STUDIES IN

Experimental Trypanosomiasis in Dogs

Caused by

Trypanosoma evansi (Steel, 1885)

A Thesis

Submitted to the Faculty of Veterinary Science and Animal Husbandry, Magadh University, in Partial Fulfilment of the Requirements for the Degree of Master of Science (Veterinary)

BY

L. N. Mandal

Post-Graduate Department of Parasitology
Bihar Veterinary College, Patna

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Dr. A. K. VARMA, Ph. D. (London),
Director, Livestock Research Station,
Bihar, PATNA,
&
Professor & Head of the Post-graduate
Department of Parasitology,
Bihar Veterinary College, Patna.

Patna,

D/ 24.11.1965.

Certified that the work described in this Thesis entitled " STUDIES IN EXPERIMENTAL TRYPANOSOMIASIS IN DOGS CAUSED BY Trypanosoma evansi (Steel, 1885)" is the bonafide work of LAKSHMI NARAYAN MANDAL, carried out under my guidance and supervison.

A. Varma 24.11.65

(A. K. VARMA)

A B S T R A C T

Title:- Studies in experimental Trypanosomiasis in dogs caused by Trypanosoma evansi (Steel,1885).

This thesis embodies the results of observations made on the progressive course of the disease, symptomatology, haematology, biochemistry, gross pathology and biology along with morphology of Trypanosoma evansi in experimental dogs. Altogether nine dogs were used in this investigation. First two dogs were inoculated with T. evansi maintained in guineapigs and in the rest subsequent passages were done. Trypanosomes in doses of 1,25,000 were inoculated in this experiment in each case.

The experiments commenced with five sets of pre-infectional readings on haematological and biochemical observations. Thereafter post-infectional haematological, biochemical and number of parasites per ml. of blood were studied daily until death. Day to day multiplication of the parasites, clinical symptoms and temperature were also recorded.

T. evansi was always found to be monomorphic, slender in forms and carrying a free flagellum. The parasite was found to multiply by longitudinal binary fission with no division of the flagellum. In wet smears the parasites were seen to move towards the

posterior direction with sluggish movement. The virulance of the parasite was found to increase on subsequent passages.

The trypanosomes appeared in the blood circulation of the experimental dogs after a prepatent period of 3-5 days. The number of parasites in blood fluctuated irregularly reaching a peak of 2 to 4 lacs per ml. and then suddenly disappearing from the circulation. The range of temperature also showed a periodic rise and fall. Lachrymation, conjunctivitis, oedema, loss of body-weight, inco-ordination of limbs, anaemia, dyspnoea followed by death were the cardinal symptoms observed. The animals died between 14 to 43 days after infection.

The blood picture showed fall in erythrocyte count, packed cell volume and the haemoglobin content. Mean Corpuscular volume increased in all the dogs except in nos. 1 and 5 in which there was drop in M.C.V. in later stage. Decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration was also noticed in some dogs. Increase in buffy coat layer was observed during Parasitaemia. The total count of white blood cells showed irregular rise and fall. In the initial stage of the infection, there was neutrophilia with a decrease of lymphocytes. The condition reversed

at the peak of infection and neutrophilia predominated during the period of death. Eosinophils were always present in lower number than in the normal dogs. The reduction of monocytes was observed with the pregress of the disease except in dog nos. 3 and 5.

Biochemical studies showed decline in the level of blood sugar to the extent of 21 to 40 mgms per 100 ml. as against the normal of 80 to 104 mgms.

The observations of gross pathological condition showed the presence of enlarged and congested spleen with reddish granular appearance on the surface. Right ventricle of the heart was found to be overdilated. Lungs were congested pneumonic and few small consolidated areas were present in patches and liver was hypertrophied with haemorrhages on the surface.

The blood smears from the lungs, liver, heart and kidneys showed the presence of large number of trypanosomes on examination.

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L. N. MANDAL.

INTRODUCTION

The significant discovery of Trypanosoma evansi as the causative agent of "Surra" in horses, camels and mules dates back to the year 1880. The first mammalian trypanosome was recorded in Russia in 1845, but it did not attract much attention probably because the strain was nonpathogenic.

Trypanosomiasis has since been recorded in many animals, such as donkey, elephant, cattle, buffalo, dog, fox, tiger and goat, though dog has been found to be very susceptible to the infection of T. evansi. Records of natural infection in dog are rather scanty. Trypanosomiasis in animals is invariably transmitted through blood-sucking vectors. The dogs in addition may pick up the infection by eating offal and meat of infected animals. This baffling phenomenon appears to be confined to dogs only. There is a solitary record of T. evansi infection in man by sucking the infected blood of guineapig (Annon, 1946-47). It has been stated that at the time of sucking the infected blood the person concerned was suffering from tonsilitis.

This disease has been recorded from Africa, South Russia and several countries of Asia. In India it is known as Surra and it is known to occur in all the States showing seasonal distribution, the majority of outbreaks occurring between July & October, the incidence coinciding with the onset of the monsoon.

Workers had held the view that the death in Surra was brought about due to the heavy consumption of carbohydrate by the parasites, which led to the failure of liver function and to glycopyruvic intoxication. Dyspnoea due to blocking of the lungs-capillaries and production of lactic acid have also been suggested as one of the causes of death. The other factors associated are deficiency in haemoglobin and failure of production of erythrocytes in the host.

From times immemorial dog has been a constant companion of man. Some of its well developed senses, such as sense of smell and hearing, have been exploited by man for the purpose of hunting, detecting crimes and acting as messenger in times of war. Besides, dog has also been used for pulling load, guiding blind, destroying rodents, looking after cattle and sheep and watching the property. In faithfulness no creature can compete with dogs and some one has rightly said "the more I see the dog the more I hate the man".

Considering the importance of the dog, it is imperative to visualise some of the important protozoan diseases of these animals. Among their protozoan diseases the most important are Babesiosis, Leishmaniasis and Surra. So far much attention has not been paid to the prevalence of Surra in dog. Therefore, in order

to acquire better knowledge and understanding of the disease process in dogs it was considered worthwhile to study the progressive course of the disease, symptomatology, clinical pathology, gross pathology along with the morphology and biology of the parasites.

***

REVIEW OF LITERATURE

Historical account:- Trypanosomes are of great medical and Veterinary importance. They parasitise man, animals, birds, reptiles, amphibea and fish. They are transmitted through leeches in the case of aquatic vertebrates and biting insects in land vertebrates. The disease caused by them has been the subject of vital importance due to intimate association between man, animals and invertebrates. The first trypanosome was discovered by Valentin of Bruce in the blood of Trout (Salmofario) in 1841. In the next two years three papers on the trypanosome of frog were published by Gluge of Brussels, Mayer of Bonn, and Gruby of Paris. It was Gruby (1843) who introduced the name Trypanosoma for the parasites of frog. Gross (1845) found trypanosomes in the mammalian host in Russia for the first time. Chaussat (1877) found them in the black rat but mistook them to be nematode larvae. Attention was drawn to the trypanosomes of mammals only when Lewis (1878) described the common trypanosome of rat in Calcutta naming it Trypanosoma lewisi. But, the actual recognition of T trypanosomiasis began with the discovery by Evans (1880) of the first pathogenic traypanosome causing "Surra" in the Indian livestock. He found the parasite in the blood of horses, camels and mules suffering from "Surra" in Dera Ismail

Khan (Punjab), now in Pakistan near the Indus. First he mistook it to be a Spirillum, because of its filiform and actively motile character. But, soon afterwards he recognised it as a flagellate. He succeeded in producing the disease in dog and horse by subcutaneous inoculation of the organisms. Steel (1885) found similar organisms in the blood of mules in Burma and named them Spirochaeta evansi. He also transmitted the disease into the monkey and dog. Crookshank (1886) in London examined the blood films of camel sent by Evans and described in detail the chief characters of the parasite and changed the name to Trypanosoma evansi (Steel 1885). Later, in India Trypanosoma equiperdum, which causes "Dourine", was recognised by Pease (1903) for the first time in the Punjab. It was studied in detail by Lingard (1903) and Mott (1906). Baldrey (1905) stated that this disease was prevalent in Baluchistan, Bombay Presidency and the United Province. Trypanosoma theileri was also recorded in the blood of cattle by Lingard (1904) and Rao et al (1934). Stirling (1921) found trypanosomes in the blood of a bullock in India and regarded them as Trypanosoma congolense; and Mudaliar (1945) described a small trypanosome from the blood of a female buffalo and called it T. evansi var ravi; but their findings have not been confirmed as yet.

Surra is an important disease of our livestock mostly found in the Punjab, United Province, Central Province, Rajasthan, Andhra Pradesh, Madras, Bombay, Bengal, Assam and Bihar as reported by Lingard (1899), Chetti (1922), Cross and Patel (1922) Ajawani et al (1933), Swaminathan (1933-1934), Rao & Mudaliar (1934), Mahajan (1934), Rajagopalan (1937), Mudalier and Ray (1947), Raju and Swaminathan (1947) and Manjrekar (1950).

Once Surra had its endemic home in India, but owing to the movement of the horses from one country to another, it has now been reported from the French China, Cochin China, Siam, Java, Bulgaria, Philippines, Cambodia, Mauritius, Madagascar, Persia, South Russia, Egypt, Sudan, Palestine, Nigeria, Syrian, and Iran (Laveran and Mesnil, 1907; Knowles, 1927; Van Dulm and Le Coultre, 1932; Yutuc, 1935; Zeiss, 1937; Granovillit and Do-Van, 1938; Adam and Lionnet, 1938; Jauffret, 1939; Pavlon and Guenev, 1939; Poursines et al, 1943; Castillo and Joaquin, 1955; Godfrey and Killick - Kendrick; 1962).

Morphology:- Lingard (1907) examined in detail the morphology of different parts of T. evansi and reported the maximum, minimum and mean measurements of eighty trypanosomes. Length measured from 19.35 to 35.26 /u with mean of 27.85 /u. The width measured 1.31 to 1.64 with mean of 1.54 /u . Rao et al (1934) studied in general the morphological characters of T. evansi and

agreed with earlier observations that it was monomorphic with a well developed undulating membrane and a free flagellum. It measured from 18 to 33 /u in length and 1.5 to 2.0 /u in width. The average length was about 25 /u. Hoare (1938) in his paper gave the importance of kinetoplast for the differenciation of certain groups. He suggested that in Brucei and Evansi groups it was 0.6 /u and usually rod-shaped. It was sub-terminal in the majority of cases and occasionally marginal. He described that T. evansi were usually monomorphic species in which all the individual possessed a free flagellum. Hoare and Bennett (1938) encountered an akinetoplastic strain of T. evansi in camels in Sudan. By sub-inoculation into the camels, horses and donkey they found it to be a pathogenic strain. Hoare (1949) revised the clasification of trypanosomes of medical and veterinary importance. According to the characters and position of the free flagellum, kinetoplast, nucleus, undulating membrane and development of trypanosomes in vertebrate and invertebrate hosts he divided them into five main groups viz Lewisi group, Vivax group, Congolense group, Brucei group and Evansi group and placed T. evansi in Evansi group on the ground that organisms of this group differed from those of the Brucei group in not showing polymorphism. Stumpy forms are recorded rarely. Most of the trypanosomes of this group resemble the long form of T. brucei. But

no cyclical development has been reported as yet in Evansi group. Ray et al (1955) observed a new structure in Trypanosoma evansi at the inner border of the undulating membrane and called it costa or basal fibre of the flagellates. Hoare (1956) revised the morphology and taxonomy of the Surra group of organisms. He examined 26 strains and four sub-strains of T. evansi from blood smears of camels, horses, mules, cattle, buffaloes and dogs and confirmed the monomorphic character of T. evansi. Only .05% of the stumpy form was detected. Out of this, three strains were maintained in mice and examined repeatedly over several years. Many times stumpy forms were seen in the blood circulation of stained slides. These observations also confirmed the polymorphism in T. evansi. In this experiment stumpy, intermediate and slender forms were seen and their measurements were taken. Typically, T. evansi measured 15-34 /u with a mean of 24 /u. Stumpy forms measured 16.8-19.6 /u intermediate 19.5 - 20.7 /u and slender 23.0 to 24.9 /u. The free flagellum measured 5-6 /u. Later on, Hoare (1957) in his recent classification grouped Evansi and Brucei groups together on the ground that slender and intermediate forms were always present and stumpy forms were also seen occasionally. Godfrey and Killick-Kendrick (1962) also found the stumpy forms of T. evansi in the blood smear of camels in Nigeria and confirmed the findings of Hoare (1956).

Multiplication:- Holmes (1904) agreed with Bradford and Plimmer that the trypanosomes conjugated and only the female forms divided thereafter. He described that paired forms joined by their posterior ends as conjugation forms or forms in copula. He also believed that in paired forms the individual having a very elongated end was a male. According to Laveran and Mesnil (1907) T. evansi divides by simple longitudinal binary fission like T. brucei. Division begins from kinetoplast. Then flagellum, nucleus and undulating membrane divide into two and finally the protoplasm also divides from the anterior end of the parasite. But division of the protoplasm has also been reported from the posterior extremity. Sometimes further division takes place before the original protoplasm divides. In such cases trypanosomes are found containing four nuclei and four centrosomes. Hoare (1949) reports that first of all kinetoplast divides into two. There is never division of flagellum. One of the daughter kinetoplasts retains the old flagellum, while the new flagellum arises from one of them. Then nucleus begins to divide into two and finally the protoplasm divides from the anterior end of the parasite. This results into two trypanosomes. He states that there is no satisfactory evidence on the occurrence of sexual phenomena in any of the haemoflagellates.

Motility:- Trypanosomes move towards the anterior extremity of the body. The movement of the trypanosomes are only due to their undulating membrane and free flagellum. (Laveran and Mesnil, 1907; Knowles, 1927; and Hoare, 1949).

Susceptible Hosts:- This disease is known to occur in horses, camels, mules, donkeys, elephants, cattle, buffaloes, and rarely in dogs, fox, tiger and goat. Once Surra was regarded as harmless for cattle and buffaloes, but it is now known to cause serious outbreak amongst these animals. In horses Surra is extremely fatal. The infected animals show fever, loss of appetite, anaemia, oedematous swelling and die in a week or may linger to six months. In camel the disease runs a chronic course and the duration may be as long as three years; hence it has been called "Tribarsa". In bovine it takes hyperacute as well as chronic form. In hyperacute form it is very virulent and generally occurs in the form of an outbreak causing heavy mortality upto 90%. In chronic or milder type, the affected animal, though apparently healthy, shows the parasites in the peripheral circulation at varing intervals and sometimes may recover or remain carriers. Dogs are very susceptible (Lingard, 1895; Chetti, 1922; Knowles, 1927; Mahajan, 1934; and Singh Bachan, 1936;).

Experimentally Surra has also been proved pathogenic for number of other mammals like mice, bat, rat, guineapig, rabbit, cats, sheep and pig. (Laveran and Mesnil, 1907; Knowles, 1927; Krijgsman, 1933; Kraneveld and Mansjoer, 1947; Ray and Harbans, 1948; Yutuc and Sher, 1949; and Castillo and Joaquin, 1955). Cardona, (1937) inoculated two hens, two pigeons, and two turtle-doves from an infected guineapig. The birds remained in good condition and they did not show trypanosomes in the blood stream. But T. evansi has been successfully cultivated in chick, pigeon and duck embryos by Longley at al (1939), Venden Berghe (1941), and Alwar (1958). Alwar (1958), successfully transmitted T. evansi in day old pigeons, squabs, duckling and turkey chicks.

