

AD-A047 618

ARMY MEDICAL RESEARCH UNIT (KENYA)
STUDIES ON AFRICAN TRYPANOSOMIASIS.(U)
SEP 75 I E MURIITHI, D E WYKOFF, R M KOVATCH

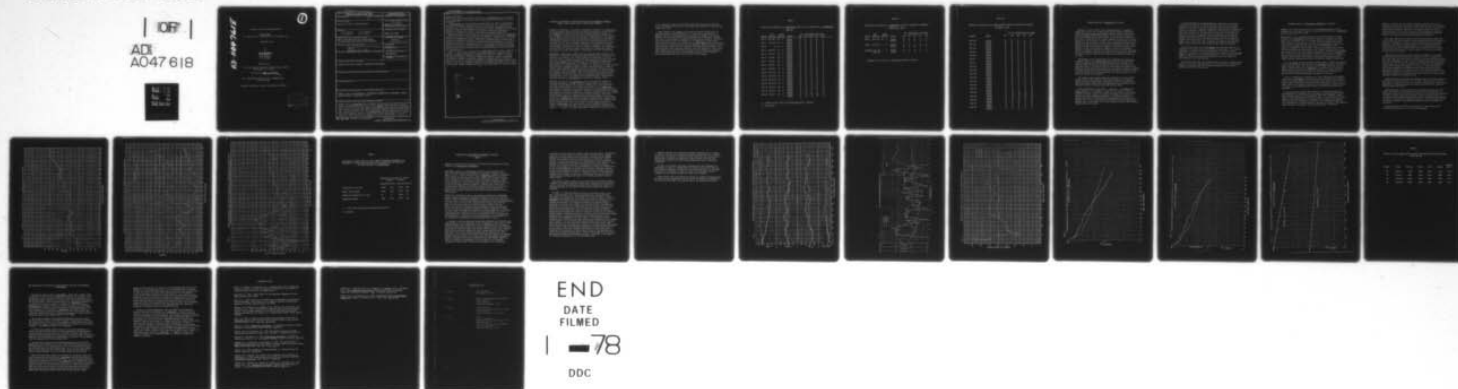
F/G 6/13

DAMD17-75-G-9398

UNCLASSIFIED

NL

AD-A047 618



END
DATE
FILMED
1 - 78
DDC

AD-A047618

①

Studies on African Trypanosomiasis

FINAL REPORT
(for the period 1 September 1974 to 31 August 1975)

September 1975

By

I. E. Muriithi
D.E. Wykoff
R. M. Kovatch
B. T. Wellde

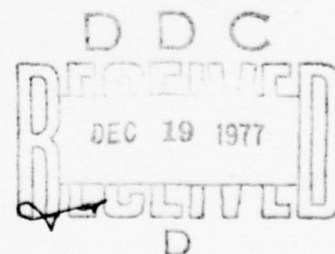
Supported by

U.S. Army Medical Research and Development Command
Washington, D. C. 20314

Contract No. DAMD 17-75-G-9398

U.S. Army Medical Research Unit (WRAIR) Kenya
Nairobi, Kenya

Approved for public release; distribution unlimited



UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Studies on African Trypanosomiasis		5. TYPE OF REPORT & PERIOD COVERED Final Report 1 Sep 74 - 31 Aug 75
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) I. E. Muriithi R. M. Kovatch D. E. Wykoff B. T. Wellde		8. CONTRACT OR GRANT NUMBER(s) DAMD17-75-G-9398
9. PERFORMING ORGANIZATION NAME AND ADDRESS THE US ARMY MED RSCH UNIT (WRAIR) KENYA HQ WALTER REED ARMY INST RES WASH DC 20012		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62759A 3A762759A831.00.096
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Develop. Command Washington, D. C. 20314		12. REPORT DATE 30 Sep 75
		13. NUMBER OF PAGES 31
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) APPROVED FOR PUBLIC RELEASE. DISTRIBUTION UNLIMITED		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) AFRICA, KENYA, TRYPANOSOMIASIS, TRYPANOSOMA RHODESIENSE, CONGOLENSIS, BRUCEI, PATHOPHYSIOLOGY, IRRADIATED, CATTLE		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Isolates of <u>T. rhodesiense</u> were collected from patients in Lambwe Valley, South Nyanza, Western Kenya. Parasites of the <u>T. brucei</u> group were also collected from cattle in the same area. When these parasites were tested by neutralization with antisera collected from bovines which had undergone long term infections with various isolates, 12 of 16 <u>T. rhodesiense</u> isolates reacted with the same antiserum indicating antigenic similarities which had persisted for at least four years. When isolates of <u>T. brucei</u> from cattle were tested, 4 of 19		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

Item 20 continued.

reacted with the same antiserum used in the T. rhodesiense tests. Isolates of T. rhodesiense from Ethiopia and Tanzania were not neutralized by this antiserum.

