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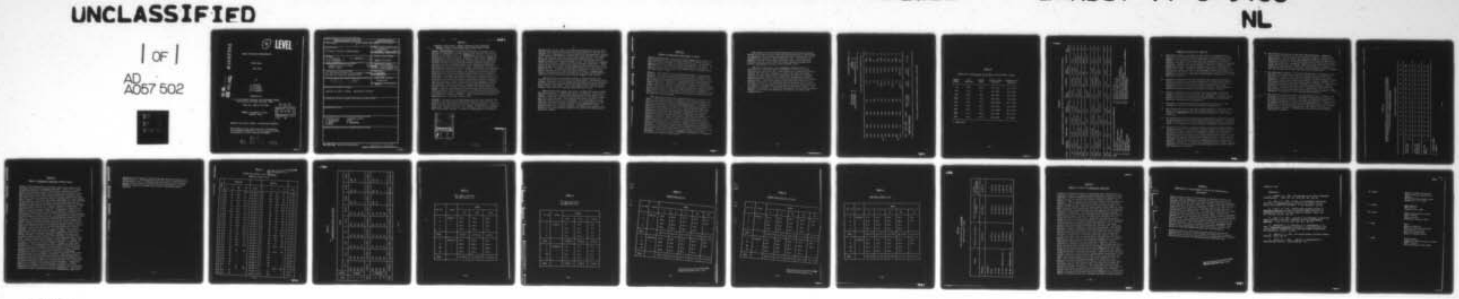

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STUDY OF AFRICAN TRYPANOSOMIASIS

Final Report

July 1978

by

I. Muriithi
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Supported by

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SECTION A

ANTIGENIC RELATIONSHIPS BETWEEN ORGANISMS OF THE TRYPANSOMA BRUCEI SUBGROUP IN THE LAMBWE VALLEY, SOUTH NYANZA, KENYA

PROGRAM AND BACKGROUND: The objective of this project is to determine the extent of antigenic variability in trypanosomes of the T. brucei group collected from man and animals in the Lambwe Valley. T. rhodesiense is endemic in the Valley causing periodic disease in man. Domestic and game animals harbor the morphologically indistinguishable T. brucei. This project is also designed to study reactions of various antisera to determine the variability of different antigenic types within the parasitic population. The findings will, in part, determine whether or not immunization could be a practical means of controlling the disease. Trypanosomes collected from adjacent countries will also be studied to determine the geographic extent of similar antigenic types. The antigenic relationship between the parasites of man and animals of these areas will also be examined. Immunization against African trypanosomiasis appears to be dependent in large part on the number of antigenic types of the parasite found in a given area. Gray (1970) examined the same herd of cattle for five years in Nigeria and reported the presence of numerous different antigenic types of T. brucei. However, while working with T. gambiense isolated from different endemic areas in Nigeria, he found similarity of basic antigenic types (Gray 1972, 1975). In Lambwe Valley human rhodesian sleeping sickness is endemic and domestic and game animals harbor T. brucei. Since the trypanosomes of man and animals are morphologically indistinguishable, their relationship to each other remains questionable. In hearby Alego Station T. brucei-like organisms were isolated from cattle and transmitted to human volunteers. These people developed typical T. rhodesiense-like infections (Onyango 1966).

PROGRESS: Isolates of the T. rhodesiense parasites were collected from patients at the Homa Bay Hospital on Lake Victoria, western Kenya, by members of the Kenya Medical Department. Blood was injected IP into rats which were then transported to us for study. Two strains of T. rhodesiense from Gambella, Ethiopia, were collected by the US Navy Research Unit, Addis Ababa, Ethiopia. Isolates of trypanosomes were tested by

neutralization (Soltys 1957) with antiserum collected from bovines which had undergone long-term infections with various isolates. To date, 46 isolates of T. rhodesiense have been acquired from trypanosomiasis patients in the valley. The dates of isolation range from 1970 until the present time. Two antisera have been used to assay these isolates: one from an animal infected with an isolate made in 1972 and the other from an animal infected with a strain obtained in 1974. The duration of infection in the two serum donor animals was 227 and 279 days respectively. Twenty two of thirty five isolates (62.8%) tested were neutralized by the first antiserum while the second antiserum neutralized 23 of 31 isolates (74.2%) tested thus far. Eighteen isolates were neutralized by both antisera while 5 isolates did not react with either antiserum. T. rhodesiense isolates from Ethiopia and two different areas in Kenya do not appear to react with either antiserum prepared against Lambwe Valley isolates.