It is thought that Surra is not transmitted to man as the causative trypanosome is of animal origin. According to Laveran and Mesnil (1907) Schat pricked himself several times with needles infected with the blood of Surra. But he never developed this disease. Annon (1946 - 1947) reported the incidence of Surra in man, as one of the historical interest of this world. Professor Lanfranchi in April, 1912, was studying animal trypanosomes, T. brucei and T. evansi. He was drawing up blood with a pipette from infected guineapig suffering from Surra. The cotton plug which was in the pipette,

dislodged in it and soaked in blood and entered his mouth. At that time he was suffering from tonsilitis. Thereafter he suffered with fever, insomnia, tachycardia, pain in the thumb and forearms, and enlargement of liver, spleen and lymphgland. His blood was examined and found trypanosomes. Professor Mesnil, by measurement and serological test concluded that the trypanosome found in his blood was T. evansi. He was treated with atoxyl and became free from this infection.

Prepatent Period:- Laveran and Mesnil (1905) inoculated two dogs with T. evansi and observed 3 days prepatent period. According to the Laveran and Mesnil (1907) Lingard infected subcutaneously and intravenously altogether eight dogs with Surra and reported 4 to 7 days prepatent period. Gaiger (1908) also inoculated Surra in Periah dog and dog showed trypanosomes in his blood on the 4th day. Cabrera and Lui (1956) stated that prepatent period in dog was 3-8 days and animal terminated fatally within next 4-32 days. Prepatent period varied depending upon the susceptibility of host, the quantity of inoculum and virulence of the strain. Haiba (1962) inoculated four puppies of 3 months age intraperitoneally with infected blood of dog suffering from Surra and found that the prepatent period was 2-5 days.

Parasitaemia:- According to Laveran and Mesnil (1907) Lingard adopted arbitrary method to study the

degree of parasitaemia in dog. Sen et al (1955) classified the degree of Parasitaemia in rats by counting trypanosomes per 1000 R.B.C. in stained blood films. Desowitz & Watson (1951) classified parasitaemia with another arbitrary method by counting number of trypanosomes present per microscopic field.

Clinical Symptoms (a) Natural Infection:- In dogs Surra has often been seen as a natural disease. Lingard (1894) reported the occurrence of this disease among sporting and Pariah dogs in Bombay, the Punjab and Madras. He noticed among the affected dogs elevation of temperature, anorexia, Oedema of the head and throat, injection of the conjunctivae and in some cases effusion into the joints and corneal opacity leading to partial or total blindness. During fever trypanosomes were found in the blood on microscopic examination. Ajawani et al (1933), Mohteda (1940), Rao (1941), Rao (1952), Sheshadri (1955) and Bhardwas et al (1962) reported trypanosomiasis in dogs from different parts of India. Besides other symptoms, Sheshadri (1955) observed periodical attack of fever, anorexia, loss of hair, progressive weakness, inco-ordination of limbs, circling movement and bilateral opacity of cornea. In one dog he did not find any sign of oedema anywhere in the body. Other symptoms observed were staring and rough coat, good appetite, oedema of the muzzle, sub-maxillary space, chest and scrotum. Natural cases of Surra in dogs have also been reported

from Indo-china, Egypt, and Mauritius, (Laveran and Mesnil, 1907 and Haiba, 1962).

(b) Experimental Infection:- Experimentally disease has been also produced in dog to study the susceptibility, symptoms and course of infection. Pease (1904) inoculated three pariah dogs with T. evansi. The dogs died in 35, 22 and 20 days respectively after inoculation. Laveran and Mesnil (1905) studied Indian Surra over two dogs with a view to compare the effects observed with those produced by Mauritian trypanosomes. The duration of the disease was 12 and 13 days ending fatally. The temperature continued till death ranging between 102.2 to 104°F. Trypanosomes were numerous in the blood except in the middle of the disease. There was loss of weight. According to the Laveran and Mesnil (1907) Lingard infected subcutaneously and intravenously altogether eight dogs with T. evansi. They died in 14½, 21, 27½, 29, 34, 36, 47 and 97 days after inoculation with blood obtained from different animals. In all cases experimentally produced disease showed the same symptoms as the natural disease. The number of trypanosomes in the blood varies. At times they are completely absent, again appeared suddenly in the blood circulation. This latent period was always observed in all the dogs except two in which parasites were present throughout the course of the disease. During the last days of the disease, trypanosomes are very numerous in the blood. Average

duration of the disease is 28 days. They also observed remittent and intermittent type of fever. The high temperature noticed was between 103 - 106°F. The relation of temperature and trypanosomes in the blood circulation was not found coinciding always. Delpy and Rafyi (1947) experimentally produced Surra in five dogs. One died after 81 days, three developed chronic infection and one died on the eleventh days after splenectomy. Cabrera and Lui (1956) produced Surra experimentally in dogs and stated that dog died between period of 4-32 days. Haiba (1962) inoculated four puppies of 3 months age intraperitoneally with infected blood of dog suffering from Surra and found the similar symptoms stated above.

Virulence:- Laveran (1908) proved that T.evansi when passaged through a series of guineapigs may become very virulent. He also stated that virulence of the same strain was exalted when passaged through dog. To-pacio (1935) inoculated single trypanosome (T.evansi) into rats, mice, guineapigs, dogs and one horse. Infection resulted in all the species with one cell of trypanosome. Ray and Harbans (1948) suggested that Pantothenic acid exerted and influence on the rate of multiplication of the trypanosomes in rats. Although Sen et al (1955) experimentally proved that the effect of starvation in rats did not produce any significant

effect in the prepatent period and the course of infection.

Haematology:- Trypanosomes are haemoflagellates. The changes produced by them in the blood picture reveal the disease even when the parasites are absent therefrom. Della vida and Verdoz-zi (1906) stated that there was gradual decrease in total count of red blood cells and increase in leucocytes followed by marked leucopenia. Neutrophils increased throughout the infection. Laveran and Mesnil (1907) observed in dogs suffering from "Nagana" (caused by T. brucei), diminution in the number of red blood corpuscles with the first appearance of the trypanosomes at times falling to one third of their original number. Usually the red blood corpuscles appeared normal but there were at times changes in their shape (Polychromatophilia). The red corpuscles were deficient in haemoglobin. There was increase in the number of leucocytes except in the eosinophils. In the beginning the number of polymorphs increased while the mononuclear cells diminished relatively in dogs. But, it was also observed in the same dog that sometimes mononuclear cells increased from 15-48%. He also reported increase of the buffy coat during parasitaemia. Krijgsman, (1933) recorded decrease in the number of erythrocytes in mice and rats infected with T. evansi and such changes persisted

throughout the course of the disease. French (1937) found that sedimentation was much more rapid in donkeys than in cattle and sheep. In cattle infected with T. congolense the rate increased in the beginning of the infection and than returned to normal. Poindexter(1939) infected rats with T. equinerdum and studied the mononuclear response. He found a marked increase in large lymphocytes. Nicolle and Simons (1939) inoculated guineapig with T. equiperdum, T. brucei and T. evansi. Observations on sedimentation rate were made and in all cases rate was found markedly increased. Hoppe (1945-1946) conducted experiments to investigate the cause of death in the albino rat infected with T. equinerdum. He found progressive anaemia, decrease of the red blood cells, haemoglobin, packed cell volume and platelets. Kaltenbach (1954) studied the responses of leucocytes in natural Surra cases of camels and horses and experimental Surra of rabbits and mice. In natural chronic infection the degree of regenerative leucocytosis and number of large lymphocytes ran f parallel to the number of circulating trypanosomes. There was no change in monocytes. In rats the infection was acute and it was first characterised by an increase of neutrophils followed by regenerative leucocytosis and finally lymphocytosis. Piennes (1954) observed the changes in the blood picture after T.congolense infection in cattle. He found severe anaemia in acute

stage which became more pronounced at the time of crisis due to haemolysis. The anaemia in acute stage was macrocytic while it was microcytic in chronic stage. Haemoglobin was low. Edward et al (1956) stated rapid fall of erythrocyte count in sheep, goat and horses infected with T. vivax, T. congolense and T. brucei. Moreover, in other experiments the erythrocyte sedimentation rate was found normal in goats but increased in sheep after inoculation with T. brucei. Cabrera and Lui (1958) observed reaction of leucocytes in dogs and pigs infected with Surra. In the prepatent period he found monocytosis, eosinophilia and reduction of lymphocytes in pigs, whereas in dogs there was diminution of lymphocytes and eosinophils and increase of neutrophils. Edward et al (1957) studied the haematological changes produced in horses by infection with T. vivax, T. congolense and T. brucei. They found reduction in red blood cell count, packed cell volume and haemoglobin after the infection. There was an increase in the erythrocyte sedimentation rate and also in bilirubin level of the plasma. There was also marked Haemolysis. Samadar et al (1962) examined the blood of goats experimentally infected with Surra and noted decrease in erythrocyte count, packed cell volume, haemoglobin percentage and increase in neutrophils throughout the infection. They also reported decrease in the mean corpuscular volume, leucopenia and microcytic hyperchromic

anaemia. Srivastava (1965) examined the blood of dog suffering from Surra after 10 days interval for haematological examination and found reduction in the red blood cell counts, decrease in the packed cell volume and indicated the macrocytic type of anaemia. He also reported increase in the sedimentation rate and neutrophilia throughout the infection.

Biochemical:- Kliger et al (1929) observed that glucose favoured the growth of trypanosomes and they were responsible for the production of lactic acid. Gaiger et al (1930) reported that T. evansi consumed glucose and produced lactic acid in both vivo and vitro. There is also evidence of drop in the blood-sugar content immediately prior to death in rats, mice and horses infected with T. evansi (Krijgsman, 1933 and Randall, 1934). Randall (loc. cit.) stated that in one animal the blood-sugar content was not markedly decreased, while in another it fell down to 27 mg/100 c.c. in 24 hours before death. Schern (1937) detected histologically the absence of glycogen in the livers of affected animals. He also stated that due to absence of sugar, protein decomposition products were excreted which led to intoxication. Von Brand (1938) reported that pathogenic species of trypanosomes consumed more sugar than non-pathogenic ones. The sugar was partially oxidised and the end product was pyruvic acid. He also concluded

that there was no doubt that trypanosomes consumed twice their own weight of sugar in 24 hours. Christophere et al (1938) reported on glucose-utilisation by trypanosomes and the formation of acid products. The Oxygen intake was large. Hoppe (1945-1946) and Hoppe & Chapman (1947) observed that rats infected with T. equiperdum died of hypoglycaemia due to consumption of glucose by the parasites. Castillo and Joaquin (1955) noted decrease in blood-sugar level in rats infected with T. evansi. Sen et al (1955) and Srivastava (1965) also observed the decrease in glucose-level of blood in rabbit and dog suffering from Surra.

Gross Pathology:- In Surra enlargement of spleen was found in mice, rats and dogs and rarely in rabbits. Apart from the splenic enlargement pulmonary congestion and small subpleural ecchymosis was also found. In buffaloes there was enlargement of the lymphatic glands and liver. Spleen was rarely or slightly enlarged. (Laveran & Mesnil 1907). Gaiger (1909) conducted the postmortem examination of dog died due to T. evansi and observed the patches of congestion in the lungs and enlargement of liver and spleen. Stomach, large and small intestine were slightly inflammed. Sen et al (1956) observed and reported on enlarged and congested spleen in guineapigs. They also found that liver and kidney were pale and the heart was flabby. The lungs were congested. The bone

marrow was slightly reactive and dark red in colour. The brain was pale and anaemic. Lymph glands were found apparently normal.

MATERIALS AND METHODS

1. Strain:- Laboratory strain of T. evansi brought from Indian Veterinary Research Institute was maintained by serial needle passage in guineapigs and used in this investigation.

2. Experimental animals, their care and management:- (a) Pariah dogs, between four to six months of age, were purchased locally and used in the experiments. Their weight varied between 10½ to 14½ lbs. In all nine dogs were used. They were fed on cooked-liver, milk, rice, bones and plenty of water. Before starting the experiments, their blood, stool and urine were examined and they were dewormed. The animals were weighed and their day-to-day temperature and clinical symptoms were recorded before and after the experimental infection.

(b) Also, guineapigs were used, which were purchased locally. They were given a diet of green grass and gram with plenty of water.

3. Site of blood collection:- (a) Dog:- Blood samples were drawn from the radial or saphenous veins. Each time about 2.5 c.c. of blood was drawn using 5 c.c. syringe fitted with a needle of 20 gauze size and kept immediately in a small vial containing dried oxalated solution. This oxalated blood was used for all haematological and biochemical studies. The counting of

trypanosomes daily per cubic centi-meter was done either with oxalated blood or fresh blood collected from the ear-vein. But, citrated blood was used for the inoculum for subsequent passage. For differential counts blood smears prepared from the ear-vein.

(b) Guineapig:- Guineapig showing teeming trypanosomes in its peripheral blood was selected for taking the blood by aspiration from the heart.

Tubes for collecting blood were prepared by pipetting 0.5 ml. of a solution of Potassium oxalate and Ammonium oxalate (Pot. oxalate -0.8 gm. Ammonium oxalate -1.2 gms and water 100 c.c.) into a small empty penicillin vial and then evaporating to dryness in a hot air oven.

4. Dose and site of inoculum:- The trypanosomes were counted with the help of a standard haemocytometer using Turk's fluid (Glacial acetic acid -3 c.c., 1% aqueous gentian violet - 1 c.c. and distilled water 100 cc.) diluent. Infective inocula containing 1,25,000 trypanosomes were injected intraperitoneally to each dog. First two dogs were inoculated with the strains maintained in guineapig and in the rest disease was produced by serial ∅ needle passage from dog to dog.

5. Study of Parasitaemia, Morphology and Biology of the Parasite:- (a) Wet blood smears:- were examined

daily to study the presence of parasites, their motility and division of the parasites under the dry high power lens.

(b) Stained smears:- Blood smears made from the inoculated animals were examined daily to study the morphology and different stages of the parasites. Smears were made from the ear-vein and stained by Penoptic method (combined Leishman's and Giemsa's) of staining. Measurements of the parasites were taken with the help of micromer. Lingard (1906) method was adopted for measuring the parasites.

(c) Counts of Trypanosomes per c.c.:- Blood samples containing the parasites were diluted in Turk's fluid and in the proportion of 1:20 the trypanosomes were counted daily by haemocytometer.

6. Haematological studies:- (i) Observing the colour of Plasma:- Blood was collected and plasma colour was seen by naked eye after centrifuging the blood samples.

(ii) Counting of total leucocytes:- For leucocytes each blood sample was diluted 1:20 in Turk's fluid in white blood diluting pipette. Counts were made in counting chamber with improved neubauer ruling and the total count per cubic centimeter was finally calculated.

(iii) Differential leucocytic count:- Blood smears were stained by Penoptic method and were examined for white blood corpuscles. Two hundred cells were counted for each determination.

