STUDIES ON THE RESISTANCE OF CHICKS TO TRYPANOSOMA EQUIPERDUM

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INTRODUCTION

The resistance of a host to infections is a very complex phenomenon affected by a variety of host mechanisms which provide varying degrees of protection against the great number of infectious agents to which the host may be exposed. No general theory satisfactorily accounts for all the complexities involved in resistance, and particularly lacking is a general theory explaining the intricacies of antibody formation. Continued effort must be made to clearly understand why a given parasite species finds it possible to live in complex association with a given host species, and why one host resists the invasion and development of a particular parasitic organism. A good example of this variation in host susceptibility is the relatively great difference observed in resistance to Trypanosoma equiperdum infections in birds and mammals; many mammals being highly susceptible and most birds being highly resistant.

Immunological mechanisms were believed to be a possible explanation of the resistance of the chick to T. equiperdum infections since the developing chick embryo is easily infected; but the infection, if not fatal, is eliminated after hatching. Therefore, corticosteroids were used in this study to inhibit mechanisms associated with immunity.

Although corticosteroids may affect resistance in several ways, these compounds are known to inhibit the development of acquired resistance as well as resistance which occurs natively.

REVIEW OF THE LITERATURE

An inspection of the literature readily revealed that the study of trypanosomes is intriguing, revealing and apparently without end. Similar experience was expected and encountered when the literature was reviewed for the adrenocortical hormones. A rather terse review of the investigations concerned with the parasite, the adrenocortical hormones, and some immunological aspects of the host under study follows.

The trypanosome used in this study was first seen by Rouget in 1894 (Watson, 1920). It was described and named by Doflein in 1901 as Trypanosoma equiperdum (Wenyon, 1926). After some years of controversy among veterinarians of Europe, T. equiperdum was accepted as the specific etiological agent of dourine of hourses and asses. Dourine as a disease had been known in certain countries in Asia and Africa for centuries. Dourine was first suspected in the United States in 1885 (Mohler, 1913), and it was recognized in Canada in 1904 (Watson, 1920).

Watson (1920) reviewed the history, research, and suppression of dourine in Canada between the years of 1904 and 1920. Serum diagnosis of dourine was standardized as early as 1911 by Winkler (1911) and by Watson (1912). Complement fixation was described as a safe and satisfactory method of diagnosis.

Agglutination and precipitation tests were also considered useful aids.

Dourine, or maladie du coit, is directly transmitted from the diseased animal to the healthy animal through coitus. Sergents in 1906 with Tabanus nemoralis and Schuberg and Kuhn in 1911 with Stomoxys calcitrans obtained mechanical transmissions of Trypanosoma equiperdum (Wenyon, 1926). T. equiperdum has been found in Europe, Asia, North and South America, and Africa; however, it has been largely eradicated from temperate climates.

Natural dourine is essentially chronic and intermittant and almost never observed in an acute form. Climatic conditions influence the progress of the disease. Cold seems to retard its development and heat appears to favor it, as does stress of breeding, heavy work, and exhaustion (Watson, 1920).

Symptoms of the disease as described by Watson (1920) are edema, tumefactions, and alterations in the genital organs; followed by plaques and skin eruptions; and finally paralyses, anemia and cachexia. Duration of the disease in this animal is between one and two years. Among naturally infected animals representative of different breeds of horses, the mortality does not exceed 50%.

In naturally infected animals T. equiperdum does not live as a true blood parasite. It lives in mucous membranes, skin, and in the serosities of local edemata. Diagnosis by microscopical examination is rare.

Trypanosoma equiperdum is yet to be cultured on an artificial medium. The organism does grow well in several laboratory animals. It has been known for many years that the course of experimental trypanosomiasis varies with different species of trypanosomes and with different species of hosts (Taliaferro and Taliaferro, 1922). White mice and rats have very low resistance to infection with T. equiperdum. Hood (1949a) found the incubation period for these animals to be about 1 and 2 days respectively when she used standardized inocula. In contrast to infections in rats and mice, infections in rabbits, guinea pigs, and deer mice were of the subacute or chronic type. The blood of rabbits rarely, if ever, contained large numbers of T. equiperdum. Cats, dogs, and sheep are also known to be susceptible to experimental infections with this organism. Duration of the infection in white mice and rats is three or four days when average inocula are used, and it is terminated with the death of the hosts. The other animals mentioned above die within one to three months as a result of an infection with T. equiperdum. Sheep and rabbits exhibit gross lesions and suffer cachexia.

It is not clearly understood how T. equiperdum effects pathogenicity in the sundry susceptible hosts; however, the methods by which trypanosomes damage their hosts should require a physiological approach according to von Brand (1951).

There have been many attempts to infect avian species with mammalian trypanosomes (Bruce, 1895; Rouget, 1896; Ziemann, 1902; Schilling, 1904; Goebel, 1906; Mesnil and Martin, 1906; Goebel, 1908; Bruce, Hammerton, and Bateman, 1911; Mesnil and Blanchard, 1912; Corson, 1931; Corson, 1932; Clark and Dunn, 1933; Mesnil, Leger, and Perard, 1936; Kuigumgift, 1937; Seager, 1944; Hood, 1949b). Bruce, Hammerton, and Bateman (1911) were unable to infect fowls with Trypanosoma gambiense by using the natural vector. There is evidence that at least temporary infection of both fowls and geese can follow inoculation of infected blood. Schilling (1904), and Mesnil and Martin (1906) infected geese with Trypanosoma brucei, but failed to infect fowls. Goebel (1906 and 1908), however, inoculated T. brucei into twenty-three fowls. Although he never found trypanosomes in the circulating fowl blood, or in smears of fowl organs, Goebel obtained infections in guinea pigs inoculated with the blood from 21 of the 23 fowls. The birds were infected with the trypanosomes for at least 81 days as indicated by guinea pig inoculations. As a rule the fowls exhibited no appreciable symptoms. Mesnil and Blanchard (1912) found that fowls

could be infected with T. gambiense and T. rhodesiense. Trypanosomes were not seen in blood films, but the blood was shown to be infective by sub-inoculating rats every ten or twelve days. Three of the fowls infected with T. gambiense died on the 28th, 38th, and 75th days. Two of those infected with T. rhodesiense died on the 56th and 62nd days.

Corson in 1931 and 1932 found a small percentage of guinea fowl and francolins to be susceptible to experimental infections with T. rhodesiense. Trypanosomes could not be found by microscopic examination, but the infection was transmitted to white rats when sub-inoculations were made. Corson also found two muscovy ducks to be susceptible to experimental infections with T. rhodesiense. Seager in 1944 reported that he transmitted T. equiperdum to the duck. Kuigumgift in 1937 failed to infect fowls and pigeons with T. equiperdum. Hood in 1949 reported failure to infect chicks with T. equiperdum, T. brucei, and T. hippicum.

It is hardly correct to state that fowls and geese are completely insusceptible to trypanosomal infections with mammalian trypanosomes in light of the above investigations, but rather the parasitemia rarely reaches a high level and the infection is difficult to demonstrate.

Avian embryos have been proven to be highly receptive

to infections with a variety of mammalian trypanosomes. Biocca in 1938, as reported by Pipkin (1960), inoculated T. brucei into the eggs of chicks, ducks, and guinea fowl. He failed to infect the embryos of ducks and guinea fowl, but his attempts to infect 8- to 14-day old chick embryos with T. brucei met with success. In years that followed most of the mammalian trypanosomes were cultivated with success in the chick embryo. Pipkin in 1960 reviewed the literature concerned with the cultivation and study of parasitic protozoa in avian embryos. Among the species of mammalian trypanosomes cultured in the chick embryo as reported by Pipkin (1960) are: T. brucei, T. cruzi, T. equiperdum, T. evansi, T. gambiense, T. hippicum and T. rhodesiense. When Trypanosoma congolense was inoculated via the supra-chorioallantoic membrane by Rodhain and van den Berghe in 1943, the organisms were not found in the blood of the embryo but were found in the chorioallantoic fluid. Embryo to embryo passage was not achieved. Longley et al. (1939) and Chabaud (1939) unsuccessfully attempted to infect chick embryos with T. lewisi as reported by Pipkin (1960).

Biocca, in describing the infection in chick embryos with T. brucei, reported that the infections died out after the seventh or eighth day. In 1943 van den Berghe found that if embryos after 10 days incubation were infected with T. evansi,

instead of dying out, the parasites persisted through hatching and sometimes showed transitory parasitemias in the hatched chicks. Mitchell, Walker, Heath and McKertcher (1939) found active forms of T. equiperdum in the blood of chicks three days after they were hatched. The chicks died on the 5th day. Chabaud in 1939 described massive septicemias with T. rhodesiense in chick embryos about the 7th or 8th day after they were infected with the organisms. The infection then steadily declined during the last week of incubation. Hood in 1949b cultivated T. brucei, T. hippicum and T. equiperdum in chick embryos. She found chicken embryos infected with T. brucei and T. hippicum which hatched either overcame the infections within forty-eight hours or the chicks succumbed to the infections within four to six days. The embryos infected with T. equiperdum usually died. On rare occasion a chick was heavily infected with T. equiperdum when it hatched, and the infection persisted for five days before disappearing.

It has been suggested that the rapid disappearance of the trypanosomes in young chicks is due to the development of antibodies (Pipkin, 1960). Hood (1949b) indicated that the susceptibility of the chick embryo to infections with mammalian trypanosomes is replaced within forty-eight hours after hatching by a solid natural immunity. Ortega y Torres (1958) managed

to hatch a chick which had been infected as an embryo with T. equiperdum. They reported a rise in parasitemia for ten days after the chick hatched. This was followed by a very sudden disappearance of the trypanosomes from the blood. They again found trypanosomes after 33 more days. The death of the chick occurred the following day.

Cortisone is one of a series of crystalline adrenal cortical hormones. It was isolated in the laboratory of E. C. Kendall from extracts of the cortex of beef adrenal glands and described in 1936 by Mason, Myer and Kendall (Wintersteiner and Smith, 1938). Independent descriptions were made by Reichstein as substances Fa and Wintersteiner and Pfiffner as compound F (Wintersteiner and Smitn, 1938). Since its isolation, cortisone and a variety of related compounds have been synthesized.

The secretions of the adrenal cortex are intrinsic in the body's ability to readjust its economy to a steady state in response to external and internal changes in its environment (Kass and Finland, 1953). Evidence suggests (Tepperman, et al., 1943) that animals undergoing stress produce larger amounts of cortical hormones than do those animals at rest. Ingle (1951) concluded that production of adrenocortical hormones is linked to peripheral utilization. According to Ingle (1950) and Sayers (1950), the secretory activity of the cortices

varies in accordance to need; insufficiency or excess of these hormones cause changes in the organic metabolism, in most if not all, of the body tissues and functions. The adrenal hormones are known to be regulatory in the metabolism of carbohydrate, fat and protein.

The interrelationships of the endocrine glands are of such a nature as to cause some frequent difficulty in the distinction of the direct effect of a hormone from the indirect effects of that same hormone on other glands which might cause disturbances which appear to be unrelated in function (Sayers, 1950; Conn and Fagans, 1952). Some uncertainty remains concerning the precise composition of the adrenal cortical secretions. Hechter as reported by Kass and Finland (1953) suggested that at least in the oxen the adrenal glands secrete corticosterone compound B of Kendall and Hydrocortisone (17 hydrocorticosterone) or compound F of Kendall.

It has been clearly demonstrated that cortisone and related compounds affect the basic physiological mechanisms involved in diseases whose etiologies are quite different. By studying selected infectious diseases it was possible to formulate some of the effects of the cortical hormones in infection and immunity, and these effects were excellently summarized by Kass and Finland in 1953. Thomas, the same year, also published an excellent review.

Studies have been made of the effects of adrenocortical hormones on the course of diseases in patients with typhoid fever, brucellosis, Rocky mountain spotted fever, leptospirosis, tuberculosis, viral pneumonia, pneumococcal and other bacterial pneumonias, tetanus, viral hepatitis, mumps, small pox, and trichinosis. In general the administration of ACTH or cortisone to patients with febrile illnesses or with illnesses characterized by malaise, anorexia, and evidence of generalized toxicity frequently showed prompt defervescence, and lessening of symptoms. Bacteriological findings indicated no improvement or even a definite impairment on the capacity of the patient to dispose of the organisms. Even in the absence of the clinical evidence of adverse effects, bacterial counts rose in exudates and affected tissue, lesions spread, and bacteriemia developed or persisted. It was realized that cortisone has the property of disorganizing the natural host parasite relationship--the outcome in favor of the parasite. This has been shown in many experimental infections with viruses, bacteria, fungi, protozoa, helminths, and in tumor transplants (Kass and Finland, 1953; Thomas, 1953). The mechanisms involved are unclear. It is thought that cortisone inhibition is exerted early in the sequence of events which lead to the formation of antibody. Taliaferro (1957) therefore suggested that in this respect the production of

inhibition resembles that which is produced by x-irradiation.

Hosts in which the effect of cortisone enhances infection include rabbits, mice, rats, guinea pigs, hamsters, monkeys, human beings, and chick embryos (Kass and Finland, 1953).

There is conflicting evidence concerning the mediated release of antibody protein, however, it has been conclusively demonstrated that the amount of circulating antibody is decreased in animals treated with ACTH, cortisone, and hydrocortisone (Germuth, 1950; Bjornoeboe, 1951; Fischel, 1952; and Dougherty, 1952) as reported by Kass and Finland, 1953 and Thomas, 1953.

