascular permeability-increasing factor was demonstrated in aqueous extracts of the parasite when inoculated intradermally following systemic introduction of Evans' blue dye.

*T. brucei* was studied in rabbits using ear chambers, contrast media with X-ray films and India ink (23). The endothelium of venules and capillaries became irregular and blood flow was impeded. Many large macrophages which lined the vessels contained carbon particles. Trypanosomes appeared cyclically in spaces between vessels, and were often seen when the parasitemia was low. Just before death the vessels became "sticky" and were lined with leucocytes. When vascular circulation ceased, the vessels disintegrated within a few hours. Fiennes described vascular lesions in the bovine species that resembled the vascular lesions in rabbits (14).

It has been established that mammalian hosts develop antibody titers against trypanosomes, and that in some unknown way, the antigenic character of the trypanosomes is altered (27, 40, 49). The ability of trypanosomes in mammalian hosts to change in antigenic character has been offered as an explanation for the fluctuations in parasitemias which are commonly observed. Recently it has been reported that although chick embryos infected with *T. rhodesiense* or *T. brucei* developed neither agglutinating antibodies nor antibodies detectable with fluorescent antibody techniques, fluctuations in trypanosome numbers occurred (22).
Antibody levels in cattle with *T. brucei* have been related to increased kinin activity following each peak of parasitemia, and to a decrease of the precursor of kinin in the blood (4). The kinins are 10 times as active as histamine, causing increased vasodilatation and capillary permeability with resulting edema and pain.

Schroeder and Ristic demonstrated a heat-stable opsonin in calves infected with *Anaplasma marginale* (44). Titers appeared to be correlated with intensity and persistence of anemia and with the amount of erythrophagocytosis in the bone marrow. An autoimmunization mechanism was proposed as the cause of the anemia.

Zuckerman proposed 4 models to account for the various evidence of autoimmunization in protozoa (52): 1. The infectious agent may share an antigen with the host cell, with antiparasitic antibody then combining with the heterogenetic antigen in the host cell. 2. The target host cell might be coated with parasitic antigen, which attracts antiparasitic antibody, followed by cellular damage. 3. The target host cell may be directly coated with antiparasitic antibody and be sensitized. 4. The target host cell may be so modified by an infectious agent, or its products, or by a drug used in treating the infectious agent, as to become autoantigenic. Zuckerman stated that protozoan diseases, in which it was suspected that autoimmunity had occurred, were all diseases of the blood and tissues where the organisms had intimate contact with cells, and often were intracellular in
nature. Intracellular forms of *T. vivax* have not been demonstrated as they have with *T. cruzi*, in which development occurs in a series of mesenchymal cells, preferentially in cardiac tissue.
CHAPTER III

MATERIALS AND METHODS

The isolate of *T. vivax* which was used in the experiment was obtained from a clinically ill cow near Neiva, Colombia, and was identified by its non-infectivity for laboratory mice, by its behavior in culture and by its morphology. The isolate was passed once in sheep to eliminate *Babesia spp.* and then in a calf which was treated with Oxytetracycline HCl* intravenously at the rate of 12 mg./kg. for 12 days and with B-W Drug No. 356-C-61** intravenously at the rate of 5 mg./kg. for 3 days to eliminate *Anaplasma marginale*. Serum from this calf remained free of complement fixing antibody titers for *Anaplasma* and *Babesia* antigens.

Eleven intact male and 4 female Holstein-Friesian calves 4 to 6 months were used. All of the calves were raised on pastures located in the Sabana of Bogota, an area considered to be trypanosome free. The calves were examined hematologically for trypanosomiasis and serologically for anaplasmosis and babesiosis and found to be free of these diseases. The calves were maintained as a group in one section of a barn, and were not allowed to mix with other cattle. Prior to their inoculation with trypanosomes, 5 cc. of blood from each of the 15 experimental calves was

*Chas. Pfizer Co., Inc., New York, N.Y.

**Burroughs-Wellcome Co., Research Park Triangle, N.C.
inoculated intravenously into a susceptible splenectomized calf, cultured on enriched blood agar (51), inoculated intraperitoneally into 2 laboratory mice at the rate of 1.0 cc. each, and examined in wet mounts.

One calf served as an experimental control in which the continued absence of blood parasites throughout the experiment indicated that contamination from outside sources was not likely. The control calf was subjected to necropsy last, and the tissues served as negative controls for the immunofluorescence study.

Oxylated blood from the carrier calf was examined daily, and a relative scale for quantitating the degree of parasitemia was established. In each test, 10 microscopic fields of fresh blood in a wet mount slide were examined at 400 magnification. If no trypanosomes were found, the test was considered negative, and if 1 trypanosome was found in a total of 10 fields it was designated a "1 plus". If an average of 1 trypanosome was found in each of 10 fields it was designated a "2 plus". When there was more than 1 trypanosome in each of 10 fields it was designated a "3 plus", and if a high number of trypanosomes were present in each of 10 fields it was designated a "4 plus".

Each of the 14 experimental calves was given intravenously 5.0 cc. of citrated blood containing approximately 100,000 organisms per cc. A sample of infective blood was frozen in glycerol using the technique of Cunningham, et al. (8). The sample was stored at -79 C, insuring a stabilate of trypanosomes (as defined
by Lumsden) for future work (35).

Beginning 5 days prior to the inoculation of the calves and continuing throughout the experiment, rectal temperatures were measured each morning and afternoon. Samples of blood from each calf were collected on each of 4 days before inoculation and subsequently on each day of scheduled necropsy. Aliquots of the blood were treated with sodium fluoride or sodium oxalate and other aliquots were allowed to clot. Blood glucose concentrations (38), packed cell volumes, hemoglobin concentrations, sorbitol dehydrogenase levels (19), serum glutamic oxalacetic transaminase concentrations (41), total serum proteins (2, 12), bilirubin concentrations (20), total and differential leucocyte counts and serum protein electrophoretic patterns (5) were determined. These data obtained from the 14 infected calves are summarized in Figures 1, 3 and 4. The group mean and standard deviation are shown on each day of observation.