(iv) Counting of total erythrocyte:- Blood sample was diluted exactly 1:200 with a special diluting pipette using Hayem's fluid (Mercuric chloride 0.5 gm. Sod. chloride 1.0 gm, Sodium sulphate 5.0 gm, and distilled water 200 c.c.). The diluted blood then placed in a special counting chamber and cells were counted in a measured volume and calculated per cubic centimeter.

(v) Estimation of haemoglobin:- Sahli's standard technique was used. In this method N/10 HCL was placed into the graduated tube upto the level of 2 gms or 10% mark. The special Sahlis pipette was filled to the mark 20 with oxalated blood. Immediately the contents of the pipette were discharged into the tube containing acid. Mixture in the graduated tube was diluted with distilled water until it had the same colour-tinge and intensity as the standard tube. Haemoglobin was estimated in gram per 100 c.c. Haemoglobin estimation was done within an hour of the collection of the blood.

(vi) Determination of erythrocyte sedimentation rate:- The erythrocyte sedimentation rate was determined with oxalated blood samples using Wintrobe haematocrit

tubes. Approximately 1 c.c. blood was pipetted into the haematocrit tube which was filled to the m/ zero mark and placed vertically at room temperature. The amount of sedimentation in millimeter was noted after one hour. Oxalated blood was used immediately after collection.

(vii) Determination of Packed cell volume and buffy coat:- The packed cell volume of fresh oxalated blood was determined, using Wintrobe haematocrit tubes. Blood was pipetted into the tube filled up to 10 mark. The tubes were centrifuged for an hour at 3,000 r.p.m. and the packed cell volume, buffy coat and plasma colour were noted.

(viii) Determination of mean corpuscular volume:- To determine the M.C.V. in cubic micron, the volume of packed cell per 100 ml. of blood was divided by the number of million of erythrocytes per cubic m.m. and multiplied by 10.

(ix) Determination of mean corpuscular haemoglobin:- To determine the M.C.H. in micro-micrograms, the haemoglobin in gram per 100 ml. was divided by erythrocytes in million per cubic m.m. and multiplied by 10.

(x) Determination of mean corpuscular haemoglobin concentration:- Haemoglobin in gms. per 100 ml. of blood

multiplied by 100 and divided by percentage packed cell volume gave mean corpuscular haemoglobin concentration. It was expressed in percentage.

7. Determination of Blood sugar in mg/100 c.c.:-
The blood sugar was estimated by Hagedorn and Jensen (1923) method, which was based on the following principle:-

The blood is deproteinised by heating with sodium hydroxide and zink sulphate and then deproteinised blood filtrate is heated in an alkaline solution for a definite time with a known excess amount of Potassium Ferricyani-de. Some of the Ferricyanide, $Fe(CN)_6^{3}$ is reduced by glucose of the blood to Ferrocyanide, $Fe(CN)_6^{4}$. The reverse (oxidative) reaction taking place in presence of oxygen in air is prevented by precipitation of ferrocyanidetion as double Potassium zink sulphate $K_2Zn_3(Fe(CN)_6)_2$ in presence of $ZnSO_4$. The excess of unchanged ferricyanide, $Fe(CN)_6^{3}$ is then reduced by iodide solution in an acid medium to liberate iodine which is then titrated with standard thiosulphate solution using starch as indicator. This back titration gives a measure of the amount of ferricyanide originally reduced by the sugar of the blood. The chemical reactions in the process are as follows:-

$2H_3Fe(CN)_6 + 2HI = 2H_4Fe(CN)_6 + I_2$

$2K_4Fe(CN)_6 + 3ZnSO_4 = K_2Zn_3(Fe(CN)_6)_2 + 3K_2SO_4$.

Reagents used:-

(1) 0.45% Zn So4, 7 H_2O.

(2) N/10 Naoh solution.

(3) Alkaline Potassium Ferricyanide solution:- This was prepared by dissolving 1.65 gms of pure crystals of Potassium ferricyanide and 10.6 gms of anhydrous sodium carbonate in 1000 c.c. of distilled water. The solution thus prepared was protected from light.

(4) Sulphate chloride solution:- The solution was prepared by dissolving 12.5 gms of zink sulphate and 62.5 gms of sodium chloride in 250 c.c. of distilled water and filtered.

(5) Potassium iodide solution:- 15 gms of Potassium iodide was dissolved in 100 c.c. of distilled water. The solution was kept in dark.

(6) .005 N-Potassium Iodate Solution:- This was prepared by accurately dissolving 0.1783 gms of water-Free potassium iodate (A.R.) in 1000 c.c. of distilled water. It was a permanent solution meant for checking the strength of .005 N-sodium thiosulphate solution.

(7) 3% Acetic Acid Solution:- This was prepared by dissolving 3 c.c. of glacial acetic acid (A.R.) in 100 c.c. of distilled water.

(8) 0.005N - Sodium Thio Sulphate Solution:- This was prepared by dissolving 0.7 gms of sodium thio sulphate in 500 c.c. of distilled water. This was an approximate solution and its strength.

(9) Starch solution:-1 gm of soluble starch was dissolved in 100 c.c. of saturated solution of sodium chloride.

Chemicals of highest purity were used throughout the experiment as far as practicable.

Analytical Procedure:- The blood was collected from the animal which was sacrificed. Potassium oxalate was used as anti coagulent. Soon after the collection the tube was shaken well and 0.1 c.c. of the blood was drawn for the estimation of blood sugar with a 0.1 c.c. pepette well dried by a mixture of ether and alcohol and put in test tube containing a mixture of 5 c.c. of 0.45% Zn SO_4, $7H_2O$ and 1 c.c. of N/10 NaOH solution as deproteinishing substance. The pipette was washed 2 to 3 times with the solution and blown empty. The sample were taken in duplicate. The blood sample thus taken in the deproteinishing solution was put in boiling water bath for 3 minutes and filtered on a micro funnel using iron free filter paper, washing the filter paper and the original test tube 2-3 times with water to be sure that all the glucose of the blood is transferred in the filterate which was collected in a

long 75 c.c. hard glass test tube. To the filterate thus collected was poured 2 c.c. of alkaline potassium ferricyanide and the solution was heated in boiling water bath for 15 minutes after which it was cooled under tap water and titrated with 0.005 N-sodium thio sulphate solution, after adding 3 c.c. of sulphate chloride solution, 0.5 c.c. of Potassium iodide and 2 c.c. of 3% acetic acid one by one and using starch as indicator.

Standardisation of .005 N Sodium-thiosulphate:

The approximate solution of the above was titrated with exact .005N potassium iodate (KIO_3) using the above reagents and starch as indicator. The factor (f) was thus determined for sodium-thiosulphate which was used to multiply the reading of the thiosulphate all the time.

Calculation for blood sugar :

It was done by running a blank experiment side by side. The reading of the thiosulphate for the unknown blood sample was substracted from the blank and the reading thus obtained was multiplied by the factor of the thiosulphate and then by 0.177 to obtain the glucose amount in milli-grams for 0.1 c.c. of blood, as 1 c.c. of .005 N Sodium-thiosulphate was equivalent to 0.177 mg. of glucose. And thus the amount of glucose per 100 c.c.

of blood sample was determined by multiplying the amount of glucose present in 0.1 c.c. of blood by 1000.

Pathological Studies:

Macroscopical:- After natural death Postmortem of all experimental dogs was conducted. Different organs were examined and macroscopical changes noted.

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OBSERVATIONS.

Morphology:- In the present study Trypanosoma evansi has always been found to be monomorphic. It is slender and carries a free flagellum. Posterior end of the parasite is blunt or sometimes slightly pointed. Kinetoplast is situated quite near the posterior extremity and it is dot-like measuring 0.3 /u. Nucleus is rounded or oval in shape and is generally situated about the middle of the body. In a few cases it was, however, found situated just anterior to the middle of the body. It measures from 1.45 to 2.90 /u. In exceptional cases when the tryps are dividing, the nucleus measures upto 5.8 /u. Flagellum originates from the kinetoplast (blepharoplast + parabasal body). It is a clear filament, which runs along the edge of the undulating membrane prolonging beyond the end of the body as a free flagellum. The flagellum is divided into the three parts, first part, which is very short, starts from the kinetoplast (centrosome) and extends to the undulating membrane and is called 'axonema'; the second, which runs along the edge of the undulating membrane; and the third part is the free flagellum which measures from 4.35 /u to 7.25 /u with mean length of 5.86 /u . The parasites, including the flagellum, measure from 18.84 to 26.65 /u in length and 1.45 to 2.90 /u in width. The average length and

breadth of the parasites vary between 23.47 /u to 1.79 /u . In dividing form breadth varies from 2.19 to 4.03 /u (Table no. I).

There is a well developed undulating membrane, which appears as a ridge extending laterally from the body of the parasites. It is very thin and extends along the greater part of the body. These parasites have 2 to 4 well developed folds of membranes. The flagellum has the same thickness right upto the end. The nucleus, kinetoplast and flagellum take deep, reddish purple colour when stained with Panoptic method of staining (Fig. 1,a).

The protoplasm often contains granules. They are generally little and irregular in shape. They are very scanty in T. evansi as compared to T. brucei. At the time of death, the parasites are less vigorous and their protoplasm contains more granules than in the case of normal parasites.

BIOLOGY.

Division:- The infected blood, when inoculated into the susceptible host, begins to multiply by longitudinal binary fission. First of all the kinetoplast which is a dot-like structure placed posteriorly begins to elongate and lengthen longitudinally and finally

separating into two halves. Resulting kinetoplasts are situated one infront of the other. The original flagellum generally remains attached to the posterior kinetoplast and new flagellum arises with the anterior kinetoplast. In few cases the original flagellum may remain attached to the anterior kinetoplast and a new flagellum originates from the posterior kinetoplast. The new flagellum increases in length. After this stage the nucleus enlarges, becomes rod-shaped, and finally divides into two equal parts. The new flagellum continues to increase in length forming undulating membrane with ridges in lateral side of the parasites. Now the dividing parasites have two kinetoplasts, two flagella with undulating membranes and nuclei. Finally the protoplasm divides and the two individual parasites separate out. Division of the protoplasm was always seen starting from the anterior part of the body. Such type of division has also been observed in wet smear. But in one case division of the protoplasm was also seen taking place from the posterior part of the parasite. Sometimes further division takes place before the original protoplasm divides and in such cases, which are rather rare exceptions to the rule of equal binary fission, a large trypanosome containing four centrosomes and four flagella are seen. Multiplication of the parasite occurred throughout the infection.(Fig. I to VIII and Plate No. 1).

Fig – I

Fig – II

Fig — I

Fig — II

Fig – III

Fig – IV

Fig – III

Fig – IV

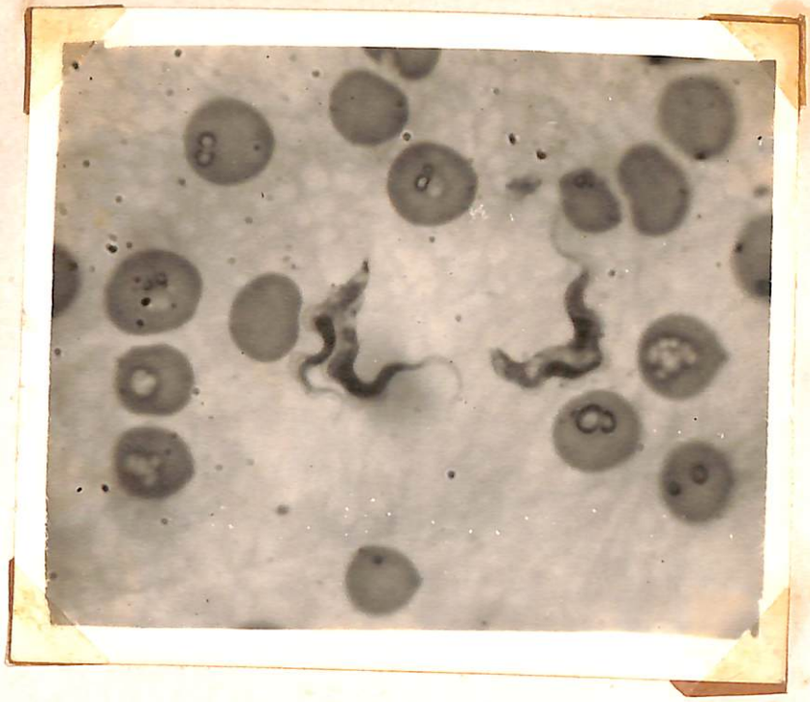

Fig – V

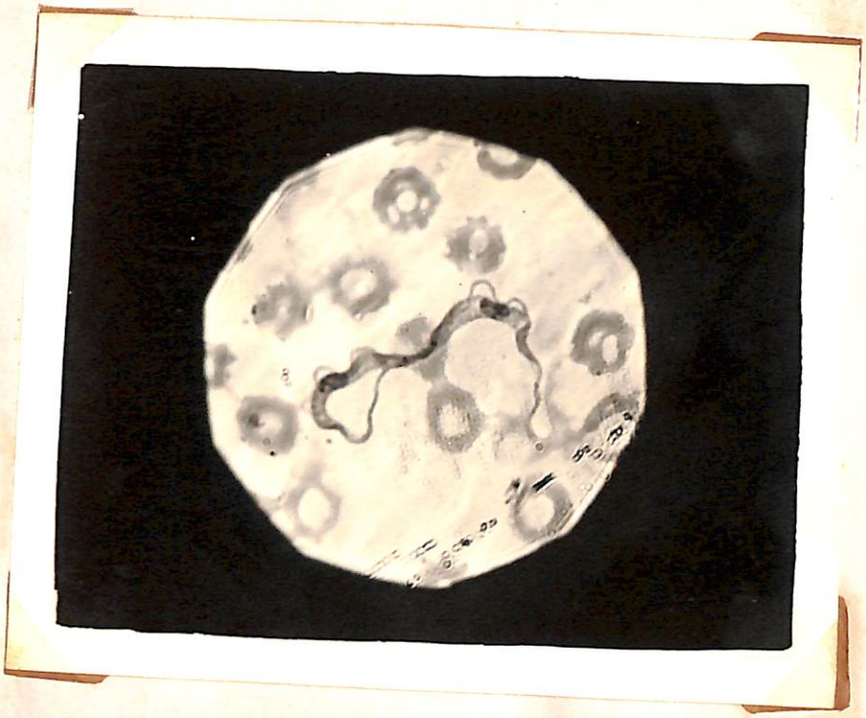

Fig – VI

Fig — VII

Fig — VIII

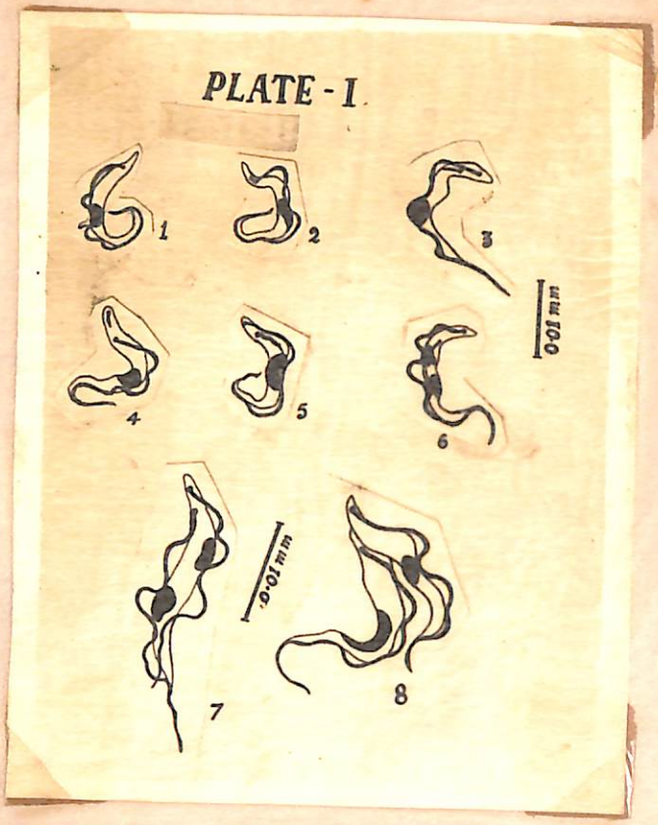

PLATE — I

Nutrition & Movement:- During this experiment unlike other trypanosomes no digestive vacuoles were seen in T. evansi. They derive their nutrition by osmosis. The sugar enalysis has proved that parasites mainly live on glucose diet, as the sugar level decreased positively on the appearance of the parasites in the blood circulation.