Halpern et al. (1951) investigated the effects of cortisone on antibody titer. Rabbits were immunized to ovalbumin. These rabbits were then administered a sufficient amount of antigen to reduce the circulating antibody to zero levels. When cortisone was administered to a group of rabbits treated in the same manner, they responded with a rapid increase in circulating antibody. whereas the antibody titers of animals which did not receive the hormone remained at zero level until 48 hours. Cortisone increased the antibody titer immediately, but also prevented the development of a greater amount found at later periods in the non-hormone treated animals. Halpern et al. concluded that the rapid increase in antibody titer in the cortisone treated group was preformed antibody released

from the lymphocytes. It is therefore concluded that cortisone exerts two effects: (1) it enhances antibody release, and (2) tends to diminish the rate of antibody synthesis.

Cortisone inhibits the secondary response (Halpern, 1951; Fischel, 1952), but it does not enhance the disappearance of antibody passively administered to rabbits. It seems therefore rather clear that inhibition of antibody synthesis and not increased catabolism is the basic mechanism by which adrenocortical hormones suppress the amount of circulating antibody.

Webster in 1923 (Clark et al., 1949) indicated in his study on experimental epidemiology that there is constancy in the virulence of a given strain of a pathogenic organism. It must therefore be concluded that the variation in malignity and host susceptibility is to be accounted for by assuming that the resistance of the host is modifiable by varietal conditions. Some of the agents which modify the resistance of mammals to trypanosomiases are: exposure in water and sunlight (Kligler and Weitzman, 1926); and low temperatures (Kalabuchov and Levinson in 1936 protected bats from experimental infections with T. equiperdum by placing the bats in ice boxes at 10°C or 3-4° C. These bats developed no immunity but the organisms disappeared.) The effect of climatic conditions on dourine of asses and horses has been considered above.

The effects of X or neutron irradiation, India ink blockade, or splenectomy on innate immunity against Trypanosoma duttoni in mice were studied in 1959 by Jaroslow. He concluded that the innate immunity to reproductive activity of the trypanosomes was decreased by injury to the lymphoid-macrophage system, whereas trypanocidal activity was decreased only when the host was more extremely injured. Friebel in 1952 experimentally infected cortisone treated mice with trypanosomes and concluded that the hormone interfered with antibody formation and also gave rise to excessive mobilization of macrophages which interfered with their adverse influence on the progress of the infection.

Agosin in 1952 infected cortisone treated mice with Trypanosoma cruzi. He found an increase in the number of parasites in the blood and a lower survival rate in comparison with controls. The inflammatory phenomena were much less marked in animals which had received cortisone.

Rats which have been infected with trypanosomes and cured are temporarily resistant to reinfection due to an actively acquired immunity (Ehrlich and Shiga, 1904). Cantrell studied the immunity of rats which had been infected with Trypanosoma equiperdum and subsequently cured with oxophenarsine (1955). Cortisone interfered with the action of oxophenarsine on T. equiperdum in two ways depending on the dose of oxophenarsine

(Cantrell, 1955). At low dosage levels of oxophenarsine, cortisone caused a delay in the disappearance of trypanosomes from the blood. At high dosage of the drug cortisone administration was associated with earlier relapse and a higher rate of death than those rats which had been treated with oxophenarsine alone. Cortisone had no effect on the course of T. equiperdum in the rat in the absence of oxophenarsine (1956). Cantrell (1959) studied the rate of increase of T. equiperdum in the rat during the logarithmic phase of growth, the deceleration of the parasitemia curves at high levels of parasitemia, and the translocation of trypanosomes from the peritoneal cavity to the blood following intraperitoneal inoculation. He found the above attributes of T. equiperdum infections to be unaffected by the administration of large doses of cortisone. His results supported the view that this infection is characterized by a very low degree of resistance in the rat.

Ashcroft (1959) studied the effect of cortisone on T. rhodesiense infections of white rats. He suggested that cortisone has several actions, some of which may inhibit the defense mechanisms of the rat and enhance a trypanosome infection. Other actions which lead to changes in the composition of blood may act to inhibit infection. He concluded

that the changes in an infection resulting from the administration of cortisone will depend on which effects are predominant. These effects in turn depend on the dose of cortisone, the time when it is given, the response of the rat and the susceptibility and adaptability of the trypanosome.

Zinnser cautioned (1920) that there exists danger in the application to any species of animal reasoning or theories deduced from experimental observations upon another species. Most of the experimental work in immunology has been with mammalian species. Further, very few investigators have studied the effects of corticosteroids on the protective mechanisms utilized by avian species.

Wolfe (1942) and earlier workers have found the chicken to be excellent producers of antibody though the use of their antisera has revealed that in some respects they cannot be handled the same as antisera produced in rabbits. Wolfe and Dilks (1948) studied the variations in the chick to produce precipitin and correlated the production of antibody by the chick with the age of the animal. Newly hatched chicks and chicks of various ages up to 12 weeks were studied. They found that newly hatched chicks gave low-titered antisera to bovine

serum. Slightly less than half of the chicks injected on the first and third days after hatching gave detectable precipitins. The highest titers were with a 1:50 antigen dilution. The percentage of chicks of one week of age that yielded precipitins was similar to that of the day old group but the titers of those that responded were considerably higher. The range in this group varied from an antigen dilution of 1:200 to 1:16000. Nineteen out of twenty-nine chicks responded in the 2-week old group with an increase in the ability to produce precipitin. Almost all birds responded at 5 weeks of age. Chickens 6 to 12 weeks old gave titers similar to the 5-week-old ones. It was suggested therefore that serological "maturity" was reached at 5 weeks of age as far as precipitin against serum proteins was concerned. Wolfe and Dilks in 1949 compared the antibody response of eight avian species. They found that the chick constantly produced yields of excellent precipitating antisera when compared with the owl, pheasant and partridge; all of which were classified as good producers but with average and maximum titers below that of the chick. Ducks and turkeys produced antisera with low precipitating titers. No precipitins could be demonstrated in the guinea fowl and pigeon antisera.

Goodman, Wolfe and Norton (1951) varied the concentrations

of NaCl n the reaction of chicken antisera with serum protein antigens using turbidimetric and quantitative nitrogen techniques. They found that determinations of maximum antibody content could not be made at salt concentrations of less than about 8%. In 1952 Goodman and Wolfe found a similarity of pheasant and owl antibeef-albumin precipitins to that of the chickens. They found maximal precipitates formed in 8% to 12% saline.

Chang, Glick and Winter in 1955 reported in an abstract that the bursa of Fabricius is directly or indirectly involved in the production of antibodies in birds. The bursa of Fabricius is an avian structure and may be described as a blind sac connected by a duct to the dorsal portion of the cloaca. It is sometimes called the "cloacal thymus." When "O" antigen of Salmonella sp. was injected into bursectomized hens, 6 died and 3 survived but produced no antibodies against the organisms (Chang, Glick and Winter, 1955). The authors reported that antibodies were demonstrated in normal birds which were given the same antigen.

Glick in 1955 reported that the maximum size of the bursa was attained in white leghorns between 4 1/2 and 6 weeks. He further reported a reduction of the size of the bursa in chicks which were administered cortisone acetate. He suggested therefore that the bursa was lymphatic in nature. In 1956

Glick, Chang and Japp demonstrated the development of antibodies to S. typhimurium in only 8 out of 75 bursectomized birds which had been infected with the organism. Antibodies were demonstrated for 63 out of 73 controls which had been injected with the antigen. They also found that the white leghorn breed has a greater resistance to S. pullorum than do Rhode Island Reds during the first two weeks after hatching. The more rapid growth rate and larger size of the bursa in white leghorns was demonstrated. It is suggested that the resistance to disease in these animals may be associated with the rate of growth and the size of the bursa during the period when the birds first develop the capacity to produce many of its antibodies. Once the bird has developed the ability to produce antibodies the ability is maintained throughout life.

In 1957 Glick found that a total dosage of 7.5 mg of cortone (1.25 mg per injection) significantly reduced the size of the bursa of Fabricius. Glick reported in 1958 that a single injection of 25 or 15 mg of cortisone after 3 hours reduced significantly the relative percent of lymphocytes and increased the relative and absolute count of heterophils in the blood of 3 week old chicks. Multiple injections caused the involution of the bursa of Fabricius and in general paralleled the effects of the hormone in the mammal.

According to Zarrow, Greenman, and Porters (1961), the bursa of Fabricius responds to a wide variety of hormones and appears to be concerned with the formation of antibodies. These workers confirmed and extended the observations of others that the adrenal corticoids as well as androgens and estrogens cause an involution of the bursa of Fabricius. Although cortisone and hydrocortisone were found to cause involution of the bursa, only hydrocortisone effectively inhibited stilboestrol induced growth of the oviduct. This agreed with the reported failure of cortisone to induce hyperglycemia in the bird while hydrocortisone was effective. There then are apparent differences in the bird with regard to the effects of these two glucocorticoids that are not apparent in mammals.

The above review indicates a number of unsolved problems concerning the parasite, Trypanosoma equiperdum, its natural and unnatural hosts and the corticosteroids used in the present study. The enigmas that impressed the author in the very beginning of this investigation did not wane but grew with constancy. No other reasons need be given to justify attempts to infect with T. equiperdum chicks which have been treated with corticosteroids.

MATERIALS AND METHODS

Trypanosoma equiperdum used in this study was obtained from a stock culture maintained in rabbits. Blood was taken from an infected rabbit and inoculated intraperitoneally into rats and maintained in rats by syringe passage. A few drops taken from the tail of infected rats were diluted in physiological saline and inoculated intraperitoneally into a new group of rats on every second or third day of the infection. Rats so infected provided a ready source of large numbers of organisms easily isolable from the hosts' blood.

Rats which were heavily infected but not moribund were bled from the heart. Cardiac blood containing the trypanosomes was either heparinized or transfered to flasks containing glass beads and shaken in order to prevent coagulation. The infected blood was then transfered into Wasserman tubes and centrifuged at approximately 2500 rpm (International Centrifuge size 2) for 10 minutes. The top buffy layers containing the trypanosomes were transfered to other tubes and washed in either physiological saline or in Hanks' balanced salt solution. This mixture was then centrifuged for 3 minutes. The portion containing the trypanosomes was transfered to Wintrobe tubes with Pasteur pipettes and centrifuged for 5 minutes. Complete separation of trypanosomes

from erythrocytes was effected by this method. Subsequent washings were made in Wasserman tubes with normal saline or Hanks's balanced salt solution. Three minutes centrifugation satisfactorily settled the organisms during washings. The last wash was followed by four minutes centrifugation and the supernatant was removed. The trypanosomes used in the preparation of dead antigens were centrifuged and washed in physiological saline or Hanks' balanced salt solution a total of not less than five times.

Trypanosomes and trypanosome infected blood were diluted with normal saline and counted in hemocytometers.

Trypanosoma equiperdum was cultured in chick embryos for eight passages over a period of 37 days. Eggs were candled to ascertain their fertility and prepared for inoculation as described by Hood (1949b). The embryos were originally infected by inoculating 0.1 ml of rat blood which contained 7×10^5 trypanosomes per cmm into the allantoic cavity of chick embryos which were 14 days old. The trypanosomes were maintained in embryos by transfering heavily infected embryonic blood mixed with embryonic fluids from embryos on the fourth or fifth day of the infection into the allantoic cavity of 12- to 14-day old chick embryos.

A modification of the technique described by Hood (1949b) was used to determine the presence of the trypanosomes in the

blood of the embryos. The infected embryos were candled and an area over one of the smaller blood vessels was marked on the egg shell with a pencil. A small hole was made through the shell with a rotary drill without piercing the shell membrane. The area was painted with metaphen and with a sterilized dissecting needle, the blood vessel was punctured. The drop of blood which formed was touched with a glass cover slip or slide without touching the shell. The blood was mixed with normal saline and examined for trypanosomes under the microscope. The embryonic blood which flowed from the puncture coagulated readily. The area was again painted with metaphane and the hole sealed with paraffin. By using this technique eggs could continue the course of development until hatching or death of the embryos.

Corticosteroids used in this study were commercial preparations. Cortisone acetate, a product of the Nutritional Biochemicals Corporation, was crystalline; it was prepared for injection by suspending 150 mg in 12 ml of sterile physiological saline. Prednisolene (Delta-Cortef) was supplied by Upjohn Co. Each cc of Delta-Cortef contained: Prednisolone (delta-1-hydrocortisone), 10 mg; with Sodium Citrate, 4.5 mg; Polyethylene Glycol 4000, 120 mg; Polyvinylpyrrolidone, 1 mg; preserved with myristyl-gamma picolinium chloride, 0.2 mg and neomycin sulfate, 5 mg (equivalent to 3.5 mg neomycin

base). Dexamethasone (Azium) was supplied by Schering Corporation. Each cc of commercial suspension contained 0.5 mg dexamethasone, 300 mg polyethylene glycol 4000, 9 mg benzyl alcohol, 1.8 mg methylparaben and 0.2 mg propylparaben as preservatives.

Chicks used in this study were white leghorns obtained on the day of their hatch and quartered in chick brooders. Food and water were available ad libitum. These chicks were wing banded and/or separated in tiers of the brooders. Chicks were injected intraperitoneally either with trypanosome infected rat blood, trypanosomes separated from rat blood and mixed with chick serum, or, as in one instance, with trypanosome infected embryo blood.