The packed cell volume was determined by standard laboratory methods. The hemoglobin concentration was determined by the Hycel* cyanmethemoglobin technique. Serum bilirubin content was assayed by the technique described by Gibson and Goodrich (20). Total serum protein was determined by a hand held temperature compensated Goldberg refractometer (A-O TS meter).** Serum protein

*Hycel Co., Houston 2, Texas

**American Optical TS Meter, Buffalo, N.Y.
electrophoresis on cellulose polyacetate membranes was done using a Gelman deluxe electrophoresis chamber, Model 51210.* Aliquots of 5.0 lambda's of serum were applied to cellulose acetate membranes soaked in fresh cold barbital buffer having pH 8.6 with an ionic strength of 0.05. Electrophoresis was continued for 45 minutes at 1.5 to 1.8 milliamperes at a constant direct current voltage of 350 volts maintained by a Gelman voltage supply, Model 38201.* The cellulose polyacetate membrane was stained for 5 minutes in a solution of 0.1% Ponceau S with 5% trichloracetic acid, differentiated in 5% acetic acid (1 minute in each of 3 baths) and cleared for 30 seconds in a mixture of 10% glacial acetic acid and 90% methanol. It was dried on glass slides at 60°C for 20 minutes and quantitated by scanning on a Gelman automatic recording and integrating scanner, Model 39372.*

Serum glutamic oxalacetic transaminase levels were measured by a modification of the original Reitman and Frankel method (41). Blood glucose concentrations were determined using a photometric adaptation of the Somogyi method (38).

One calf, selected by random sampling, was euthanatized by electrocution at each of the post inoculation times of 12 hours, 1, 2, 3, 5, 8, 12, 17, 23, 30, 38, 45 and 88 days. One calf was necropsied on day 37 following death due to trypanosomiasis. The control calf was the last calf to be killed.

As each calf was dissected at necropsy, triplicate sets of tissues were collected. One set was preserved in buffered 10% formalin and tissue sections were stained with hematoxylin and eosin, using standard techniques. The other 2 sets were frozen onto wooden blocks using Cryoform* and stored in separate freezers at -20 C for immunofluorescence studies.

Emphasis was placed on the histologic study of sections of tissues from the major organs and lymph nodes, although tissues from each of the following anatomical sites were examined: lip, tongue, dental pad, ear, eye, nasal mucosa, mandibular lymph node, mesenteric lymph node, prefemoral lymph node, hemal lymph node, lung, heart, aorta, bone marrow, adrenal, kidney, urinary bladder, spleen, pancreas, rumen, reticulum, abomasum, duodenum, ileum, colon, liver, gall bladder, ovary, uterus, penis, testicle, cerebrum, cerebellum, medulla oblongata, choroid plexus, pituitary, spinal cord, bone, semilunar ganglion, semitendinous muscle, diaphragm and mediastinal lymph node. All samples of a given organ except those samples representing gross lesions were taken from similar locations within the organ.

Bone marrow biopsies were taken from the ribs of 3 calves on each scheduled day of necropsy. The 3 calves included the calf to be dissected, plus 2 other calves selected at random. After all the calves had been subjected to 1 biopsy, the selection

process was repeated, using ribs from the opposite sides of the calves.

On day 23 a quantity of blood was withdrawn from an infected calf selected at random and allowed to clot in order to obtain serum containing antibodies against T. vivax. The 112 cc. of serum was put in dialysis tubing with an air pocket at each end and then placed in a 1000 cc. beaker containing half saturated (NH₄)₂SO₄ solution. The solution was agitated overnight with a magnetic stirrer in a refrigerator set at 4°C. The solution was changed twice. After 24 hours the serum volume had been reduced to 46 cc. An equal volume of physiological saline containing 1 drop of 1.0 N NaOH per liter was added. To this was added, drop by drop, 56 cc. of saturated (NH₄)₂SO₄, using a magnetic stirrer. After 1 hour of stirring, it was centrifuged at 10,000 rpm for 15 minutes using a refrigerated Sorvall* Model RC2-B centrifuge. The supernatant was discarded, and the precipitate was washed with half saturated (NH₄)₂SO₄. The centrifugation-wash cycle was repeated 2 more times. The precipitate was then redissolved in a minimum quantity of 0.5 molar Na₂CO₃-NaHCO₃ solution at pH 9.0 (1 part 0.5 molar Na₂CO₃ and 4 parts 0.5 molar NaHCO₃). The re-suspended material was placed in new dialysis tubing and dialyzed against 0.05 molar NaHCO₃ solution at pH 7.5 in a 1000 cc. beaker. The solution in the beaker was changed twice overnight. The

*Ivan Sorvall, Inc., Newtown, Conn.
material in the dialysis tubing, now having a volume of 51 cc., contained 4.9% protein. A sample of it was examined by cellulose acetate zone electrophoresis and contained gamma globulin with no albumin. The 51 cc. of solution was expanded to 100 cc. by mixing with 38 cc. of cold physiological saline and then slowly adding 11 cc. of buffer solution pH 9.0 (1 part 0.5 molar Na₂CO₃ and 4 parts 0.5 molar NaHCO₃). One milligram of fluorescein isothiocyanate (FITC)* was used for each 20 milligrams of protein in the solution. The FITC was dissolved in 2 cc. of acetone and was added slowly to the swirling solution which was then stirred overnight in the refrigerator. The conjugated preparation, which had a pH of 8.9, was passed through 2 columns containing Sephadex** G-25 (Course), and the gamma globulin containing portion was collected in fractions and tested for protein concentration. It was filtered, using a Swinny filter holder containing a Milipore*** filter with a 0.45 micron pore size, and stored in 1.0 cc. aliquots at -20 C.

Positive control slides for the fluorescent antibody study were prepared as follows. Blood from an infected calf having a 4-plus parasitemia was collected in tubes containing the sodium salt of ethylenediaminetetraacetic acid (EDTA). The blood was

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*Nutritional Biochemicals Corp., Cleveland, Ohio

**Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

centrifuged at approximately 500 rpm until the supernatant was clear. The supernatant was collected in a separate tube containing a very small quantity of the erythrocytes from the original tube. This material was mixed by swirling, and 1 drop was placed on each glass slide, spread evenly across the surface and allowed to air dry. The slides were fixed for 10 minutes with acetone at -20°C and then allowed to dry. They were stored in slide boxes surrounded by plastic bags containing a small quantity of CaCl₂ in a freezer at -20°C.

Sections of uninfected tissues were cut from frozen blocks of mediastinal lymph node and kidney taken from the control calf. Sections for FA studies were cut at 4 microns. Those tissues which were examined by FA techniques included those of the ear, cerebrum, thymus, heart, lung, mediastinal lymph node, prescapular lymph node, liver, spleen, kidney and bone marrow. The sections were mounted on new glass slides that had been previously cleaned in acetone and air dried. The tissues were fixed in acetone at -20°C.

