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CLINICOPATHOLOGICAL STUDY ON EXPERIMENTAL *TRYPANOSOMA BRUCEI* INFECTIONS IN HORSES

PART 1. DEVELOPMENT OF CLINICALLY RECOGNIZABLE NERVOUS SYMPTOMS IN NAGANA-INFECTED HORSES TREATED WITH SUBCURATIVE DOSES OF ANTRYPOL AND BERENIL

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ABSTRACT

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Studies on the pathogenesis and symptomatology of the acute and chronic forms of human sleeping sickness and those appearing in equine nagana caused by *Trypanosoma brucei* Plimmer & Bradford, 1899 are given. In man the initial invasion of the blood stream and lymph nodes by either *T. rhodesiense* Stephens & Fantham, 1910 or *T. gambiense* Dutton, 1902 is invariably followed by parasites entering the cerebrospinal fluid and eventually extending to the brain and producing symptoms of meningo-encephalitis. In horses the invasion of the blood stream and lymph nodes by *T. brucei* results in the development of a peracute, acute or chronic disease which nearly always terminates fatally without clinical evidence of an involvement of the central nervous system.

Consideration of the relatively short reaction periods of 2 to 3 months in *T. brucei* infections when compared with those of 9 months to several years in human trypanosomiasis, suggested that prolongation of the course of nagana in horses by subcurative treatments with Antrypol and Berenil would allow the parasite sufficient time to enter the cerebrospinal fluid and then to exert its pathogenicity on the central nervous system. It was found that such treatments resulted both in the extension of the course and in the appearance of nervous symptoms in two of the five treated horses. The involvement of the central nervous system was confirmed at necropsy by a mild hydrocephalus, oedema of the brain, thickening of the meninges and the detection of *T. brucei* in the cerebrospinal fluid.

Evidence is presented that in common with *T. rhodesiense* and *T. gambiense*, *T. brucei* under certain conditions exerts its invasive potential for the cerebrospinal fluid.

INTRODUCTION

African equine trypanosomiasis, caused by *Trypanosoma brucei* Plimmer & Bradford, 1899 is a peracute, acute or chronic insect-borne disease. It is characterized by pyrexia, progressive anaemia, emaciation, muscular atrophy, lachrymation and nasal hydrorrhoea, oedematous swelling of the external genitalia, lower abdomen and chest, ventral region of the neck, face, sometimes supraorbital fossae in peracute cases and limbs, and in advanced cases "lumbar paralysis" (Knuth & Du Toit, 1921). Depending upon the virulence of the pathogen, the animal may die within a few weeks or several months after the onset of symptoms. Macroscopic pathological changes are not pathognomonic. There appear to be no studies directed specifically to the nervous system of naturally or experimentally infected solipeds (Innes & Saunders, 1962). The only references to histopathological studies of the nervous system in domestic animals appear to be those conducted by Sauerbeck (1906) and Mönckeberg & Simons (1918) on dogs suffering from an artificially induced *T. brucei* infection. A valuable contribution towards the studies of equine trypanosomiasis has nevertheless been made by Martini (1905). He found polymorphic trypanosomes in the CSF immediately after the death of experimentally infected solipeds. The two trypanosome strains, which he used for his investigations, were isolated from horses in Togo, West Africa. He refers to them as "tsetse-parasites" but Laveran & Mesnil (1907) consider them to be *T. brucei*.

African human trypanosomiasis is caused by either *T. gambiense* Dutton, 1902, which causes the chronic form lasting up to several years, or *T. rhodesiense* Stephens & Fantham, 1910, which produces the acute form of sleeping sickness lasting 3 to 9 months. The initial stages of infection are characterized by an invasion of the blood stream and lymph nodes by trypanosomes, while in advanced stages the nervous system becomes involved, the parasite first penetrating the cerebrospinal fluid (CSF) and eventually extending to the brain and producing symptoms of meningo-encephalitis (Hoare, 1949).

Clinically, sleeping sickness is characterized by an irregular, intermittent fever, lymphadenopathy, localized oedemas and erythemas of the face, eyelids and ankles and evanescent congested or erythematous patches on the face, trunk or limbs. Anaemia, general weakness and wasting are not prominent at first but gradually increase as time progresses (Laveran & Mesnil, 1907). Although apathy is one of the chief features, Mense (1930) states that it may be preceded or, alternatively, interrupted during the early stage by a short period of excitability. This is invariably followed by drowsiness which progresses to coma and ultimately to death, if untreated.

Consideration of the meningo-encephalitic lesions in both forms of human trypanosomiasis prompted us to produce a chronic form of *T. brucei* infection by administering subcurative doses of Antrypol to infected horses. It was hoped that during the subdued course of

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nagana, the protozoon would penetrate the CSF and thereafter the brain. When it became apparent that this form of treatment failed to produce recognizable nervous symptoms in one horse, subcurative doses of Berenil were included in the chemotherapeutic programme. It was believed that Berenil would prevent acute deaths and extend the course of the disease and thus afford it an opportunity to invade the CSF. This approach was successful and resulted not only in the development of pronounced subcutaneous oedemas but also in readily recognizable nervous symptoms.

Observations on the parasitaemia, symptoms, haematology, serology, chemotherapy and macroscopic lesions, conducted over a period of a year, are given here. The histological examination of various organs, with special reference to changes in the nervous system, will be described in Part 2.

Materials and methods

Trypanosoma spp.: The *T. brucei* strain was kindly supplied by Mr. W. P. Boyt, Chief Veterinary Officer, Trypanosomiasis Research Section, Branch of the Tsetse Fly and Trypanosomiasis Control, Salisbury, Rhodesia in December, 1963.

The *T. equiperdum* Doflein, 1901 strain was obtained through the courtesy of Dr. F. Hawking, Division of Chemotherapy and Parasitology, National Institute for Medical Research, London, England.

Animals: Six horses were used for these experiments. They were immunized against anthrax and horsesickness. The complement fixation test was used to determine whether they were susceptible to trypanosomiasis.

All horses were infected with *T. brucei* either by the subcutaneous or the intravenous route. Of these, two served as the untreated control group, one as the Antrypol treated group and the remaining three as the Antrypol and Berenil treated group.

Guinea pigs and white rats, used for the maintenance of the two *Trypanosoma* spp., production of the *T. equiperdum* antigen and biological tests, were bred at Onderstepoort. In order to avoid complications by various pathogens in the experimental horses, infected guinea pigs were used as the source of *T. brucei*.

Serology: Only the complement fixation test was used and the technique was essentially the same as that employed by Robinson (1926) using the anti-sheep corpuscles haemolytic system. *T. equiperdum* antigen was used for all the tests. Serum samples were inactivated at a temperature of 58°C for 30 minutes and such sera never gave non-specific reactions.