During the past year a number of cases of trypanosomiasis were reported from an area in Kenya near the Uganda border. We obtained three isolates from humans and 8 T. brucei isolates from cattle in the same area. An antiserum prepared against one of the human isolates neutralized all three isolates from humans. When tested against four of the T. brucei isolates from cattle, the antiserum neutralized all of them.

When isolates of T. brucei from cattle in Lambwe VAlley were tested, the antiserum showed a strong effect on 5 of 25 isolates. No parasites appeared in mice given trypanosomes incubated with immune serum at any dilution. Two other isolates were neutralized at 10^3 and below, however mice at 10^4 were positive. The nature of this partial reaction is unknown but these isolates will be retested. It is reasonable to assume that these reacting parasites from cattle are T. rhodesiense since the neutralization test is variant specific. The percentage of cattle parasites which react with the antibody is surprising and indicates that cattle may be a more important aspect in the cycle of the disease than previously thought.

SECTION B

STUDIES ON TRYPANOSOMA RHODESIENSE IN CATTLE

PROGRAM AND BACKGROUND. During our initial immunological studies of the irradiated vaccine and antiserum production in cattle, we noted that some animals underwent a severe form of disease. In general T. rhodesiense has been reported as being non-pathogenic for cattle. We have therefore carried out additional experiments to confirm our original findings and to compare the disease process in bovines with that which occurs in man.

PROGRESS. Ten animals in our study developed disease characterized by weight loss, fever, pleocytosis and CNS disorders. Uncoordinated movements, circling and opisthotonus were observed (Table 1B). Fever and leucopenia were common during the onset of patent parasitemia and were followed by a leucocytosis. Terminal WBC levels were somewhat reduced (Table 2B). Generally, there was an increase in packed cell volumes early in the disease although mild to moderate anemia developed as the infection progressed. Cerebrospinal fluid from infected animals had increased levels of leucocytes made up primarily of lymphocytes. Total protein levels of cerebrospinal fluid were also increased. Gamma globulin was detected by electrophoresis and spinal fluid was positive for complement fixing antibody.

Five additional bovines were autopsied during the period of this report. Gross observations as documented in our previous progress report included thickened dull grey meninges over the dorsal aspects of the brain and prominent lymph and hemolymphadenopathy. Histologically severe meningoencephalitis remains the salient histological feature. The results are summarized in Table 3B. The most severe changes are noted in the white matter of the central nervous system where mixed perivascular plasmacytic and lymphocytic infiltrates and focal and diffuse gliosis are important features. Demyelination is almost exclusively limited to perivascular areas. Although involvement of the spinal and peripheral nerves is minimal, limited infiltrates of inflammatory cells in the neural sheath and perivascular spaces can be found on thorough search. Of interest, in three animals autopsied between 84 and 108 days post inoculation a moderate to severe pancarditis was found. Myocytolysis and sarcolemmal cell hyperplasia accompanied by infiltrates of macrophages, lymphocytes and plasma cells were evident. The epicardium and endocardium also had infiltrates of inflammatory cells. The lungs of these animals had large numbers of hemosiderin laden macrophages in the alveolar walls. Pulmonary hemosiderosis is frequently associated with cardiac insufficiency.

The presence of severe meningoencephalitis with the most extensive lesions in the white matter of the central nervous system in our material is compatable with the leukoencephalitis observed in humans with chronic trypanosomiasis (Caldwell, 1937; Manuelidis, 1967). The findings of myocardial lesions in our animals that died after a relatively short clinical course is similar with the cardiac syndrome associated with acute T. rhodesiense infections of man (Ormerod, 1970).

Detection of parasites in T. rhodesiense infected cattle is difficult after the fourth month of infection if one relies on sub-inoculation of blood into rats. Small quantities of lymph node aspirate (usually less than 0.1cc expanded in 1.0cc of 10% fetal calf serum) injected into rats appears to be a more effective method of isolating the parasite late in the course of infection. Examination of Giemsa-stained lymph node smears is also less efficient than lymph node aspirate subinoculation. The results between 15-30 months post inoculation are included in Table 4B. The lymph node aspirate technique may be a useful adjunct in the detection of infections in the field and the detection of infection in animals to be used in future immunization studies. The detection of parasites in lymph node aspirates of our animals for lengthy periods is not unlike the persistence of infection in chronic human trypanosomiasis.