Tissues from each infected calf were stained and examined as a group along with at least 1 positive control slide and 1 each of the negative control slides. The slides were allowed to warm to room temperature. The conjugate was thawed and diluted 1:50 with 0.01 molar NaH₂PO₄ buffered saline with pH 7.5, and carefully added to the tissue so that its entire area was covered. Each preparation was placed on supports in a separate covered
Petri dish containing moistened filter paper and incubated for 30 minutes at 37°C. Then the sections were removed from the incubator and washed for 15 minutes in each of 3 successive changes of phosphate-buffered saline that had been chilled previously. The sections were air dried and cover slips were applied, using a non-fluorescing glycerin solution at pH 9.0. They were examined within 1 hour, using a Leitz Ortholux binocular microscope equipped with 10X oculars and 10X, 40X, 54X oil immersion and 100X oil immersion objectives and a dark field condenser. Non-fluorescing immersion oil was employed. The light source was an Osram Mercury super pressure HBO-200W lamp with a diffusion filter, a BG 12 excitor filter and a K 470 barrier filter.
CHAPTER IV

RESULTS

Clinical Manifestations

The first clinical sign was an increase in the morning rectal temperature which averaged 2.5 C above normal (Fig. 1). The fever was at a maximum 24 hours after inoculation and had decreased by day 3. The average afternoon rectal temperatures are shown in Fig. 1.

Changes in the appearance of the calves were detectable approximately 2 weeks post inoculation. The calves gradually became emaciated and their hair coats became dry. At approximately 4 weeks post inoculation the calves appeared more docile, their heads drooped, and general weakness gradually became apparent. At 5 weeks post inoculation submandibular edema could be detected in some calves (Fig. 2). The edema receded in all animals as death became imminent and in 2 calves the edema had regressed completely before death. Dehydration and lacrimation were apparent and the abdomens became gaunt after 5 weeks on experiment. Appetite remained near normal, but there was a general lack of vigor in this phase. The calves moved more sedately toward their feed and appeared to eat in a more languid manner.

Clinical Pathology

The parasitemia became evident on day 5 and reached...
Fig. 1. Morning and afternoon rectal temperatures and hematological data. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased because of selective serial necropsy. Arrow indicates time of intravenous inoculation with *Trypanosoma vivax*.
Fig. 2. Calf infected 6 weeks previously with *Trypanosoma vivax*. Note submandibular edema, lacrimation and generally depressed attitude. Death was expected following the onset of these signs.
maximum (4-plus level) on day 7 (Fig. 1). Thereafter, the degree of parasitemia in a given calf varied from zero to a maximum relative value of 4-plus.

The infected calves became anemic as the experiment progressed. Hemoglobin levels and packed cell volumes decreased steadily (Fig. 1). There was an overall decrease in the myeloid: erythroid (M:E) ratio (Fig. 1). Total and direct bilirubin levels increased slightly, and then fluctuated thereafter (Fig. 3). The SGOT levels increased in the last half of the experiment and the standard deviation increased (Fig. 3). Serum sorbitol dehydrogenase levels increased and then regressed (Fig. 3).

The level of total serum proteins had decreased by day 8 but remained constant thereafter (Fig. 3). A slight decrease in the absolute amount of serum albumin occurred. Although there was a relative increase in the percent of globulins, there was actually a slight decrease in the absolute amount of globulins present due to the decrease in total serum proteins. The albumin:globulin (A:G) ratio decreased slightly (Fig. 3). The absolute amount of gamma globulins increased, while the absolute amount of alpha and beta globulins decreased (Fig. 3).

Blood glucose levels remained normal until after day 30, when the mean values decreased to one half those of the pre-inoculation values (Fig. 4).

Total leucocyte counts decreased by day 8 and remained fairly constant until day 30 when there was an abrupt increase.
Fig. 3. Hematological data. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased because of selective serial necropsy. Arrow indicates time of intravenous inoculation with Trypanosoma vivax.
Fig. 4. Hematological data. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased because of selective serial necropsy. Arrow indicates time of intravenous inoculation with *Trypanosoma vivax*.
to a level above normal (Fig. 4). The leucopenia was influenced by the development of neutropenia in the acute phase (Fig. 4). This condition persisted until near the end of the experiment, when a neutrophilia developed.

There was a slight decrease in the number of lymphocytes by day 8 (Fig. 4). However, there was a relative lymphocytosis due to the considerable decrease of total leucocytes. The absolute number of lymphocytes was approximately normal at the end of the experiment. A very slight eosinopenia and monocytopenia developed gradually throughout the course of the experiment (Fig. 4).

Gross Pathology

The tissues and organs of the calves killed at 12 hours, 1, 2, 3 and 5 days were not observed to have lesions except for an occasional small area of consolidation in the apical or cardiac lobes of the lungs.

In the calf killed on day 8 the lymph nodes were slightly edematous and the hemal lymph nodes were slightly enlarged.

The abdominal cavity of the calf killed on day 12 contained approximately 30 cc. of amber fluid. One prescapular lymph node contained multiple petechiae. The thymus appeared edematous and the spleen was slightly enlarged.

The lymph nodes were enlarged and edematous in the calf killed on day 17. The hemal lymph nodes were slightly enlarged. The thymus was reduced in size.
The lymph nodes of the calf killed on day 23 were moderately enlarged and slightly edematous. The hemal lymph nodes were normal in size. The thymus was small, but of normal consistency. One small abscess containing thick greenish exudate was present in 1 tonsil. Red bone marrow extended half way down the femoral shaft, contrasting with red marrow that occupied approximately the proximal 20% of the femur in calves killed previously.

The lymph nodes of the calf killed on day 30 were slightly enlarged and edematous. One prescapular lymph node contained multiple ecchymoses. The hemal lymph nodes were greatly enlarged. The thymus was not evident. The abdominal cavity contained approximately 40 cc. of amber fluid.

An experimental calf in a weakened condition died on day 37 after routine blood samples were taken. Although not detected on external examination of this calf, the connective tissue of the intermandibular space was found to be edematous. All of the lymph nodes were moderately enlarged and edematous and hemorrhages were present in the prescapular and renal lymph nodes. The thymus was small and edematous. The epicardial surface contained multiple petechiae, the right ventricle was rounded and there was serous atrophy of pericardial fat. The pericardial sac contained approximately 30 cc. of amber fluid. The liver capsule and parenchyma were slightly more difficult to cut than normal. The kidneys had multiple petechiae on their surfaces and there was serous atrophy of perirenal fat. The cortices of the adrenal glands
were congested.