As a rule the CF tests were conducted at weekly but sometimes at shorter intervals. The reason why these tests were conducted throughout the entire course of the disease was because Craig (1948) had pointed out that CF tests with antigens prepared from *T. gambiense* and *T. rhodesiense* had not proved to be successful in the diagnosis of African sleeping sickness. Whether or not such a phenomenon would appear in the chronic form of *T. brucei* needed to be determined.

CF tests in the six horses were negative for approximately 2 weeks after infection. This was followed by a partial fixation of complement for periods varying between 2 to 6 weeks and thereafter by complete fixation until death supervened. The state of the parasitaemia and the reactions to serological tests agreed well and confirmed the conclusions of other investigators that *T. equiperdum* antigen is satisfactory from a diagnostic standpoint.

Trypanocidal drugs: Antrypol is effective against the *T. gambiense - evansi* group and is used as a curative and a prophylactic. It does not readily penetrate the CNS and is therefore of no value as a curative when trypanosomes have invaded the CSF (Findlay, 1950). Hawking (1963) states that in animals this drug may have a toxic effect on the kidneys and that this may be accompanied by minute haemorrhages and degenerative changes in the liver, lung and CNS. When administered in large doses to horses it may cause laminitis. In equine *T. brucei* infections it is prescribed as a curative at the rate of 10 mg/kg and persists in the blood for long periods. Two to three subcurative treatments with 5 mg/kg of Antrypol, repeated after fairly long intervals, were given to four horses and toxic effects were not expected.

Berenil is a curative when given at the rate of 7 mg/kg to horses suffering from *T. brucei*. It is readily excreted and hence its prophylactic action is limited. In doses approaching toxic levels acute symptoms result from the effects of the drug on the central nervous system, namely tremors, nystagmus and ataxia (Hawking, 1963). Subcurative doses administered to three horses varied from 1 to 3 mg/kg and toxic symptoms were not expected.

In attempts to produce the cerebral form of the disease in horses infected with *T. brucei,* treatment with Antrypol was followed by that with Berenil in three. Although Antrypol may persist in the blood of animals for 3 months and longer, it was not anticipated that the synergic action of these drugs would have a curative effect even when the interval between the injections was as short as 19 days as in Mare 5588 (Table 6a). On the contrary the chronicity persisted and terminated with an involvement of the CNS.

Haematological observations: Erythrocytic, leucocytic and differential white cell counts were conducted on four treated horses initially at weekly intervals but when it was suspected that the anaemia would become more severe they were repeated at shorter intervals. Special attention was paid to the degree of anaemia so that timely intervention with subcurative doses of Antrypol or Berenil could be applied to extend the chronicity of the infection. In doing so, it was hoped that *T. brucei* would have an opportunity to invade the CSF and produce lesions similar to those occurring in human sleeping sickness.

Blood and tissue smears were fixed by May-Grünwald solution and stained with a 5 per cent solution of Giemsa stain for 35 minutes.

Experiments and results

Experiment 1

In order to determine the degree of virulence of the Rhodesian *T. brucei* strain, Geldings 5484 and 5480 were injected with infective guinea pig blood by the subcutaneous route. Observations on the course of the disease are recorded in Tables 1a and 1b for Gelding 5484 and in Tables 2a and 2b for Gelding 5480. In both horses the incubation period was 4 days and the duration of the reaction 50 days. Blood smears examined daily remained positive throughout the course of the disease. Clinically, they showed a progressive anaemia, subcutaneous oedema of the external genitalia and ventral region of the abdomen and thorax, unsteady gait and finally prostration for 2 days before death. An attempt to prolong the life of Gelding 5480 (Table 2) by administering a curative dose of Berenil together with an infusion of gluco-saline on the 47th day of the reaction was of no avail. This horse was killed *in*

extremis two days later and Gelding 5484 died. Nervous symptoms were absent in both horses.

Serologically, 5484 showed a partial fixation of complement from the 13th to 47th day and complete fixation on the 52nd day after infection. In Gelding 5480 there was a partial fixation of complement from the 13th to 54th day of reaction.

At necropsy the main lesions of both horses were a generalized anaemia, emaciation, gelatinous infiltration of the subcutaneous tissues and degenerative changes of the liver.

Consideration of the severity and duration of the clinical symptoms showed that the Rhodesian *T. brucei* strain is highly virulent. The failure of Gelding

TABLE 1a *Gelding 5484. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection	Date of treatment	Clinical symptoms and signs	Lesions at necropsy
27.12.63 sc, 10 ml blood from a guinea pig harbouring *T. brucei*	4	31.12.63 to 18.2.64	—	30.12.63 to 6.1.64 - subcutaneous oedema, 8 cm in diameter, at site of injection on left side of neck; 28.1.64 to 18.2.64 - moderate oedema of scrotum and prepuce with an extension to the ventral region of abdomen; 11.2.64 to 16.2.64 - general weakness and unsteady gait; 17.2.64 to 18.2.64 - prostrated; 19.2.64 - died	P M 42231 - Slight post mortem changes; generalized anaemia; decubital lesions on hip, elbow and face; subcutaneous gelatinous infiltration of scrotum, prepuce and ventral region of abdomen; subepi- and subendocardiai petechiae; degenerative changes of liver; yellow pigmentation of small intestine

Legend - Tables 1a to 6a
IP = incubation period
iv = intravenously
sc = subcutaneously
CF = complement fixation

TABLE 1b *Gelding 5484. Observations on the parasitaemia, haematology and serology*

Date	*T. brucei*	RCC 10^6/mm^3	WCC 10^3/mm^3	CF tests
18.12.63	•	•	•	—
27.12.63	•	•	•	—
2.1.64	+	•	•	—
9.1.64	4+	•	•	+
15.1.64	4+	•	•	+
20.1.64	+	4,18	8,5	•
23.1.64	+	•	•	+
29.1.64	2+	•	•	+
5.2.64	+	•	•	+
12.2.64	2+	•	•	+
17.2.64	+	•	•	++

Legend - Tables 1b to 6b

T. brucei — = negative
„ „ + = present
„ „ 2+ = rare
„ „ 3+ = fairly frequent
„ „ 4+ = frequent

CF test — = negative
„ „ + = partial fixation
„ „ ++ = complete fixation
„ „ • = not done

A = Antrypol
B = Berenil

RCC = red cell count
WCC = white cell count
PCV = packed cell volume

TABLE 2a. *Gelding 5480. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection	Date of treatment	Clinical symptoms and signs	Lesions at necropsy
27.12.63 sc, 10 ml blood from guinea pig harbourring *T. brucei*	4	31.12.63 to 18.2.63	17.2.63 Berenil 6 mg/kg i m; intravenous infusion of 2 litres 10% glucosaline	30.12.63 to 7.1.64 - subcutaneous swelling, 8 cm in diameter, at site of injection on left side of neck; 16.1.64 to 27.1.64 - moderate oedema of scrotum and prepuce; 28.1.64 to 19.2.64 - marked oedema of external genetalia with extension to ventral region of abdomen, chest neck and face; 14.2.64 to 17.2.64 - general weakness and unsteady gait; 18.2.64 to 19.2.64 - prostrated; 19.2.64 - killed *in extremis*	P M 42232 - Generalized anaemia; subcutaneous gelatinous infiltration of scrotum, right flank, ventral region of abdomen, chest, neck and face; gelatinous infiltration of mediastinal, renal, cardiac and pelvic fat; degeneration of liver; fibrinous peritonitis; petechiae in mucosa of bladder; brain lacerated following bullet wound

5480 to respond to treatment during the advanced stage of the disease made it evident that, to produce a chronic form of trypanosomiasis, subcurative medication would have to be applied during the early stage of the infection and repeated as required.