PATHOGENIC INFECTIONS OF TRYPANOSOMA RHODESIENSE IN CATTLE

	An. No.	Infecting strain	Clinical signs	Duration of Disease (Days)	DETECTABLE PARASITES*		
					Blood	Lymph node	CSF
1.	268	Wellcome	1,3,5,6**	179	Neg.	N.D.	N.D.
2.	6882	LVH-1	1,6	227	Pos.	Pos.	Neg.***
3.	243	LVH-1	1,3,4,6	582	Neg.	N.D.	N.D.
4.	7304	LVH-2	1,4,6	714	Neg.	Pos.	Neg.
5.	7307	LVH-9	1,4,6	279	Pos.	Pos.	Pos.
6.	8601	LVH-12	1,3,4,6	703	Neg.	Pos.	Neg.***
7.	7859	LVH-12	1,4,6	301	Neg.	Pos.	Pos.
8.	16	LVH-28	1,6	108	Pos.	Pos.	Pos.
9.	8888	LVH-29	1,6	92	Pos.	Pos.	Pos.
10.	8901	LVH-29	1,6	84	Pos.	Pos.	Pos.

* At necropsy by subinoculation into mice

** Clinical signs:
1. Unco-ordinated movements
2. Circling
3. Tremor
4. Hypersensitivity
5. Opisthotonos
6. Weight loss

*** Spinal cord positive

TABLE 2B

LEUCOCYTES IN TRYPANOSOMA RHODESIENSE INFECTED CATTLE (x1000)

ANIMAL NUMBER	PRE INFECTION	SECOND WEEK	HIGHEST VALUE (AND DAY)	TERMINAL VALUE (AND DAY)
16	11.9	16.3*	17.9 (25)	15.1 (108)
243	9.6	7.7	34.3 (51)	18.7 (551)
268	14.6	12.6	18.6 (36)	10.8 (179)
6882	11.2	9.1	18.8 (26)	9.4 (227)
7304	12.6	7.9	20.8 (558)	20.7 (714)
7307	15.4	23.4	25.8 (14)	15.6 (279)
8601	14.6	9.3	37.2 (586)	18.2 (703)
8888	16.7	19.5	19.9 (25)	16.1 (91)
8901	10.5	12.9*	21.9 (58)	15.5 (84)

* THIRD WEEK

TABLE 3B

SUMMARY OF THE HISTOLOGICAL CHANGES IN THE CNS OF CATTLE INFECTED WITH TRYPANOSOMA RHODESIENSE

ANIMAL	CERVICAL SPINAL CD.	CEREBELLUM	MIDBRAIN	CORTEX-OCCIPITAL LB.	BASAL GANGLIA	MIDCORTEX	CORTEX-OLFACTORY	PITUITARY	SEE COMMENT
16	1,3,5	1,3,5	1,3,5,6	1,3-5,8	1,3-6,8	1,3,5,8,9	1,3,5,8	1,3,7	A
243	1,3,4,7	1,3-8	1,3-5,7,9	1-9,11	1-11	1-9,11,12	1-5,7,8	3,4,8,10	B
268	N/A	1,3-5,7,8	1,3,5,7	N/A	1-8,10,12	1-3,5,7,8	N/A	3,4,8,13	C
6882	3	1,3,5,7	3,7	1,3	3	N/A	N/A	3,8,13	D
7304	1,3,4	1,3-5,8,10	1,3-5,7	1-10	1-3,5-8, 10-12	1-5,7-9,11	1-9	3,4,8,13	E
7307	1,3	1,3,5,7,8	1,3,6,7,11	1,3-5,8	1,3	1-5,8,9,11	1,3-5,8	3,13	F
7859	1,3,5,7	1,3,5-8	1-8	1-10	1-11	1-3,5-10,12	1-10	1,3,7	G
8601	1,3-5,7,14	1,3-8	1,3-5,7,8, 14,15	1-5,7-9,15	1,3-9,15	1-11,15	1-9,15	1,3,4,8	H
8888	1	1,3	1,3,5	1,3-5	1,3,5	1,3,5	1,3-5	1,3,7	I
8901	N/A	1,3	1,3	N/A	N/L	N/A	1	1,3,7	J