T. rhodesiense has been found to be pathogenic for cattle. Trypanosomes isolated from humans produced disease in cattle which was characterized by weight loss, fever, pleocytosis and CNS disorders. Uncoordinated movements, circling and opisthotonos were observed. Gross alterations in the brain included thickened dull grey meninges, while the salient histological picture was a moderate to severe non-purulent meningoencephalitis.

Thrombocytopenia has been found to be a prominent feature of T. congolense infections in cattle. This condition is dependent on high levels of trypanosomes in the peripheral blood. Chronically infected animals with lower blood levels of trypanosomes develop a less severe thrombocytopenia. When trypanosomes are at very low levels in these animals, thrombocytes are at normal or increased levels. Chemotherapeutic cure of animals with severe thrombocytopenia results in a rapid elevation of thrombocyte levels to higher than normal values. Leukocyte levels follow a pattern similar to that described for thrombocytes.

Early in the course of anemia in T. congolense-infected cattle there is an increase in mean corpuscular volume and mean corpuscular hemoglobin while the mean corpuscular hemoglobin concentration does not change. A limited reticulocyte response was evident during this period. Later, the erythrocyte indices return to normal even though the anemia persists. Chrome 51 labelling of bovine erythrocytes indicates an appreciable shortening of half life in cells in the infected animals with increased clearance of the isotope in the urine. Plasma clearance of Fe_{59} is also more rapid in infected animals.

ADDITIONAL TO:	
HTIC	Write Section ☒
BDC	Ref: Section ☐
UNANNOUNCED	☐
IDENTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
SIN	AVAIL. AND/OR SPECIAL
A	

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

ANTIGENIC RELATIONSHIPS BETWEEN ORGANISMS OF THE TRYPANOSOMA BRUCEI
GROUP IN THE LAMBWE VALLEY, SOUTH NYANZA, KENYA

PROBLEM: To determine the extent of antigenic variability in trypanosomes of the T. brucei group collected from man and animals in 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 designed to study reactions of various trypanosome isolates with various antisera to determine the variability of different antigenic types within the 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 in these areas will also be examined.

BACKGROUND: 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 types of T. brucei. He postulated that this heterogeneity made immunization attempts impractical. It appears to us that the techniques employed were not specific enough to detect all variants of a given isolate thus giving an exaggerated number of basic antigenic types. 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 identical, their relationship to each other remains questionable. In near by 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 T. rhodesiense were collected from patients at the Homa Bay Hospital on Lake Victoria, western Kenya, by members of the Kenya Medical Department. Blood was injected I.P. into rats which were then transported to us for study. Two strains of T. rhodesiense from Gambella, Ethiopia, were collected by the U.S. Navy Medical Research Unit Number 5, 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. The first series of experiments was undertaken with antiserum against the initial strain of T. rhodesiense (LVH-1) collected in Lambwe Valley, August 1972. During the past year 12 new isolates from humans have been collected and tested, bringing the total to 16 (Table I). Three other strains of known T. rhodesiense were also tested, two from southern Ethiopia and a laboratory strain (Wellcome) which had been isolated in Tanzania and maintained in rodents for many years. These findings are shown in Table II.

It is noteworthy that 12 of the 16 isolates from man were neutralized by the antiserum against LVH-1, indicating a persistence of these similar types since 1972.

When isolates of T. brucei from cattle were tested (Table III) the antiserum showed a strong effect on 5 of 19 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 species and even variant specific. The percentage of cattle parasites which react with the antibody is surprising and indicates that cattle may be a more important reservoir host than believed to now. While game animals have borne the onus of being the most important reservoirs, the close proximity of man and his domestic cattle in the Valley may pose a more important aspect in the cycle of the disease.

TABLE I

EFFECTS OF ANTISERUM TO T. RHODESIENSE (LVH-1) ON 16 ISOLATES OF T. RHODESIENSE
FROM MAN

STRAIN	DATE ISOLATED	DONOR		SERUM	NO. TRYPANOSOMES PER MOUSE				
		AGE	SEX		10 ⁴	10 ³	10 ²	10 ¹	10 ⁰
LVH 1	16-8-72	52	M	Immune	0*	0	0	0	0
				Normal	5	5	1	1	0
LVH 2	17-12-72	37	F	Immune	0	0	0	0	0
				Normal	5	5	2	0	0
LVH 3	73	?	?	Immune	0	0	0	0	0
				Normal	5	5	5	3	0
LVH 4	20-5-74	10	F	Immune	0	0	0	0	0
				Normal	5	5	5	5	5
LVH 5	18-7-74	55	M	Immune	0	0	0	0	0
				Normal	5	5	5	5	5
LVH 6	3-8-74	52	F	Immune	0	0	0	0	0
				Normal	4	5	2	2	0
LVH 7	3-8-74	22	F	Immune	0	0	0	0	0
				Normal	5	5	4	3	3
LVH 8	8-8-74	25	M	Immune	0	0	0	0	0
				Normal	5	5	5	5	5
LVH 9	28-8-74	41	M	Immune	5	5	4	5	5
				Normal	5	5	4	5	5
LVH 10	2-10-74	50	M	Immune	0	0	0	0	0
				Normal	3	4	4	4	0
LVH 11	28-10-74	28	F	Immune	4	3	4	3	1
				Normal	5	5	4	3	4
LVH 12	24-12-74	50	M	Immune	0	0	0	0	0
				Normal	5	4	5	4	4
LVH 13	22-1-75	30	F	Immune	0	0	0	0	0
				Normal	5	5	4	5	2
LVH 14	23-4-75	42	M	Immune	5	4	3	0	0
				Normal	4	4	5	3	1
LVH 15	26-6-75	60	M	Immune	0	0	0	0	0
				Normal	5	5	2	1	1
LVH 16	1-7-75	42	M	Immune	5	**	**	**	**
				Normal	5	**	**	**	**