The following methods were used in the examination of chick blood for the presence of trypanosomes. Cardiac blood from chicks was injected into white mice and these mice were examined for trypanosomes. The time of the first observation of T. equiperdum in the peripheral blood of mice and the death of mice were noted. Cardiac blood and peripheral blood obtained from the claws of the birds were examined directly as wet mounts in physiological saline. Blood films of cardiac and peripheral blood were made and stained with Wright's stain and examined for T. equiperdum. Wet mounts and blood films were designated as positive or

negative for trypanosomes. If trypanosomes were found with some frequency the findings were recorded as number of trypanosomes per 1000 erythrocytes. Blood was also drawn into heparinized capillary tubes and centrifuged in an International Clinical Centrifuge. The capillary tubes were then broken just above or below the top layers of cells. These top layers along with some of the plasma were placed on glass slides covered and searched for the presence of trypanosomes. All direct examinations of wet mount preparations and blood films stained with Wright's stain were performed with no consideration for the amount of time spent on a given specimen. The whole preparation was scanned with low and high power objectives. Oil immersion was used depending on observations made with lower magnifications.

INFECTION OF CHICKS TREATED WITH CORTICOSTEROIDS

EXPERIMENT I. Infection of Chicks Treated with Prednisolone

Since the literature indicated that the effect of corticosteroids on the protective mechanisms of an animal depends upon dosage and time, the first experiment was performed in order to observe what effect Prednisolone has on the refractiveness of chicks to infection with T. equiperdum when it is administered at various dosage levels and periods prior to attempted infections.

Chicks approximately four days old were alloted into two groups of 15 and two groups of 24. A fifth group of 13 chicks was originally alloted as a control group, but due to a laboratory accident cannot be considered. The first experiment is therefore without proper controls.

The chicks weighed approximately 59 grams at the beginning of the experiment. The corticosteroid was administered intramuscularly and intraperitoneally. The right and left thigh muscles were alternated for the intramuscular injections, and the intraperitoneal route was juxtaposed with the intramuscular routes.

The treated groups of chicks were labeled as A, B, C, and D. Groups B and D were each composed of 24 chicks six days old. Group B was treated with 0.5 mg of Prednisolone (10 mg /kg body wt) *per diem* for 5 days prior to being injected with *T. equiperdum*. The chicks in group D were treated with 0.25 mg of Prednisolone (5 mg/kg body wt) for 5 days prior to receiving the inocula of trypanosomes. Groups A and C were each composed of 15 chicks and were not treated with the corticosteroid until they were 9 days old. Six-tenths mg of Prednisolone (approximately 10 mg per kg body weight) was administered *per diem* to chicks in group A two days prior to their receiving the injections of *T. equiperdum*. Group C was treated with 0.3 mg of Prednisolone (approximately 5 mg/

kg wt) *per diem* for two days prior to the injection of *T. equiperdum*.

Trypanosoma equiperdum was separated from infected rat blood and suspended in a solution of one part chick serum and normal saline. One-tenth cc portions were injected intraperitoneally into each chick. In this and subsequent experiments the trypanosomes were injected in such a way to minimize a descrepancy of time between groups of chicks. One chick from each group was injected with the organisms in rotation according to groups so that the inoculation of all groups was completed at approximately the same time.

Treatment was continued in each group for two days post infection (see Table 1).

Twenty-four hours after the chicks were injected with trypanosomes the blood of four chicks from each group was examined for presence of the protozoan. Wet mount preparations in normal saline and Wright's stained smears were scanned. On the following day three chicks from each group were bled from the heart. Smears for Wright's stains were made and blood was heparanized and pooled according to groups. Four-tenths cc of blood was injected into each of two white mice from each group of chicks. Cardiac punctures of the chicks and sub-inoculations into white mice were repeated on the third and fourth day after the injection of the trypanosomes.

TABLE 1

TREATMENT OF CHICKS WITH PREDNISOLONE IN EXPERIMENT I

Group	A	B	C	D	Route day of experiment
Number of chicks	15	24	15	50	
Average weight of chicks	50 g	50 g	50 g	50 g	
Initial dosage Prednisolone	10 mg/kg wt	10 mg/kg wt	5 mg/kg wt	5 mg/kg wt	
Mg of Prednisolone per chick in 0.1 cc of sterile physiological saline and manufacturers' suspension		0.5 mg		0.25 mg	1.M. 1
		0.5 mg		0.25 mg	1.M. 2
		0.5 mg.		0.25 mg	1.P. 3
	0.6 mg	0.6 mg	0.30 mg	0.30 mg	1.M. 4
	0.6 mg	0.6 mg	0.30 mg	0.30 mg	1.M. 5
Total mg. Prednisolone prior to inoculation of *Trypanosoma equiperdum*	1.2 mg	2.7 mg	0.60 mg	1.35	mg 5
Injection of *T. equiperdum* in 50% chick serum and physiological saline	0.1 cc	0.1 cc	0.1 cc	0.1 cc	1.P. 5
Treatment after injection of trypanosomes:					
Mg of Prednisolone	0.6 mg	0.6 mg	0.30 mg	0.30 mg	1.P. 6
	0.6 mg	0.6 mg	0.30 mg	0.30 mg	1.M. 7
Total mg of Prednisolone after injecting trypanosomes	1.20 mg	1.20 mg	0.6 mg	0.6 mg	
Total mg of Prednisolone per chick	2.40 mg	3.90 mg	1.20 mg	1.95 mg	

No mouse inoculations were made on the fifth day. Chicks were again bled from the heart on the sixth and seventh day post-infection. Peripheral blood samples of three or four chicks were examined daily from each of these groups as wet mounts in normal saline and as Wright's stained blood smears. The career of the trypanosomes in the chicks was not followed after the seventh day of the infection.

EXPERIMENT II. Infection of Chicks Treated with Cortisone Acetate

Six-day old chicks which weighed approximately 50 g were alloted into three groups. Group A consisted of twelve chicks which were administered 2.5 mg of cortisone acetate (50 mg/kg body wt) intraperitoneally in 0.2 cc of sterile physiological saline *per diem* for seven days. On the 7th day of treatment these chicks were injected intraperitoneally with a 0.1 cc suspension containing 20,000 trypanosomes per cmm in a mixture of chick serum and normal saline. Cortisone treatment was continued for two more days (eighth and ninth day of the experiment) after the injection of *T. equiperdum*. See Table II.

Group B, which also consisted of 12 chicks, was treated with cortisone acetate and inoculated with the trypanosomes as in group A; however, additional injections of 2.5 mg of

TABLE II

TREATMENT OF CHICKS WITH CORTISONE ACETATE IN EXPERIMENT II

Group	A	B	C	Day of experiment
Number of chicks	12	12	24	
Average weight of chicks	48 g	48 g	48 g	1
Initial dosage of cortisone acetate	50 mg/kg wt	50 mg/kg wt	0	
Milligrams of cortisone acetate per chick in 0.2 cc of sterile normal saline	2.5 mg	2.5 mg	0	1
	2.5 mg	2.5 mg	0	2
	2.5 mg	2.5 mg	0	3
	2.5 mg	2.5 mg	0	4
	2.5 mg	2.5 mg	0	5
	2.5 mg	2.5 mg	0	6
	2.5 mg	2.5 mg	0	7
Total mg of cortisone acetate prior to inoculation of T. equiperdum	17.5 mg	17.5 mg	0	7
Injection of T. equiperdum in 50% chick serum and physiological saline, 20,000 mm^3	0.1 cc	0.1 cc	0.1 cc	7
Treatment after injection of trypanosomes:				
Mg of cortisone acetate	2.5 mg	2.5 mg	0	8
	2.5 mg	2.5 mg	0	9
		2.5 mg	0	12
		2.5 mg	0	13
Total mg of cortisone acetate after injecting trypanosomes	5 mg	10.0 mg	0	
Total mg of cortisone acetate/chick	22.5 mg	27.5 mg	0	

cortisone acetate were given on the twelfth and thirteenth days of the experiment.

Group C consisted of 24 untreated chicks which were injected intraperitoneally with 0.1 cc of the trypanosome suspension used in groups A and B.

Direct microscopic examinations were made daily of blood taken from the claws of at least four birds of each group. These examinations began on the first day after the injection of the organisms and continued until the experiment was terminated.

On the seventh day after the chicks had been injected with the trypanosomes, one from each group was bled from the heart and 0.5 cc of heparinized blood was injected into white mice. Sub-inoculations were again made on the ninth, tenth, and twelfth days after the chicks received the organisms in group A. Sub-inoculations were made into white mice from group B on the seventh, nineth, twelfth, and sixteenth days after chicks had been injected with the trypanosomes.

Sub-inoculations were made from group C on the second, fourth, sixth, eighth, tenth, twelfth, twenty-eighth, thirty-fifth, and forty-fifth days after the chicks had been injected with T. equiperdum.

EXPERIMENT III. Infection of Chicks Treated with Delta-1-hydrocortisone

Twenty-four chicks were treated with 0.5 mg (10 mg per kg weight) of prednisolone *per diem* for five days. On the fifth day of the treatment a 0.1 cc suspension containing *T. equiperdum* 20,000 cmm was injected intraperitoneally. A group of 24 controls was similarly injected with the trypanosomes. The control group was labeled group C. The treated chicks were divided into two groups of 12. Group A was administered 0.5 mg (10 mg/kg body wt) of the corticosteroid for two more days after receiving the organisms. Group B was administered the corticosteroid the first two days post-infection, then three injections separated by 1 day and finally 2 more injections separated by two and four days. See Table III.

EXPERIMENT IV. The Infection of Dexamethasone-Treated Chicks with *T. equiperdum*

On day one and two of this experiment, 40 chicks 1 to 3 days old weighing 40 to 45 g, were injected intraperitoneally with 0.1 cc of dexamethasone which was commercially prepared to contain 0.5 mg of the compound per cc. On the third, fourth, and fifth days of the experiment the chicks were injected with 0.05 cc of the dexamethasone. On the fifth day of the treatment the chicks were divided into two groups of

TABLE III

TREATMENT OF CHICKS WITH PREDNISOLONE IN EXPERIMENT III

Group	A	B	C	Day of experiment
Number of chicks	12	12	24	
Average weight of chicks	50 g	50 g	50 g	1
Initial dosage of prednisolone	10 mg/kg wt	10 mg/kg wt	0	
Mg of prednisolone per chick in 0.1 cc of sterile physiological saline and manufacturer's suspension	0.5 mg	0.5 mg	0	1
	0.5 mg	0.5 mg	0	2
	0.5 mg	0.5 mg	0	3
	0.5 mg	0.5 mg	0	4
	0.5 mg	0.5 mg	0	5
Total mg of prednisolone prior to inoculation of Trypanosoma equiperdum	2.5 mg	2.5 mg	0	5
Injection of T. equiperdum in 50% chick serum and physiological saline, 20,000 trypanosomes mm^3	0.1 cc	0.1 cc	0.1 cc	5
Treatment after injection of trypanosomes:				
Mg of prednisolone	0.5 mg	0.5 mg	0	6
	0.5 mg	0.5 mg	0	7
	0	0.5 mg	0	9
	0	0.5 mg	0	11
	0	0.5 mg	0	13
	0	0.5 mg	0	16
	0	0.5 mg	0	21
Total mg of prednisolone after injecting trypanosomes	1.0	3.5 mg	0	
Total milligrams of prednisolone	3.5 mg	5.5 mg	0	

20 and labeled A and B. Two groups, each consisting of 20 normal untreated chicks of the same hatch as A and B, were labeled C and D.

On the fifth day of the experiment, group A (composed of 20 chicks treated with the corticosteroid) and group C (composed of 20 untreated chicks) were given 0.5 cc of rat blood which contained 760,000 T. equiperdum per cmm.

Dexamethasone (0.05 cc) was administered intraperitoneally to groups A and B on the sixth, seventh, eighth, and ninth days of the experiment. No treatment was given on the tenth day of the experiment. The corticosteroid was administered on the eleventh, thirteenth, and fifteenth days of the experiment. The last injection of dexamethasone was given on the twenty-first day of the experiment. See Table IV.

EXPERIMENT V. Superinfection of Normal Chicks with T. equiperdum.

Six normal chicks 24 to 48 hours old each weighing approximately 40 g were injected intraperitoneally with 1 cc of rat blood containing 2.1×10^6 T. equiperdum per cmm. A white mouse weighing 20 g was inoculated intraperitoneally with 1 cc of the same blood. Another 20 g mouse was inoculated with 0.5 cc of the infected rat blood. The chicks and mice were examined for trypanosomes by microscopically searching for their presence in a few drops of peripheral blood mixed with

TABLE IV

TREATMENT OF CHICKS WITH DEXAMETHASONE IN EXPERIMENT IV

Group	A	B	C	D	Day of experiment
Number of chicks	20	20	20	20	
Average weight of chicks	40 g	40 g	40 g	40 g	1
Mg of dexamethasone per chicks 0.5 mg per cc of manufacturer's suspension	0.05 mg	0.05 mg	0	0	1
	0.05 mg	0.05 mg	0	0	2
	0.025 mg	0.025 mg	0	0	3
	0.025 mg	0.025 mg	0	0	4
	0.025 mg	0.025 mg	0	0	5
Total mg of dexamethasone prior to inoculation of Trypanosoma equiperdum	0.175	0.175	0	0	5
Injection of rat blood containing 760,000 T. equiperdum mm^3	0.5 cc	0	0.5 cc	0	5
Treatment after injection of trypanosomes:					
Mg of dexamethasone	0.025 mg	0.025 mg	0	0	6
	0.025 mg	0.025 mg	0	0	7
	0.025 mg	0.025 mg	0	0	8
	0.025 mg	0.025 mg	0	0	11
	0.025 mg	0.025 mg	0	0	13
	0.025 mg	0.025 mg	0	0	15
	0.025 mg	0.025 mg	0	0	21
Total mg of dexamethasone after injecting trypanosomes	0.175	0.175	0	0	
Total mg of dexamethasone	0.350	0.350	0	0	

physiological saline. Each of the chicks and mice was examined and diagnosed as positive or negative for the trypanosomes 15 minutes, 1, 2, 4, 8, 12, 24, 30, 36, 40, and 48 hours after they were injected with the parasite.