The calf killed on day 38 was weak, and its eyes were sunken. Submandibular edema was conspicuous. All of the lymph nodes were moderately enlarged and edematous, and 1 prefemoral lymph node had several ecchymotic hemorrhages. The hemal lymph nodes were greatly enlarged and prominent, and the thymus was small and edematous. Depot fat in the animal was minimal. The cut surface of the ventricular myocardium was pale, and the pericardial sac contained approximately 10 cc. of amber fluid. The liver was pale, and the capsule and parenchyma were more resistant to cutting than normal. The abdominal cavity contained approximately 25 cc. of amber fluid. The capsular surfaces of both kidneys contained multiple petechiae. The thymus was gelatinous in consistency.

The lymph nodes of the calf killed on day 45 were moderately enlarged and edematous, and the hemal lymph nodes were greatly enlarged. Although no free fluid was observed in the body cavities, considerable fibrin was present in the ventral abdomen. The mesentery of the spiral colon contained a large quantity of edema. Serous atrophy of fat was evident. The thymus was small and edematous. The right ventricle of the heart was moderately rounded. The apical lobes, the ventral portions of the cardiac lobes and small ventral portions of the diaphragmatic lobes of the lung were red and firm. The trachea contained white foam. The hepatic capsule and parenchyma were moderately resistant to
cutting. Red bone marrow extended more than half way down the length of the femur.

The last calf was killed on day 88. This calf had chronic cystitis. The gross, microscopic and clinical pathological data did not reflect uncomplicated chronic trypanosomiasis, therefore data from this calf are not included.

Microscopic Pathology

Lungs

Within 12 hours after inoculation, hypertrophy was evident in the endothelium of the pulmonary arterioles. There appeared to be an excess number of mitotic figures in the endothelium of arterioles. Within 24 hours post inoculation some alveolar spaces contained serofibrinous exudates and erythrocytes. Intraalveolar aggregations of macrophages were seen by day 8, and in sections of lung tissue collected from calves killed subsequently, there were similar aggregations of macrophages in blood vessels. Some small blood vessels observed in longitudinal section contained multiple small dark bodies. By day 23 some alveolar walls were slightly thickened and contained eosinophilic material. The interalveolar lesion became more prominent with time (Fig. 5). This lesion was randomly distributed throughout the parenchyma within pulmonary lobules. Typically, an affected lobule was located adjacent to a normal lobule, and therefore the affected tissue was dispersed in a random pattern. By the end of the
Fig. 5. Lung from calf infected 38 days previously with Trypanosoma vivax. Innumerable debris-laden macrophages accounted for most of the increased thickness of the interalveolar tissue. No deposition of extravascular material was demonstrable with special stains. H&E. 350X.
experiment the interalveolar tissue was seen to contain numerous macrophages, some of which apparently contained engulffed material. Lung sections collected on days 30, 37 and 38 and processed with Periodic Acid Schiff or Masson's Trichrome Stain* indicated the basement membranes of the vessels were normal, and there was no evidence of deposition of excess material along the vessels.

Heart

Evidence of progressive changes were found in the myocardium as early as day 2. At that time small lymphocytes had infiltrated the interstitial tissue around blood vessels and lymphatics. Larger lymphocytes were recognizable by day 5 and day 12. The endothelial cells of arterioles were hyperplastic. After a month there were many plasma cells in the interstitial tissue along the blood vessels, lymphatics, nerves and between muscle bundles (Fig. 6). Some myocardial nuclei were altered, with the chromatin being arranged in a long, thin, wavy line along the central axis of the cell, such as is described for the "caterpillar cell" of certain human cardiac myopathies (43). Sarcosporidia were common in heart muscle as well as in skeletal muscle.

Liver

Initial changes in the liver were observed by day 3. There

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*PAS & Masson's Trichrome Stains: AFIP Protocol. 1957
Fig. 6. Heart from calf infected 38 days previously with *Trypanosoma vivax*. Cellular infiltration at this stage involved numerous plasma cells as well as lymphocytes. Some myocardial nuclei resembled "caterpillar nuclei" seen in certain human myopathies. H&E. 150X.

Fig. 7. Liver from calf infected 38 days previously with *Trypanosoma vivax*. Periacinar congestion masked an occasional dying hepatocyte. Fatty metamorphosis was predominant in midzonal areas. H&E. 130X.
were areas of acute focal necrosis which were infiltrated with neutrophils. Some of the hepatocytes located near terminal hepatic veins had undergone fatty metamorphosis. A few lymphocytes had infiltrated into portal tracts by day 5, and they were more numerous by day 8. By day 23 there were large aggregations of macrophages in terminal hepatic veins, and a few neutrophils had infiltrated into the parenchyma. Kupffer cells scattered throughout the tissue were filled with globules of hemosiderin. Periacinar congestion was marked by the fifth week, and in most, but not all calves there were many degenerated hepatocytes around terminal hepatic veins, with fatty metamorphosis being present in the midzonal regions (Fig. 7). Periacinar fibrosis could not be demonstrated by special staining techniques.

Kidneys

In the kidneys of the calf killed on day 3 there was excessive proteinaceous material in the proximal convoluted tubules, and the glomerular tufts were slightly hypercellular. The degree of proteinuria had decreased to a low but persistent level by the end of the second week. Endothelial hypertrophy was evident in arterioles oriented near glomeruli. The lumens of some of these arterioles were very small during the second week. Perivascular infiltration of lymphocytes, plasma cells and other mononuclear cells became more prominent as time elapsed. At the end of 3 weeks, large accumulations of cells were present around the
arcuate arteries.

The degree of hypercellularity of the glomerular tufts increased and at the end of 5 weeks the tufts completely filled some of the Bowman's spaces. Macrophages filled with hemosiderin were located predominantly in the medulla. Accumulations of neutrophils in the proximal convoluted tubules and elsewhere in the kidney were visible on day 38.

Lymph nodes

In general, by 12 hours post inoculation mitotic activity was at a high level in the germinal centers of most lymph nodes. During the experiment most lymph nodes had evidence of lymphoid or reticuloendothelial hyperplasia, however, toward the end of the experiment some germinal centers appeared exhausted. Occasionally small hemorrhages and edema were seen. Macrophages containing hemosiderin were scattered throughout the lymphatic tissue during the latter portion of the experiment.

The mediastinal lymph nodes were typical of those nodes in which lymphoid hyperplasia occurred. During the first week there was a moderate degree of hyperplasia and many necrotic cells were present in germinal centers. In the second week there apparently was an increase in the number and size of the germinal centers. Marked lymphoid hyperplasia was evident during the last 4 weeks of the experiment.

In the prescapular lymph nodes a mild reticuloendothelial
cell hyperplasia was evident by the end of the first week, and the condition developed to a moderate or marked level during the second week. The condition regressed considerably near the end of the experiment. Concurrently, the concentration of lymphocytes appeared slightly decreased on day 2 and on day 12 there was moderate depletion, although the concentration of lymphocytes was approximately normal at the end of the experiment.