Trypanosomes are extra-cellular parasites found in the plasma of blood displacing the red corpuscles by their movement. The movement of trypanosomes is seen chiefly due to their undulating membrane and free flagellum. It has been observed that free flagellum has the lashing movement, oscillating alternately from right to left. Trypanosomes are seen moving from place to place with the help of free flagellum and sometimes seen to travel across the field of the microscope. In fresh wet smear they are seen to move towards the anterior end of the body with the help of the free flagellum. But, when the movement becomes sluggish the same parasite is seen to move towards posterior direction with the help of only undulating membrane.

Infectivity and Virulence:- Trypanosomes were inoculated into the body of the experimental animals in definite doses. They developed in the body by their power of multiplication. This power continued throughout the period of illness except in the latent period.

Fig — A

Power of reproduction coincides generally with the febrile attacks. The parasites appeared in each case in the experimental dogs from 3 to 5 days after the infection. The dog no. 1 & 2 were given inoculum from guineapig source and they died after 43 and 38 days respectively. The same strain was passaged through series of dogs and it became more virulent. They died in 18, 21, 22, 21, 18 and 14 days after inoculation showing symptoms of acute phase. Dog no. 4 was killed to study the location of parasites during latent period of infection. (Table No. 2).

CLINICAL SYMPTOMS

Dog No. 1

(A) Parasitaemia, Temperature & Prepatent period : Weight 14½ lbs. On March 29, 1965 diluted blood of an infected guineapig containing trypanosomes was injected intra-peritoneally. Temperature before inoculation was 102°F. On April 1, a few parasites were seen in the blood and at the same time there was rise of temperature to 104°F. From April 2 to April 11 fever continued with slight remission and trypanosomes rapidly increased with the maximum of 2 lacs per c.c. in the blood on the 5th and then the number of parasites diminished. At times they were few and also completely disappeared from the blood circulation. After the 11th the temperature fell to 101 - 102°F and suddenly it rose to 106°F. Trypanosomes were present in the blood from 6th May onward till death of the dog. (Graph No. A & Table No. 4).

Fig — 1

Fig — 2

Fig – 3

Fig – 4

(B) Clinical Symptoms:- Noted were anorexia, high temperature, conjunctivitis and swelling around both the eyes which appeared on 10th April. On 21st April oedema of the head and throat developed. Oedematous swelling of all four limbs specially joints and paws appeared on 6th May. Inco-ordination of the limbs was also observed. Corneal opacity with partial blindness was seen. Respiratory distress and increased heart beat were more prominent in the last stage. The dog was dull, weak and emaciated till death. (Table No. 2 & Fig. 1-4).

Dog no. 2

(A) Parasitaemia, Temperature & Prepatent period:

Weight 12 lbs. This dog was also injected on March 29, 1965 with diluted blood of guineapig containing trypanosomes. On that day temperature was 101°F. Parasites appeared in circulation on 2nd April. Temperature began to rise with the appearance of the parasites, and it rose up to 105°F, Trypanosomes became numerous during the period and it rose to more than 2 lacs on 7th April per c.c. in the blood. After that temperature and trypanosomes concentration fell with abrupt rise and fall. Sometimes parasites were so scanty that they were not easily detectable even in stained smear. From 29th April till the date of death, parasites were very

Fig – 5

Fig – 6.

Fig – 7

Fig – 8

numerous in blood. It rose to more than 3 lacs per c.c. and dog died on 6th May, 1965. (Graph No.A and Table no. 6).

(B) Clinical Symptoms:- The dog was off-feed during high temperature, and it had the tendency to drink more water during parasitaemia. On 8th April congestion of the conjunctivae and swelling around the upper eyelids appeared. Oedematous swelling around both the eyes and keratitis became more prominent after 20th April. Swelling of the head, pharyngeal region, throat, muzzle, joints of right hind and left forelimbs was also seen. Respiratory distress, increased heart beat, weakness and emaciation were more prominent. The animal was unable to move. One day before death it weighed 8 lbs. (Table no. 2 & Fig. no. 5 to 7).

Dog no. 3

(A) Parasitaemia, Temperature & Prepatent period: Weight 11½ lbs. The animal was inoculated on 7th April, 1965 with infected diluted blood of dog no.2. Temperature before inoculation was 102°F. Parasites appeared in the peripheral circulation on 10th April, although the temperature rose to 104°F on 11th and it continued with slight remission till 17th April. Parasites rapidly increased in number from 11th to 15th April and reached a concentration of more than 2 lacs

Fig – 9

Fig – 10.

per c.c. of blood. After that the number of the parasites diminished rapidly. At times they were seen even one in number in the whole of the stained blood films. The decrease of the parasites was followed by fall of temperature. Again from 22nd the number of the parasites and the temperature increased simultaneously. One day preceding death the temperature rose to 104°F and the parasites increased to 4 lacs per c.c. of blood. The animal died at 6 a.m. on 25th April, 1965. (Graph no. A & Table no. 8).

(B) Clinical Symptoms:- Anorexia, dullness and tendency to drink more water was noticed during high temperature. Conjunctivitis and opacity of cornea had been the predominant symptoms in the early stage of the disease. Swelling around the upper and lower eyelids of both the eyes appeared on 23rd April. This led to partial blindness till 25th April. Oedematous swelling of the throat, lower jaw, forelimbs and sub maxillary spaces appeared and persisted till death. The animal became weak and emaciated. The body coat was rough and weighed 10 lbs before death. (Table no. 2 & Figs. 8 to 10).

Dog No. 4.

(A) Parasitaemia, Temperature & Prepatent period : Weight 11 lbs. On 25.4.65 this dog was inoculated with diluted blood of infected dog no. 3. The temperature

began to rise and touched 104°F on 2nd May 1965. Parasites appeared in blood on 30th April and increased in number on 2nd and 6th May, 1965. There was periodical rise and fall in the number of the parasites and the temperature. The number of trypanosomes diminished on 3rd and 9th May, 1965. Temperature fell to 102°F on 6th May, 1965 and then remained almost stationary until the 10th May. When the temperature fell to 100°F and parasites disappeared from blood circulation, the dog was killed to study the location of parasites during latent period. (Graph No. A & Table No. 10).

(B) Clinical Symptoms:- Conjunctivitis and opacity of cornea appeared on 6th May, 1965. Oedematous swelling of the upper eye lid also appeared. Animal weighed 10 lbs. just before sacrifice. (Table No. 2).

Dog No. 5.

(A) Parasitaemia, Temperature & Prepatent period: Weight 10½ lbs. On 4th May, 1965 diluted blood of infected dog no. 4 was injected. Temperature before inoculation was 101.5°F. On 8th May temperature rose to 104°F and parasites also appeared in the blood circulation. Parasites rapidly increased in number and reached more than 3 lacs per c.c. on 16th May and then began to diminish and disappeared almost completely from the blood on 21st May 1965. A fresh rise occurred on 22nd May.

Fig – 11

Fig – 12.

Fig – 11

Fig – 12.

From 8th to 19th May 1965 fever continued with slight remission. Temperature suddenly fell to 100°F on 22nd May and again rose to 104.1°F on 23rd May, when the dog died. The parasites were then very numerous in the blood circulation. (Graph No. A & Table No. 12).

(B) Clinical Symptoms:- There was anorexia and dullness during high temperature. Conjunctivitis and swelling of the face particularly around the both eyes appeared on 17th May, 1965. Emaciation and respiratory distress were more marked after 18th May. The animal weighed 8½ lbs at the time of death. (Table No. 2 & Fig.No. 11).

Dog No. 6.

(A) Parasitaemia, Temperature & Prepatent period : Weight 13 lbs. Diluted blood of infected dog no. 5 was taken and injected on 23rd May, 1965. Temperature before inoculation was 101°F. On 26th May, few parasites were seen in the blood circulation and at the same time there was a considerable rise of temperature. From 27th to 31st May 1965, fever continued with slight remission. Trypanosomes rapidly increased in number and reached about 4 lacs per c.c. in blood on 31st May 1965. Trypanosomes after that began to diminish and disappeared completely from the blood circulation on 1st June 1965. Again there was slight increase in the number and disappeared completely from the blood circulation on

7th June 1965. This latent period remained till 9th June. Parasites were numerous during the last few days of the disease. It reached upto 4 lacsper c.c. From the first June onward there was either intermittent or remittent fever. Two days preceding death temperature was 104°F. The dog died on 14th June 1965. (Graph No. A & Table No. 14).

(B) Clinical Symptoms:- There was periodical attack of fever. Conjunctivitis and swelling of the upper right eye lids appeared on 6th June 1965. The swelling continued till 10th June and disappeared after that. There was no other symptoms except dullness. Appetite was normal. The animal weighed 11 lbs at the time of death. (Table No. 2).

Dog No. 7.

(A) Parasitaemia, Temperature & Prepatent period: Weight 14 lbs. This dog was also inoculated with infected diluted blood of dog no. 5 on 23rd May 1965. Temperature before inoculation was 101°F. Trypanosomes appeared in the blood on 27th May 1965. After that the temperature rose to 104°F and trypanosomes increased to about 4 lacs per c.c. on 29th May 1965. On 30th May temperature touched 105°F and parasites began to diminish and disappeared completely from blood on 1st & 2nd June 1965. With the disappearance of the parasites

temperature also fell and again rose to 105°F. After that there was slight increase of parasites and it remained stationary for few days and disappeared again on 7th & 8th June 1965. Before death parasites were numerous in blood and reached 4 lacs per c.c. From 4th June onward there was remittent type of fever varying from 102 to 105°F. The dog died on 13th June 1965. (Graph No. A & Table No. 16).

(B) Clinical Symptoms:- There was dulness and anorexia during temperature. Emaciation, respiratory distress and increased heart beat were observed. There were never any eye lesions and oedematous swelling. Animal weighed 12 lbs at the time of death. (Table No.2).

Dog No. 8.

(A) Parasitaemia, Temperature & Prepatent period: Weight 14 lbs. This dog was injected on 14th June 1965 with diluted blood from infected dog no. 6. Before inoculation the temperature was 102°F. On 17th June temperature rose to 103°F and parasites also appeared in the blood. From onward there was always rise and fall of temperature varying generally between 102°F to 104.5°F. But on 24th June and the date of death, the temperature fell to 99°F. Parasites rapidly increased in number from 18th to 21st June, 1965 and suddenly disappeared from blood on 23rd June, 65. Parasites

again began to increase from 25th June and reached about 5 lacs per c.c. in blood one day preceding death. At the time of death parasites were numerous in blood. The animal died on 2nd July, 65. (Graph no. A & Table No.18).

(B) Clinical Symptoms:- There was loss of appetite, dullness and distress. During febrile condition parasites were always present in the blood. First clinical symptom appeared on 23rd June 65, with lachrymal discharge and conjunctivitis. Later on swelling of the muzzle and right upper eye lids developed. Animal weighed 11 lbs at the time of death. (Table no. 2 and Fig. 12).

Dog No. 9.

(A) Parasitaemia, Temperature, & Prepatent period: Weight 13½ lbs. On 20th June 1965, this dog was inoculated with diluted blood from infected dog no. 8. Temperature before inoculation was 102°F. Parasites appeared in the blood on 23rd June 65 and at the same time there was considerable rise of temperature to 103°F. From 24th onward there was remittent and intermittent fever till death. Parasites gradually increased from 24 to 26th June and disappeared suddenly on 27th June from peripheral circulation. Parasites reappeared in the blood after two days and began to increase in number and reached more than 4 lacs per c.c. on 4th June 1965.

The dog died in the same night. (Graph No. A and Table No. 20).

(B) Clinical symptoms:- There were never any eye lesions and oedematous swelling of any part of the body. Only anorexia, dullness and dyspnoea were observed. The animal weighed 11 lbs at the time of death. (Table No. 2).

HAEMATOLOGICAL STUDIES

Before starting the experiments all the dogs were examined thoroughly. Haematological studies and sugar analysis of their blood were also carried out for comparative studies. (Table Nos. 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21).

Total Red Cell Count:- Erythrocyte count in all the dogs showed abrupt fall in the beginning on appearance of the parasites in the peripheral circulation. In the middle of the infection there was gradual fall and during the last stage the total cell count decreased to 1.78 millions per c.c. in dog no. 2 and in other cases it fell down to less than 3 millions per c.c. except dog no. 1. In which the total count remained almost stationary after the first decrease or showed tendency to increase few days before the death of the animal. (Table No. 4, 6, 8, 10, 12, 14, 16, 18, 20 and 21).

Total White Blood Corpuscles:- Leucocytes study in nine dogs revealed that there was marked increase of total leucocytes counts after infection in almost all the dogs except dog no. 9 and it reached to even more than 21,000 per c.c. in dog no. 8. As regards dog no. 9 which did not show any rise of total leucocytes even after infection, which had an average 23,600 leucocytes counts before infection. Two days after the appearance of the parasites, particularly when there was an abrupt rise in the number of parasites, the leucocytes count was found to have decreased to a considerable number, so much so, it reached to 6,200 per c.c. Later it was found that there was either increase or decrease in the total count of leucocytes throughout the period of their infectivity. Sometimes it was observed that it touched to normal level in all the dogs except dog no. 3, in which there was always leucopenia during the course of the infection. This stage of leucopenia was in all the dogs noticed generally during the period of rise of parasites in the blood exception to this was dog no. 1 in which leucopenia was seen in the latent period. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18 and 20).

Haemoglobin Estimation:- Haemoglobin estimation revealed that there was general decrease of haemoglobin percentage in all the dogs. The variation in the

decrease ranged from 3.5 to 3.8 gms percent in dog nos. 2, 5, 6 and 7 and 5 gms percent in dog nos. 1, 3, 8 and 9 at the time of their death. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18, 20 and 21).