Four of these six chicks were successfully given 1 cc of rat blood intraperitoneally containing 9.2×10^5 T. equiperdum per cmm 74 hours after they had been injected with trypanosomes for the first time. Two of the six birds immediately died after being injected. The blood of the four chicks was microscopically examined for the presence of trypanosomes every four hours for sixteen hours and then 24 and 48 hours after the second injection. These same birds after 48 hours were given 0.5 cc of Hanks' solution which contained 1.5×10^6 T. equiperdum per cmm. The blood of each chick was again examined for trypanosomes after 4, 8, 12, and 24 hours by microscopically scaning a few drops of blood taken from the claws and mixed in normal saline. Sub-inoculations were made into white mice from 2 of these chicks 48 hours after they were last injected with trypanosomes.

EXPERIMENT VI. Trypanosome Elimination

This experiment had as its concern the comparison of time of the elimination of T. equiperdum over a 48-hour period in normal and dexamethasone treated chicks.

Group 0 consisted of six one-day old chicks which were inoculated with 1 ml of rat blood containing approximately 2×10^6 T. equiperdum per cmm. The blood of these chicks was examined for trypanosomes 15 minutes after they had been injected with the organisms by searching under the microscope for their presence in wet mounts of blood made from the claws of the chicks. Chicks were again examined in this fashion after 1, 8, 12, 24, 36, 48, and 60 hours. Cardiac punctures were made on all of the chicks at random some time between the twelfth and sixtieth hour post-inoculation. Cardiac blood was put into heparinized capillary tubes and centrifuged in a clinical centrifuge. The capillary tubes were broken just above the cells and the top layers were examined for the presence of trypanosomes.

Group A was composed of 13 normal 3- and 4-day-old chicks. One ml of pooled rat blood containing 1.1×10^6 T. equiperdum per cmm was injected intraperitoneally. Observations similar to those on group 0 were made 4, 8, 12, 24, 36, and 48 hours after the organisms had been injected. In addition to examining wet mounts of blood, blood films were made and stained with Wright's stain. The number of trypanosomes observed were recorded per 1000 erythrocytes.

Group B was composed of 10 chicks which had been treated with dexamethasone. On the first day of hatching, these

birds were injected with 0.1 ml of dexamethasone intraperitoneally. This dosage was repeated by the same route on the second day. The chicks were injected intraperitoneally with 0.5 ml of the compound on the fourth and fifth days after hatching. On the third day the birds were also injected intraperitoneally with 1 ml of the infected rat blood given to group A. Observations were made as outlined under group A.

Group C was treated with dexamethasone as in group B, but the birds were not injected with the trypanosomes.

Group D was uninfected, and was untreated.

SEROLOGY

AGGLUTINATION

To determine the agglutinating and immobilizing activity of sera, serial 2 fold dilutions were prepared and mixed with T. equiperdum. All experimental tubes contained 1 ml final dilution of the reagent mixtures. All serum dilutions refer to final dilutions in each tube. The antigen was the last addition to each tube. Experiments were carried out at room temperature and in 37° water baths. Trypanosoma equiperdum was collected from rats in the manner described above. After the last wash and centrifugation, the trypanosomes were resuspended in normal saline or in Hanks' balanced salt solution plus 0.2% glucose if the organisms were used alive. If the antigens were preserved the organisms were killed in twice their volume of a preserving fluid consisting of 90 parts 0.85% NaCl, 10 parts neutral glycerine, and 0.1 part formalin (Watson, 1912). The preserved antigens were kept under refrigeration at 5°C. Preserved antigens were centrifuged, and the preserving fluid removed and substituted with fresh salt solution to obtain the proper consistancy just prior to being used in tests. Nine volumes of suspending medium were added to one volume of centrifuged trypanosomes. Antigens which showed so-called autoagglutination were discarded.

Agglutination tests were performed by mixing 0.25 ml of serum or serum dilutions with 0.25 ml of trypanosomes suspended in salt solutions. Readings were made after 15 minutes standing at room temperature by examining a drop of the mixture from each tube on a Börner slide under a microscope. Large clumps of organisms were recorded as positive. Small scattered clumps were recorded as plus minus. No clumping was recorded as negative. Trypanosomes were noted as motile or non motile. The percent of motility was estimated with the aid of counts obtained with a hemacytometer.

Tests were performed on normal sheep serum, anti- T. equiperdum sheep serum, normal chick serum, serum from dexamethasone treated chicks, serum from untreated chicks which had been injected with the trypanosome, and serum taken from chicks which had been treated with the corticosteroid and then infected with trypanosomes.

If there were no reaction observed after 15 minutes standing at room temperature they were examined again after 30 minutes, 1 hour, 2 hours, 3 hours, and after 4 hours. Similar periodic examinations were made on those tests which were conducted at 37°C. If the reaction was negative after four hours the reagents were incubated overnight and the results of the test read within 12 hours.

PRECIPITIN RING TEST

Trypanosomes were prepared as for the agglutination tests in a suspension of normal saline or Hanks' balanced salt solution. The suspensions of trypanosomes were minced in a VerTis as follows. The microcup was continually bathed in a bath of salt water with ice. The suspension of trypanosomes was kept at a temperature of no more than -5°C. The VerTis was run at full speed for a total of 5 minutes. The material was then removed from the microcup and examined microscopically. Very few intact trypanosomes could be distinguished but structures resembling flagella and nuclei were observed. The material was repeatedly centrifuged until it was absolutely clear. This trypanosome extract was then immediately used or it was frozen at -18°C. During this study there was noted no apparent difference in frozen antigen and that antigen which was immediately used. Antigen kept frozen was generally used within a week.

Tubes 5 mm long and 0.5 mm in diameter were calibrated to contain approximately 0.5 ml. With a tuberculin syringe and a 20 gauge needle, 0.25 ml of clear antigen was added to the tubes. To each of these an equal amount of the antisera to be tested was added with Pasteur pipettes. The point of the pipette was passed through the antigen and rested upon the bottom of the tube.

The serum was released slowly and displaced the antigen fluid upward without mixing. The tubes were observed for the appearance of a thin white ring at the interface of serum and antigen after 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and after 12 hours. Three dilutions were used for each serum or pooled sera tested. They were undiluted, 1:5, and 1:10 dilutions.

HEMAGGLUTINATION

A modification of the hemagglutination test of Middlebrook and Dubos (1948) was used. One volume of a 2 percent suspension of normal chicken blood cells in Hanks' balanced salt solution was added to an equal volume of the trypanosome extract as prepared for the precipitin ring test. The mixture was incubated at 37°C for 1 hour, during which time the tube was shaken at 20-minute intervals. The cells were then washed once in saline, and finally resuspended in the original volume again constituting a 2 percent cell suspension. One drop of this suspension was added to each tube of the two fold dilutions of the test sera made up in 0.5 cc quantities. The tubes were shaken and left at room temperature overnight or for at least six hours. All tubes were than incubated at 37°C for 30 minutes in a waterbath and afterwards gently shaken to observe for clumps of cells. If clumps of cells

were not obvious the tubes were centrifuged in an International Clinical Centrifuge for 3 minutes. A clumping of cells was considered positive. If the cells rose evenly dispersed upon shaking the tube the test was considered negative. If the agglutination formed a rather uniformly thin carpet of cells that was relatively easily dispersed but did not rise evenly upon shaking, the test was considered plus minus. A drop of untreated chick cells was added to the undiluted serum and to the 1:4 dilution of serum. Normal chick serum, dexamethasone treated chick serum, serum from chicks which had been injected with T. equiperdum, serum from chicks which had been treated with dexamethasone and injected with T. equiperdum were tested.

NEUTRALIZATION

Sera of normal chicks and chicks which had been injected with T. equiperdum but no longer harbored the organism were studied for the presence of neutralizing properties. Sera were collected and pooled. The trypanosomes were titrated in Hanks' balanced salt solution with 0.2 percent glucose. The protozoans were harvested from the blood of heavily infected rats and washed in normal saline. They were examined for vitality and 10 fold dilutions were rapidly made in the glucose Hanks' balanced salt solution. The first tube contained approximately 5 ml of a suspension of approximately

2×10^{7} trypanosomes per ml and the last tube contained 5 ml of approximately 2×10^{-2} trypanosomes per ml.

Since it had been shown that sera of certain animals have antibodies against trypanosomes without having had direct contact with the organisms, or trypanocidal agents which were not considered antibodies (Thiroux, 1909 as reported by Taliaferro and Olsen in 1943; Culbertson, 1935; Warren and Borsos, 1959) preliminary tests were carried out on the sera of normal 6 day old chicks. Later tests were conducted on normal rat sera and sera from chickens which had been injected with T. equiperdum 48 hours before exsanguination but showed no signs of harboring the trypanosomes.

To 3 sets of 10 tubes, each tube containing 1 ml of pooled normal rat sera or 1 ml of pooled sera of chicks, or 1 ml of Hanks' balanced salt solution, was added 1 ml of the dilutions of trypanosomes making a series of final dilution of 1:2 of all sera tested, and a range of dilutions in series of trypanosomes from 1×10^{7} in the first tubes to 1×10^{-2} in the tenth tubes. These were incubated at 37°C for 1 hour. Twenty-five hundredths ml of the contents of each tube w[illegible] injected intraperitoneally into each of four white mice which individually weighed approximately 20 g. Each mouse was therefore given from the first tube of each group approximately 2.5×10^{6} trypanosomes.

The number of trypanosomes injected from the last tubes into each mouse would then be 2.5×10^{-3}. Mice were examined after 24 hours for the presence of T. equiperdum. The number of days before the trypanosomes were observed in the tail blood of mice and the deaths of mice in the test was recorded in Table XIII.

RESULTS

CULTURES OF T. EQUIPERDUM IN CHICK EMBRYOS

When T. equiperdum was cultured, in chick embryos in this study, it was observed that the organisms increased in number until about the fourth or fifth day. At this time the embryonic blood teemed with trypanosomes. The organisms appeared unusually fast in their movement. Also on the fourth or fifth day of infection trypanosomes began to clump and appear vacuolated; further dead trypanosomes in obvious numbers were seen floating about giving an appearance of "dead fish." Though no one embryo was examined daily, daily random examination strongly indicated that the cultures of T. equiperdum grown in chick embryos exhibit a diminution from the fifth day post-infection until the day of hatching or, as more frequently was the case, the day of the embryo's death. These observations were not quantitatively established, rather, qualitative comparisons were made of embryonic blood examined beginning the third day post-infection until death of the host or hatching occurred.

The eight serial passages were carried out with ease. Out of a total of 171 embryonated eggs injected with T. equiperdum during the passages, twenty-one chicks hatched uninfected. On two occasions only two eggs examined on the

fourth day post-infection were found negative for trypanosomes. Both of these embryos later became positive. This strongly indicated that although all of the 171 eggs were not examined, the 21 embryos that hatched free of infections were in all probability infected as embryos.

EXPERIMENT I

Chicks in this experiment were divided into groups and were administered prednisolone at various dosage levels and periods prior to the attempted infection. The chicks were examined in this experiment 48 hours after they had been injected with T. equiperdum for the presence of this parasite in their blood. No trypanosomes were observed in the wet mount preparation of peripheral blood or cardiac blood, nor could the parasite be detected on blood films stained with Wright's stain. As indicated in Table V, the trypanosomes were detectable by sub-inoculations of blood into white mice. T. equiperdum was isolated by this method from the blood taken from chicks of groups A, B, and C as early as 48 hours after the trypanosomes had been injected into the chicks. The latest the trypanosomes were detected to have been extant in chicks was 7 days at which time the course of the infection ceased to be followed.

TABLE V

RESULTS OF INOCULATING WHITE MICE WITH BLOOD OF CHICKS WHICH HAD BEEN TREATED WITH PREDNISOLONE AND INJECTED WITH T. EQUIPERDUM (EXPERIMENT I)

Group	Number of days after injection of trypanosomes into chicks before subinoculation	Number of days before T. equiperdum observed in peripheral blood of mice		Number of days after injection of chick blood before the death of mice	
A		Mouse$_1$	Mouse$_2$	Mouse$_1$	Mouse$_2$
Chicks treated with	2	7	-	9	-
prednisolone 10 mg/	3	6	6	6	6
kg wt per diem 2	4	5	5	6	7
days pre- and 2 days	6	6	-	7	-
post-infection	7	3	3	5	5
B					
Chicks treated with	2	7	7	7	12
prednisolone 10 mg/	3	6	6	6	7
kg wt per diem 5	4	5	5	6	6
days pre- and 2 days	6	3	3	4	4
post-infection	7	3	3	5	5
C					
Chicks treated with	2	7	7	7	7
prednisolone 5 mg/	3	6	6	6	7
kg wt per diem 2	4	5	5	6	6
days pre- and 2 days	6	3	3	4	4
post-infection	7	3	3	5	5
D					
Chicks treated with	2	-	-	-	-
prednisolone 5 mg/	3	6	6	6	6
kg wt per diem 5 days	4	5	5	5	5
pre- and 2 days post-	6	3	3	4	4
infection	7	3	3	6	6

EXPERIMENT II

Chicks in experiment II were treated with cortisone acetate. No trypanosomes were observed in the daily direct microscopic examinations of peripheral and cardiac blood of these chicks. Trypanosomes were recovered from groups A and B of this experiment from blood taken as early as 7 days and as late as 9 days after the organisms had been injected into the chicks.