Experiment 2

Having determined the degree of virulence of the *T. brucei* strain it was decided that an attempt should be made to produce a chronic form of nagana by administering a subcurative dose of Antrypol during the

TABLE 2b *Gelding 5480. Observations on the parasitaemia, haematology and serology*

Date	*T. brucei*	RCC 10^6/mm³	WCC 10^3/mm³	CF tests
18.12.63	—	•	•	—
27.12.63	—	•	•	—
2.1.64	+	•	•	—
9.1.64	3+	•	•	+
15.1.64	+	•	•	+
20.1.64	+	4,17	6,7	•
23.1.64	+	•	•	+
29.1.64	+	•	•	+
5.2.64	+	•	•	+
12.2.64	3+	•	•	+
17.2.64	2+	•	•	+
18.2.64	+	•	•	+
19.2.64	+	•	•	+

TABLE 3a. *Gelding 2287. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection	Date of treatment	Clinical symptoms and signs	Lesions at necropsy
20.2.64 iv, 10 ml blood from guinea pig harbouring *T. brucei*	6	26.2.64 to 13.3.64 15.4.64 to 8.6.64	13.3.64 Antrypol 5 mg/kg iv 8.6.64 Antrypol 5 mg/kg iv	Progressive anaemia followed by a transient improvement (Table 3b) after first treatment; second treatment of no value and died 2 days later; 3.3.64 to 15.3.64 - slight oedema of scrotum and prepuce; 16.3.64 - oedema receding; 19.3.64 to 15.4.64 - condition apparently normal; 16.4.64 to 10.6.64 - oedema of external genitalia with extension to ventral region of abdomen and thorax; inappetence and emaciation; 1.6.64 to 7.6.64 - unsteady gait; 25.5.64 to 10.6.64 - oedema of limbs below elbows and hocks; 10.6.64 - died	P M 42261 - Generalized anaemia and icterus; subcutaneous gelatinous infiltration of scrotum, prepuce, ventral region of abdomen, thorax and limbs; atrophy of fat; hydrothorax, hydropericardium; subpleural and subendocardial petechiae; congestion and oedema of lungs; slight tumor splenis: generalized lymphadenitis; cirrhosis of liver; hypertrophy of bone marrow; mucocatarrhal gastritis; congestion of mucosa of colon

TABLE 3b *Gelding 2287. Observations on the parasitaemia, haematology and serology*

Date	*T. brucei*	RCC 10^6/mm³	WCC 10^3/mm³	PCV	Differential WCC					CF tests
					M	L	N	B	E	
21.2.64	—	•	•	•	•	•	•	•	•	—
25.2.64	—	•	•	•	•	•	•	•	•	—
5.3.64	+	5,50	9,4	24	5	34	55	3	3	+
11.3.64	3+	5,23	9,7	23	3	37	59	1	0	+
13.3.64	A	•	•	•	•	•	•	•	•	•
18.3.64	—	4,52	7,4	23	3	39	53	1	4	—
25.3.64	—	4,96	11,1	27	2	29	64	2	3	—
1.4.64	—	5,96	6,3	33	2	32	54	0	12	—
8.4.64	—	5,86	6,5	30	2	85	10	0	3	++
15.4.64	—	6,71	7,8	33	1	22	72	0	5	++
22.4.64	+	5,74	6,7	28	3	44	51	0	2	++
29.4.64	2+	7,40	6,8	27	1	39	59	0	1	++
6.5.64	+	3,91	6,7	22	3	38	50	2	7	++
13.5.64	+	2,56	5,9	15	1	53	46	0	0	++
20.5.64	+	2,81	4,0	16	1	53	41	0	5	++
27.5.64	+	3,28	3,9	19	1	43	55	0	0	++
3.6.64	+	2,74	4,8	17	0	37	62	0	1	++
8.6.64	+	2,21	7,1	15	0	35	65	0	0	++
8.6.64	A	•	•	•	•	•	•	•	•	•

early stage and repeating treatment at a later stage of the disease if necessary. It was hoped that nervous symptoms would develop if the duration of the disease could be extended beyond the period of 54 days which the two horses in Experiment 1 survived. This would not only offer *T. brucei* a better opportunity to invade the CSF but would also permit the parasite to exert its pathogenicity over a longer period.

Gelding 2287 was infected with *T. brucei* by the intravenous route. Observations on the course of the disease are listed in Tables 3a and 3b. The incubation period was 6 days and the duration of the reaction 105 days. A parasitaemia of *T. brucei* and a moderate swelling of the external genitalia were seen up to the 16th day of reaction when a subcurative dose of Antrypol was administered by the intravenous route. Parasites could not be demonstrated during the ensuing 33 days and the oedematous swellings receded within 3 days. This was followed by a parasitic relapse on the 50th day, reappearance of the oedemas on the 51st day, commencement of a progressive anaemia on the 70th day, an unsteady gait on the 97th day and prostration on the 103rd day of reaction. These symptoms persisted until death supervened on the 105th day. The horse failed to respond to a second Antrypol treatment which was given on the first day of prostration.

Serologically it was found that a partial fixation of complement was present from Day 14 to 20 after infection, no fixation during the ensuing 13 days and complete fixation from Day 48 until the animal died. Absence of fixation during the 13 day-period may have been influenced by the administration of Antrypol.

At necropsy the usual nagana lesions were accompanied by hyperplasia of the bone marrow (Table 3a).

No satisfactory explanation can be given as to why this chronic form of nagana, which persisted for 105 days, was not associated with recognizable nervous symptoms. Absence of these symptoms suggested that *T. brucei* had not invaded the CSF.

At this stage of the investigation the failure to produce nervous symptoms in Gelding 2287 was tentatively attributed to the fact that the duration of the chronic infection was inadequate for the protozoon to produce lesions in the CNS. It was believed that, by extending the chronicity period beyond 105 days, conditions would become favourable for the production of nervous symptoms. An attempt to extend the period by repeated injections of Antrypol was dismissed on the grounds that the accumulation of this compound in the blood would eventually reach such a concentration that the activity of the parasite would be suppressed to a very low level and its invasive potential reduced. For this reason Berenil, which is readily excreted within 2 days, was selected to assist Antrypol in extending the chronicity period of nagana.