N/A: Not available or tissue sections not identified
N/L: No lesions
1: Meningeal infiltrations
2: Subpial gliosis
3: Perivascular infiltrates
4: Mott's cells
5: Vasculitis
6: Periarterial edema
7: Gliosis, focal
8: Gliosis, diffuse
9: Gemistocytic astrocytes
10: Malacia or Gitter cells
11: Vacuolation and/or cyst formation
12: Subependymal gliosis and/or malacia
13: Colloid cysts adenohypophysis
14: Neuronal degeneration in areas of intense inflammation
15: Perivascular hemosiderin

ADDITIONAL COMMENTS FROM TABLE 3B

A. The salient feature in the brain of this case was a lymphoplasmacytic infiltrate in the meninges and perivascularity at all levels of the CNS examined. Only a few Mott's cells were found. Gliosis was irregularly diffuse in the subcortical white matter with gemistocytic astrocytes limited to a single section. The pituitary had a marked glial-inflammatory infiltrate limited to the neurohypophysis. Additional changes included marked srous atrophy and chronic inflammation of fat in the coronary groove of the heart. A mixed infiltrate of lymphocytes and plasma cells were noted to be most intense in the myocardial interstitium adjacent to the epi and endocardium. All lymphoid organs were reactive with follicular hyperplasia and marked accumulation of lymphocytes and plasma cells in the medullary sinusoids.

B. Lesions were most severe in the subcortical white matter but extensive lesions were present in the grey matter as well. Marked cystic cavitations primarily surrounding blood vessels near the external capsule of the basal ganglia, extensive astrocytosis of the white matter and exaggerated sulci indicates marked cerebral atrophy. The inflammatory reaction was mainly plasmacytic and Mott's cells were numerous.

C. Lesions in the brain sections examined were most severe in the white matter of midbrain and basal ganglia. The inflammatory reaction was primarily lymphocytic. Mott's cells were infrequent. The stroma of the chorid plexus of the 4th ventricle was edematous and the perivascular areas were infiltrated with lymphocytes.

D. The least severe histological changes were found in this case. The inflammatory reaction in the CNS was primarily lymphocytic.

E. The inflammatory reaction was mixed plasma-lymphocytic with a few neutrophils, and most severe in myelinated areas of the brain. There was extensive subependymalgliosis, malacia and vacuolation near the lateral ventricles.

F. The inflammatory reaction was primarily lymphocytic and much more extensive in the myelinated portions of the CNS than in the grey matter. Peripheral nerve involvement was noted in the optic, trigeminal, sciatic and brachial nerves. It consisted of a mild perivascular and neural sheath infiltrate.

G. Lesions were most severe in the subcortical white matter, basal ganglia and white matter of the cerebellum. Salient features included marked perivascular infiltrate, composed of lymphocytes, plasma cells, histiocytes and irregular diffuse gliosis. Gemistocytic astrocytes were numerous. Occasional gitter cells were found in areas most severely involved. Large lakes of proteinaceous fluid surrounded several arteries. The gliosis in the cortex was most frequently focal. Mott's cells in this case were not numerous. Also in areas of intense inflammation reaction, degeneration and glial satellitosis of neurons were occasionally found. Marked reactive hyperplasia of all lymphoid organs and mild pancarditis were other important histological alterations.

H. The inflammatory reaction in the brain was mixed plasma - lymphocytic with a severe reaction at all levels of the CNS examined. Mott's cells were numerous. The most severe reactions were found in the subcortical white matter where the gliosis appeared generally diffuse and perivascular infiltrates extensive. In the cortex focal glial infiltrates were found. In the spinal cord the inflammatory reaction was most severe in the distal lumbar and sacral portions. Spinal nerve involvement was limited to a mild pial and perivascular infiltrates. Of the peripheral nerves examined, only the brachial had a single small perivascular infiltrate.

I. The inflammatory reaction in the brain was limited to a moderately severe meningeal and perivascular infiltrate of lymphocytes and plasma cells. Few Mott's cells were found. The most interesting change was a severe pancarditis. Extensive interstitial infiltrates in the myocardium appeared especially intense surrounding Purkinge fibers. Myofibril degeneration with marked sarcolemmal cell proliferation and the presence of large numbers of cardiac histiocytes indicates a reaction of long standing. Chronic steatitis is evidenced by an infiltrate of lymphocytes and plasma cells and clusters of reticuloendothial cells. The alveolar walls of the lung were thickened and contained abundant iron positive brown pigment. Reactive hyperplasia was prominent in all lymphoid organs examined.