Bone Marrow (femur)

Specimens of bone marrow from the first 2 calves killed consisted mostly of depot fat with some evenly distributed hemopoietic cells. After day 3 the blood forming tissue increased at the expense of the fatty tissue and by day 12 there were confluent areas of hemopoietic tissue. Cells of the erythroid series were generally more numerous than cells of the myeloid series. It was estimated that there were about twice as many erythroid cells as myeloid cells on day 23. After this time reticuloendothelial (RE) cells were numerous, and in some areas they had aggregated in discrete foci. Congestion was apparent in tissue collected after 3 weeks, and tissue collected on day 38 contained many cells of the neutrophilic series.

Ear

Endothelial hyperplasia was evident in a few subcutaneous arterioles by day 3, but this was not evident in calves killed
toward the end of the experiment. A slight to moderate infiltration of eosinophils, lymphocytes and macrophages had occurred in the interstitial tissue along blood vessels and lymphatics. The concentration of infiltrating cells in the subcutaneous tissue remained at a fairly constant level during the course of the disease.

**Thymus**

During the first week there was a slight increase in numbers of eosinophils and neutrophils in the interstitial tissue between lobules. The cortices of the lobules appeared thin by day 12, and by day 23 there was a definite depletion of thymocytes. No parenchyma remained by day 30, and on days 37 and 38 all that remained was supporting connective tissue and vessels, with no distinctly discernable boundary between cortex and medulla. There was a moderate degree of atrophy present in some of the thymus tissue collected on day 45 but in other areas the tissue was normal.

**Spleen**

There was moderate congestion and moderate to marked hemosiderosis in sections of spleen collected after the first week, and this condition persisted throughout the experiment. Neutrophils collected in the red pulp, mostly near Malphigian corpuscles, in the late stages of the disease.
Hemat Lymph Nodes

There was a variable degree of congestion.

Fluorescent antibody stained tissue

Trypanosomes fluoresced with a typical apple green color in smears from infected blood (Fig. 8). Smears were improved by removing most, but not all, of the erythrocytes as less time was required per slide. The optimum dilution of the conjugate was 1:50.

Trypanosomes with somewhat less fluorescence than that of the controls were detected in the mediastinal lymph node and spleen of the experimental calf killed on day 23. The parasite seen in the mediastinal lymph node was intact, and the nucleus and kinetoplast both had a strong fluorescence. The rest of the parasite was easily visible, but fluorescence was weak. The second trypanosome, seen in splenic tissue, possessed similar fluorescing qualities as the one described in the lymph node. It was intact except for a small portion of the anterior extremity. In neither case was it possible to say if the parasites were located in vessels. Because the organisms were essentially of the same shape as the trypanosomes observed in blood films, they probably were lying free in the interstitial fluid. Almost certainly they were not intracellular. An examination of tissue cut from the same frozen blocks and examined by light microscopy
Fig. 8. Blood smear from *Trypanosoma vivax* infected calf incubated 30 minutes (37°C) with FITC-conjugated immune serum. Large oval nucleus and smaller kinetoplast (left extremity of organism) exhibited identical quality of apple green fluorescence. Three erythrocytes are present. Ultraviolet light with dark field condenser. 1620X.
failed to locate any portions of parasites.

Microscopic structures having an evenly distributed apple green fluorescence were observed in tissue from certain organs of the experimentally infected calves but not from tissue of the control calf. In cardiac tissue collected at 12 hours a blood vessel was seen to be surrounded by an area of evenly distributed fluorescence. In renal tissue taken later in the experiment there was a similar type of fluorescence that was evenly distributed through the glomerular tufts of some Bowman's spaces and in the interstitial tissue around vessels associated with the convoluted tubules and to a lesser extent with descending tubules.

In tissue from the control calf and most infected calves non-specific fluorescence was seen in the form of multiple, even-sized circular granules which were not trypanosomes. The granules had an apple green fluorescence and were sufficiently small that as many as 200 were estimated inside some cells. The cells were suspected of being macrophages. Usually, however, the number in a cell was much less, although the size of the granules remained fairly constant. Organs most often affected included lymph nodes, spleen and thymus, while the cerebrum was rarely involved.

Granules similar to those seen intracellularly were observed intercellularly in bone marrow of at least 1 infected calf. In some areas the granules were packed tightly together in the intercellular spaces and often were sufficiently numerous as to form a syncytium which tended to outline the normal cellular structures.
CHAPTER V

DISCUSSION

Calves inoculated with *T. vivax* developed fever within 24 hours, which was similar to results reported with *T. vivax* (15, 30) and *T. congolense* (13) in Africa, while inoculation of *T. vivax* in Venezuela rarely caused fever (34). During the rest of the experiment the average morning and afternoon rectal temperatures did not deviate markedly from the normal range, which also was similar to the African work. It was difficult to demonstrate relapsing temperatures with *T. congolense* infections except by calculating a 3-day average in order to "smooth" the temperature curve, or else by using a virulent strain of parasite in cattle of very young age (13, 15). It is well known that trypanosomes undergo changes in antigenic composition (27), which may be related to the decrease in fever. It is possible that a mild persistent fever did occur which was sufficiently variable as to be indistinguishable from normal individual variation of rectal temperature in a small group of animals.

The afternoon temperatures were consistently higher than those recorded in the morning, with the exception of the initial fever, because the calves were regularly exposed to strong afternoon sunlight. Some rectal temperature values were low near the end of the experiment because 1 or more calves were in a moribund state.
Each calf reacted similarly, gradually becoming weak and listless, although its appetite remained normal. Submandibular edema, which developed during the latter part of the experiment, probably was related to the failing heart and to decreased osmotic pressure of the blood due to loss of albumin and possibly other blood proteins via the kidney. Albumin is considered to account for 45% or more of the osmotic pressure of the blood and is important in fluid balance (9). The edema tended to regress as death became imminent, and its appearance, followed by regression was interpreted as an unfavorable prognostic sign. It was likely that dehydration in the terminal stage was a factor in the regression of the edema, although quantitation of water intake was not monitored. A similar report of submandibular edema was made in the T. vivax infected cattle in Venezuela (34), but not in the African work (15, 30).