Experiment 3

Tentative reasons have been given above for the inclusion of Berenil in the subcurative treatment programme. By its application it was hoped that more favourable conditions would be created for *T. brucei* to invade the CSF and to exert its pathogenicity on the CNS.

TABLE 4a. *Gelding 5065. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection		Date of treatment	Clinical symptoms and signs	Lesions at necropsy
		T. brucei	*B. equi*			
18.2.64 iv, 8 ml blood, from a guinea pig harbouring *T. bruvei*	3	21.2.64 to 3.3.64 31.3.64 to 21.5.64 29.6.64 to 17.7.64 2.9.64 to 18.9.64 6.10.64 to 20.10.64 After death *T. brucei* was found in liver and CSF	20.3.64 4.6.64 to 1.7.64 11.8.64 27.10.64 13.11.64	6.3.64 Antrypol 5 mg/kg iv 18.5.64 Berenil 1 mg/kg im Methionin 12.5 mg/kg iv 22.5.64 Berenil 1 mg/kg im 17.9.64 Antrypol 5 mg/kg iv 20.10.64 Antrypol 5 mg/kg iv	Progressive anaemia followed by a transient improvement (Table 4b) after each of the five treatments; 25.5.64 to 22.6.64 and 19.10.64 to 9.11.64 - a transient rise in the WCC (Table 4b); 3.3.64 to 3.9.64 oedema of scrotum and prepuce; 10.3.64 - extension of oedema to ventral region of abdomen; 20.3.64 to 29.3.64 - oedema receding; 30.3.64 to 13.5.64 - condition apparently normal; 14.5.64 - oedema of scrotum and prepuce; 18.5.64 - oedema pronounced with extension to ventral region of abdomen and thorax; 17.7.64 - oedema of external genitalia, abdomen and thorax receded; 10.9.64 to 21.9.64 - oedema of supraorbital fossae and lips; apathy, forelimbs extended side ways, head and neck depressed; 22.9.64 - oedema of supraorbital fossae and lips receded; 20.10.64 to 13.11.64 - oedema of supraorbital fossae and lips; pronounced apathy; forelimbs extended side ways and head and neck depressed; 12.11.64 - unsteady gait; 10.11.64 to 11.11.64 - prostrated; 12.11.64 - unable to rise; 13.11.64 - electrocuted	P M - 44359 - Generalized anaemia and icterus; oedema of supraorbital fossae and lips; superficial erosion of right eyelid; emaciation; thrombosis of the pulmonary arteries and worm nodules in right lung; hydropericardium; icteric pigmentation of liver; verminosis of large intestine; hyperplasia of bone marrow of femur; mild hydrocephalus, oedema of brain and moderate thickening of meninges

Gelding 5065 was infected with *T. brucei* by the intravenous route. Observations on the course of the disease are presented in Tables 4a and 4b. The incubation period was 3 days and the duration of the reaction 267 days. Antrypol was administered on Day 15, Berenil on Days 87 and 91 and Antrypol again on Days 209 and 242 in order to suppress the activity of T. *brucei* in tissues other than those of the CNS.

Observations made during various stages of the reaction may briefly be summarized as follows:

(a) Microscopic *T. brucei* infections were observed on Days 1 to 12, 40 to 91, 129 to 148, 195 to 211 and 239 to 243.

(b) Relapses of *Babesia equi* (Laveran, 1901), which apparently did not influence the course of nagana, appeared on Days 30, 105 to 132, 173, 250 and 267.

(c) Oedematous swellings of the external genitalia [Plate 1 (2 and 3)] and ventral regions of the abdomen and thorax [Plate 1 (1)] were visible on Days 12 to 38 and 84 to 148.

(d) Oedemas of the supraorbital fossae and lips were evident on Days 203 to 214 and 243 to 267 [Plate 1 (4 and 5)].

(e) Periods of marked progressive anaemia were observed on Days 48 to 119 and 137 to 166 (Table 4b).

(f) Leucocytosis persisted from Days 96 to 123 (Table 4b).

(g) Nervous symptoms in the form of apathy, accompanied by a lateral extension of the forelimbs and depression of the head and neck, persisted from Days 203 to 267 [Plate 1(1)].

(h) Serological tests showed a partial fixation of complement from Days 16 to 50 after infection and thereafter a complete fixation during the remaining period of the disease.

(i) Necropsy not only revealed lesions commonly seen in equine nagana but in addition hyperplasia of the bone marrow [Plate 1 (6)], a mild hydrocephalus, oedema of the brain and a moderate thickening of the meninges. No

TABLE 4 b *Gelding 5065. Observations on the parasitaemia, haematology and serology*

Date	a. *T. brucei* b. *B. equi*		RCC 10^6/mm³	WCC 10^3/mm³	PCV	Differential WCC					CF tests
	a	b				M	L	N	B	E	
18.2.64	—	—	•	•	•	•	•	•	•	•	—
5.3.64	+	—	8,08	7,2	24	3	36	57	3	1	+
6.3.64	A	•	•	•	•	•	•	•	•	•	•
11.3.64	—	—	4,62	7,6	22	3	41	51	1	4	+
18.3.64	—	—	6,00	7,9	29	2	38	54	1	5	+
20.3.64	—	+	•	•	•	•	•	•	•	•	•
25.3.64	—	—	6,47	7,5	31	1	38	49	2	10	+
31.3.64	+	—	5,92	8,2	30	3	51	40	0	6	+
8.4.64	+	—	5,28	8,4	32	7	47	42	1	3	++
15.4.64	+	—	4,35	9,8	25	1	47	47	0	5	++
22.4.64	—	—	4,30	10,0	22	1	45	49	0	5	++
27.4.64	2+	—	3,70	6,6	21	1	44	55	0	0	++
6.5.64	+	—	2,20	1,9	13	6	55	39	0	0	++
13.5.64	+	—	2,15	2,9	13	3	67	30	0	0	++
18.5.64	B	•	•	•	•	•	•	•	•	•	•
20.5.64	+	—	1,31	4,4	9	3	39	58	0	0	++
22.5.64	—	—	1,56	8,6	10	1	41	58	0	0	++
22.5.64	B	•	•	•	•	•	•	•	•	•	•
26.5.64	—	—	1,97	16,3	13	•	•	•	•	•	++
27.5.64	—	—	2,18	12,9	9	0	14	85	0	1	++
29.5.64	—	—	2,30	18,0	14	3	11	80	3	3	++
3,6.64	—	—	2,99	20,6	19	1	15	83	0	1	++
8.6.64	—	+	3,68	13,8	24	0	15	81	0	4	++
18.6.64	—	+	4,08	10,7	23	0	23	67	0	10	++
22.6.64	—	+	5,27	11,4	28	0	35	53	2	10	++
29.6.64	—	+	5,30	7,4	29	0	27	72	1	0	++
6.7.64	2+	—	3,38	8,4	22	•	•	•	•	•	++
11.7.64	2+	—	3,78	5,2	20	3	63	34	0	0	++
20.7.64	+	—	2,97	6,1	18	0	63	30	0	7	++
27.7.64	—	—	5,70	8,5	24	0	42	51	0	7	++
4.8.64	—	—	4,10	9,2	25	1	63	36	0	0	++
10.8.64	—	—	5,44	7,0	29	0	57	38	0	5	++
11.8.64	—	+	•	•	•	•	•	•	•	•	•
17.8.64	—	—	5,72	7,8	30	0	27	64	1	8	++
24.8.64	—	—	6,31	7,5	33	0	32	56	0	12	++
31.8.64	—	—	6,45	7,0	34	1	38	58	0	3	++
8.9.64	—	—	5,12	5,0	26	1	57	36	1	5	++
14.9.64	+	—	•	•	•	•	•	•	•	•	++
17.9.64	+	—	3,95	3,3	20	0	45	54	0	1	++
17.9.64	A	—	•	•	•	•	•	•	•	•	•
21.9.64	—	—	4,85	6,6	23	0	63	29	3	5	++
28.9.64	—	—	5,48	9,6	26	0	48	46	0	6	++
5.10.64	—	—	5,25	6,3	28	0	28	69	1	2	++
12.10.64	—	—	4,82	6,6	24	•	•	•	•	•	++
19.10.64	+	—	4,16	10,4	20	0	21	69	0	10	++
20.10.64	A	•	•	•	•	•	•	•	•	•	•
26.10.64	—	+	5,20	12,8	35	0	22	75	0	3	++
2.11.64	—	—	5,60	12,6	28	•	•	•	•	•	++
9.11.64	—	—	5,48	12,5	27	0	33	60	0	7	++