J. The lesions in the brain in this case were minimal with the meninges and perivascular spaces in the brain containing a mild multifocal infiltrate of plasma cells and lymphocytes. The heart was extensively damaged and the lesions characterised by focal myofibril degeneration with accumulation of engorged macrophages and proliferated sarcolemmal cells. Infiltrates of plasma cells and lymphocytes were also noted multifocally in the mycardium as well as the epi and endocardium. The lung was congested with alveolar walls thickened. Macrophages in the walls contained abundant iron positive pigment.

TABLE 4B

RELATIVE EFFECTIVENESS OF DIRECT LYMPH NODE SMEAR EXAMINATION COMPARED TO IP INOCULATIONS USING BLOOD AND LYMPH NODE ASPIRATES FOR DETECTION OF CATTLE HARBORING TRYPANOSOMA RHODESIENSE

DURATION OF INFECTION IN MONTHS

		15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
BLOOD (5ml) IP TO RATS	I*	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
	C**	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
LYMPH NODE ASPIRATE IP TO RATS/MICE	I	4/9	4/9	3/9	3/9	2/9	3/9	3/9	3/9	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8
	C	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
LYMPH NODE SMEARS	I	4/9	2/9	2/9	2/9	1/9	2/9	1/9	1/9	1/8	0/8	1/8	1/8	1/8	1/8	1/8	1/8
	C	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

* INFECTED ANIMAL

** CONTROL

SECTION C

ANEMIA IN TRYPANOSOMA CONGOLENSE INFECTED CATTLE

A total of 22 experimental and 13 control cattle were used to study the anemia caused by T. congolense infections. Early in the course of infection decreases in packed cell volume, erythrocyte concentration and hemoglobin occurred and coincided with increases in both mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). The indices reached the highest levels during the period between eight and twelve weeks after infection and were accompanied by a reticulocyte (Ret.) response. By week 20 these values (MCV, MCH, Ret.) had decreased to pre-infection levels even though the anemia persisted (Table 1c). The apparent half life of chromium-51 labelled erythrocytes in infected animals was approximately 40% of that of controls at eight weeks. In surviving animals the half life gradually returned to normal as the parasitemia level decreased (Table 2c). Chromium was excreted in the urine of infected animals at higher levels than that of controls indicating an excessive destruction of labelled erythrocytes and excretion of chromium by the kidney (Table 3c). No differences in chromium levels in fecal samples were found between experimental and control animals (Table 4c). Total blood volumes of infected and control animals did not differ significantly throughout the course of infection (Table 5c). Plasma volumes of infected animals increased and these animals underwent a corresponding decrease in erythrocyte volume (Tables 6c and 7c). Serum iron levels which were elevated at eight weeks post infection decreased to low levels by week 28. In surviving animals or after treatment, the serum iron levels returned to normal. Early during the infection the plasma iron turnover (PIT) measured with iron-59 was greater than that of controls indicating increased erythrocyte production; however, by week 28 the PIT was only 38% of that occurring in controls indicating the presence of a severe dyshemopoeisis at this time. Iron-59 incorporation followed a similar pattern. Whereas 50% of the injected dose of iron reappeared in controls in an average of 6.9 days, it took only 4.9 days to appear in animals at eight weeks. Later, however (28 weeks), eight days were required before 50% of the injected dose of iron reappeared in erythrocytes in infected animals. In surviving and treated animals PIT's returned to normal. Thrombocytopenia was found to be a prominent feature of T. congolense infections in cattle and appears to be inversely related to the level of trypanosomes in the peripheral circulation. Chronically infected animals with low levels of parasites had less severe thrombocytopenia and thrombocytes were usually found in normal or elevated numbers when parasites could not be observed. Decreased survival time of chromium-51 labelled thrombocytes was found in infected animals. Chemotherapeutic cure of animals with thrombocytopenia resulted in a rapid elevation of thrombocyte levels to higher than normal values. Leucocyte concentrations followed a similar but less marked course. Preliminary coagulation studies indicated that partial thromboplastin times were

extended after six weeks of infection and that protamine sulfate tests became positive. Fibrinogen levels were lower but no change was noted in prothrombin times. It appeared that the spleen did not contribute greatly to the breakdown of erythrocytes since splenectomized calves developed anemia at a similar rate to that of non-splenectomized controls.