* Number of mice (out of 5) developing patent infection

** Not tested

TABLE II

EFFECTS OF ANTISERUM ON T. RHODESIENSE PARASITES ISOLATED IN DIFFERENT
GEOGRAPHICAL AREAS

STRAIN	DATE ISOLATED	DONOR		SERUM	NO. TRYPANOSOMES PER MOUSE				
		AGE	SEX		10^4	10^3	10^2	10^1	10^0
ETH-1	30-10-73	30	F	Immune	5*	5	5	1	0
				Normal	4	5	5	5	3
ETH-2	26-11-74	?	M	Immune	5	5	4	5	4
				Normal	5	5	5	5	5
WELLCOME	over 20 years ago	?	?	Immune	5	4	4	2	0
				Normal	5	5	4	5	1

* Number of mice (out of 5) developing patent infections

TABLE III

EFFECTS OF ANTISERUM ON T. BRUCEI-GROUP PARASITES ISOLATED FROM CATTLE
IN LAMBWE VALLEY

ISOLATE	SERUM	NO. OF TRYPANOSOMES PER MOUSE				
		10^4	10^3	10^2	10^1	10^0
LVB 15A	Immune	0*	0	0	0	0
	Normal	5	5	5	5	5
LVB 16A	Immune	0	0	0	0	0
	Normal	5	5	2	1	0
LVB 18A	Immune	5	1	1	0	0
	Normal	4	3	5	0	1
LVB 3B	Immune	3	3	2	0	0
	Normal	4	2	3	2	0
LVB 24B	Immune	5	5	5	3	0
	Normal	5	5	5	5	5
LVB 2B	Immune	5	0	0	0	0
	Normal	5	5	4	5	3
LVB 4B	Immune	5	5	5	3	3
	Normal	5	5	5	5	5
LVB 10B	Immune	5	5	4	1	0
	Normal	5	5	5	5	3
LVB 36B	Immune	5	5	5	0	2
	Normal	5	5	5	2	5
LVB 16B	Immune	0	0	0	0	0
	Normal	5	5	5	4	0
LVB 12C	Immune	0	0	0	0	0
	Normal	5	5	4	5	5
LVB 36C	Immune	4	3	0	0	0
	Normal	5	4	1	1	0
LVB 42C	Immune	5	0	0	0	0
	Normal	5	5	4	4	**
LVB 43C	Immune	5	4	4	5	5
	Normal	5	5	4	5	4
LVB 65C	Immune	0	**	**	**	**
	Normal	5	**	**	**	**
LVB 66C	Immune	3	**	**	**	**
	Normal	5	**	**	**	**
LVB 72C	Immune	5	2	0	0	0
	Normal	5	5	2	3	0
LVB 77C	Immune	5	5	3	1	0
	Normal	5	5	5	5	4
LVB 78C	Immune	4	4	2	1	0
	Normal	4	5	5	5	1

OBSERVATIONS ON T. RHODESIENSE IN CATTLE

During our early studies of the immunogenicity of irradiated trypanosomes in cattle, we noted that control animals challenged with viable Trypanosoma rhodesiense (Wellcome strain) underwent a mildly pathogenic course of infection which resulted in self cure. Fever and leukopenia were noted early in the course of infection but after a brief period the animals appeared to suffer no untoward reaction to the infection. However, in a later experiment one animal (268) which was infected with this strain suffered a severe form of disease characterized by a lack of growth and terminally by a rapid weight loss and a severe episode typical of central nervous system disorder. Since these symptoms occurred in only one of approximately 10 animals infected with this strain, we were not convinced that these symptoms were entirely due to the trypanosome infection although we had not observed this behavior in any other animal on our farm.

Subsequently 4 of 5 animals which were infected with recently isolated strains of T. rhodesiense from Lambwe Valley experienced a disease pattern similar to that described above. To date, two (243 and 6882) of the four diseased animals have been killed and autopsied while the remaining two are still under observation. Cerebrospinal fluid obtained from three of ₃ these animals showed a pleocytosis ranging from 330 to 512 WBC per mm³. Trypanosomes have also been isolated from the CSF by subinoculation IP in mice. Bacterial cultures of the CSF were negative.