difficulty was experienced in removing 100 ml of CSF by the atlanto-occipital puncture. The fluid appeared turbid and a number of active *T. brucei* parasites were readily detected in wet preparations. Giemsa-stained films showed a fairly large number of leucocytes and parasites with typical morphological features. In tissue smears, prepared from other organs, parasites, in relatively small numbers, could be found only in the liver.

(j) Each of the three rats, that received an intraperitoneal injection of 3 ml of CSF, failed to develop a *T. brucei* infection. No explanation can be offered for this failure.

Consideration of the observations made it apparent that subcurative treatments with Antrypol and Berenil were followed by a chronic form of nagana which lasted for 267 days. Nervous symptoms were first recognised on Day 203 of the reaction and persisted for 64 days. The horse repeatedly banged its head on the stable floor during the throes of death. It was electrocuted. The involvement of the CNS was confirmed at necropsy by the presence of hydrocephalus, oedema of the brain, thickening of the meninges and demonstration of *T. brucei* in the CSF.

While this experiment was in progress two additional *T. brucei* infected horses were subjected to a similar form of subcurative medication. Observations on the successful termination of these tests are described in the ensuing two experiments.

Experiment 4

Stallion 5259 was infected with *T. brucei* by the intravenous route. Observations on the course of the disease are listed in Tables 5a and 5b. The incubation period was 6 days and the duration of the disease 270 days. Antrypol was administered on Day 5, Berenil on Days 86 and 155 and Antrypol again on Day 193 in order to suppress the activity of *T. brucei* in tissues other than those of the CNS.

Observations made during various periods of reaction may briefly be summarized as follows:

(a) Microscopic *T. brucei* infections were observed on Days 1, 53 to 85, 148 to 155, 186 to 192 and 257 to 270.

(b) Oedematous swellings of the external genitalia [Plate 1 (8)] and ventral region of the abdomen and thorax were visible on Days 66 to 135 and 199 to 250.

(c) Oedema of the supraorbital fossae persisted from Days 208 to 270.

(d) Marked anaemia was present from Days 64 to 120 and a moderate form from Days 218 to 270.

(e) Nervous symptoms, initially in the form of apathy and later interrupted by bouts of marked excitability, when handled, were observed from Days 219 to 270.

(f) Serological tests showed a partial fixation of complement on Days 9 to 43 after infection and thereafter complete fixation until death.

TABLE 5a. *Stallion 5259. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection	Date of treatment	Clinical symptoms and signs	Lesions at necropsy
21.4.64 iv, 3 ml blood from a guinea pig harbouring *T. brucei*	6	27.4.64 to 29.4.64	1.5.64 Antrypol 5 mg/kg iv	Progressive anaemia followed by a transient improvement (Table 5b) after each of the four treatments;	P M 44374 - Moderate generalized icterus; anaemia; right eye blind; contusion and swelling of right eyelid; oedema of external genitalia, ventral region of abdomen and supra-orbital fossae; hydropericardium; hyperplasia of splenic Malpighian corpuscles; petechiae in cortex and ecchymoses in pelvis of kidneys; mild hydrocephalus and oedema of the meninges; thrombosis of blood vessels of left testicle and both spermatic cords
		18.6.64 to 20.7.64	21.7.64 Berenil 1 mg/kg im	1.7.64 to 14.7.64 - oedema of scrotum and prepuce; 14.7.64 to 27.7.64 - marked anaemia (Table 5b)	
		21.9.64 to 28.9.64	28.9.64 Berenil 3 mg/kg im	15.7.64 - extension of oedema to ventral region of abdomen and thorax; 20.7.64 to 9.8.64 - oedema pronounced;	
		29.10.64 to 4.11.64		10.8.64 - oedema and anaemia receding; 8.9.64 to 10.11.64 - condition apparently normal; 11.11.64 to 22.11.64 - oedema of external genitalia; 20.11.64 - oedema of supraorbital fossae; 23.11.64 - extension of oedema to ventral region of abdomen;	
		8.1.65 to 21.1.65	5.11.64 Antrypol 5 mg/kg iv	24.11.64 to 21.1.65 - oedema of external genitalia, ventral region of abdomen and supraorbital fossae pronounced; 1.12.64 to 21.1.65 - periodic yawning and paresis of hindquarters; 10.12.64 to 21.1.65 - nervous symptoms alternating from apathy, when left alone, to excitability, when handled, and terminating in an attempt to climb up on the stable wall and exhaustion; 21.1.65 - escaped from stable, ran about aimlessly, excitable, coaxed back to stable when exhausted, tranquilized and electrocuted	
		After death *T. brucei* was found in spleen, heart blood, lung and CSF			


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Necropsy not only revealed lesions commonly seen in equine nagana but in addition hyperplasia of the marrow of long bones, hydrocephalus, oedema of the brain and cloudiness and thickening of the meninges [Plate 2 (9 and 11)]. No difficulty was experienced in obtaining 250 ml of CSF by the atlanto-occipital puncture. The fluid appeared turbid and a readily detectable number of active parasites were observed in wet preparations: Giemsa-stained films showed a fairly large number of leucocytes and several parasites with typical morphological features. Blood and tissue smears from the spleen, liver and lung revealed a small number of trypanosomes.