TABLE 1c

PACKED CELL VOLUMES IN T. CONGOLENSE

INFECTED CALVES ± 2S.E.

Weeks	Infected					Control				
	7	8	8B	10	Mean	5	6	3	9	Mean
0	34	36	34	34.5	34.6±0.9	29	35	31	33	32.0±2.6
1	30	34	28.5	31	30.9±2.3	29	35	29.5	31	31.1±2.7
2	26	29	26	30	27.7±2.1	32	33.5	33.5	31	32.5±1.2
3	20	27	23.5	27.5	24.5±3.5	30	29	29	33	30.2±1.9
4	23	25	22	30.5	25.1±3.8	33	30	32.8	33.3	32.3±1.5
5	22	21	24.5	29.5	24.2±3.8	29	27	33.5	37.5	31.7±4.7
6	22	25	18.5	24.5	22.5±3.0	30	27	32	34.5	30.9±3.2
7	23	20.5	19.5	25	22.0±2.5	32	31	32	35	32.5±1.7
8	24	21	17.5	22.5	21.2±2.8	35	38	31	33	34.2±3.0
9	22	22	18.5	22.5	21.2±1.8	33	32	30	34	32.2±1.7
10	24	21	18.0	21.5	21.1±2.5	34	34	30	34.5	33.1±2.1
11	24	23	15.5	20	20.6±3.8	34	38	30	34	34.0±3.3
12	24	21	19.0	23.5	21.9±2.3	30	33	33.5	33	32.4±1.6
13	24	22	22	24.5	23.1±1.3	32	35	33	31.5	32.9±1.5
14	24	22	24	26.5	24.1±1.8	32	35	33	31.5	32.9±1.5
15	26	22	21.5	27.5	24.2±3.5	32	36	34.5	31.5	33.5±2.1
16	26	22	21	27.5	24.1±3.1	32	35	34.5	31	33.1±1.9
17	27	20	18.5	24	22.4±3.9	32	34	30	28	31.0±2.6
18	27	19	18	25	22.2±4.4	33	36	31	30	32.5±2.6
19	25	19	22.5	29.5	24.0±4.4	31	33	36.5	34.5	33.7±2.3
20	25	23	-	-	24.0±2.0	30	31	-	-	30.5±1.0
21	27	26	22	28	25.7±2.6	28	31	36.5	38	33.4±4.7
22	25	22	-	-	23.5±3.0	28	30	-	-	29.0±2.0
23	27	23	-	-	25.0±4.0	29	29	-	-	29.0± -
24	28	27	-	-	27.5±1.0	32	31	-	-	31.5±1.0
25	27	25	-	-	26.0±2.0	28	28	-	-	28.0± -
26	27	23	-	-	25.0±4.0	31	30	-	-	30.5±1.0
27	-	-	22	28	25.0±6.0	-	-	36.5	38.0	37.7±1.2
28	28	27	-	-	27.5±1.0	33	35	-	-	34.0± -
29	26	23	-	-	24.5±3.0	28	28	-	-	28.0± -

TABLE 2c

^{51}Cr ERYTHROCYTE SURVIVAL ($T_{\frac{1}{2}}$) $\pm$2S.E.

An. No.	Group	Weeks								
		0-2	2-4	4-6	6-8	10-12	12-14	18-20	22-24	28-30
5	Control	324	346	331	322	348	360	355	323	299
6		319	336	283	310	334	341	314	338	310
3		326	312	317	339	318	347	-	-	-
9		319	376	355	381	360	332	-	-	-
Mean		322$\pm$3.6	342.5$\pm$26.5	321.5$\pm$30.1	338,0$\pm$31.0	340.0$\pm$18.1	345.0$\pm$11.7	344.5$\pm$41.0	330.5$\pm$15.0	304.5$\pm$11.0
7	Infected	286	166	134	172	192	165	288	290	265
8		260	223	120	121	105	125	151	194	222
8B		246	160	72	102	87	108	-	-	-
10		301	265	185	147	139	145	-	-	-
Mean		273$\pm$24.8	203.5$\pm$49.9	127.8$\pm$46.5	135.5$\pm$30.5	130.8$\pm$46.2	135.8$\pm$24.7	219.5$\pm$137.0	242$\pm$96.0	243.5$\pm$43.0

TABLE 3c

^{51}Cr URINARY EXCRETION
RBC EQUIVALENTS (ml.)