The extent of the nervous signs seen terminally varied between animals. Animal 6882 exhibited only uncoordinated leg movements. Animal 268 had uncoordinated leg movements, circling to the right and opisthotonos. Animal 243 had uncoordinated leg movements and a continuous unilateral periorbital and muzzle tremor.

Gross alterations in the brain included thickened dull gray meninges in all animals. In addition, the brain of 243 had exaggerated sulci, submeningeal scars and small cystic cavitations near the external capsule. The salient histological feature was a moderate to severe meningoencephalitis. It was least severe in animal 6882 where the perivascular and meningeal infiltrates were usually 1 to 2 cells thick and were comprised mainly of lymphocytes admixed with a few plasma cells. Focal areas of gliosis were randomly distributed in the brain.

A severe generalized meningoencephalitis was found in the brain of animals 268 and 243. In animal 268 wide cuffs of an infiltrate-proliferate of lymphocytes and plasma cells were randomly dissiminated in the brain. Meningeal involvement with the same inflammatory cells was moderately extensive. Diffuse and focal areas of gliosis were most prominent in the gray matter. Lesions in animal 243 were more extensive with the perivascular and meningeal infiltrate-proliferate primarily plasmocytic. Large numbers of Mott's cells or mature plasma cells were a prominent component of the inflammatory reaction and were found in all levels of the brain, brain stem and spinal cord sections that were examined. In addition, periarterial edema, cystic cavitations and extensive gliotic scars were found.

The Wellcome strain of T. rhodesiense has been maintained in the laboratory by syringe passage in rodents for many years. It would be reasonable to assume that its pathogenicity for cattle would be reduced as a result of its laboratory history. However, the T. rhodesiense of Lambwe Valley origin has been shown to be a parasite of cattle in the Valley and would presumably, as we have found, be more pathogenic for these animals.

The lesions described are commonly found in chronic trypanosomiasis in man but have not been described in the CNS of bovines. Additional study of the bovine model may elucidate mechanisms important to definition of the disease in man.

PATHOPHYSIOLOGY OF TRYPANOSOMA RHODESIENSE IN BOVINES

PROBLEM: To attempt documentation of the pathophysiological process occurring in a systematic study of bovines inoculated with T. rhodesiense recently isolated from a human.

BACKGROUND: In this laboratory bovines were inoculated with certain strains of T. rhodesiense isolated from humans as controls for other studies or in attempts to obtain large amounts of convalescent serum. This immune serum was used in the definition of antigenic relationships between morphologically identical T. rhodesiense collected from humans and T. brucei isolated from animals in the Lambwe Valley (details elsewhere in this report). Some of the experimentally infected bovines showed an unexpectedly severe chronic disease process late in the course of infection. Three of the animals were killed in extremis. The common and salient histological finding was a moderate to severe non-purulent meningoencephalomyelitis. In two additional experimentally infected bovines that remain alive, examination of cerebral spinal fluid revealed a marked pleocytosis, 254 and 512 WBC/mm³ respectively.

Of 19 T. brucei isolates from the Lambwe Valley, 5 (26%) were neutralized by the antibody obtained from a T. rhodesiense (LVH-1) experimentally infected bovine. This indicates that an agent the same, or very similar antigenically to T. rhodesiense, is present in the cattle in Lambwe Valley. The bovine is therefore likely to be highly important in the epidemiology of the natural disease occurring in humans. It may also be an important pathogen for cattle in that area.

There is considerable controversy in the literature on the significance of T. rhodesiense infection in bovines. Some (Kleine and Kunert; Wilde and French) report patent infections that induce a mild clinical disease which eventually self-cure. Others (Carmichael; Bruce et al.; Duke) report more virulent infections in experimentally infected cattle. In either case, pathophysiologic aspects of infection have not been described.

To systematically study these aspects, 10 bovines 5-8 months of age comprised of 5 Herefords and 5 Aryshires, weighing 220-400 pounds were selected from the Veterinary Department herd at Kabete, Kenya. They were inoculated with the LVH-12 strain, 1×10^4 parasites per 200 pounds. Four young bovines, 2 Herefords and 2 Aryshires, of similar size and age were maintained as controls. This report describes the first 23 weeks of a study designed to define the clinical, hematological, serum chemical, serological and histopathological alterations.

RESULTS: In one of the ten infected animals a single parasite was found in a thick blood film on day 3 post-exposure (PE). The remaining 9 infected animals were positive on thick films examined on day 4 PE. Peak fevers occurred on day 5 PE in two animals, day 6 PE in five animals and day 7 PE in three animals. Peak parasitemias calculated from the number of parasites per 100 WBC varied between 21,700 and 434/mm³. The mean of the peak parasitemias was 9,756/mm³.

The daily temperature variations for infected animals during the first 2 weeks PE, and the weekly average temperatures for infected and controls thereafter are shown in Fig 1. Other clinical observations include a mild depression that occurred during the period of peak parasitemia and fever. Lymphadenopathy (3 to 4 times normal) was noted 30 days PE and persisted during the remainder of the 23 weeks of infection. The summary of weekly weights of infected and control animals is shown in Fig 2.