Three rats, which received an intraperitoneal injection of 3 ml of CSF, failed to develop a *T. brucei* infection. No explanation can be offered for this failure.

Consideration of the observations made it apparent that subcurative treatments with Antrypol and Berenil were followed by the development of the chronic form of nagana which persisted for 270 days. Nervous symptoms were observed on Day 219 of the reaction and lasted 51 days. The involvement of the CNS was confirmed at necropsy by macroscopic lesions in the meninges and brain [Plate 2 (9 and 11)] and the presence of active parasites in the CSF. In contrast to the apathetic behaviour over a period of 73 days by Gelding 5065, Stallion 5259 exhibited apathy for 30 days but thereafter this phase was interrupted by periods of excitability when handled. As time progressed the degree of excitability reached a dangerous level. Cleaning the stable and feeding became a dangerous task for the servant who had attended the horse during the entire course of the disease. The horse was therefore tran-

TABLE 5b *Stallion 5259. Observations on the parasitaemia, haematology and serology*

Date	*T. brucei*	RCC 10^6/mm³	WCC 10^3/mm³	PCV	Differential WCC					CF tests
					M	L	N	B	E	
22.4.64	—	9,87	8,8	39	1	34	55	0	10	—
27.4.64	2+	8,05	6,8	36	0	10	90	0	0	—
1.5.64	A	•	•	•	•	•	•	•	•	•
6.5.64	—	5,48	6,4	27	0	44	52	0	4	+
13.5.64	—	6,29	9,6	30	0	26	68	1	5	+
18.5.64	—	6,97	6,9	31	0	49	37	0	14	+
26.5.64	—	7,08	7,2	32	0	35	55	1	9	+
3.6.64	—	6,83	7,7	33	1	34	56	3	6	+
8.6.64	—	7,69	5,7	34	1	42	53	0	4	+
22.6.64	+	7,23	5,7	36	0	58	39	1	2	++
29.6.64	+	5,15	5,9	27	1	36	63	0	0	++
6.7.64	+	4,15	8,9	22	•	•	•	•	•	++
14.7.64	2+	2,64	5,4	15	2	73	24	0	1	++
20.7.64	—	2,34	5,1	15	0	60	40	0	0	++
21.7.64	B	•	•	•	•	•	•	•	•	•
27.7.64	—	3,32	5,6	18	2	72	26	0	0	++
4.8.64	—	4,58	9,8	28	0	49	49	1	1	++
10.8.64	—	5,52	8,9	28	0	41	55	0	4	++
17.8.64	—	6,22	10,7	32	1	39	60	0	0	++
24.8.64	—	6,49	7,2	33	0	52	39	0	9	++
31.8.64	—	6,54	7,4	33	1	49	48	0	2	++
8.9.64	—	8,58	6,5	31	0	48	49	0	3	++
14.9.64	—	5,86	6,0	32	1	43	51	1	4	++
21.9.64	—	6,22	7,7	33	0	45	51	0	4	++
28.9.64	+	5,10	9,8	26	0	45	55	0	0	++
28.9.64	B	4,90	11,7	26	1	53	42	0	4	++
5.10.64	—	4,90	11,7	26	1	53	42	0	4	++
12.10.64	—	7,58	10,4	37	•	•	•	•	•	++
19.10.64	—	7,27	10,4	38	1	38	60	0	1	++
26.10.64	—	6,24	9,5	30	•	•	•	•	•	++
2.11.64	2+	4,93	8,0	25	0	51	45	0	4	++
5.11.64	A	•	•	•	•	•	•	•	•	•
9.11.64	—	5,49	9,3	27	1	51	41	1	6	++
18.11.64	—	6,84	12,5	33	0	46	46	1	7	++
23.11.64	—	6,48	7,8	32	1	50	42	1	6	++
30.11.64	+	4,55	10,6	24	1	28	70	0	1	++
7.12.64	—	4,46	11,3	22	0	40	59	0	1	++
14.12.64	—	3,82	8,3	18	0	53	40	0	7	++
21.12.64	—	5,12	10,1	24	1	36	57	0	6	++
4.1.65	—	5,84	10,1	29	0	50	50	0	0	++
12.1.65	+	3,79	9,1	19	1	49	49	1	0	++
19.1.65	+	4,98	9,2	23	0	57	42	0	1	++

PLATE 1 Clinical symptoms of chronic *T. brucei* nagana induced by subcurative treatments with Antrypol and Berenil

1. Gelding 5065: Apathy and emaciation
2. Gelding 5065: Oedema of scrotum and prepuce
3. Gelding 5065: Muscular atrophy and oedema of scrotum
4. Gelding 5065: Oedema of supraorbital fossae and eyelids resembling "Dikkop" horsesickness
5. Gelding 5065: Oedema of lips
6. Gelding 5065: Bone marrow hyperplasia throughout most of the diaphysis of the femur
7. Mare 5588: Oedema of vulva
8. Stallion 5259; Pronounced oedema of scrotum and prepuce