Group C of this experiment was not treated with cortisone acetate but was given the inocula of trypanosomes. The same methods were used in attempts to recover the trypanosomes as in groups A and B, however, their presence was not detected. The birds in this group remained negative throughout the experiment. All of the birds listed in Table VI were observed for a period of eight weeks. There was an apparent slower rate of development of chicks in groups A and B when compared with the developmental rate of the chicks in group C. All chicks recovered from their infection and presented no signs of relapse.

EXPERIMENT III

Chicks in this experiment received prednisolone. The post-infection treatment was varied for groups A and B. Group C was not given any of the corticosteroid.

In group A no trypanosomes were seen when blood taken from the claws or hearts of these was observed as wet mounts under the microscope. As indicated by transfer of the organisms to white mice, T. equiperdum was isolated from the blood of chicks in this group as early as 2 days and as late as 10 days after the parasites had been introduced into the peritoneal cavity of the birds. Twelve days after the chicks had been injected with the trypanosomes, they were observed to be negative for the parasite. See Table VII. Chicks that were known to be positive for the trypanosomes recovered without relapse. The growth of chicks in this group was retarded when compared to birds which had not been treated with the prednisolone.

The presence of trypanosomes was directly observed in the blood taken from the claws of two birds in group B only on two days. Direct microscopy of peripheral blood of chick #5295 and chick #5340 revealed that these birds were heavily enough infected after 12 days that the trypanosomes were noted approximately every fourth field under high power objective. Trypanosoma equiperdum was again found the next day in these two birds. The characteristic movement appeared unchanged in the wet mount. The trypanosomes were encountered with rarity on the blood films made on the twelfth and thirteenth days after these birds had been initially infected with this

TABLE VI

RESULTS OF INOCULATING INTO WHITE MICE BLOOD OF CHICKS WHICH HAD BEEN TREATED WITH CORTISONE ACETATE AND INOCULATED WITH T. EQUIPERDUM

Group A: Chicks treated with 50 mg of cortisone acetate per kg wt seven days pre- and two days post-infection with T. equiperdum

Chick number	No. of days after injection of trypanosomes into chicks before sub-inoculation	No. of days before trypanosomes observed in tail blood of mice	No. of days after injection of chick blood before death of mice
5718	7	4	6
5732	9	3	4
5380	10	-	-
5800	12	-	-

Group B: Chicks treated with 20 mg of cortisone acetate per kilogram weight seven days pre- and a total of four days post-infection with T. equiperdum

4632	7	3	4
5704	7	3	5
4650	9	2	4
5733	9	2	4
5724	12	-	-
5753	12	-	-
5509	16	-	-

All chicks examined from group A 10 days after they had received trypanosomes were negative and remained so during six weeks of observation.

All chicks examined from group B 12 days after they had received the injections of trypanosomes remained negative during six weeks of observations.

Group C: All chicks in this group were untreated but T. equiperdum was inoculated as in group A and B. No trypanosomes were recovered from their blood.

organism. There was no apparent difference in the morphology of the trypanosomes grown in the chicks which had been treated with the corticosteroid and the trypanosomes that had originally been given in the inocula to chicks in this group.

Chicks in group C were not treated with any corticosteroid. The chicks were injected with T. equiperdum as in groups A and B. No trypanosomes were observed by direct microscopic examination. Sub-inoculations into white mice 48 hours after the trypanosomes were injected into the chicks resulted in no transference of the trypanosomes to the mice. Periodic sub-inoculations of blood taken from 12 different chicks over a period of 45 days revealed no trypanosomes. See Table VIII. Direct microscopic examinations were made on blood of all 24 chicks in this group, though only the blood of 12 chicks was used for mouse sub-inoculations.

When blood obtained from chicks 5321, 5329, 5378, and 5334, all of which were positive for trypanosomes, was injected into normal chicks, none of the chicks became infected with the organism.

TABLE VII

RESULTS OF INOCULATING INTO WHITE MICE BLOOD OF CHICKS WHICH HAD BEEN TREATED WITH PREDNISOLONE AND INOCULATED WITH T. EQUIPERDUM IN EXPERIMENT III

Chick number	No. of days after injection of trypanosomes into chicks before sub-inoculation	No. of days before trypanosomes observed in tail blood of mice	No. of days after injection of chick blood before death of mice
Group A:			
Chicks treated with 10 mg of prednisolone per kilogram weight 5 days pre- and two days post-infection with T. equiperdum			
5374	2	6	7
4648	2	4	5
5319	6	4	7
5357	8	4	5
5295	10	4	5
4649	12	-	-
5208	14	-	-
5718	16	-	-
5794	21	-	-
5728	28	-	-
5701	35	-	-
5357	45	-	-

TABLE VII. Continued

Group B: Chicks treated with 10 mg of prednisolone per kilogram weight per diem 5 days pre-infection. Similar dosage was given on two consecutive days post-infection followed by three injections of prednisolone separated by one day and then two more injections separated by two days and four days. See Table III

5321	6	4	8
5329	8	4	7
5378	10	3	6
5342	12	3	5
5334	12	3	4
5769	14	8	9
5340	16	5	7
5205	16	5	7
6540	21	-	-
6503	28	-	-
5324	35	-	-
5315	45	-	-

Group C: This group of chicks was not treated with the corticosteroid but was inoculated with T. equiperdum

5599	2	-	-
5292	4	-	-
6451	6	-	
5281	8	-	-
6510	10	-	-
5116	12	-	-
5309	21	-	-
5762	28	-	-
5703	35	-	-
5701	35	-	-
5296	45	-	-
5367	45	-	-

EXPERIMENT IV

Trypanosoma equiperdum was transfered to 2 white mice which had been sub-inoculated with blood taken from a chick in group A only 24 hours after the bird had been injected with the trypanosomes. The prepatent period in these mice was 4 days. Ten chicks were bled daily from the claws. The blood was drawn into heparinized capillary tubes (1.4 OD, 75 mm long) and centrifuged in an International Clinical Centrifuge. Trypanosomes were found in the top layers of cells when the capillary tubes were broken just above or below the area of separation of blood cells and plasma and subsequently examined under the microscope. Since by this method trypanosomes were successfully demonstrated in the blood of chicks, sub-inoculations were resorted to only when no trypanosomes were observed in centrifuged blood of chicks.

Trypanosomes were found in the blood which had been centrifuged as early as 2 days after the chicks had been infected. The parasites were found as late as 13 days after the birds had been infected with T. equiperdum. Blood sub-inoculated into white mice from this group revealed that the chicks were still infected as late as 15 days post-infection.

The results of the infection in group A are outlined in Table IX. Some of the chicks in the early part of the experiment were considerably less active than others. The deaths

of individual chicks could be predicted. Morbid chicks appeared very sleepy, were lame and isolated themselves from the rest of the flock, and appeared to have suffered from a conjunctivitis. Massive numbers of trypanosomes were never observed in the blood of such chicks, however, the organisms could be detected without centrifugation. Blood taken from moribund chicks did not show large numbers of trypanosomes. It in fact was difficult to find trypanosomes in the centrifuged blood of birds which were dying or those which had just recently died. Most of the chicks showed no symptoms and in general carried on activities which exemplified healthy birds. Many of the birds after the experiment had been in progress for approximately 2 weeks became hyperactive, and some of them even flew out of the brooder when the grating was removed, suprisingly uninhibited by the presence of their examiner. Growth of the birds and development of feathers were considerably less in this group than in groups C and D which did not receive the corticosteroid.

Group B was treated with dexamethasone only. No deaths occurred in this group. The chicks appeared very active and often escaped from the brooder en masse when it was opened.

Group C was injected with the trypanosomes but was not treated with the corticosteroid. No trypanosomes were observed in centrifuged blood. No mice were positive for

trypanosomes after they had been sub-inoculated with blood taken from the hearts of the chicks. Mice were sub-inoculated 1 day after the injection of trypanosomes into the chicks. These were repeated 12, 14, 15, 17, 19 and 21 days after the birds had been injected with the parasites.

Group D was composed of normal untreated uninfected chicks. They simply waxed during the experiment.

EXPERIMENT V

Trypanosomes were found in the peripheral blood of chicks 15 minutes after the organisms had been inoculated. The trypanosomes looked in every way similar to those found in the tail blood of the mice which were also found 15 minutes after they had been injected with similar inocula. All the chicks remained alive after the first injection. The parasites were found to be active in their blood up to 36 hours in four out of the six chicks. Though the trypanosomes were present and active in their blood, it was noticed that the parasites had begun to appear vacuolated and clumped together. The motility of the organisms was noticeably lessened. Many of the trypanosomes were observed floating like "dead fish" in the mixture of blood and normal saline. Blood taken from 2 of the chicks at this time was still infective for mice. Trypanosomes were first observed seven days after the rodents had

TABLE IX

RESULTS OF EXAMINATIONS OF CENTRIFUGED CHICK BLOOD FOR THE PRESENCE OF TRYPANOSOMES, THE CUMULATIVE MORTALITY OF CHICKS TREATED WITH DEXAMETHASONE AND SUBSEQUENTLY INFECTED WITH T. EQUIPERDUM, THE PREPATENT PERIOD IN DAYS AND DEATH OF MICE SUB-INOCULATED WITH INFECTED CHICK BLOOD

Number of days post-infection of treated chicks	Cumulative mortality of chicks	Percent chicks positive for trypanosomes	Prepatent period		Day of death after inoculation	
			$Mouse_1$	$Mouse_2$	$Mouse_1$	$Mouse_2$
1	1		4	4	5	5
2	2	60				
2	3	80				
4	3	80				
5	4	60				
6	5	100				
7	6	100				
8	8	40				
9	8	20				
10	8	40				
11	8	20				
12	8	0	5	5	5	5
13	8	1				
14	8	0	6	6	8	8
15	8	0	6	6	7	7
16	8	0	-	-	-	-
17	8	0	-	-	-	-
19	8	0	-	-	-	-
21	8	0	-	-	-	-

been sub-inoculated. The mice died the following day.

There were no trypanosomes found in the blood of these chicks after 40 hours. White mice sub-inoculated with blood taken at this time remained negative. The trypanosomes had completely disappeared after 40 hours according to tests used for the detection of T. equiperdum in this study.

Only four chicks were successfully infected intraperitoneally with T. equiperdum 74 hours after the first injection using 6 chicks. Two of the 6 immediately died after the second inoculation. The chicks first appeared choked, they regurgitated and suffered spasms for a very short time. The remaining 4 birds suffered no such effects. Trypanosomes were observed after 4 hours by direct microscopic examination of the blood taken from the remaining four birds. Trypanosomes were not found again during a 49 hour period of observation.

Forty-eight hours after the second injection of parasites, the chicks were injected with 0.5 ml of trypanosomes contained in Hanks' balanced salt solution. No trypanosomes were found by direct microscopic examination within 48 hours. Two chicks were bled from the heart at this time and their blood sub-inoculated into white mice. The mice remained negative for 3 weeks and were discarded. The chicks remained healthy for another two weeks, and periodic examination revealed no trypanosomes. The chicks were discarded after 2 weeks.

TABLE X

RESULTS OF EXPERIMENT VI. THE NUMBER OF TRYPANOSOMES PER THOUSAND ERYTHROCYTES AND THE TIME OF DEATH OF INDIVIDUAL CHICKS

Chick number	Time in hours post-infection						
	4	8	12	18	24	36	48
	Number of trypanosomes per thousand erythrocytes						
	Group A (treated with dexamethasone)						
370	5	5	3	4	3	3	2
371	15	10	0				
372	10	9	10	8	9	7	8
373	9	8	6	6	5	6	6
374	7	8	6	6	5	4	5
375	7	3	5	1	1	4	3
376	6	1	1	1	3	2	3
377	8	5	6	5	5	4	5
378	0	1	0	0	0	1	0
379	6	7	6	9	Dead		
	Group C (no treatment)						
201	7	5	0	2	0	0	0
202	7	4	4	0	0	0	0
203	0	0	0	0	0	0	0
204	1	1	1	0	0	0	0
205	5	4	5	4	0	0	0
206	15	15	13	13	Dead		
207	1	0	0	0	0	0	0
208	8	7	8	5	0	0	0
209	9	9	7	7	0	0	0
210	10	9	10	8	0	0	0
211	0	0	0	0	0	0	0
212	7	8	6	6	7	0	0
213	2	1	1	0	0	0	0

EXPERIMENT VI

Trypanosomes were found in the blood of most birds in group 0 up until 24 hours after they had been injected. Three of the chicks were positive for trypanosomes after 36 hours and one of these remained positive for as long as 48 hours. When trypanosomes were found in the chicks after 36 hours they appeared vacuolated, clumped, and many were inactive.

Trypanosomes were found in the blood of most birds in groups A and C over a period of 18 hours. After 18 hours the trypanosomes began to disappear very rapidly from group C. It was noted that a decline in number of trypanosomes continued at a slower rate in chicks treated with dexamethasone. After 24 hours the decline ceased. The course of the infection along with the deaths of individual chicks may be seen in Table X. Figure 1 represents the average parasitemia suffered in groups A and C.

No deaths occurred in group B which was only treated with dexamethasone. Birds in group D were uninfected and untreated. They remained quite healthy.