Packed Cell Volume:- With the onward progress of the infection, there was abrupt decease in the percentage of P.C.V. in all the dogs. After that there was gradual fall in dogs nos.3, 4, 5, 6 & 7, till the date of death, in which it came down to 18, 28, 15, 20.5 & 20.5% respectively. While in dog nos. 1, 2, 8 & 9 there was slight increase of P.C.V. percentage in the middle course of the infection, it showed again tendency towards decrease and fell down to 18, 16, 21 & 19.5% respectively. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18, 20 & 21).

Mean Corpuscular Volume:- Mean Corpuscular volume increased in all the dogs and remained as such till death except dog nos. 1 & 5. In both the dogs there was also increase of M. C. V. in the early and middle course of the infection. It started decreasing and finally came down to 58 and 57.6 cubic micron on the last day when the dogs died. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18, 20 & 21).

Mean Corpuscular Haemoglobin:- Decrease of M.C.H. in dog nos. 2, 4, 6, and 7 was noted; while in other

dogs no significant changes was observed. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18 & 20).

Mean Corpuscular Haemoglobin concentration:- The value of M.C.H.C. decreased markedly in dog nos. 2, 6 and 7 and slightly in dog nos. ~~&~~ 4, 5, 8 & 9, while no significant changes were observed in dog nos. 1 & 2 (Table nos. 4, 6, 8, 10, 12, 14, 16, 18 & 20).

Erythrocyte sedimentation Rate:- Almost all the dogs showed slight increase of E. S. R. but this increase was seen only when there was high temperature. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18 & 20).

Buffy Coat:- There was increase in buffy coat layer in all the dogs when the parasites-concentration was highest in the blood circulation. In dog nos. 8 & 9 buffy coat from 5 to 5.2 m.m. in thickness was recorded during parasitaemia. After centrifugation of infected blood, it was observed that the upper layer of buffy coat was rich with parasites than the lower layer of the sedimentation.

Plasma Colour:- Haemolysis was generally noted in the plasma ~~colour~~ during high temperature and period of parasitaemia.

Differential Leucocytic Count:- After inoculation of trypanosomes, the percentage of neutrophils

increased with the corresponding decrease of lymphocytes percentage in all the dogs. There was also reduction of eosinophils except dog no. 4. Later on lymphocytosis with the reduction of neutrophils were also observed in-variably in all the dogs. But this stage of lymphocytosis only lasted from 24 to 48 hours. If lymphocytosis persisted as in dog nos. 1, 2, 3, 6, 7, 8 & 9 the latent period of the parasites increased. Last day or few days prior to death neutrophilia was again noted in all the dogs. There was also decrease in the monocyte percentage except dog no. 3. (Table nos. 4, 6, 8, 10, 12, 14, 16, 18,20 & Graph Nos. B &. Fig. nos. IX to XVI).

Biochemical studies:-

Sugar:- The blood sugar level fell down gradually in all cases after the appearance of the parasites in the blood circulation. Few days prior to death of the animal marked decrease of sugar percentage was noticed. Finally it came down to 21, 32, 31, 35, 30, 28 & 36 mgs in case of dog nos. 2, 3, 5, 6, 7, 8 & 9 respectively. In this experiment it was observed that death occurred due to hypoglycaemia. (Table Nos. 4, 6, 8, 10, 12, 14, 16, 18, 20, 21 & Graph nos. A).

Macroscopical changes:- The spleen was enlarged and congested with reddish granular appearance on the surface. Right ventricle was over dilated producing broad shaped

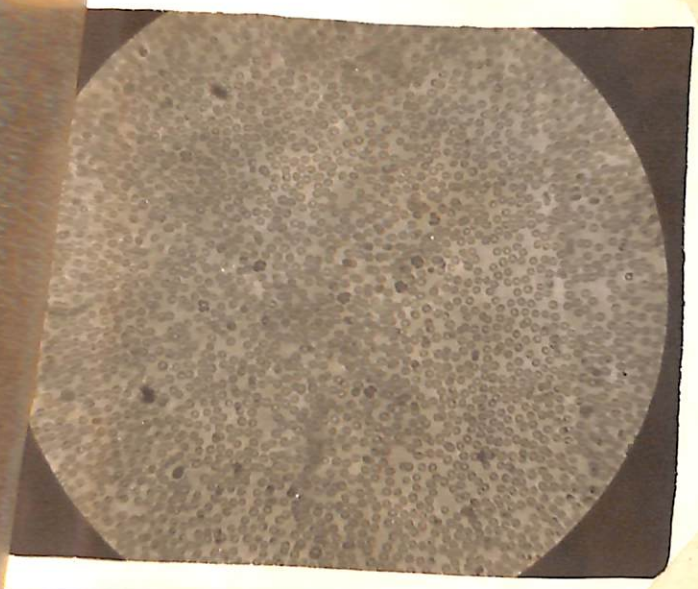

Fig — IX

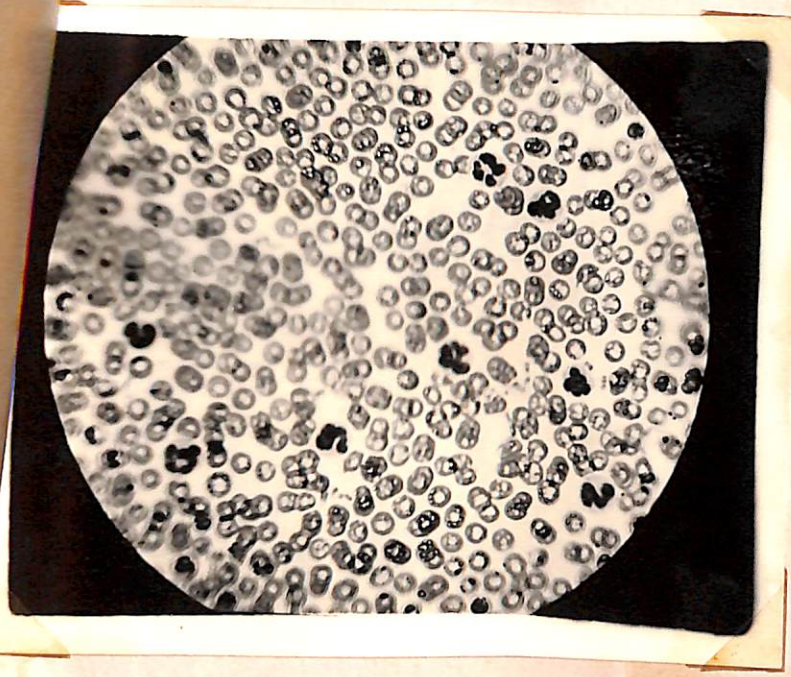

Fig — X

Fig — IX

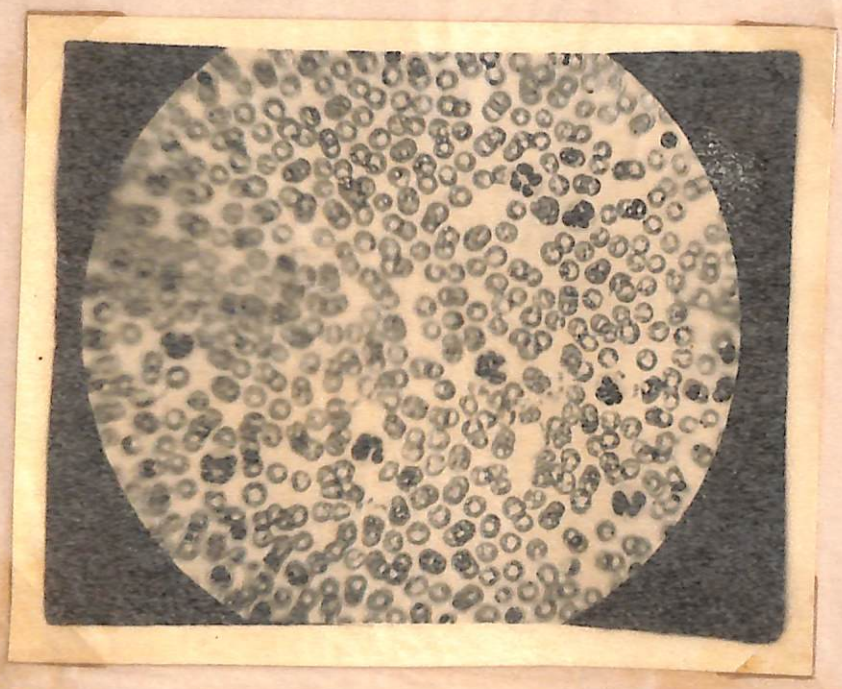

Fig — X

Fig – XI

Fig – XII

Fig — XIII

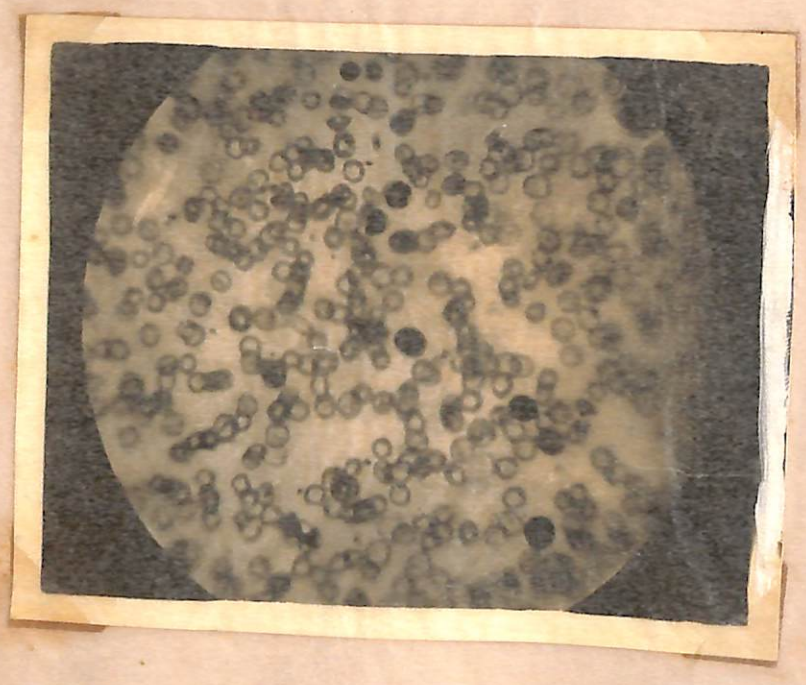

Fig — XIV

Fig — XV

Fig — XVI

heart. Sometimes pleural and peri cardial exudation, were also present. Trachea and bronchi contain frothy exudate. Lungs were congested, pneumonic and few small consolidated areas were present in patches. Liver was swollen and hypertrophied. Edges were rounded. Consistency friable, pale in appearance & indicating degenerative changes with petechial haemorrhages on the surface in few cases only. Gall bladder swollen and full of greenish yellow bile. Kidney looked normal. Bone marrow was highly reactive and dark red in colour. Brain was pale and anaemic. Lymph glands were found apparently normal. (Table no. 22 & Fig. nos. 13 to 16).

After postmortem examination smears were prepared from lungs, liver, heart, kidneys, spleen, bone marrow, brain and lymph glands. They were stained and examined for parasites. On examination number of trypanosomes were found in stained smears of lungs, liver, heart and kidneys of all dogs except dog no. 4 which was killed during latent period. Smears taken from other organs were found negative for the parasites. While smears of spleen, bone marrow, and brain of dog no. 5 proved positive for few degenerated trypanosomes. Dog no. 4 did not reveal parasites from any organ.

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Fig – 13

Fig – 14

Fig – 14

Fig – 14

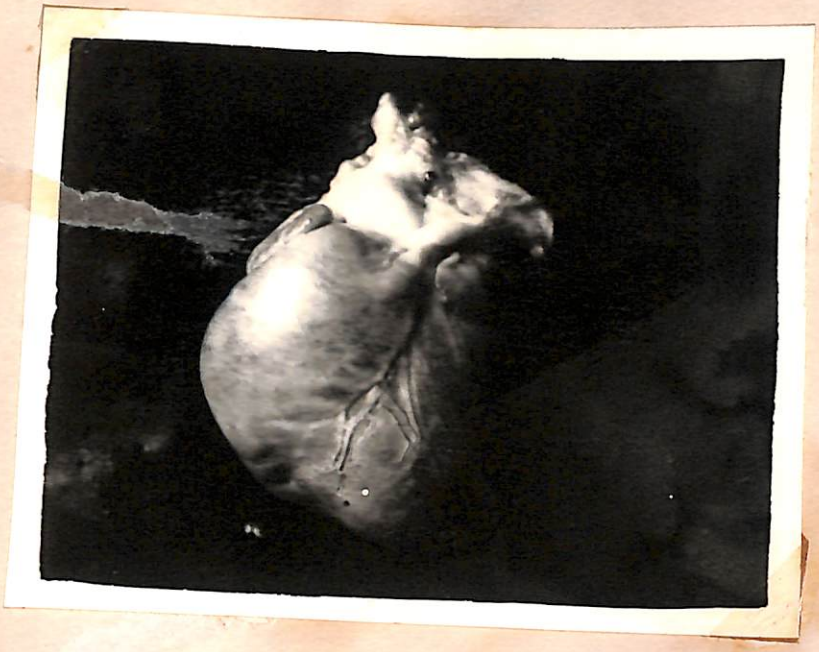
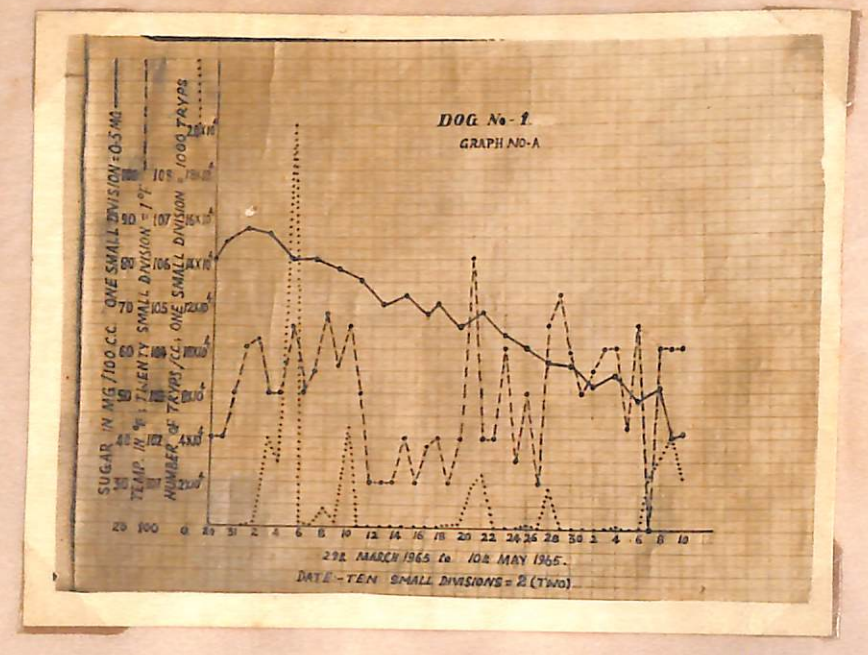
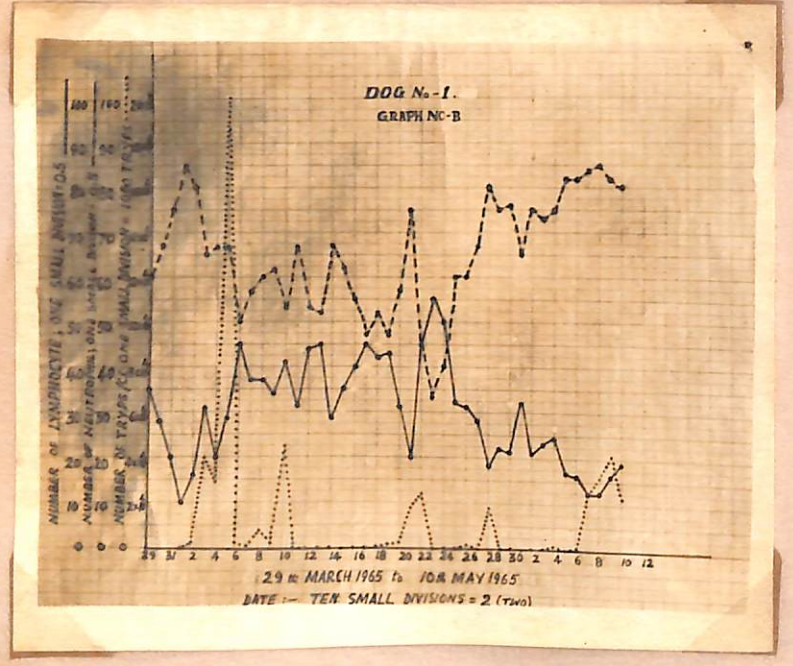
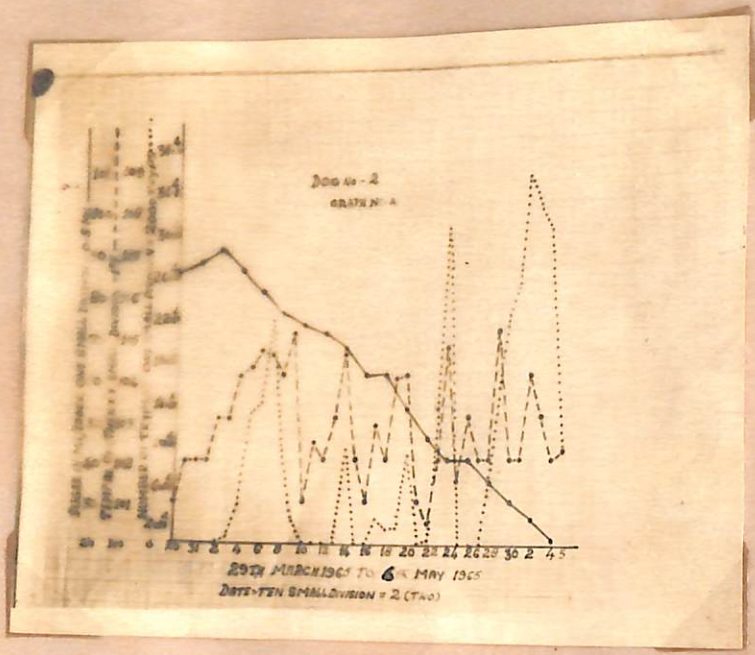