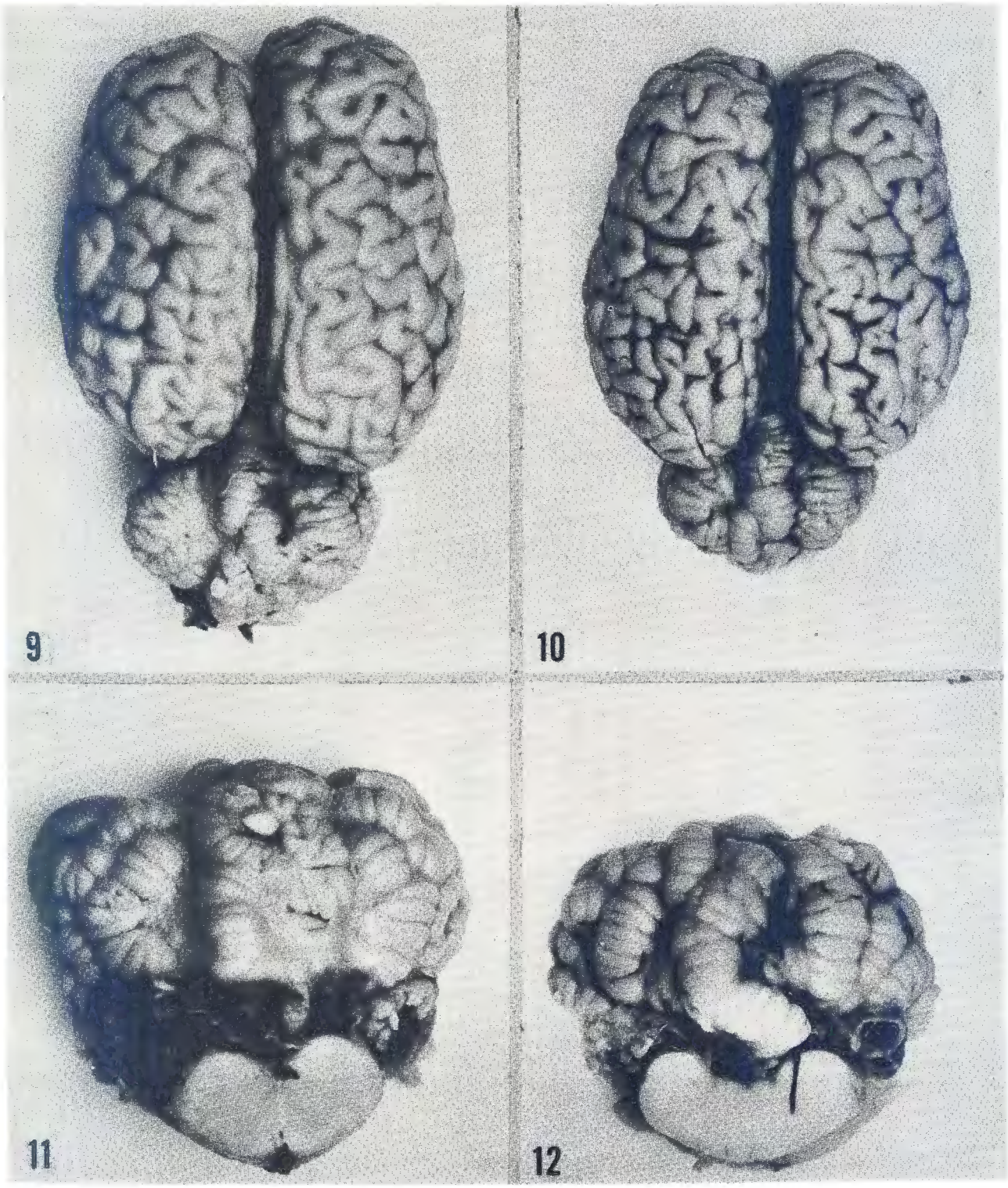

PLATE 2 Macroscopic appearance of the affected brain of Stallion 5259 compared to that of a normal horse brain. (Photographed after fixation in a 10% formalin solution).

9. Dorsal view of the affected brain showing an opaque appearance and thickening of the meninges
10. Dorsal view of a normal horse brain showing sharply defined gyri and sulci for comparison with that presented in the preceding photograph
11. Posterior view of the cerebellum, choroid plexus of the fourth ventricle and medulla oblongata. Note the thickened meninges which are very prominent in the paravermal fissures
12. Posterior view of the cerebellum, choroid plexus of the fourth ventricle and medulla oblongata of normal horse. Note the clearly defined convolutions

quillized and electrocuted. It should be mentioned that this horse was partially blind in the right eye and that the impaired vision could have contributed a great deal towards its excitable behaviour.

Experiment 5

Having produced a chronic form of *T. brucei* nagana with involvement of the CNS by treating two horses with Antrypol, then with Berenil and in the advanced stage of the chronicity again with Antrypol, an attempt was made to determine whether or not the last medication was essential for the creation of favourable conditions that would encourage the parasite to invade the CSF and to produce nervous symptoms. Exclusion of the Antrypol therapy after treatment with Berenil was regarded as being necessary in order to obtain more information on the pathogenesis of the artificially produced meningo-encephalitis.

Mare 5588 was infected with *T. brucei* by the subcutaneous route. Observations on the course of the disease are recorded in Tables 6a and 6b. The incubation period was 6 days and the duration of the disease 130 days. Antrypol was administered on Days 12 and 80 and Berenil on Day 99 of the reaction.

Observations made at various periods of the reaction may briefly be summarized as follows:

(a) Microscopic infections of *T. brucei* were observed on Days 1, 8 to 13, 52 to 73 and 99.
(b) Oedema of the vulva was observed from Days 71 to 130 [Plate 1 (7)].
(c) No clinically recognizable nervous symptoms became evident. The phlegmatic temperament of Mare 5588 could have masked symptoms of apathy.
(d) General weakness on Days 126 and 127 was followed by prostration on Days 128 to 130 when the mare was electrocuted.
(e) Serological tests showed a partial fixation of complement on Days 11 to 22 after infection and subsequently complete fixation until death.
(f) At necropsy there were no lesions, other than an oedema of the vulva, that would have suggested that the mare had suffered from nagana. Although no attempt was made to determine the presence

TABLE 6a. *Mare 5588. Observations on the parasitaemia, therapy, symptomatology and macroscopic pathology*

Date and method of infection	IP in days	Period of microscopic infection	Date of treatment	Clinical symptoms and signs	Lesions at necropsy
16.6.64 sc, 2,5 ml blood from a guinea pig harbouring *T. brucei*	6	22.6.64	3.7.64 Antrypol 5 mg/kg iv	20.6.64 to 30.6.64 - subcutaneous swelling, 5 cm in diameter, at site of injection on left side of neck; 14.7.64 to 12.10.64 - rise in WCC (Table 6b); 31.8.64 to 7.9.64 - moderate oedema of vulva;	P M 44354 - Oedema of vulva; thrombosis of pulmonary arteries of lungs; fibrinous pericarditis; hydropericardium; atrophy of heart; fibrinous adhesions of liver and spleen; petechiae in splenic capsule; verminosis of small and large intestines
		29.6.64 to 3.7.64			
		12.8.64 to 2.9.64	9.9.64 Antrypol 5 mg/kg iv	8.9.64 to 29.10.64 - oedema of vulva more marked; 25.10.64 to 26.10.64 - very weak, unsteady gait; 27.10.64 to 29.10.64 - prostrated;	
		28.9.64	28.9.64 Berenil 3 mg/kg im	29.10.64 - electrocuted	

TABLE 6b *Mare 5588. Observations on the parasitaemia, haematology and serology*

Date	*T. brucei*	RCC 10^6/mm³	WCC 10^3/mm³	PCV	Differential WCC					CF tests
					M	L	N	B	E	
16.6.64	—	6,02	9,1	31	0	25	66	0	9	—
22.6.64	3+	8,01	11,1	39	0	15	85	0	0	—
26.6.64	—	7,04	6,5	35	0	48	50	2	0	+
3.7.64	A	•	•	•	•	•	•	•	•	•
6.7.64	—	4,03	7,4	21	2	40	56	0	2	+
14.7.64	—	5,45	10,4	23	1	39	58	0	2	++
20.7.64	—	6,78	9,2	23	2	43	45	2	8	++
27.7.64	—	7,11	10,2	37	1	32	59	1	7	++
4.8.64	—	5,59	4,1	28	3	37	60	0	0	++
10.8.64	—	6,61	8,1	32	2	45	50	1	2	++
17.8.64	+	6,05	17,2	31	2	70	25	1	2	++
24.8.64	+	5,68	12,7	28	2	58	39	0	1	++
31.8.64	+	4,56	8,7	23	0	12	83	2	3	++
8.9.64	—	3,03	13,4	18	1	46	51	0	2	++
9.9.64	A	•	•	•	•	•	•	•	•	•
14.9.64	—	4,70	10,4	22	0	48	49	1	2	++
21.9.64	—	4,73	12,1	28	1	32	64	2	1	++
28.9.64	+	4,75	8,5	27	0	35	64	0	1	++
28.9.64	B	•	•	•	•	•	•	•	•	•
5.10.64	—	5,15	13,7	27	0	46	49	2	3	++
12.10.64	—	5,76	13,8	32	0	33	65	0	2	++
19.10.64	—	6,05	12,1	30	1	43	54	0	3	++
26.10.64	—	6,04	12,3	32	1	35	60	1	3	++

of *T. brucei* in the CSF, histopathological examination, conducted subsequently, revealed that the CNS was involved.