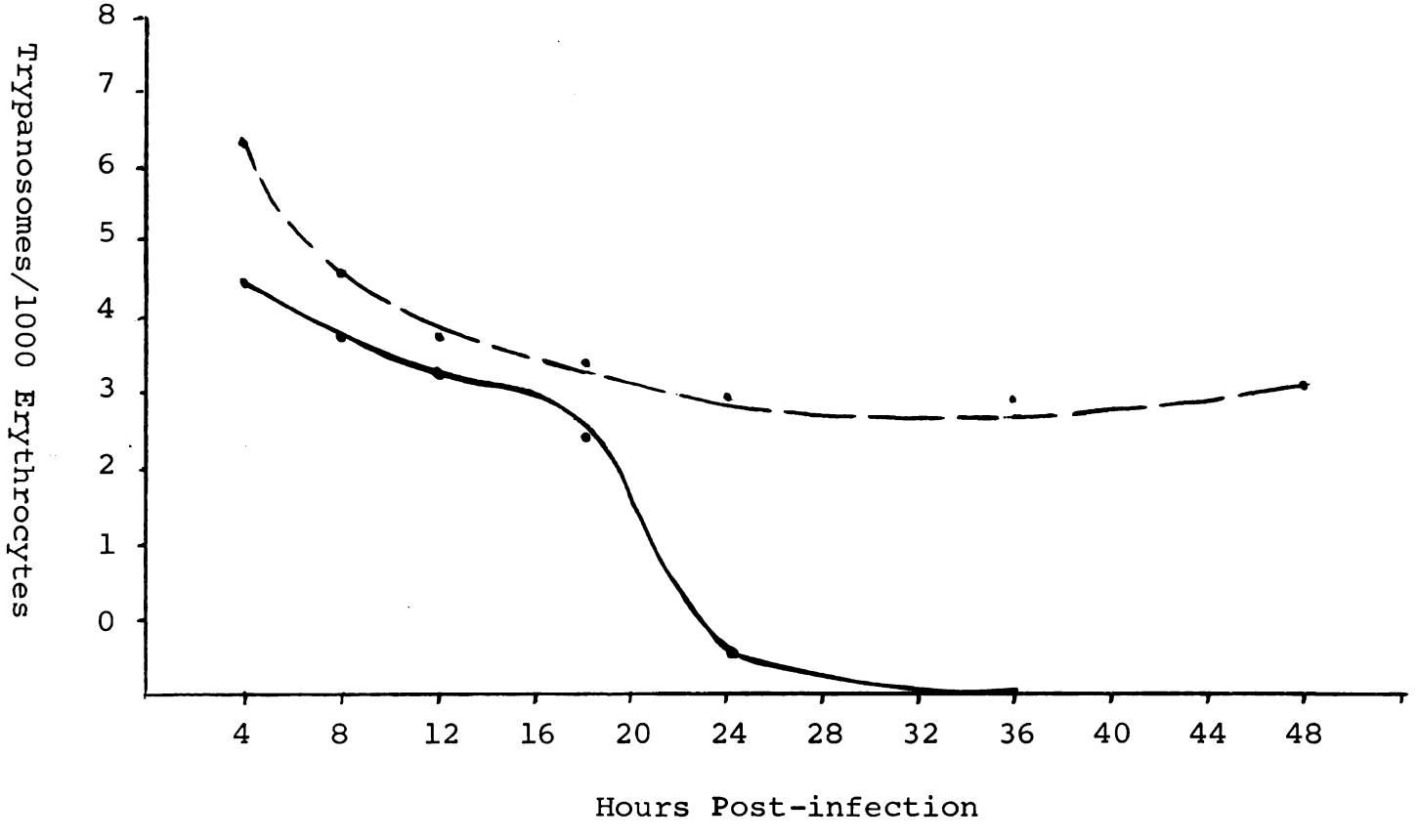

Fig. 1. Parasitemia curve of Experiment VI. The broken line represents a parasitemia in dexamethasone treated chicks. The unbroken line represents the average parasitemia in untreated chicks.

AGGLUTINATION

When preserved trypanosomes were mixed with sera taken from sheep which had been infected with the trypanosomes, a definite agglutination occurred in all dilutions of serum used in experiments carried out at laboratory temperature and at 37°C. At laboratory temperature, serum from a normal sheep and sera taken from chicks which had been injected with trypanosomes two weeks previously gave weak positive reactions in the first and second dilutions of sera and in the first sera dilution respectively. Serum taken from chicks which had been injected with the trypanosomes 48 hours before gave definite positive agglutination in the first serum dilution, but only a weakly positive reaction was observed in the second dilution, and all other serum trypanosome mixtures at laboratory temperature were negative. (See Table XI.) When reaction mixtures were incubated at 37°C, serum taken from the normal sheep agglutinated the trypanosomes in the first dilution but gave only a weak positive reaction in the second dilution. Sera taken from chicks which had been injected with trypanosomes 48 hours previously gave a definite agglutination in the first dilution but were weakly positive through the fourth dilution after which they were entirely negative. All other sera gave no reaction. (See Table XI.)

TABLE XI. SERUM AGGLUTINATION OF TRYPANOSOMA EQUIPERDUM

Serum dilutions	1:2			1:4			1:8			1:16			1:32		
	Time in minutes	Agg	Mot	Time in minutes	Agg	Mot	Time in minutes	Agg	Mot	Time in minutes	Agg	Mot	Time in minutes	Agg	Mot
TYPE SERUM	Living Trypanosomes 37° C														
Normal chick	240	-	85	240	-	85	240	-	85	240	-	40	240	-	35
"Infected chick"	240	+	10	240	+	55	240	±	55	240	±	10	240	±	1.5
Hanks' balanced salt solution plus 2% dextrose	240	-	0	240	-	0	240	-	0						
	Preserved Trypanosomes, Laboratory Temperature														
Normal sheep	720	±		720	±		720	-		720	-		720	-	
Infected sheep	30	+		30	+		30	+		30	+		30	+	
Normal chick	720	-		720	-		720	-		720	-		720	-	
Dexamethasone treated chick	720	-		720	-		720	-		720	-		720	-	
Chick infected 2 wks previously	720	±		720	-		720	-		720	-		720	-	
Chick infected 48 hrs previously	720	+		720	±		720	-		720	-		720	-	
Chick treated with dexamethasone and infected 48 hrs previously	720	-		720	-		720	-		720	-		720	-	
	Preserved Trypanosomes 37°C														
Normal sheep	720	+		720	±		720	-		720	-		720	-	
Infected sheep	30	+		720	+		720	+		720	+		720	+	
Normal chick	720	-		720	-		720	-		720	-		720	-	
Dexamethasone treated chick	720	-		720	-		720	-		720	-		720	-	
Chick infected 2 wks previously	720	-		720	-		720	-		720	-		720	-	
Chick infected 48 hrs previously	720	+		720	±		720	±		720	±		720	-	
Chick treated with dexamethasone and infected 48 hrs previously	720	-		720	-		720	-		720	-		720	-	
Normal saline	720	-		720	-		720	-		720	-		720	-	

Agg = agglutination
Mot = motility

When living trypanosomes were used as antigen, serum obtained from chicks 48 hours after they had been injected with T. equiperdum agglutinated the trypanosomes after four hours incubation at 37°C. Ninety percent of the trypanosomes had at this time become nonmotile in serum dilutions of 1:2. Lysis, as such, was questionable; however, the trypanosomes were highly vacuolated. The organisms retained enough of their morphology not to be designated as having been lysed. Definite agglutination occurred in the 1:4 dilution. Doubtful positive agglutinations were observed in dilutions through 1:16. As can be seen from Table XI the motility of the trypanosomes is greatly reduced in the sera obtained from the chicks which had been injected with the trypanosomes 48 hours previously.

No agglutination occurred in normal chick serum and motility was not obviously affected until the serum was diluted 1:16. No agglutination occurred in the salt solution and the trypanosomes died very shortly.

PRECIPITIN RING TEST

Only the serum obtained from a sheep which had an infection with T. equiperdum gave positive ring tests. Normal sheep serum was negative as were all chick sera tested.

HEMAGGLUTINATION

When sera from chicks which had been injected with T. equiperdum 48 hours previously were mixed with erythrocytes coated with extract of T. equiperdum and incubated under the conditions described, the erythrocytes agglutinated in dilutions of sera as high as 1:16, 1:32, and 1:4. Doubtful positives were registered in the next higher dilutions for each of these. Untreated cells were not agglutinated by any of the sera diluted 1:2; further, all saline controls were negative.

NEUTRALIZATION

When sera from normal chicks were pooled and incubated with Trypanosoma equiperdum at 37°C for one hour and then injected into mice, all of the mice became infected and died within 3 to 4 days.

When large numbers of trypanosomes (2.5×10^6) were diluted 10 fold down through 2.5×10^4 trypanosomes did not infect mice; however, more concentrated dilutions did produce infections in mice. (See Table XIII.) When mice were inoculated with 2.5 trypanosomes which had been incubated in rat serum half of the mice in that series became infected and died. Trypanosomes in a concentration of 2.5×10^3 which were incubated with chick sera taken 48 hours after T. equiperdum had

TABLE XII

AGGLUTINATION OF SENSITIZED RED CELLS BY THE SERA OF CHICKS WHICH HAD BEEN INJECTED WITH *T. EQUIPERDUM* 48 HOURS PREVIOUSLY

Dilutions of sera in saline	Infected chick sera			Pooled normal chick	
	1	2	3	4	Saline control
1:2	++	+++	++	-	-
1:4	+	++	+	-	-
1:8	+	++	±	-	-
1:16	±	+	-	-	-
1:32	-	+	-	-	-
1:64	-	±	-	-	-
1:128	-	-	-	-	-
1:2 with untreated red cells	-	-	-	-	-

TABLE XIII

RESULTS OF NEUTRALIZATION EXPERIMENT

Series I

Hanks' balanced salt solution + 0.2% dextrose

Day of observation	Final dilution of sera 1:2 Approximate number of trypanosomes per 0.25 ml									
	2.5×10^{6}	2.5×10^{5}	2.5×10^{4}	2.5×10^{3}	2.5×10^{2}	2.5×10^{1}	2.5×10^{0}	2.5×10^{-1}	2.5×10^{-2}	2.5×10^{-3}
	1	2	3	4	5	6	7	8	9	10
1	-	-	-	-	-	-				
2	4+/4	2+/4	-	-	-	-				
3	4+/4	4+/4	2+/4	-	-	-				
4	4D/4	1D/4	2+/4	-	-	-				
5		4D/4	4+1D/4	-	-	-				
6			4D/4	-	-	-				
7				-	-	-				
8				-	-	-				
9				-	-	-				
10				-	-	-				
Summary	4D/4	4D/4	4D/4	-	-	-				

+ = trypanosomes in blood - = no trypanosomes D = dead

TABLE XIII. Continued

Series II
Normal rat sera pooled

1	4+/4	2+/4	-	-	-	-	-	-	-	-
2	4+/4	3+/4	2+/4	-	-	-	-	-	-	-
3	4D/4	2D/4	4+/4	4+/4	1+/4	-	-	-	-	-
4		4D/4	4D/4	4+/4	4+/4	1+/4	-	-	-	-
5				4D/4	1D/4	2+/4	-	-	-	-
6					4D/4	3+1D/4	-	-	-	-
7						3D/4	-	-	-	-
8						-	2+/4	-	-	-
9						-	2+/4	-	-	-
10						-	2D/4	-	-	-
Summary	4D/4	4D/4	4D/4	4D/4	4D/4	3D/4	2D/4	-	-	-

Series III
Pooled chick sera taken 48 hours after T. equiperdum had been injected into birds

1	-	-	-	-	-	-	-	-	-	-
2	4+/4	3+/4	-	-	-	-	-	-	-	-
3	4+/4	1+/4	-	-	-	-	-	-	-	-
4	4D/4	2D/4	4+/4	-	-	-	-	-	-	-
5		4D/4	1D/4	1+/4	-	-	-	-	-	-
6			4D/4	3+/4	-	-	-	-	-	-
7				3D/4	-	-	-	-	-	-
8					-	-	-	-	-	-
9					-	-	-	-	-	-
10					-	-	-	-	-	-
Summary	4D/4	4D/4	4D/4	3D/4	-	-	-	-	-	-

been injected in the birds were infective for 3 out of 4 mice in a concentration of 2.5×10^3, and did not infect mice in the next lower concentration or any thereafter.

DISCUSSION AND CONCLUSIONS

The susceptibility of the chick embryo to laboratory infections with T. equiperdum was obvious in this study. The ease of serial transfer was in some respects a repetition of the work by Hood (1949b). Further, some of the observations on cultures of this parasite in the chick embryo extend those made by Hood and other investigators. It was observed that a similar diminution of parasitemia occurred beginning the fifth day after the chick embryos were infected with T. equiperdum, as was reported by Chabaud (1939) when he cultured T. rhodesiense in the avian embryo. Insufficient data for discussion were collected to assert that all of the twenty-one chicks which hatched from eggs that had been inoculated with T. equiperdum had known infections as embryos. It is significant that from no one of these twenty-one chicks were trypanosomes recovered. One of the objects of infecting embryonated eggs with this protozoan was to hatch chicks infected with it as did Hood (1949b) and Mitchel et al. (1939). For this reason at least four or more eggs of each series were not examined during incubation for the parasites for fear of interfering with their hatchability. Evidence strongly indicated, however, that a major portion of these hatched chicks were infected as embryos since approximately 100% of all embryos examined, but not hatched, were infected. On only

two occasions did Hood find a chick which hatched free of trypanosomes though it had as an embryo a known infection.

The chick embryo is considered immunologically immature. Its tolerance for foreign antigens supports this concept (Burnet, 1958) making it difficult to explain the clumping, vacuolation, diminution and death of trypanosomes grown in the chick embryos as reported here. Possible explanations are: (1) the embryo may not be completely inept in the recognition and destruction of foreign antigens, (2) the trypanosomes may have been responding to adverse conditions caused by morbid or moribund embryos and (3) the trypanosomes due to their increase in number may have depleted some component of the substrate present previously in the healthy embryo which was vital to the reproduction and survival of the parasite.