Anemia developed rapidly in the acute phase, based on the rapid rate of decrease of hemoglobin and PCV (Fig. 1). In the subacute phase these blood parameters decreased at a persistent but slower rate which appeared to be directly associated with the gradual development of emaciation. Anemia, rather than dilution due to an increase of plasma volume, was suggested by concurrent evidence of an erythropoietic response in histological sections of femur marrow and smears of rib biopsies and a slight but immediate increase in total and direct bilirubin levels following inoculation (Fig. 3).
When the PCV reached the range of 16% to 20%, death could be expected, as illustrated by a PCV of 17% in the calf that died. Rave, in Colombia (39), infected 5 calves of similar age and breeding to the calves used in this experiment and observed anemia in which the PCV decreased to 15% to 22%, after which a calf had to be killed on day 47. Except in cases where animals died in the acute phase, anemia was one of the most constant signs that developed in cattle infected with *T. vivax* (15, 30, 34) and anemia, rather than hypoglycemia, was suspected of being the cause of death in subacute bovine trypanosomiasis in Kenya (30). Anemia of Venezuelan cattle infected with *T. vivax* was often aggravated by blood loss from chronic intestinal parasitism (34). The present experiment indicated that trypanosomiasis in Colombian calves under favorable husbandry conditions and in the absence of other hemoparasites can be fatal, whereas apparently other native calves infected with *T. vivax* are often clinically unaffected (50). It is evident that innate resistance, husbandry practices and perhaps unknown factors are also important.

The timing of increases in serum bilirubin levels coincided with that of the first parasitemia, and apparently was the result of the release of hemoglobin from erythrocytes. However, the bilirubin levels did not exceed the limits of published normal values for calves (7). The increase in direct or conjugated serum bilirubin was indicative of normal hepatic function in response to the elevated level of non-conjugated bilirubin.
The latter is the difference between the total and the direct bilirubin, and this had increased approximately 2 fold with the first parasitemia. In contrast, data derived from African calves infected with *T. congolense* and which were anemic had normal icterus index values, although no serum bilirubin tests were made (13).

The SGOT values increased gradually from day 23 until the experiment terminated (Fig. 3), and histologic examination revealed that lesions in cardiac and hepatic tissue had developed during this time. It is known that homogenates of *T. vivax* release 10 times as much SGOT as is present in equal volumes of normal serum and that the SGOT is elevated in serum from animals that have experienced destruction of muscle and hepatic tissue (26). The rise of SGOT in the chronic phase probably was due chiefly to tissue necrosis. It is possible that the slight but rapid rise in enzyme levels during the 24 hours following inoculation was due to enzymes released from the infecting trypanosomes. The similar rate and magnitude of SGOT increase after day 5 that coincided with the first parasitemia, and that was followed by a slightly elevated plateau, was probably due at least in part to the periodic destruction of trypanosomes. The tissue destruction that occurred in the chronic phase evidently released sufficient tissue enzymes to raise the level higher, although the absolute rise was indicative of only mild tissue damage. Additional evidence of hepatic tissue damage was furnis-
ed by the slightly elevated levels of serum sorbitol dehydrogenase (SDH), an enzyme specific for hepatic tissue (19), during the third and fourth weeks (Fig. 3).

There apparently was considerable damage to the endothelium of the renal glomeruli due to trypanosomes of the first parasitemia and succeeding parasitemias, because there was evidence of a moderate degree of proteinuria in histological sections of kidney as well as a decrease in total serum proteins (Fig. 3). Total serum proteins stabilized at a lower level during the second week which likely was the result of several factors, including a reduced rate of loss of proteins through the kidney. The decreased level of proteinuria observed in histologic sections of renal tissue after the second week supported this theory. The extent of hepatic lesions was considered insufficient to account for the decrease in production of serum albumin.

There was a slight decrease in total serum globulins, but the percentage of total serum globulins was greater because of the loss of serum albumin. It is possible that some of the smaller alpha globulins also were lost via the kidney, as there was a very slight decrease of this fraction. The first proteins to leak through the glomerular membrane are albumin and alpha globulins, and if the damage is severe, the beta and gamma fractions also may be lost (3). The A:G ratio decreased slightly in the first 2 weeks and this change was apparently due to the considerable loss of albumin through the kidney rather than due to an
absolute increase of globulins.

These results agree with results from *T. vivax* infections in sheep (6, 40) and *T. brucei* in rats (31) in which there was a decrease in serum albumin. One author (31) attributed the loss to kidney damage, while another (6) stated that a primary increase of serum globulin acted as a plasma expander, resulting in dilution of albumin and beta globulins. Total serum proteins decreased and the A:G ratio was reversed, with excess albumin appearing in the urine of rabbits with *T. evansi* (47).

Gamma globulins increased gradually in the 4 weeks following the first parasitemia (Fig. 3), and this was probably related to the immune response following the protozoal infection. Similar responses were observed by others in trypanosomiasis (6, 31, 40). The increase of gamma globulins is accepted as being related to the development of immunity following exposure to infectious agents, although the magnitude of response to protozoal agents may not be as clear cut as that of bacterial infections (9).

However, in calves with eperythrozoonosis, another hemoprotozoan disease, the serum gamma globulins actually decrease and the A:G ratio increases, which is contrary to most reports (10).

The same individual calf had progressively lower blood glucose values on each of the last 2 observation days (Fig. 4), and at necropsy there was emaciation and serous atrophy of fat. Liver tissue showed moderately increased resistance to cutting and microscopically there was hepatic periacinar congestion but
no fatty metamorphosis. Fiennes (13) found no significant changes in levels of blood sugar in calves or adult cattle with *T. congolense* infections, although he indicated that hypoglycemia had been reported occasionally in cattle with chronic trypanosomiasis.

A recent report suggested that *T. brucei* infection in rats resulted in a diabetes mellitus-like condition (48). In the rat, there was a decrease of peripheral glucose utilization in the acute stage that was masked by a high rate of glucose consumption by the trypanosomes, however, hypoglycemia became apparent in the chronic stage. The condition responded to, but was not entirely restored to normal by insulin. The response described is more characteristic of adrenal diabetes than of pancreatic or pituitary diabetes (28). In this experiment, only 1 calf developed hypoglycemia, and no evidence of fatty metamorphosis was observed in the liver of this particular calf, a lesion consistently reported with diabetus mellitus (33).

Leucocytic parameters in trypanosomiasis apparently vary with the age of the cattle, species of the trypanosome, susceptibility to other diseases and unknown factors. In this experiment neutropenia occurred following the first parasitemia, but neutrophilia developed after 3 weeks (Fig. 4). This data correlated with the presence of pus in the convoluted tubules of the calf killed on day 38 and with chronic cystitis seen in the last experimental calf which was killed on day 88. Apparently an in-
crease in susceptibility to other diseases developed before the end of the experiment, probably because of a general decrease in body defenses. Mild neutropenia was also reported in adult African cattle infected with either *T. vivax* (16) or *T. congolense* (13), whereas Colombian calves infected with *T. vivax* developed mild neutrophilia (39).