An. No.	Group	Weeks		
		2-4	6-8	12-14
5	Control	40.4	32.8	46.0
6	"	39.1	34.5	43.8
3	"	36.1	46.8	53.6
9	"	42.5	58.0	46.1
Mean		39.5 ± 2.7	43.0 ± 11.8	47.4 ± 4.3
7	Infected	69.5	117.6	391
8	"	83.8	288.0	163.9
8B	"	82.3	383.0	168.1
10	"	75.2	120.1	139.4
Mean		77.7 ± 6.6	227.2 ± 131.0	215.6 ± 117.6

TABLE 4c

^{51}Cr FECAL EXCRETION
RBC EQUIVALENT (ml.)

An. No.	Group	Weeks		
		0-4	4-8	10-14
5	Control	1.7	2.5	2.4
6	"	2.2	2.7	2.6
3	"	2.8	2.8	3.0
9	"	1.4	1.6	1.9
Mean		2.0 ± 0.6	2.4 ± 0.4	2.6 ± 0.5
7	Infected	2.4	2.1	1.9
8	"	2.0	3.4	2.6
8B	"	2.3	2.3	2.2
10	"	2.7	2.7	1.9
Mean		2.4 ± 0.3	2.6 ± 0.6	2.2 ± 0.3

TABLE 5c

BLOOD VOLUME ML./KG.

An. No.	Group	Weeks				
		0	4	10	18	28
5	Control	48.8	48.0	47.1	51.9	50.1
6	"	52.6	55.7	53.7	52.2	49.5
3	"	45.1	45.8	47.5	49.7	-
9	"	46.4	46.6	48.2	48.2	-
Mean		48.2 ±3.3	49.0 ±4.5	49.1 ±3.1	50.2 ±3.0	49.8 ±0.6
7	Infected	52.4	56.5	51.9	49.3	53.2
8	"	48.8	50.2	49.7	52.8	50.4
8B	"	47.7	47.1	51.5	47.5	-
10	"	50.4	47.5	45.5	49.1	-
Mean		49.8 ±2.0	50.3 ±4.3	49.7 ±2.9	49.7 ±2.2	51.8 ±2.8

TABLE 6c

PLASMA VOLUME ML./KG. ($\pm$ 2S.E.)

An. No.	Group	Weeks				
		0	4	10	18	28
5	Control	31.2	33.6	31.1	33.7	33.6
6	"	31.6	39.0	35.0	35.3	32.2
3	"	28.6	30.0	32.5	31.6	-
9	"	27.8	30.9	32.5	29.9	-
Mean		29.8 $\pm$1.9	33.4 $\pm$4.1	32.8 $\pm$1.6	32.6 $\pm$2.4	32.9 $\pm$1.4
7	Infected	33.0	44.6	40.5	37.5	38.3
8	"	28.3	38.4	39.3	42.8	36.7
8B	"	26.5	36.0	42.8	37.0	-
10	"	33.3	32.5	35.0	35.4	-
Mean		30.3 $\pm$3.4	37.9 $\pm$5.1	39.4 $\pm$3.3	38.2 $\pm$3.2	37.5 $\pm$1.6

TABLE 7c

RED CELL VOLUME ML./KG.

An. No.	Group	Weeks				
		0	4	10	18	28
5	Control	17.6	14.4	16.0	18.2	16.5
6	"	21.0	16.7	18.7	19.9	17.3
3	"	16.5	15.8	15.0	18.1	-
9	"	18.6	17.9	15.7	18.3	-
Mean		18.4 $\pm$1.9	16.2 $\pm$1.5	16.3 $\pm$1.6	18.6 $\pm$0.9	16.9 $\pm$0.8
7	Infected	19.4	11.9	11.4	11.8	14.9
8	"	20.5	11.8	10.4	10.0	13.7
8B	"	21.2	11.1	8.7	10.5	-
10	"	17.1	15.0	10.5	13.7	-
Mean		19.5 $\pm$1.8	12.4 $\pm$1.7	10.3 $\pm$1.1	11.5 $\pm$1.7	14.3 $\pm$1.2

TABLE 8c

IRON UTILIZATION IN *T. CONGOLENSE* INFECTED CATTLE (± 2S.E.