Hematological findings include transient leucopenia at the time of peak parasitemia followed by leukocytosis (Fig 3). Differential WBC counts done on jugular blood indicate a transient absolute neutropenia and lymphopenia on day 8 PE. The average high WBC occurred during the 4th, 5th and 6th week PE. During this period an absolute lymphocytosis and mild absolute neutrophilia were observed. The packed cell volume (PCV) results are summarized in Fig 4. A mild depression in RBC values accompanied the depressed PCVs. Mean corpuscular hemoglobin concentrations, mean corpuscular volume and mean corpuscular hemoglobin indices have remained within normal limits to date. A mild transient depression in platelet counts was noted on the 8th and 12th day post exposure in infected animals.

During the first seven weeks PE Giemsa-stained thick films prepared from tail blood were positive in 70 to 100% of the infected animals when 200 oil immersion fields were examined. Subsequently, a progressive diminution of trypanosome positive smears occurred with no positives detected after day 135 PE.

Subinoculation of blood into rats was initiated during the fourth month of infection. The sub-inoculation of lymph node aspirate was initiated during the fifth month of infection. The results are summarized in Table I. These initial data indicate that the subinoculation of a small amount of lymph node aspirate (usually less than 0.1cc expanded to 0.5cc in 10% fetal calf serum) injected IP into rats appears to be an effective method of isolating the parasite late in the course of infection.

Serum preserved at -20° C for serological, serum chemical and immunoglobulin analysis has not yet been examined.