The observation of agglutination, vacuolation, and death of the parasite in the later stages of the infection in the chick embryo appear to parallel the findings of Hood (1949b) where she observed that embryos infected with T. brucei, and T. hippicum remained infected only briefly after the eggs hatched, and that the trypanosomes usually agglutinated and appeared vacuolated prior to their sudden disappearance from the young chicks.

Warren and Borsos in 1959 demonstrated by immunological methods that fowl sera contain at least two factors against the crithidia of Trypanosoma cruzi; a heat-stable factor apparently strongly agglutinating and sensitizing antibody and a heat labile factor considered to be complement. They found the antibody present in all sera of mature normal birds tested. It was transmitted by the hen to the yolk and thence to the blood of the embryo. When the birds were hatched and reared under germ free conditions, the antibody was present in their sera until shortly after hatching. Once the antibody was lost in germ free birds, it did not reappear. The lytic factor was present in the sera of germ free birds as well as normal birds.

Thus there exists the possibility that the embryo or young chick may have antibodies passively transfered to it against certain of the trypanosomes. These antibodies are due probably to a cross antigen response and not to direct contact with trypanosomes. Hosodat et al. (1955) found that antibody transfered passively by the hen appears to be available in the circulation of the blood of the embryo only during the latter stages of its development or at least increase in titer at this time. The observations of Chabaud, Hood, as well as those presented in this study may conceivably have their explanations in passive immunity; the antibody

being developed in response to common antigens. No evidence is presented here to support this idea. More experimental data than are now available must be accumulated before very much of anything can be said with certainty.

Chen et al. in 1945 studied the parasitemia and length of survival of mice infected with T. equiperdum. Hood (1949a) made comparative studies of this parasite in a variety of rodents including mice. Cantrell (1953-1959) studied the generation time, antigenic variation, mutation rate, and the duration of acquired immunity in rats infected with T. equiperdum. All of these investigators observed that in experimental infections individual variations are found within a group of susceptible hosts and that the inoculating dose of trypanosomes is recognizable by the time of appearance of the organisms in the blood of the rodents. Both rats (Cantrell, 1959) and white mice (Chen, et al, 1945) have little or no resistance to infections with T. equiperdum. The prepatent period in white mice is relatively short and the infection is fatal. Chen, et al. reported that the length of survival, the logarithm of parasitemia and the duration of infection are lineally related. They derived empirical formulae from which the survival time or degree of parasitemia might be calculated for the inoculating dose of trypanosomes. They found that individual variation in the survival time of mice infected with

T. equiperdum was independent of the dose of trypanosomes used in an inoculum. The deviation of survival time in groups of mice receiving different doses of trypanosomes was about the same. They concluded that the duration of infection was not a contributing factor to the survival time. They explained this by the fact that there is no acquired immune reaction in mice to infections with T. equiperdum. Actually, in 1904 Ehrlich and Shiga reported that if mice infected with trypanosomes are treated with trypan red, they do show resistance to reinfection. The resistance was not due to persistence of drug; rather, it was immunological.

As in rats the reproductive cycle of T. equiperdum in mice is about four hours (Chen, et al. 1945). They reported that this organism when inoculated intraperitoneally, required six to seven hours before the number of trypanosomes in circulation equaled the dose used for the infection. Reproduction followed a geometric progression.

If initially the parasitemias in white mice infected with T. equiperdum with blood taken from chicks which are infected with this parasite are dependent upon the number of trypanosomes injected, then the prepatent period in white mice is a reflection of the number of trypanosomes circulating in the blood of the chick at the time the infective blood was obtained and sub-inoculated.

In the first experimental infections of chicks there were no reliable untreated controls. However the experiment was not valueless insofar as it can be seen that when chicks infected with T. equiperdum were treated with five to ten mg of delta-1-hydrocortisone per kg weight for five to two days pre-infection and for two days post-infection, Trypanosoma equiperdum was isolated from the blood of the birds as early as 48 hours and as late as seven days post-infection. Further the prepatent period in the mice was lessened and the time of death hastened when they were sub-inoculated with blood obtained from chicks on the sixth and seventh days post-infection as compared with the prepatent period and death of mice sub-inoculated with blood obtained from chicks on the second and third days post-infection. The number of trypanosomes apparently had increased in the blood of the chicks between the second and seventh days.

The dosage levels of corticosteroid and periods of pre-infection treatment were varied in an attempt to arrive at a proper protocol for future use as well as to ascertain the differences in susceptibility of chicks so treated. The questions were not neatly answered by experiment I. There were no great differences noted in susceptibility to T. equiperdum when treatment was varied between five and two days pre-infection. Further the dosage levels appeared to have

resulted in no striking differences in susceptibility in this experiment.

In the second experiment cortisone acetate was injected into six-day chicks per diem for seven days prior to their being injected with T. equiperdum. Since in the first experiment trypanosomes were recovered from chicks as late as seven days post-infection, it was decided that no sub-inoculations in experiment II would be made prior to seven days after the birds had been injected with the parasite. Trypanosomes were first recovered from blood taken seven days post-infection. The last day on which trypanosomes were recovered from their blood was nine days post-infection. At no time did the parasitemia rise high enough to be detected by direct microscopic examination. This was consistent with the previous experiment and with the results obtained by other workers who attempted infections of various avian hosts with trypanosomes normally considered to be mammalian parasites. Though the different post-infection treatments with cortisone acetate showed no experimental difference in the duration of the infection, it was apparent that the prepatent period in mice was shorter when they were sub-inoculated with blood obtained from chicks in group B indicating the presence of a greater number of trypanosomes in the chick from which the sample was taken. The effect of continuous post-infection treatment cannot

really be determined from the data in this experiment.

At no time were trypanosomes recovered from chicks which did not receive a corticosteroid in group C of experiment II. The results obtained from this group of chicks are consistent with those of Hood (1949b) and Kuigumgift (1937). Von Brand, Tobie, and Mehlman in 1951 found that: cortisone had no detectable influence on trypanosomes in vitro, nor did it change the rate of development of the organism in vivo. Therefore the difference in host susceptibility to T. equiperdum groups of chicks treated with cortisone acetate and group C which was not treated with the steroid can only be accounted for by assuming that the resistance of the treated chicks was modified. The disappearance of the trypanosomes after nine days was probably due to the lowered level of cortisone with consequent return to the normally more resistant host system.

Further substantiation of the modification of the resistance to T. equiperdum in chicks was appreciated when this parasite was isolated from chicks which had been treated for five days pre-infection and two days post-infection with delta-1-hydrocortisone in experiment III. When mice were sub-inoculated with blood from group A, trypanosomes were recovered from chicks which had been infected for 48 hours at the earliest and 10 days at the latest. When treatment was continuous in group B of this experiment III, trypanosomes

were recovered as late as sixteen days post-infection. The highest level of parasitemia appeared to have been between the tenth and twelfth days post-infection after which there was a drop and eventual complete removal of trypanosomes as indicated by sub-inoculated mice. It is clearly evident that continued treatment with delta-1-hydrocortisone repressed resistance longer. Withdrawal of the compound was reflected by the disappearance of the organism from the chicks. This again indicated that if the level of the corticosteroid is not high enough the mechanisms of resistance are mobilized with the resultant removal of the trypanosome. This observation is in agreement with the work done by Ward and Johnson (1959).

Though chicks in group C, the untreated group, were bled as early as 24 hours and as late as 45 days after they had been injected with T. equiperdum, no trypanosomes were detected in their blood at any time during the experiment. The difference in susceptibility to T. equiperdum was repeated only more clearly between normal chicks and those treated with a corticosteroid.

When blood was obtained from chicks which had proven infections, as indicated by sub-inoculations of mice, and injected into normal chicks, no infection with T. equiperdum resulted. This indicated that the trypanosomes had not been

so modified in this experiment that they could establish infections in normal chicks. The failure of the trypanosomes to infect these normal birds lends support to the idea that the infections observed in chicks treated with the corticosteroid was not a result of a change in virulence of the organism but rather a modification of the host resistance to this parasite.

The discrepancy of growth and development of chicks treated with delta-1-hydrocortisone and chicks which were not treated with the corticosteroid may be pertinant to any explanation of the parasitological problem here involved. The inhibition of growth is just one of the many manifestations of the effect of the compound in chicks. The relationship between the inhibition of protein synthesis and inhibition of growth is obvious as is the association between protein synthesis and antibody production.

It was evident as early as the second day after the trypanosomes were injected in the chicks in experiment IV that dexamethasone was highly effective in depressing the resistance which chicks normally have against T. equiperdum.

Though the method of examining the blood of infected chicks with the aid of the centrifuge did not give quantitative values of individual infections, it did adequately determine the presence of trypanosomes and was an indication of the

extent of the infection within the group. A total of eight infected chicks died which had received dexamethasone. When the withdrawal of the compound began, the expected cessation of deaths and drop in the number of chicks in whose blood trypanosomes were found was noted. The sudden drop in observed infections on the eighth day post-infection might be expected in light of the other experiments in this study. It is significant that the blood of chicks, in which trypanosomes were demonstrable after the eighth day, was so populated with the organisms that they were found without the aid of centrifugation. There was an apparent crisis between the eighth and tenth day post-infection which removed organisms from the blood. However some trypanosomes appear to have resisted removal and though they were not found by direct observation, their presence was established by the transference of the infection to mice.

It is concluded that all three of the corticosteroids used in this study permit transient infections with T. equiperdum in chicks. The refractoriness of chicks to this trypanosome was repeated again and again in normal chicks.

It was thought that repeated exposure to large numbers of trypanosomes might depress the resistance of the chicks or be of sufficient numbers to overcome the resistance. This effect was not observed. Rather the chicks tenaciously

resisted infection when large numbers of trypanosomes were repeatedly injected. The repeated observations of clumping, vacuolation, and death in the parasite population was highly significant. The rapid disappearance of organisms in the chicks after the first and second inoculations was dramatic. It was easy to find the organisms when blood was obtained four hours after the second injection of trypanosomes and microscopically scanned. These organisms completely disappeared between the fourth and eighth hour. The organisms in the third inoculation disappeared entirely and directly. It is impossible not to observe that this experiment was an example of protection almost directly observed in action. The several injections appeared to have enhanced the resistance of the chicks to T. equiperdum. It became apparent that definite factors appear in the blood of chicks after a short time and with swift efficiency eliminate the trypanosomes. The origin and real nature of these factors is unknown. It is believed that these reactions visibly observed in the young chick, when large numbers of trypanosomes were injected, were reactions of the same type which occurred in control chicks in previous experiments when smaller numbers of organisms were injected, but more dramatically demonstrated.

Antigen elimination has been employed by many workers as a technique to indicate antibody formation. It is difficult

to imagine that the elimination of the trypanosomes in the infected chick after hatching was due to the formation of antibodies unless new antibodies can be stimulated, appear, and check an infection within 24 to 36 hours. In all the other experiments in this study it was observed that enhancement of infection occurred very early in cortisone treated animals as compared with normal or conventional animals. In the present study when antigen elimination was employed as a method to demonstrate antibody formation there was after only four hours post-infection a discrepancy between the elimination of the organisms by the untreated group of chicks when compared with the group treated with dexamethasone. Both groups showed an initial rapid decline as was expected. Adjustment periods between a host and parasite results, as a rule, in the death and destruction of some of the parasites.

The chicks in experiment VI were treated with dexamethasone three days prior to injection with the organism and would be expected to have had a high level of corticosteroid in the tissues and blood and a corresponding lowered resistance. Elimination of the trypanosomes in the treated chicks ceased between 20 and 24 hours. After 24 hours trypanosomes were no longer observed by direct microscopic examination in the blood of the untreated groups. The observation that the production of antibodies accelerates the removal of the specific

antigen was experimentally observed in 1918 by Longcope, W. [illegible] and Rakens. They found, in patients who had been treated with horse serum, that the appearance of antibodies in their sera in high concentration caused a rapid disappearance of the antigen. Mackenzie and Leake in 1921 found that individuals who were good precipitin formers against foreign serum had severe serum disease, and that there was a corresponding decrease in circulation of precipitinogen. Precipitinogen persisted in the circulation of patients in whom little or no precipitin was demonstrated. It was postulated that in such individuals the production of antibodies was prevented. Erlich suggests in formulating his side-chain theory that when cells fail to take up foreign protein, the protein apparently may remain in an individual's circulation until it is slowly disposed of by some other mechanisms (1900).

Accelerated elimination of antigen is interpreted by Gleny and Hopkins (as reported by Talmage, et al. 1951) as evidence of antibody production. Talmage, et al (1951) concluded that this method provides valuable information concerning the onset of infection and early antigen excess phase of antibody production otherwise difficult to obtain. Information concerning the quantitative production of antibody cannot, however, be ascertained by this method.

By use of antigen elimination techniques Ward and Johnson in 1959 demonstrated the inhibition of the primary response in rabbits treated with cortisone. Their data indicated that the inhibition of antibody production which they observed was exerted early in the sequence of events which lead to antibody synthesis.

While the reality of the high susceptibility of the chick embryo to infection with T. equiperdum, the powerful resistance of hatched chicks to infection with this parasite, and the modification of this resistance with cortisone acetate, delta-1-hydrocortisone, and azium cannot be doubted, the mechanisms responsible for this behavior are not clearly expressed. Kass and Findland (1953) in summarizing the mechanisms involved in the decreased resistance of infection in animals which had been subjected to cortisone and ACTH treatment lists:

I. Inhibition of the inflamation
   A. Decreased capillary permeability
   B. Decreased cellular exudation and infiltration
   C. Decreased exudation of fluid and protein
   D. Decreased phagocytosis by polymorphonuclear leukocytes
II. Inhibition of antibody production
III. Negative nitrogen balance
IV. Alteration of reticulo-endothelial function.