Group	Serum Iron (µg/100ml.)	Total Iron binding Cap. (µg/100ml.)	Plasma Iron transport mg./Day/100ml.
Controls	153.0±20.4	334.2±39.6	0.86±.22
Infected			
8 wks.	241.4±25.0	412.4±26.6	2.50±.80
17 wks.	85.00±25.0	203.5±29.6	0.95±.24
28 wks.	26.70±4.7	150.0±45.7	0.27±.04
28 wks. (treated 20 wks.)	101.00±26.0	167.0±10.0	0.88±.14
61 wks.	141.7±54.5	384.3±53.7	1.27±.34
61 wks. (treated 20 wks.)	136.0±32.0	377.0±22.0	1.06±.10

SECTION D

IMMUNITY IN CATTLE TO TRYPANOSOMA CONGOLENSE

A total of 42 Hereford cattle of various ages were infected with the trans mara strain of T. congolense and observed for evidence of an age resistance. Results showed that eight of nine cattle infected at one year of age or less survived the infection without treatment. Two animals of eight in the age range of one to two years also survived the infection. All 25 animals whose ages ranged from two to five years either succumbed to the infection or had to be treated because of the severity of the disease. When the young animals, which needed no treatment to survive, were rechallenged at periods out to one year after the last observation of patent parasitemia, they appeared to be completely refractive to infection. The older animals which required Berenil treatment to survive were also rechallenged at intervals after therapy. Three animals infected for 49 to 75 days before treatment were rechallenged 198 to 296 days later. Extensions in prepatent periods ranged from five to 13 days when compared to controls and the resulting infections were of a relapsing nature followed by self cure. Effects of this disease on clinical parameters were minimal. One animal infected for 196 days and rechallenged 501 days later had prepatent period of 14 days as compared to five days for controls. This animal developed a brief relapsing infection followed by self cure. Animals which were infected for periods of 41 to 77 days, received treatment, and were then rechallenged from 600 to 900 days later, showed some resistance to infection. Prepatent periods were extended from one to three days over those of control animals and although the resulting disease was severe, one of four animals self cured without treatment. When animals which had self cured secondary challenges were rechallenged at periods out to two years later, they were completely refractory to homologous challenge from mice. These animals developed brief infections when challenged with the relapse variants collected from a bovine chronically infected with the homologous strain. Prepatent periods were extended however, and the infections were rapidly eliminated without severe clinical disease. Since the bovine developed strong resistance to a challenge of syringe passed trypanosomes we have recently initiated a study to determine whether or not they are also immune when the homologous strain of parasite is transmitted by fly bite. Tsetse flies (Glossina morsitans) were infected by daily exposure to infected guinea pigs or bovines for a period of two weeks. Two weeks after the last exposure, the flies were fed on immune and non-immune cattle. All control animals (nine) have developed high levels of persistant parasitemia. Six immunized cattle have developed low level relapsing infections while the remaining nine immunized animals have not developed detectable parasitemias as yet. Significant anemia, leucopenia and thrombocytopenia have developed in all controls; however, immunized animals have not shown any severe clinical disease.

SECTION E

IMPROVEMENTS TO THE LABORATORY FACILITY AND PREPARATION OF NEW REGENTS

While a strong immunity to blood forms was apparent in calves who had self cured T. congolense infections and in adults who were repeatedly infected and cured, the immunity needed to be tested against metacyclic challenge from tsetse flies. We were given extra facilities at the Veterinary Research Laboratories (1,000 sq. ft.) which we reconditioned into an insectory, laboratory, small animal room and exposure room for large animals. The facility has been double screened and equipped with insectocutors. The large animal exposure room contains a stanchion whereby cattle can be exposed to fly bites indoors. Arrangements were made through Dr. Anthony Jordan at Bristol University, England, to receive 200 G. morsitans pupae per month on a regular basis. At the present time, although we are still receiving the pupae from England we are collecting approximately 1,000 pupae a month from our own rearing colony. The Trans-Mara I strain of T. congolense which had been used throughout our immunization studies was readily transmitted by the G. morsitans flies and we were able to initiate the metacyclic challenge experiments which are now in progress.

During the past year, bovine serum has been fractionated and the immunoglobulins purified. These reagents have been used to immunize goats who will provide antisera which will be conjugated with fluorescin isothiocyanate and used in immunopathological investigations.

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