FIG 2 WEIGHTS OF TEN BOVINES INFECTED WITH T. RHODESIENSE AND FOUR CONTROLS

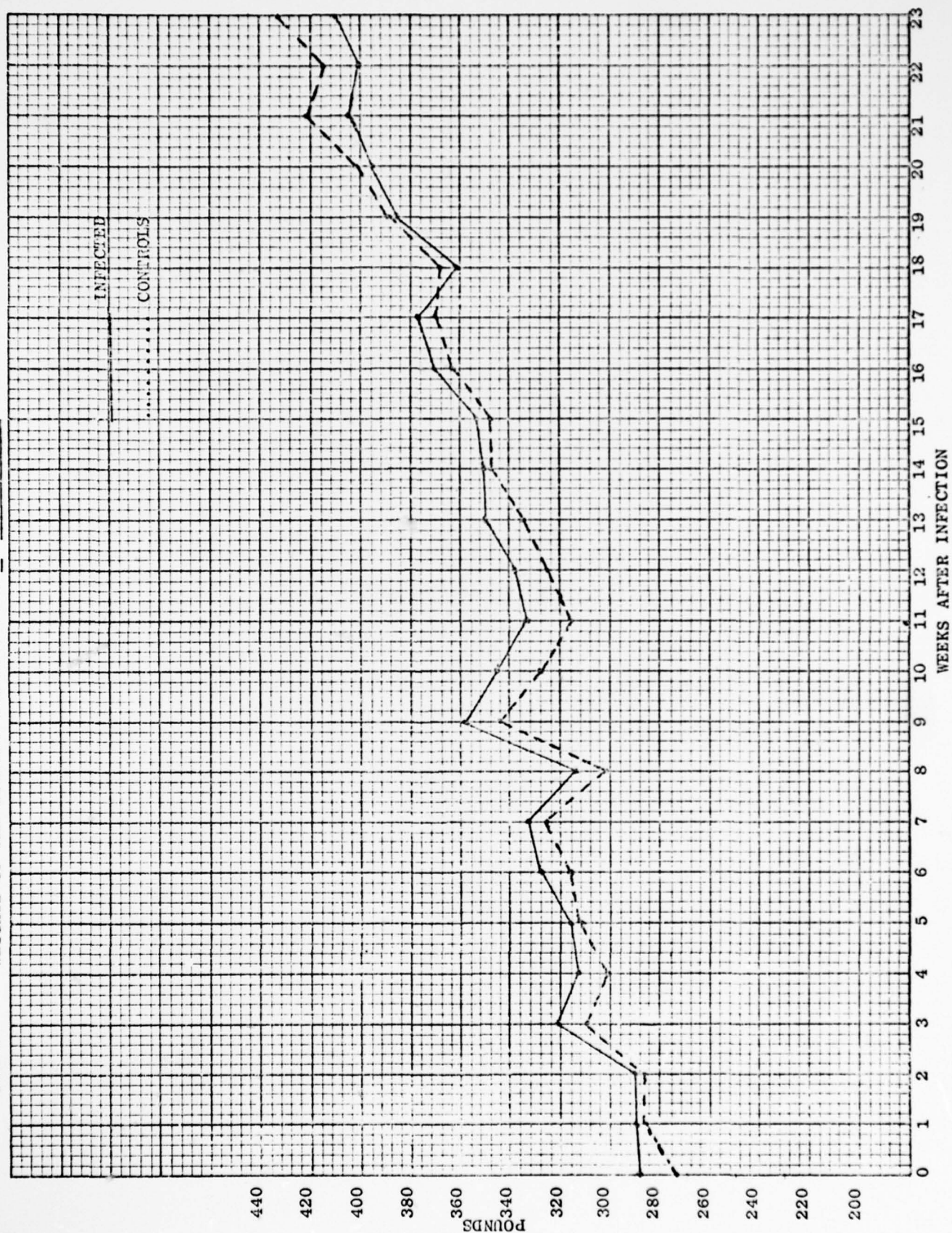


FIG 3
PACKED CELL VOLUME IN TEN BOVINES INFECTED WITH T. RHODESIENSE AND FOUR CONTROLS

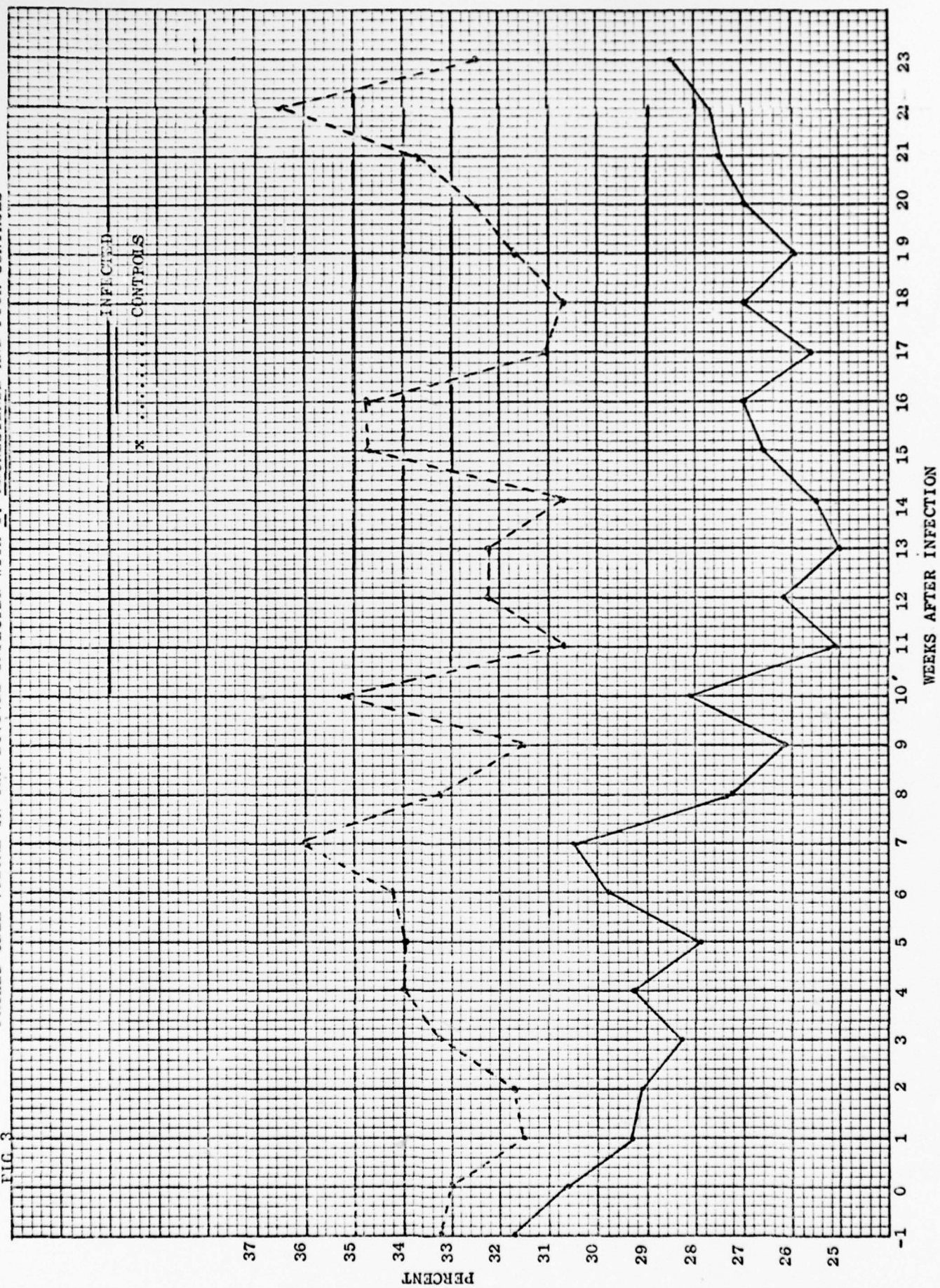


Fig 4

AVERAGE LYMPHOCYTES IN TEN BOVINES INFECTED WITH T. RHODESIENSE AND FOUR CONTROLS
 AVERAGED TWICE WEEKLY FOR THE FIRST THREE WEEKS AND WEEKLY THEREAFTER