Consideration of these results makes it apparent that subcurative treatment of nagana with two successive administrations of Antrypol and a single injection of Berenil, interspaced at adequate intervals, can also be followed by development of lesions in the CNS. It still needs to be determined whether or not other therapeutic programmes with these or other trypanocidal agents will culminate in clinical and histopathological manifestations as observed in Gelding 5065, Stallion 5259 and Mare 5588.

Discussion

Comparisons between the behaviour of *T. gambiense* and *T. rhodesiense* in man with that of *T. brucei* in a horse after biological transmissions, make it apparent that there is a striking difference in their pathogenicity. In man the initial stage of both infections is characterised by an invasion of the blood stream and lymph nodes by the pathogens. After a variable period of time, approximately a month in *T. rhodesiense* and several months in *T. gambiense,* parasites invade the CSF. In later stages of the disease, parasites penetrate the brain producing nervous symptoms and inflammatory lesions. Antrypol is very effective in curing patients before the nervous system is involved. Thereafter, it cannot produce a permanent cure because it does not penetrate the CNS.

The clinical manifestations of equine nagana differ from those appearing in human sleeping sickness. Depending upon the degree of virulence of *T. brucei* strains, infection of horses is followed by development of a peracute, acute or chronic disease. Affected horses very rarely recover. Antrypol is a very effective curative, provided medication is applied before signs of exhaustion become imminent. Parasitic relapses, attributable to an involvement of the nervous system, have not been described.

Chandler (1957) conducted observations of CSF in five oxen and two sheep infected with either *T. congolense* Broden, 1904 or *T. vivax* Ziemann, 1905. He found a single *T. vivax* in the CSF sample from one sheep but concluded that, since the sample contained erythrocytes, the trypanosome had direct origin in the contaminating blood. In his discussion he states: "It is generally understood that trypanosomes are not found in the c.s.f. of domestic animals, but results of examinations are recorded. Carmichael & Jones (1939) did not detect trypanosomes in the c.s.f. of one ox infected with *T. vivax* that was studied. Curasson & Morner (1948) report the finding of *T. vivax* in the c.s.f. of a sheep, but give no details; it is possible that their c.s.f. sample was contaminated with blood; it can be seen from the table that even a sample which is water-clear to the eye can be shown by centrifugation to contain erythrocytes. As far as the author is aware, there are no reports on c.s.f. examinations made in *T. brucei* infections; this polymorphic trypanosome may enter the c.s.f. of domestic animals and investigations on this would be of interest".

The remark made by Chandler (1957) on the possible behaviour of *T. brucei,* is supported by the investigations of Martini (1905) who had found polymorphic "tsetse-parasites" in the CSF of his experimentally infected horses.

In the present studies examination of the CSF was conducted only on two out of the six artificially infected horses. Geldings 5484 and 5480 served as controls and reacted over a period of 50 days. Gelding 2287 was treated with a subcurative dose of Antrypol and exhibited a chronic form of nagana but nervous symptoms were not observed even though the disease lasted for 105 days. Gelding 5065, Stallion 5259 and Mare 5588 received subcurative treatments of Antrypol and Berenil and developed chronic infections which persisted for periods of 266, 270 and 130 days respectively. It is unfortunate that the CSF was not examined in all instances as this would have given a better idea of the pathogenesis of *T. brucei* in two untreated and four treated horses. The examination of the CSF from Gelding 5065 and Stallion 5259 with clinically recognizable symptoms for 73 and 51 days respectively before death, revealed the presence of *T. brucei* thus clearly indicating that the CNS was involved. The involvement of the CNS in Mare 5588, which did not exhibit nervous symptoms, could be determined subsequently by demonstrating microscopic lesions in the brain.

Consideration of the development of chronic nagana in three horses by the administration of subcurative doses of Antrypol and Berenil at various stages of the reaction period suggests that favourable conditions were created not only for *T. brucei* to penetrate the CSF but that in these circumstances it would be able to exert its pathogenicity on the CNS of horses, a feature invariably disclosed by *T. rhodesiense* and *T. gambiense* in man suffering from sleeping sickness.

Summary and conclusions

1. Attention is drawn to the fact that there appear to be no studies directed specifically to the nervous system of naturally and experimentally *T. brucei* infected horses.
2. A comparison is made between the pathogenesis and symptomatology of the acute and chronic forms of human sleeping sickness and those appearing in equine *T. brucei* nagana.
3. In man the initial invasion of the blood stream and lymphatic system by either *T. rhodesiense* or *T. gambiense* is invariably followed by parasites penetrating the CSF and eventually extending to the brain and producing symptoms of meningo-encephalitis.
4. In horses the initial invasion of the blood stream and lymphatic system by *T. brucei* results in the development of a peracute, acute or chronic disease, which, in many instances, terminates fatally without clinical evidence of an involvement of the CNS.
5. The apparent absence of an involvement of the CNS suggests that by the time that *T. brucei* penetrates the CSF the animal dies from exhaustion thereby restricting the parasite from exerting its pathogenicity on the nervous system.
6. Reasons have been given why Antrypol and Berenil were selected for the production of a chronic form of *T. brucei* nagana in horses.
7. Repeated subcurative medications of three horses with Antrypol and Berenil was followed by an extension of the reaction period to 130, 266 and 270 days compared with that in control horses which persisted only for 50 days.
8. Two of the treated horses developed a chronic form of the disease associated with nervous symptoms, the presence of *T. brucei* in the CSF and macroscopically visible lesions of the meninges and brain. Although the third horse failed to re-

veal clinically recognizable nervous symptoms, histopathological studies nevertheless showed lesions in the brain.

9. Evidence has been brought forward that, in common with *T. rhodesiense* and *T. gambiense, T. brucei* possesses an invasive potential for the CSF.
10. The extent to which the CNS of the experimentally *T. brucei* infected horses was involved, is described in Part 2 of this report.

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