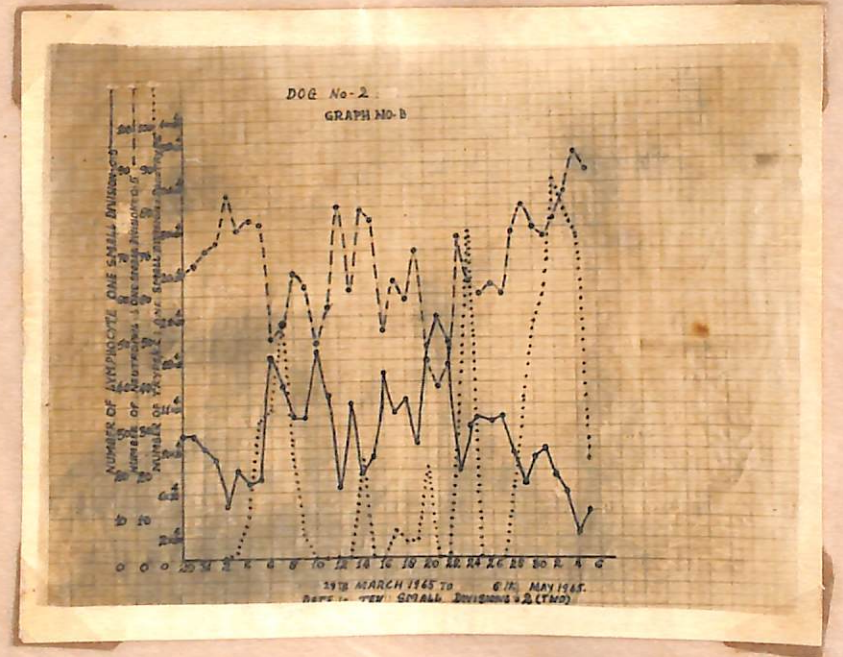
Fig – 15

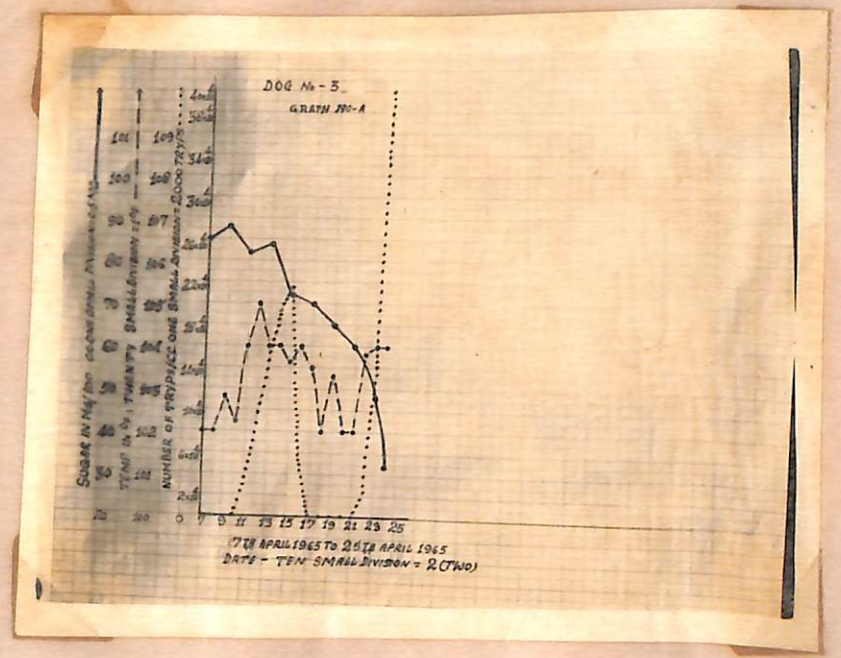
Fig – 16.

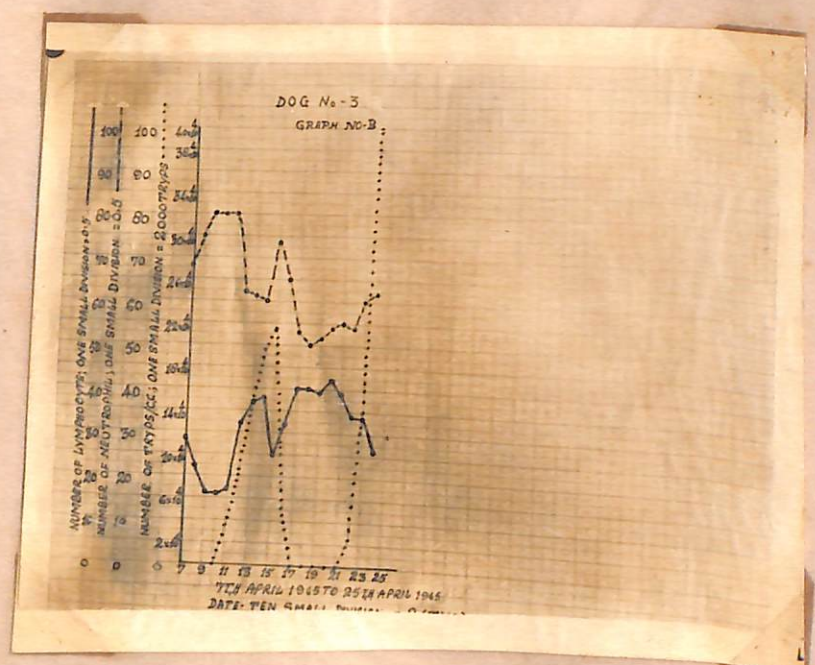

DOG No-1.
GRAPH NO-A
SUGAR IN MG/100 C.C. ONE SMALL DIVISION=0.5 MG.
TEMP. IN °F. ; TWENTY SMALL DIVISION = 1°F.
NUMBER OF TRYPS/CC. ONE SMALL DIVISION = 1000 TRYPS
29 MARCH 1965 to 10th MAY 1965.
DATE - TEN SMALL DIVISIONS = 2 (TWO)

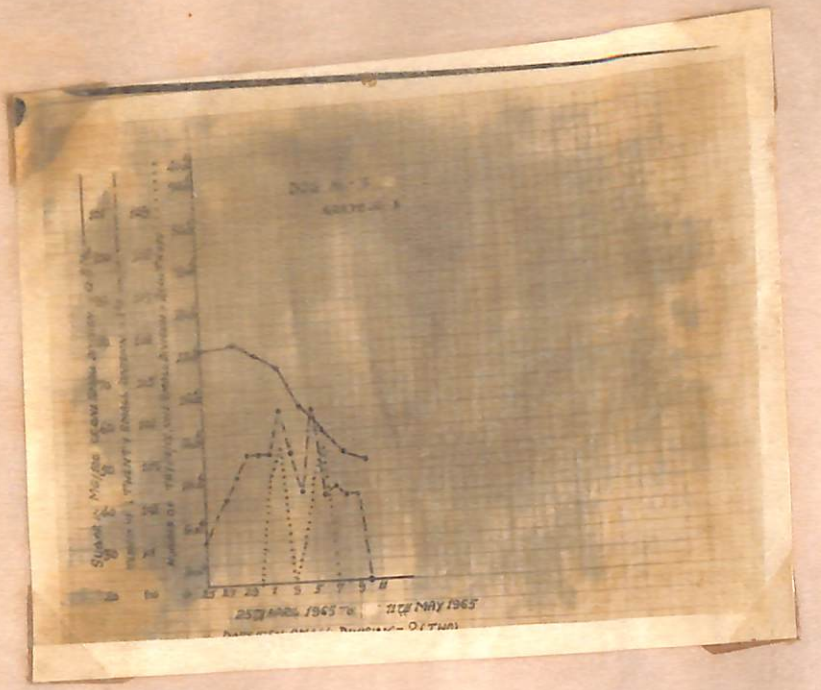

DOG No-1.
GRAPH NO-B
NUMBER OF LYMPHOCYTE ; ONE SMALL DIVISION=0.5
NUMBER OF NEUTROPHILS ; ONE SMALL DIVISION = 1000 TRYPS
NUMBER OF TRYPS/CC. ONE SMALL DIVISION = 1000 TRYPS
29 th MARCH 1965 to 10th MAY 1965
DATE :- TEN SMALL DIVISIONS = 2 (TWO)

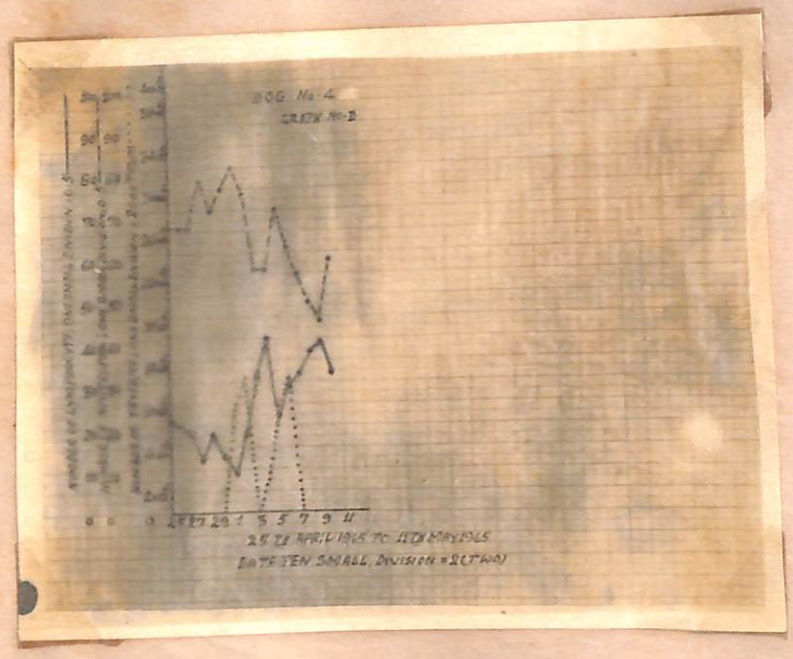

DOG No-2
GRAPH No-A
29TH MARCH 1965 TO 6TH MAY 1965
DATE-TEN SMALL DIVISION = 2 (TWO)

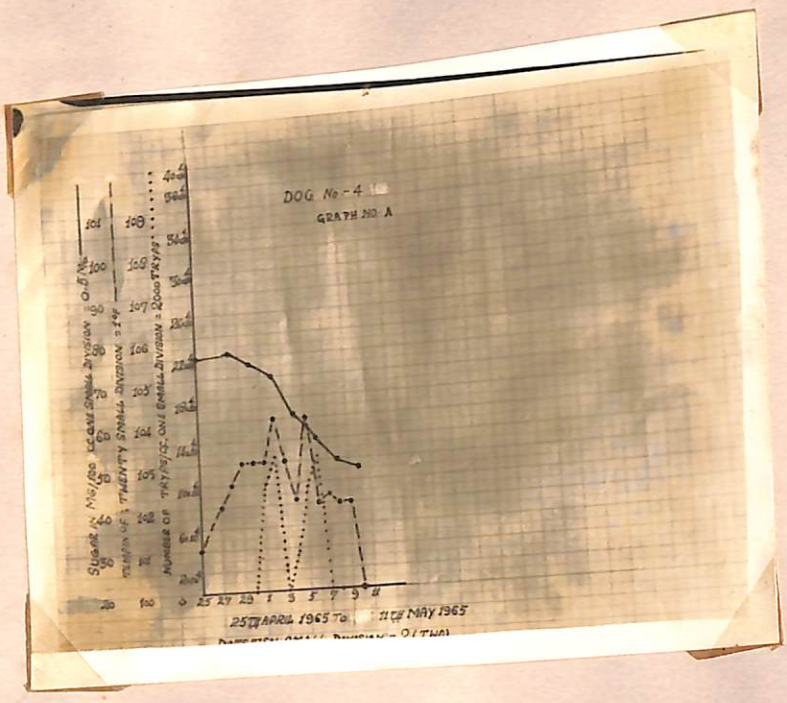

DOG No-2
GRAPH NO-B
29TH MARCH 1965 TO 6TH MAY 1965.