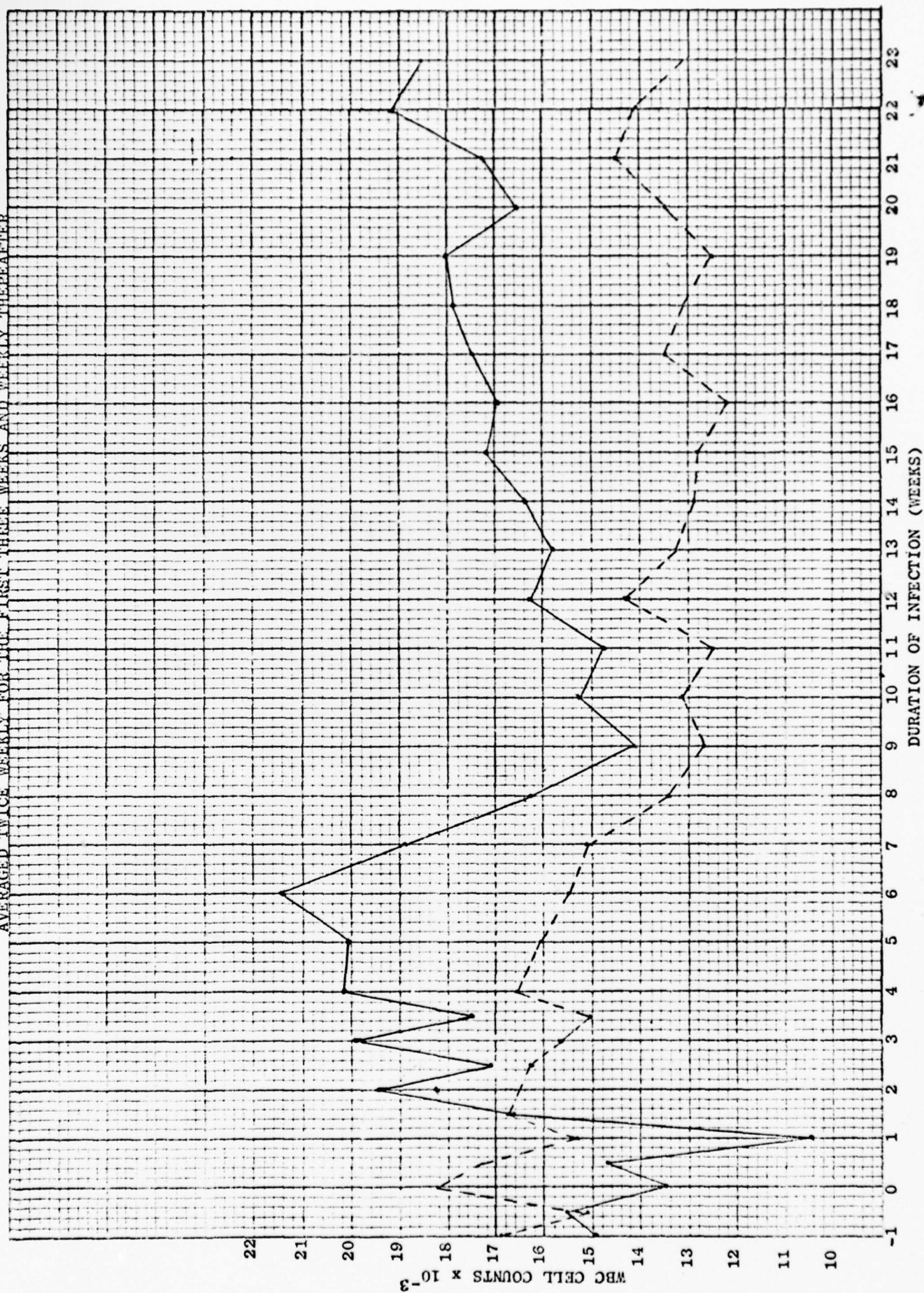


TABLE I

THE RELATIVE EFFECTIVENESS OF DIRECT SMEAR EXAMINATION COMPARED TO IP
INOCULATIONS, USING BLOOD AND LYMPH NODE ASPIRATES FOR THE DETECTION
OF CATTLE HARBORING T. RHODESIENSE-

	Duration of Infection in Cattle			
	4 months		5 months	
	Infected	Control	Infected	Control
Blood (5ml) IP to rats	8/10*	0/4	2/10	0/4
Blood, thick smears	0/10	0/4	0/10	0/4
Lymph node aspirate IP to rats	**	**	10/10	**
Lymph node smears	5/10	0/4	5/10	0/4

* 8 of 10 rats showed patent infection by day 30

** Not done

PATHOLOGY OF TRYPANOSOMA CONGOLENSE IN BOVINES

ANEMIA

PROBLEM: To define the etiology of the anemia which develops in cattle infected with Trypanosoma congolense.