There was mild lymphocytopenia after the first week (Fig. 4), although histologic examination of most lymph nodes revealed an increased level of activity in germinal centers and increased numbers of lymphocytes in the sinuses. Histological examination revealed an infiltration of lymphocytes into the interstitial tissue along blood and lymphatic vessels in most organs, apparently in response to antigens, possibly including soluble antigens released from the trypanosomes. In contrast, lymphocytosis was reported in calves infected with *T. congolense* (13) or *T. vivax* (39), although the number of calves used in the latter work was small.

There was generalized hypertrophy of endothelium and stenosis in the small blood vessels. The many small dark bodies observed in small blood vessels of the lung and other organs were suspected of being degenerated trypanosomes. Lymphocytes, plasma cells and macrophages were common in interstitial tissue along blood and lymph vessels.

Material suspected of being dead trypanosomes was seen in stained sections of the lung. Since the lung contained the first
capillary bed in the path of the trypanosomes following inoculation in the jugular vein, a portion of the initial inoculum probably became lodged in pulmonary tissue. Alterations in the integrity of the vascular endothelium and hemorrhage apparently resulted from the influence of the parasites. Whatever the process there was a gradual thickening of the interalveolar structures which was easily detectable after 3 weeks (Fig. 5). This change was not consistent throughout the parenchyma but developed in individual scattered areas, probably as a function of the distribution pattern of dying trypanosomes in the pulmonary parenchyma. It is possible that the trypanosomes of succeeding parasitemias became lodged in vessels already damaged or sensitized and provided a continuous source of irritation of tissue in focal areas. Another possible source of continuing irritation may have been from soluble antigens, which are exoantigens, and are known to develop in certain T. vivax infections (25). Apparently the interalveolar change was due principally to the numerous macrophages observed, many of which contained engulfed material. Based on the examination of lung tissue using special staining techniques,* there was no evidence of the deposition of excess amorphous material associated with interalveolar capillaries.

The efficacy of gaseous exchange between the alveoli and the

*PAS and Masson's Trichrome Stains: AFIP Protocol, 1957
surrounding capillary network probably was decreased due to accumulations of macrophages along interalveolar capillaries. Other conditions contributing to anoxia were the anemia and cardiac insufficiency. The combined influence of these 3 factors probably detrimentally influenced endothelial and myocardial viability.

The hearts from some of the calves killed last had enlarged right ventricles. This probably was due chiefly to pulmonary hypertension caused by stenosis of pulmonary arterioles and also by the accumulation of macrophages along interalveolar blood vessels. Apparently the parasites exerted a detrimental effect on the endothelial component of myocardial vessels in a manner similar to that observed in the lung, consequently, inflammatory cells were attracted into the interstitial tissue surrounding blood vessels and lymphatics (Fig. 6). This inflammatory process was more pronounced in the myocardium than in other organs.

Plasma cells were prominent in cardiac and lymphatic tissue after 1 month. Plasma cells in large numbers are usually limited to the lymph nodes, and they are particularly associated with chronic inflammation (18). It is interesting that 1 month after experimental inoculation of *T. vivax* into sheep IgG immunoglobulins appeared, whereas prior to that time the immunogenetic response had consisted of only macroglobulins (40). Changes observed in the myocardial nuclei of chronically affected calves resembled the Anitschkow myocytes or "caterpillar cells" which have been described for human cardiac myopathies (43). Cardiac insuffi-
ciency probably accounted for the chronic passive congestion of the liver and contributed to the submandibular edema and edema of the lymph nodes and other organs. However, the hypoproteinemia probably contributed to the edema also due to decreased osmotic pressure of the blood.

It is possible that the acute focal hepatic necrosis observed on day 3 was due to trypanosomes becoming lodged in the sinusoids. However, similar lesions were not present in the liver of calves killed later, although parasitemias were repeatedly demonstrated. The periacinar congestion was probably a result of impaired function of the heart. Zones of degenerating hepatocytes observed around the terminal hepatic veins and fatty metamorphosis seen in midzonal hepatocytes (Fig. 7) were also probably due to abnormal circulation, although the anemia and possibly the deposition of material around pulmonary capillaries may have been contributing factors. Mild to moderate lymphocytic infiltration of portal areas was similar to the general lymphocytic infiltration of interstitial tissue in other organs. Aggregations of macrophages were seen in terminal hepatic veins. Fiennes similarly described periacinar congestion, fatty metamorphosis and aggregations of macrophages in hepatic vessels in African cattle infected with *T. congolense* (14) and another investigator found large macrophages in the ear capillaries and venules of rabbits 2 weeks after being infected with *T. brucei* (23).

The integrity of the vessel walls of the glomerular tufts
was altered before the first parasitemia became evident and proteinuria developed. This change was apparently initiated by the relatively small number of inoculated trypanosomes. Hyperplasia of the cells of glomerular tufts eventually resulted in filling Bowman's spaces. Some of the tufts were embedded in a matrix of proteinaceous material. Neutrophils were observed in the renal tubules from the animal killed on day 38 and the last calf remaining alive, which was killed on day 88, had a chronic purulent cystitis. It is tempting to explain the decrease in serum albumin by the loss of protein through the kidneys. Macrophages, lymphocytes and plasma cells collected around arterioles and small renal arteries. Some of these perivascular infiltrations were of considerable size at the end of the experiment. Cortical and arcuate vessels were affected most frequently.

The hyperplasia of RE and lymphatic elements of lymph nodes was assumed to result chiefly from the trypanosomal infection. The plasmacytosis which developed in certain lymph nodes during the fifth week coincided with the appearance of high numbers of plasma cells in the interstitial tissue of the heart. However, the reason for the plasmacytosis at that specific time was not determined.

Hypertrophy of femoral bone marrow was evident at necropsy, and histologic examination of marrow indicated that a response in erythropoietic activity had begun during the first week post inoculation. This response coincided with the time of the first
parasitemia and with the initial decline in hemoglobin after day 5. Increased erythropoietic activity continued thereafter and there was no evidence of exhaustion of the hemopoietic system. The M:E ratio (Fig. 1), based on rib biopsies, declined moderately during the experiment, and this change was considered due principally to increased erythropoietic activity. The proliferation of RE tissue in bone marrow was considered evidence of an extreme reaction by the body defense mechanism in response to a persisting infection.