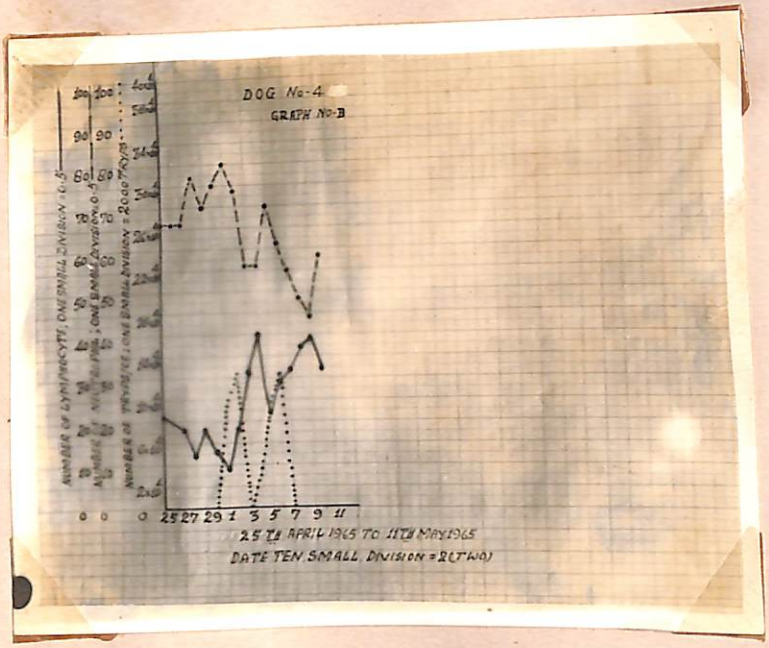

DOG No - 5
GRAPH No-A
7TH APRIL 1965 TO 25TH APRIL 1965
DATE - TEN SMALL DIVISION = 2 (TWO)

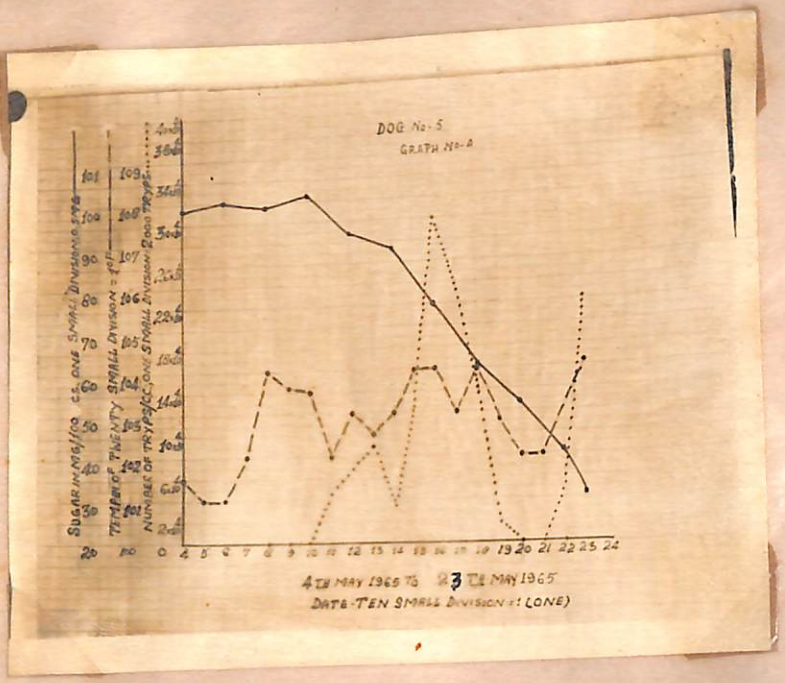

DOG No - 5
GRAPH No-B
7TH APRIL 1965 TO 25TH APRIL 1965

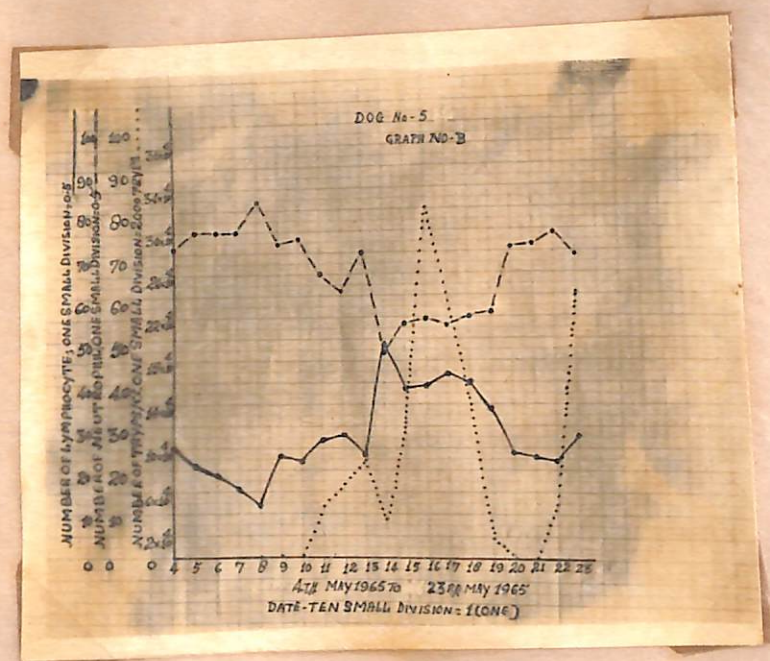

25TH APRIL 1965 TO 11TH MAY 1965

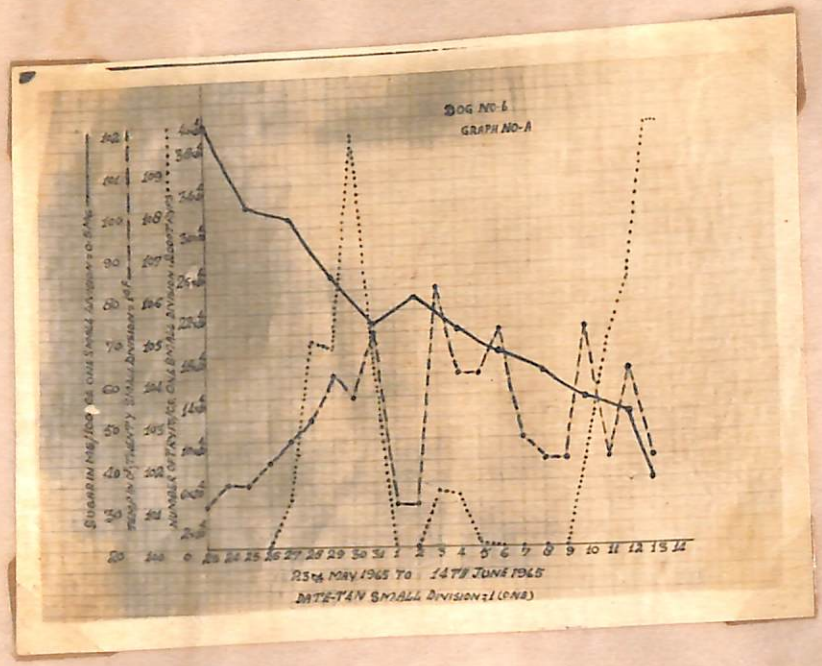

BOG No. 4
25TH APRIL TO 11TH MAY 1965
ONE TEN SMALL DIVISION = 2(1"WG)

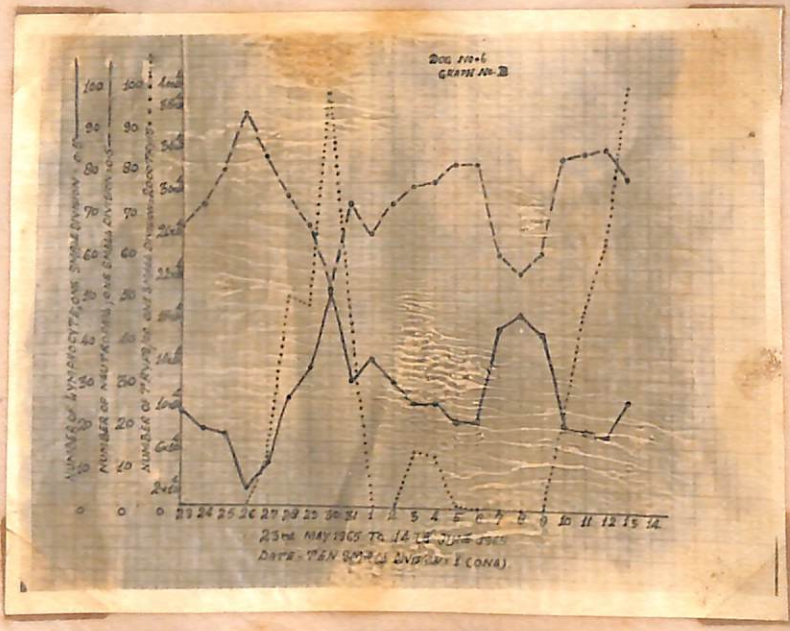

DOG No-4
GRAPH NO. A
25 27 29 1 3 5 7 9 11
25TH APRIL 1965 TO 11TH MAY 1965

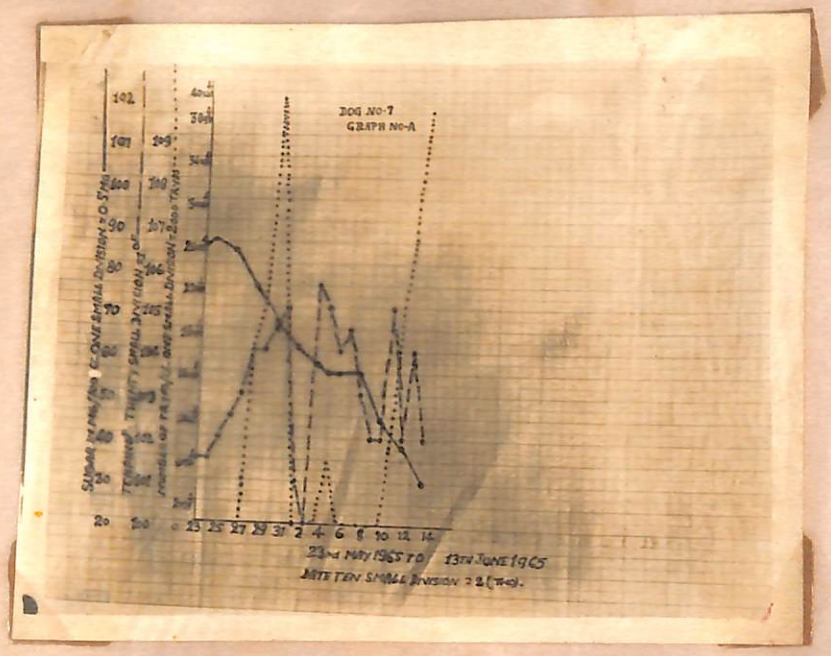

DOG No-4
GRAPH NO-B
25 27 29 1 3 5 7 9 11
25TH APRIL 1965 TO 11TH MAY 1965
DATE TEN SMALL DIVISION = 2(TWO)

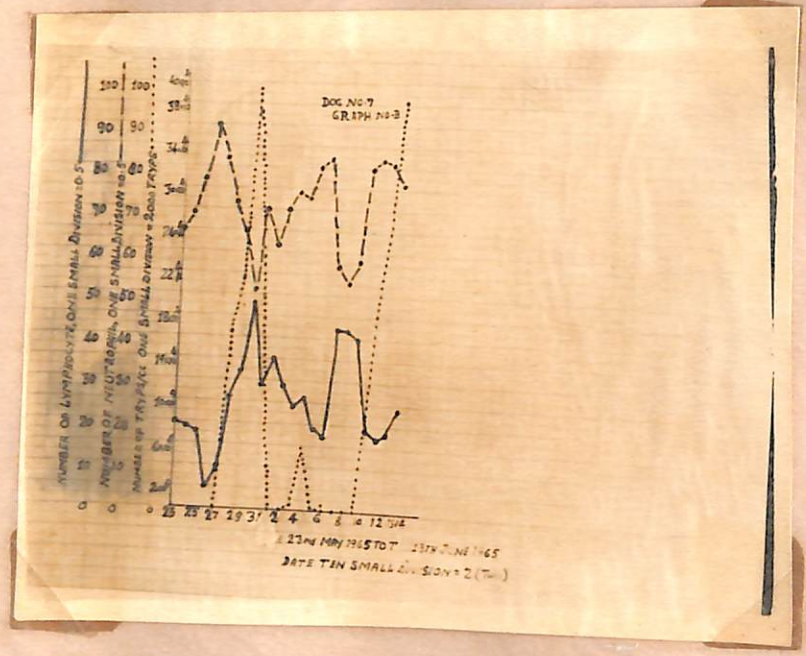

DOG No-5
GRAPH No-A
SUGAR IN MG/100 C.C. ONE SMALL DIVISION=5MG
TEMPERATURE OF TWENTY SMALL DIVISION=1°F
NUMBER OF TRYPANOSOMES ONE SMALL DIVISION=2000 TRYP.
4TH MAY 1965 TO 23 TH MAY 1965
DATE-TEN SMALL DIVISION=1 (ONE)

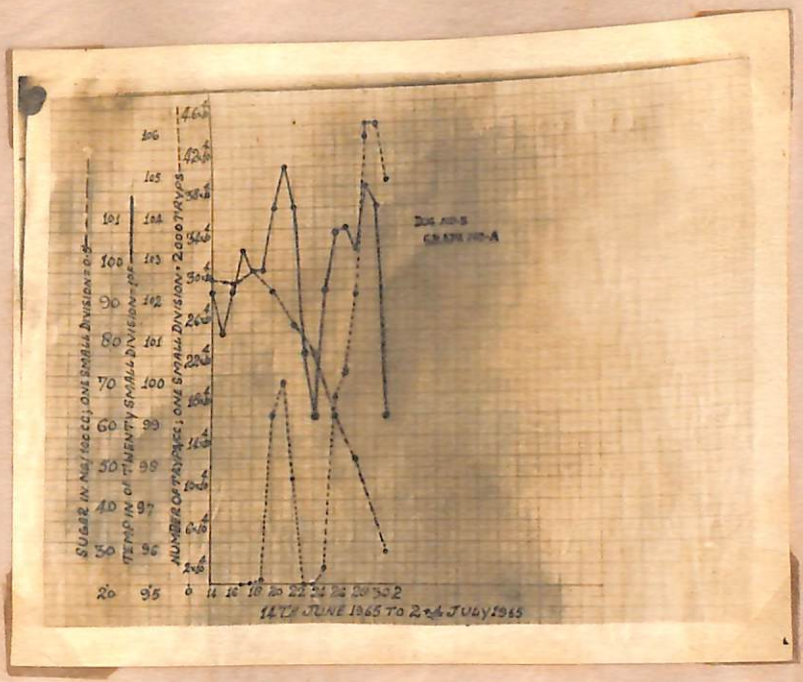

DOG No-5
GRAPH No-B
NUMBER OF LYMPHOCYTE; ONE SMALL DIVISION=0.5
NUMBER OF NEUTROPHILS ONE SMALL DIVISION=0.5
NUMBER OF TRYPANOSOME ONE SMALL DIVISION=2000 TRYP.
4TH MAY 1965 TO 23RD MAY 1965
DATE-TEN SMALL DIVISION=1 (ONE)