The literature indicates that there are alterations of the function of the reticulo-endothelial (RE) system in cortisone treated animals. The RE cells are regarded as a major

defense mechanism against infection in general. When bacteria are injected into laboratory animals the organisms are removed from the blood stream within one to two hours and engulfed by the RE cells. Not only are these cells thought to function in the mechanical removal of foreign substances; but even more important the RE tissues, under varietal influences, are believed to produce the substances which are directly or indirectly involved in mechanisms of antibody formation. The RE system is also a source of cells which are involved in inflamation (Thomas, 1953). No doubt the resistance of the chick, as it is for other hosts, is dependent to some extent upon phagocytoses of parasites by various leucocytes, particularly macrophages; and the production of new phagocytes and various reparative proliferations (Taliafferro, 1948).

That cortisone depresses the inflamation reaction is an unexplained but accepted fact. It is understandable that a local inflamatory response depressed in cortisone-treated animals may augment the spread of organisms from infected tissues to the general circulation, but it is difficult to depend upon this observation to explain how the corticosteroids enhanced the trypanosomal infection in chicks where there is no obvious local inflamation. Changes in permeability cannot be held accountable for the differences noted in the susceptibility of treated birds and untreated-birds. Alterations

of inflamation of tissues might be explained in this way but, the events of systemic protozoan infections such as trypanosomiasis do not readily lend themselves to this kind of explanation.

Kass and Finland (1952) suggested that the role of nitrogen balance is difficult to assess. Protein deficiency may increase susceptibility to infection in some diseases in which the effects of adrenocortical steroids have been studied. Clark et al. (1949) found however, that in some diseases where the etiological agents were viruses or protozoa, that evidence suggested deficiencies in nutrition actually lessen susceptibility. Specific nutrients critical to the multiplication of T. equiperdum may have been mobilized by the corticosteroids. It should be remembered, however, that von Brand (1951) reported that the rate of development of trypanosomes in vivo was not influenced by cortisone or ACTH. It should be pointed out that his experiments were conducted with susceptible hosts and therefore caution must be exercised in any attempt to translate his observations to fit the present study. The nutritional effect cannot be ruled out, though the importance of the idea that corticosteroids alter the host in such a way that it becomes nutritionally more desirable for T. equiperdum is uninviting. Sufficient nourishment appears to be available in chick embryos to support massive growth of T. equiperdum.

It is possible that the hatched chick has less of some trace nutrient needed by the parasite and that this trace nutrient is sufficiently available in chick embryos. No work has been reported where cortisone enhanced the availability of such trace nutrients. Further no description of athrepsis has been reported to include clumping, vacuolation and death in sequence for T. equiperdum. It should be noted that there was one instance in this study (experiment VI) where a normal chick was injected with a high number of trypanosomes which did not decline. This chick died.

Some preliminary attempts in the serological examination of chick blood, though lacking in elegance, stimulated an acute awareness in the author that phenomena involved in disease and infection are frequently not understood; some of these phenomena may be inadequately described and others undescribed. Concepts of natural immunity are frequently discussed. Investigators have in large part confined their experimental studies, however, to artificial or naturally acquired immunity. The practical importance and relative ease which acquired immunity lends itself to laboratory demonstration no doubt justify the myriad of works published on the subject.

That the effect of the corticosteroids upon the refractoriness of chicks is directly associated with antibody forming mechanisms is admittedly difficult to envision.

Laveran and Mesnil (1902) reported that fowls which were resistant to infection with trypanosomes, yield, after long immunization with a suspension of trypanosomes, a serum devoid of trypanocidal property. It is interesting that Laveran discovered that human serum has trypanocidal properties *in vivo* but erred with Mesnil, in their report that human serum exercises no harmful action on the pathogenic trypanosomes *in vitro*. Yorke, *et al* (1930) reported that fresh serum, *in vitro*, was rapidly trypanocidal to *T. brucei*, *T. equiperdum*, *T. equinum* and others. Human serum was found not to affect the trypanosomes pathogenic for man (*T. gambiense*, *T. rhodesiense*, or T. cruzi), the common trypanosome of newts (*T. dymyctyli*) and rats (*T. lewisi*). One of the human trypanosomes, *T. rhodesiense*, does become susceptible to the action of human serum after it has been passed through mice (Culbertson, 1935). Even if fowl are found to produce trypanocidal antibodies after injection with trypanosomes, it does not follow that chick embryos and young chicks actively do so. It is particularly difficult to imagine that chicks and chick embryos are capable of producing antibodies as rapidly as it appears to be necessary (one to two days) for the elimination of *T. equiperdum*. It should be noted that in most infections which are enhanced by cortisone, events move rapidly after the introduction of microorganisms. When rabbits that have been treated with cortisone are infected with *Streptococci* group

A in the skin an extensive septicemia is established in less than 24 hours. In normal animals a transient bacteriemia a few hours after infection was noted but, by the end of 24 hours, the blood obtained from the animals and cultured proved negative. There is some question that the prevention of septicemia in such an instance is a function of antibody formation (Thomas, 1953). Since no functional antibody forming mechanism is described for chick embryos additional evidence against antibody involvement is observed. It has been previously mentioned that enhancement of virus infections is observed in cortisone treated chick embryos.

Much of the discrepancy between the observations made may stem from a lack of a definition of an antibody, incomplete understanding concerning the mechanisms of antibody formation, the variety of methods claimed for the demonstration of antibodies, and the fact that claims of measures of antibody might more accurately be considered measures of systems involving antibody reactions.

Other agents in the serum besides those that are with agreement called antibodies are affected by cortisone. Some of these are thought to be protective and are considered part of the non-acquired immunological factors observed in man and animals. Such phenomena are long known but poorly understood. Most have low specificity. Such substances as

complement, properdin, C-reactive protein, serum mucoprotein, Tilletts bactericidal factor, and hyaluronidase inhibitor are extant but have escaped accepted understanding.

Early in this study many hours were spent observing the survival of T. equiperdum in normal chick serum in vitro. The environment of normal chick serum was without effect on the organisms. It was decided early that the resistance to T. equiperdum observed in the chick was not to be found in the existence of so-called innate antibodies. Continued efforts were nevertheless made to demonstrate deleterious effects on this parasite by normal chick serum. After observing the effects of the chick embryo on trypanosomes and the similar effects produced on this parasite when massive numbers of the organisms were injected into young chicks (i.e. clumping, vacuolation, and early death of the trypanosomes), and a corresponding lack of the above effects on the organism when trypanosomes were injected into cortisone-treated chicks, further serological tests were attempted. Between 24 and 36 hours post-injection, a definite trypanocidal effect was observed, in vivo, in normal chicks; thus in vitro examination of serum taken just after the disappearance of the trypanosomes was desirable.

When the precipitin ring test was employed all chick sera gave negative results. Sheep anti-T. equiperdum serum

gave positive results. Apparently then the antigen was a valid one. Chick serum has some peculiar properties according to Goodman et al. (1951). They reported that the effect of increasing the salt content was to cause a more complete precipitation of the antigen and antibody and that determinations of maximum antibody content could not be made at salt concentrations of less than about eight percent. Wolfe and Dilks (1949) reported in a study of comparison of antibody responses of eight avian species, that chicks constantly yielded excellent precipitating antisera. The reaction was lost however upon dilution. It is inconsistent that the ring test in this study was not positive when some of the other serological tests did give positive results. This is especially true when the hemagglutination test was definitely positive. The inconsistency can only be explained by asserting that the hemagglutination test was the more sentive, if not the more accurate of the two.

As was anticipated the sera taken from a sheep which had been infected with T. equiperdum did in all instances tested give positive reactions. This serum was used only as a positive control for the antigen preparations and was of no other interest in this study.

Correlation of age and variation of antibody response in chicks has been discussed in the introduction. Though

evidence indicates that very yong chicks are poor producers of antibody, if producers at all, this study showed that the sera taken from chicks less than a week old which had been injected with T. equiperdum gave positive reactions when mixed with this trypanosome or erythrocytes coated with its extract in vitro. This is not too surprising insofar as noticeable reactions could be seen taking place when blood of chicks containing these organisms for 24 to 36 hours was examined. Repeatedly, blood samples taken from such birds showed trypanosomes which were clumped or agglutinated, dead, and vacuolated. This reaction was repeated when live trypanosomes were incubated with sera taken from chicks which had been injected with trypanosomes. This pooled sera in low dilution (1:2 and 1:4) agglutinated, vacuolated, immobilized and apparently killed trypanosomes after four hours at 37°C. The infectivity of the organisms was seen to be slightly affected when such sera were incubated with trypanosomes for one hour as indicated by the neutralization test. Yorke, et al. 1929, found that T. equiperdum will survive for at least nine hours if 1/25 of its volume is sheep serum. The organisms survive 24 hours if the volume is 1/5 sheep serum. When normal chick serum was diluted 1:16 and 1:32 less than half the trypanosomes survived for 4 hours. This mortality was probably due to anthrepsis. Far more obvious reduction in

motility of the trypanosomes was noted in sera taken from chicks which had only recently harbored the organism. All of the serological studies except the precipitin ring test indicated a definite difference between normal chick serum and serum taken from chicks which had just two days previously been inoculated with the parasite.

Little or no reaction was observed with chick serum taken from birds which 2 weeks previously had been injected with T. equiperdum. This indicates that the agent or agents which appeared in chick serum, when chicks were inoculated with the parasite, is apparently short lived once the trypanosomes are expelled from the blood of chicks.

It is concluded that though chick embryos are highly susceptible to laboratory infections with T. equiperdum, some of the developing birds appear capable of recovering from the infection. Normal chicks are extremely resistant to infections with T. equiperdum. This resistance is alterable in favor of the trypanosome when large continuous doses of corticosteroids are injected into these birds. Though the resistance of chicks to this parasite may be a consequence of several factors, part of the explanation appears to be immunological insofar as definite anti-T. equiperdum factors were shown to appear very shortly after normal chicks had been inoculated with T. equiperdum.

SUMMARY

It has been shown that normal white leghorn chicks are refractive to infections with T. equiperdum. Evidence indicates that though twelve to fourteen day old embryonated eggs of this same bird are highly susceptible, resistance appears perfected at the time of hatching. It was noted that embryonated eggs when infected with T. equiperdum exhibit an obvious diminution of trypanosomes after the fourth or fifth day. It was at this time that clumping, vacuolation and death was observed among the parasite. Though the infection in general is lethal for chick embryos, evidence was presented indicating that some escape the lethal effects and hatch free of infections.

Though normal chicks appear refractive there was no evidence of so-called natural antibodies present in their sera. When young chicks were subjected to treatment with high pre-infection and post-infection doses of prednisolone, cortisone acetate, and dexamethasone, modification of the resistance mechanisms associated with their refractoriness to T. equiperdum was obvious, and transient infections lasted up to sixteen days. In some instances the infection appeared to have been lethal. Trypanosomes were seldom observable by direct microscopic examination of chick blood, but when blood

obtained from infected birds was sub-inoculated into white mice the infections were transfered and easily indicated by death of the mice. The prepatency in mice sub-inoculated with chick blood was thought to be a reflection of the number of viable trypanosomes in the inoculating dose of infective blood. The appearance of the organisms in the peripheral blood of the mice and the death of the rodent was hastened when blood was obtained and injected six days or more after the birds had been infected as compared with blood taken from birds 2 or 3 days post-infection. Blood specimens were also examined for trypanosomes by centrifugation in capillary tubes. This method satisfactorily indicated the presence of trypanosomes and indicated the least number of trypanosome infected chicks in a group. Blood was also examined by direct methods and on several occasions trypanosomes were in sufficient number that they were detected without sub-inoculation or centrifugation. These occasions were few.

When post-infection treatment was continued, the infection was prolonged. Withdrawal of treatment was reflected in complete recovery from infection.

When chicks were infected with numbers of trypanosomes so that their presence was observable by direct microscopic examination, parasitemias lasted for 24 to 36 hours. The disappearance of organisms from the blood stream was usually

proceeded by clumping, vacuolation and death of the parasite population. When normal birds were subjected to repeated massive infections, only transient parasitemias resulted. Each succeeding injection of trypanosomes was more rapidly removed than the one preceeding.

Preliminary serological examinations showed that although normal chick sera have no effect on this parasite, sera in low dilution (1:2 to 1:4) obtained from chicks injected with T. equiperdum approximately 48 hours previously, agglutinated, vacuolated, immobilized and apparently killed trypanosomes in vitro when the organisms and serum were mixed and incubated for four hours at 37°C. Preliminary work also showed that serum from chicks so inoculated agglutinated dead trypanosomes and chick erythrocytes coated with extract of T. equiperdum. This agglutinating property appears to be short lived insofar as sera from chicks which two weeks previously had been injected with trypanosomes had little or no effect in vitro on the organism.

The action of cortisone was discussed as it relates to infections and disease and as it might apply to this parasitological problem. The exact role of cortisone and susceptibility in more widely studied infections and diseases is still incompletely understood. It is suggested that in the present study the answers to questions raised are not to be

found only in antigen antibody reactions, particularly as is conventionally conceived. The rapidity with which the changes occurred in the sera of normal young chicks after they were infected with this organism was noted. The fast moving events observed in the differences in time in the removal of living trypanosomes from normal chicks as compared with the removal effected by chicks subjected to treatment with corticosteroids was observed. It was concluded that part of the explanation for the relationship between T. equiperdum and chicks may be immunological.

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