There are few reports in the literature describing bone marrow response in trypanosomiasis. Fiennes found that the marrow throughout the full length of long bones of calves and adults infected with T. congolense was red, but stated that in histological sections the marrow was aplastic. This redness probably indicates a state of congestion of a tissue which normally is filled largely with depot fat and hematopoietic tissue.

No immediate response was observed in the granulocytic cell series in bone marrow sections in response to decreased levels of circulating neutrophils. However, there was evidence of stimulation of the neutrophilic cell series in bone marrow sections from the last 3 calves killed which correlated with the increased numbers of circulating neutrophils in the same calves (Fig. 4).

In the ear endothelial hyperplasia of some subepithelial arterioles had occurred by day 3 but stenosis was not observed.
There was a moderate degree of cellular infiltration of interstitial tissue around blood vessels but it did not reach the intensity of the perivascular infiltration that occurred in the kidney and heart. A portion of the infiltrated cells were eosinophils, whereas in the other organs except the thymus the number of eosinophils was minimal. The influence of factors other than the trypanosome infection could not be ruled out.

Accumulations of neutrophils were observed in the red pulp of the spleen in the last several calves killed. The effect was most pronounced in tissue near the splenic corpuscles. The neutrophils possibly were attracted to that site as a result of antigens which accumulated in cells of the RE system, although normal pooling of cells due to decreasing circulation can not be ruled out.

Fluorescent antibody conjugate at a dilution of 1:50 was considered optimum. More dilute preparations resulted in inconsistent fluorescence of the trypanosomes and less dilute preparations resulted in excess non-specific fluorescence.

Positive control blood smears containing a reduced number of erythrocytes were preferred because less time was required in locating the trypanosomes, whereas the presence of a few erythrocytes facilitated initial focusing of each slide.

The color and intensity of fluorescence of the nucleus and kinetoplast within a given trypanosome was identical (Fig. 8) suggesting that components common to both structures were
involved. This effect applied both to the trypanosomes seen in positive control blood smears and in trypanosomes from infected tissue, although the fluorescence was less intense in the latter. Deoxyribonucleic acid (DNA) and nucleoproteins of DNA are known to be present in both the nucleus and kinetoplast of trypanosomes (11) and both compounds are of sufficient size as to stimulate an immune response. It is likely that one or both compounds served as antigens common to both the nucleus and kinetoplast.

Fluorescence of the external surface of the trypanosomes was of a much more subdued intensity compared to the fluorescence of the nucleus and kinetoplast. The external antigens involved were probably either a part of the external wall or were related to metabolic products that may have coated the organisms. Apparently the external antigens were less recognized as foreign antigens by the immune mechanism of the calves as compared to the endoantigens from the nucleus and kinetoplast. Metabolic excretory products of trypanosomes are likely to be of small average molecular size, and therefore the antigenic response to such products would be relatively weak.

The amorphous, non-particulate fluorescence observed in glomerular tufts, convoluted and descending tubules and interstitial tissue in the kidney was not seen in renal tissue of the control calf. It is possible that the fluorescence was related to soluble antigens to T. vivax which have been demonstrated (25, 49). Such antigens would likely be widespread in the
tissues and this may explain the diffuse distribution of fluorescence that was observed. The pattern of distribution of fluorescence in interstitial tissue along vessels was thought to be similar to the distribution of inflammatory cells seen with conventional microscopy. The source of the antigens was suspected of being the degenerated trypanosomes in or near small blood vessels or lymphatics.

Fluorescence of a particulate, granular nature was considered to be non-specific because a similar pattern of fluorescence was seen in tissue of the control calf. The number of fluorescing particles was highest in organs containing an abundance of macrophages, such as the spleen and lymph nodes, and therefore the fluorescence may have been related to breakdown products. However, granules believed to be of a similar nature because of their shape, size, and degree of fluorescence were also abundant in intercellular areas of the bone marrow.
CHAPTER VI

SUMMARY

*T. vivax* obtained from a clinically sick cow near Neiva, Colombia, South America was passed in a sheep and in a calf, and then inoculated into the jugular vein of 14 Holstein-Friesian calves. Fever occurred by 24 hours and recurring parasitemia commenced after 72 hours post inoculation. It was estimated that practically all of the 14 calves would have died spontaneously of trypanosomiasis within 3 months if none of the calves had been euthanitized.

Associated with the first and subsequent parasitemias were decreases in hemoglobin, PCV, M:E ratio, serum albumin, A:G ratio and neutropenia, and slight increases in total and direct bilirubin and SGOT levels. Later changes included slightly elevated, fluctuating total bilirubin levels, slightly increased SGOT and gamma globulin levels and slightly decreased alpha and beta globulin levels. Blood glucose levels decreased in only 1 calf after day 30.

After 2 weeks all calves exhibited a gradual loss of body condition and later submandibular edema usually became evident. The latter condition sometimes regressed and this sign along with a PCV below 20% indicated that death was near. After 4 weeks the post mortem lesions considered sufficiently consistent to be of diagnostic value included conspicuously hypertrophied, edematous
lymph nodes and hypertrophied hemal lymph nodes, emaciation, rounded right heart, palpably firm liver, atrophied thymus and hypertrophied femoral bone marrow.

Associated with the *T. vivax* of the infecting inoculum and of succeeding parasitemias was a generalized endothelial hyper trophy and mononuclear cell infiltration along blood and lymph vessels, with proteinuria and bone marrow hyperplasia. Generalized lymphatic and RE hyperplasia occurred, but was not proven to be due to the trypanosomiasis. At 3 weeks post inoculation there was an aggregation of macrophages containing engulfed material distributed along capillaries in pulmonary interalveolar tissue, and this lesion in combination with the anemia and apparent cardiac insufficiency were thought to be important in the development of anoxia and probably contributed to the single fatality observed.

Periacinar congestion and fatty metamorphosis of midzonal hepatocytes were probably related to the failing heart and indirectly to the pulmonary lesion. Cystitis and pyelonephritis in the last 2 calves to be killed probably was a reflection of generalized debility due to chronicity of the trypanosome infection.

The detection of intact trypanosomes or their residue in histologic sections, utilizing a direct fluorescent antibody technique, was of limited success. Generalized fluorescence of interstitial tissue associated, at least in part, with vessels
was observed in tissue from infected calves but not from the control calf. It was suspected that the fluorescence was related to the presence of soluble antigens to the trypanosomes.
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VITA

Charles Arthur Daley, son of Thomas Henry Daley and Lena Gunderson, was born near Shelby, Montana, on March 25, 1932. He attended elementary and high school in Shelby, Montana, and received his diploma in May, 1951.

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