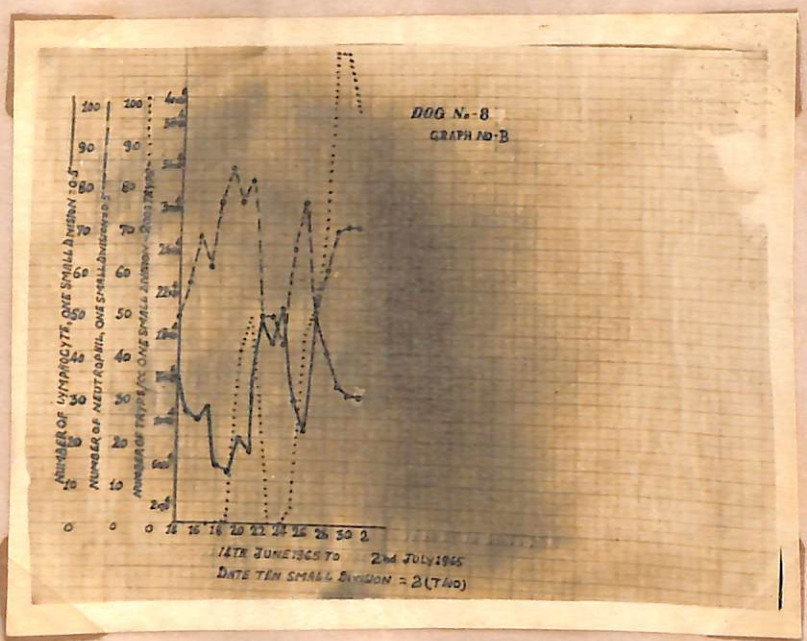

DOG NO-6
GRAPH NO-A
23rd MAY 1965 TO 14TH JUNE 1965
DATE-TEN SMALL DIVISION=1 (ONE)


DOG NO-6
GRAPH NO-B
23rd MAY 1965 TO 14TH JUNE 1965
DATE-TEN SMALL DIVISION=1 (ONE)


DOG NO-7
GRAPH NO-A
23RD MAY 1965 TO 13TH JUNE 1965
DATE TEN SMALL DIVISION = 2 (TWO).


DOG NO-7
GRAPH NO-B
23RD MAY 1965 TO 13TH JUNE 1965
DATE TEN SMALL DIVISION = 2 (TWO).


DOG No-8
GRAPH NO-A
14TH JUNE 1965 To 2nd July 1965


DOG No-8
GRAPH NO-B
14TH JUNE 1965 To 2nd July 1965
DATE TEN SMALL DIVISION = 2 (TWO)

DISCUSSION & CONCLUSION

Morphology:- The general morphological features in respect of dimensional measurements, disposition of nucleus, kinetoplast, undulating membrane and free flagellum agree with those given by Lingard (1907), Rao & Mudaliar (1934) and Hoare (1956 & 1957) for T. evansi except the occurrence of stumpy forms reported by Hoare (1956). Hoare reported for the first time polymorphism in T. evansi and placed this parasite in Brucei group. He described three forms, viz slender, intermediate and stumpy. Later Godfrey and Killick-Kendrick (1962) also reported the occurrence of stumpy forms without free flagella in camel and confirmed the findings of Hoare (1956).

In the present investigation no stumpy form devoid of flagellum was encountered, though the measurements of some of the parasites agreed with those given by Hoare (1956) for stumpy and intermediate forms. The author, therefore, feels reluctant in the absence of free flagellum, to record polymorphism in T. evansi on the basis of dimensional differences alone. Because the measurements given by Hoare for all the three forms fall under the range given by Lingard (1907) and others advocates of monomorphisim.

Multiplication:- The pattern of division was found to be by longitudinal binary fission. First, kinetoplast divides into two, i.e. an anterior and a posterior one. The original flagellum remaining attached in most cases with the posterior kinetoplast. The new flagellum originates and develops from the remaining one. Then the division of nucleus takes place into two and finally the protoplasm divides in most cases from the anterior end of the body, though in a few cases it also divides from the posterior end. Dibinary fission was also observed during the investigation in a few cases, i.e. an individual dividing form resulting finally into four individuals.

Thus, the modes of division observed during present study does not support the observations of Laveran & Mesnil (1907) regarding the division of flagellum into two halves but it agrees with the findings of Hoare (1949). The occurrence of multiple division reported by the former authors are, however, confirmed in this study. According to Hoare (1949) the division of protoplasm takes place only from the anterior end but in this study in a few cases it was observed that the protoplasm divided from the posterior end of the body. This is in confirmity with the findings of Laveran & Mesnil (loc. cit) who also observed the division of protoplasm from the posterior end in a few cases.

Motility:- In wet preparations the active trypanosomes were found to move towards the anterior end of the body

with the help of the free flagellum and undulating membrane, an observation agreeing with that of Laveran & Mesnil (1907), Knowles (1927) and Hoare (1949). But an interesting observation was recorded during this investigation regarding the movement of trypanosomes towards the posterior extremity, which appears hitherto unreported. In wet smears when the parasites became sluggish after a period of active movement, they were found to show backward movement with the help of their undulating membrane.

Virulence:- During the course of subsequent passage of the trypanosomes in nine dogs, the severity of the clinical symptoms was found to increase gradually while the period of survival to decrease. This shows that subsequent passages of the trypanosomes in dogs with definite doses increase their virulence, which was observed by Laveran and Mesnil (1908) also in guineapigs and dogs.

Prepatent period:- Nine pariah dogs were inoculated with T. evansi and the parasites appeared in the peripheral circulation in all the dogs in 3 to 5 days of the infection. The present study thus confirms the observations of Laveran & Mesnil (1905), Gaiger (1908), Cabrera & Lui (1956) and Naiba (1962), who also reported the prepatent period to be 2-8 days.

Parasitaemia:- Parasitaemia, i.e. appearance of the trypanosomes in the infected dogs, was studied by actual counting of the trypanosome per c.c. of blood

at 24 hourly intervals. This method was adopted in order to make the observation more objective and findings more accurate. Because the method adopted hitherto by other workers, viz Lingard cited by Laveran & Mesnil (1907), Sen et al (1955) and Desowitz & Watson (1961), to study the parasitaemia has been arbitrary with expression of the numbers symbolically.

Parasites appeared in the blood in 3 to 5 days, gradually increasing in number reaching the peak in 5 to 8 days with a count of nearly 2 to 3 lacs per c.c. This was followed by sudden disappearance of the parasites from the circulation and reappearance after 1 to 4 days with a peak count of nearly 2 to 4 lacs per c.c. This trend of appearance of the parasites in circulation followed by sudden disappearance with 2 to 4 such repetitions was observed in all the nine experimental dogs. But the duration of parasitaemia and also the period of disappearance varied from dog to dog. Similarly the number of repetitions of parasitaemia in latent phase cycle also differed from dog to dog. During the last days of the disease trypanosomes were very numerous in the blood circulation. These findings are in conformity with those of Pease (1904), Lingard cited by Laveran & Mesnil (loc. cit.), Delpy & Rafyi (1947) and Haiba (1962) in dogs.

The correlation between the rise of temperature and parasitaemia is interesting. Generally the temperature

rose up with the appearance of the parasites in the circulation and fell down with their disappearance therefrom. But sometimes variations in certain cases were also observed, though the parasites in the circulation were numerous, the temperature remained normal or slightly above normal. Such variations were also observed by Lingard cited by Laveran & Mesnil (loc. cit.). The high range of temperature varied from 104 to 106.2°F while the lowest temperature recorded was 99°F.

Clinical Symptoms:- The clinical symptoms observed in the experimental dogs were broadly classified as acute and chronic. In the acute form the animals did not survive more than three weeks whereas in the chronic one they survived for as long as 43 days. In the acute cases the animals generally showed periodical rise and fall of temperature, dullness, anorexia, conjunctivitis, swelling around the eyes, throat and lower jaw. Weakness, emaciation and dyspnoea were also observed. But in dog no. 3 the body coat was found to be rough and in dog no. 9 no swelling was observed.

In chronic form, in addition to the above symptoms, progressive emaciation, partial blindness, opacity of cornea and nervous symptoms with paralysis of hind limbs were observed. The present study is thus, corroborative of the observations of Peas (1904), Laveran & Mesnil (1905), Lingard cited by Laveran & Mesnil (1907) Ajawani et al(1933),

Sheshadri (1955), Bhardwaz et al (1962) and Haiba (1962).

HAEMATOLOGY

The blood picture was studied daily in order to find out the exact trend of variations in the blood constituents. So far as the author is aware no worker has studied this aspect of trypanosomiasis, in such detail with observations made at such short intervals.

Total Red Cell Count:- The trend adopted by the erythrocyte count was sudden fall after the appearance of the trypanosome in the circulation. Then a gradual drop, followed by again sudden drop just before death. This observations agrees with the findings of Della Vida and Verdozzi (1906), Lingard cited by Laveran & Mesnil (1907), Krijgsman (1933), Hoppe (1945-46), Edward et al (1956), Samadar et al (1962) and Srivastava (1965).

Total White Blood Corpuscles:- After infection leucocytosis was observed during the prepatent period followed by leucopenia, which continued till death in one (dog no.3) of the nine dogs. This agrees with the findings of Della Vida and Verdozzi (1906) and Samadar et al (1962).

In 8 other dogs the behaviour of leucocytes differed from the reports of the above workers. After leucocytosis, the leucopenia occurred but did not continue till death as reported by these workers. But showed an alternate rise

and fall coinciding with the alternate parasitaemia and latent phase respectively till death.

Haemoglobin & Packed Cell Volume:- Decrease in haemoglobin percentage and packed cell volume were recorded in all the experimental dogs which confirms with the findings of other workers like Lingard cited by Laveran and Mesnil (1907), Hoppe (1945-46), Edward et al (1957), Samadar et al (1962) and Srivastava (1965).

Mean Corpuscular Volume:- As regards mean corpuscular volume there was an increase of M.C.V. in all the dogs and remained as such till death except in dog nos. 1 and 5 which showed drop in the later stage of infection. Thus in clinical parlance it may be said that macrocytic anaemia was observed in all dogs except nos. 1 and 5 where anaemia in the early period was macrocytic and later became microcytic. The later observation agrees with the finding of Fiennes (1954).

Mean Corpuscular Haemoglobin & Mean Corpuscular Haemoglobin Concentration:- M.C.H. dropped in four experimental dogs which agrees with the findings of Samadar et al (1962) who worked on the experimental goats infected with T. evansi. In other dogs no significant changes were observed. M.C.H.C. also decreased in most of the animals except two where it remained stationary.

Erythrocyte Sedimentation Rate:- No significant change in erythrocyte sedimentation rate was observed and this confirms with the findings of Edward et al (1956)

part of the disease in rats infected with T. evansi. But the above findings do not support the observations of Della Vida & Verdozzi (1906) & Samadar et al (1962). They reported neutrophilia and decrease of lymphocytes throughout the course of the disease.

A drop in the eosinophil count was also observed which continued thoughout the whole course of the infection. This also confirms with the findings of Laveran & Mesnil (1907) and Cabrera and Lui (1956) who reported diminution of eosinophils in dogs infected with T. brucei and T. evansi. There was also decrease in the monocyte percentage except dog no. 3 and 9. But Kaltenbach (1954) reported no change in the monocyte percentage of camels and horses in natural Surra cases.

BLOOD SUGAR

In the course of investigations the changes in the blood sugar content were studied on every alternate day. The blood sugar level dropped gradually with the progress of the infection till the terminal stages. The animal showed marked hypoglycaemia. From the present observations it can be seen that death occurred in all the experimental dogs when there was sudden fall in blood sugar level.

The present findings are in agreement with those of Gaiger et al (1930), Randall (1934), Castillo and

Joaquin (1955) and Sen et al (1955), who have reported hypoglycaemia in mice, horses, rats and rabbits infected experimentally with T. evansi. Christophers et al (1938) also observed deficiency in blood sugar contents and formation of acid products. Von Brand (1938) reported that pathogenic species of trypanosomes consumed more sugar than the non-pathogenic ones. This is also confirmed by the present findings.

Gross Pathology:- During postmortem examination enlargement of spleen, hypertrophy of liver and patches of congestion in lungs were seen in all the dogs, which confirms with the observation of Laveran & Mesnil (1907) in mice, rats and dogs, Gaiger (1909) in dogs and Sen et al (1956) in guineapigs infected with T. evansi.

In the present investigation bone marrow was also slightly reactive, brain was pale and anaemic, kidneys and lymph glands were found normal. Stomach, large and small intestines were slightly inflammed. As regards changes in bone marrow, brain, lymph glands the findings are in agreement with that of Sen et al (loc.cit) and in regard to stomach, large and small intestines with that of Gaiger (1909). In chronic cases right ventricle was always found overdilated. Smears of lungs, liver, heart, kidneys, spleen, bone marrow, brain and lymph glands were examined. Except lungs, liver, heart and kidneys smears of other organs were found negative for the parasites.

SUMMARY

Experimental Trypanosomiasis in dogs caused by T. evansi (Steel, 1885) was studied. Nine dogs were used in this experiment and observations were made on progressive course of the infection, clinical symptoms, haematology, biochemistry and gross pathology. The morphology and biology of the parasites were also studied.

T. evansi was always found to be monomorphic, slender in form and carrying a free flagellum. The parasite was found to multiply by longitudinal binary fission. The division starting from the kinetoplast and ending with that of the protoplasm. There was no division of the flagellum. In wet smears the parasites were also seen to move towards the posterior direction with sluggish movement. On subsequent passages the virulence of the parasite was found to increase.

The trypanosome appeared in the blood circulation of the infected dogs after a prepatent period of 3 to 5 days. After appearance the number of the parasites present in the blood fluctuated irregularly reaching a peak of 2 to 4 lacs per c.c. and then suddenly disappearing from the circulation. Similarly the temperature also showed a periodical rise and fall. Symptoms observed were lachrymation, conjunctivitis, oedema wasting inco-ordination of limbs, anaemia dysponea and finally death between

14 to 43 days after inoculation.

The blood picture showed fall in erythrocyte count, packed cel volume and the haemoglobin content. Mean corpuscular volume increased in all the dogs except dog nos. 1 & 5 in which there was drop in M.C.V. in later stage. The count of white blood corpuscles showed irregular rise and fall. In the initial stage of the infection, there was neutrophilia with a decrease of lymphocytes. This was followed by lymphocytosis and decrease in neutrophils. At the time of death there was neutrophilia. A drop in the eosinophil count was also observed with the progress of the disease.

Biochemical studies showed decline in the level of blood sugar to the extent of 21 to 40 mgms. per 100 c.c. as against the normal of 80 to 104 mgms. Gross pathology showed enlarged and congested spleen with reddish granular appearance on the surface. Lungs were congested and pneumonic. Liver was hypertrophied. Right ventricle of the heart was found to be overdilated. Smears of lungs, liver, heart, kidneys, spleen, bone marrow, brain and lymph glands were examined. Except lungs, liver, heart and kidneys smears of other organs were found negative for the parasites.

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