BACKGROUND: Anemia of undetermined nature is a characteristic of most infections caused by pathogenic trypanosomes. Previous work by Wellde, et al. (1974) indicated that T. congolense produces a rapid and severe anemia in Hereford cattle, these animals therefore being good study models. Baseline factors including erythrocyte indices, thrombocyte, reticulocyte and leukocyte levels have been determined. Bone marrow biopsies of infected and control animals have also been done in an effort to judge erythrocyte potential. Radioisotopic labels have recently been employed to determine erythrocyte survival and sites of sequestration. Iron kinetics are also being studied to determine plasma iron clearance rates and utilization in the production of new erythrocytes.

RESULTS: As indicated in Fig 1, an increase in both mean corpuscular volume and mean corpuscular hemoglobin occurred in the five infected animals. Mean corpuscular hemoglobin concentration did not differ from control values. A limited reticulocyte response was evident beginning about the 30th day after infection, and persisted through the 100th day. However, only one animal had a reticulocytosis greater than 1%. After this period of limited erythroid response the mean corpuscular volume and the mean corpuscular hemoglobin levels appeared to return to lower values although the hematocrit remained around 20%. A striking thrombocytopenia occurred with the onset of parasitemia. Levels of thrombocytes were reduced to as low as $30,000/\text{mm}^3$ from the normal mean of $492,000/\text{mm}^3$. We have found the thrombocytopenia to be dependent on the numbers of trypanosomes in the peripheral blood (Fig 2).

Leukocyte levels are generally depressed throughout the infection and they appear to be also dependent on the trypanosome levels (Fig 2). When infected animals were treated with Berenil thrombocyte levels rapidly became elevated over preinfection values. Leukocyte levels also returned to normal (or somewhat higher than normal levels) shortly after therapy. These results strongly suggest that when trypanosomes are present in the blood, there is an increased consumption of thrombocytes. This phenomenon is consistent with diffuse intravascular coagulation although grossly infected animals show minimal signs of hemorrhage. Histopathological examination of tissues indicates that some vessels appear to contain leukocytes, trypanosomes and other material in plug-like formations. Erythrocytes, however, do not appear to be a prominent

component of these formations (Kainer 1974, Kovatch unpubl). Thrombocytopenia has been described in man and animals infected with T. rhodensis and in these infections the detection of fibrin split products as well as decreased levels of fibrinogen tend to indicate a coagulopathy. It has also been shown that trypanosomes in the presence of specific antibody and complement attract platelets which adhere to them. Whether or not this process of immune adherence can occur in vivo as well as in vitro has not been established but could well be involved in the initial clumping of thrombocytes and their subsequent removal by the reticuloendothelial system. After curative therapy packed cell volumes do not return to normal at a rapid rate (Fig 3). The erythroid response after therapy is also not characterized by a strong reticulocyte response although the packed cell volumes gradually return to normal levels.

Bone marrow biopsies taken early in the course of infection showed a normal or somewhat hyperactive appearance. Normoblasts were plentiful as were cells of the leucocytic series. These observations confirm our previous findings in bone marrow obtained early during the course of infection.

In order to study erythrocyte survival in infected animals, four male Hereford-Boran calves weighing approximately 175 pounds each were used in an experiment. Two animals were infected with 1×10^4 T. congolense (Trans Mara) while the other two served as controls. Prior to infection, all animals had received transfusions of homologous Cr^{51} -labeled erythrocytes. The calves were housed in metabolism cages and blood, urine and fecal samples were collected daily, measured and aliquoted for radioactivity determinations. The animals were also followed by routine hematology, levels of parasitemia and temperature. Since these experiments have only been recently initiated, only preliminary data are available. These do indicate a trend toward increased plasma volumes, decreased erythrocyte survival times and increased splenic sequestration of labeled erythrocytes in the infected calves. Fig 4 indicates the disappearance of whole blood in one control and one infected animal. The onset of patent parasitemia corresponds with a change in the slope of the line indicating blood radioactivity in the infected calf (control: 14.5 day half life; infected: 7 day half life). Fig 5 shows the erythrocyte disappearance curves for the same animals. The change in the slope of the line denoting disappearance in the infected animal is not evident until about 14 days after that of whole blood. This indicates that a dilution of RBCs occurred early in the infection as a result of an expanded plasma volume. Later, however (day 26) the control and infected lines cross indicating an actual decrease in the number of circulating erythrocytes in the infected animal.

Surface counting over selected body sites indicated that the spleen of infected animals was sequestering labelled erythrocytes to a greater degree than the spleens of control animals (Table I). All animals were bled on day 33 and their erythrocytes again labelled with Cr_{51} and retransfused. Results from this second transfusion have not yet been calculated.

In order to determine the plasma clearance rate and subsequent incorporation of Fe_{59} into erythrocytes 4 infected cattle and 2 controls were injected with Fe_{59} . Plasma half time clearance rates for one experimental and one control are shown in Fig 6. The clearance rate was increased in the infected animal over that of the control.

These studies have been restricted because of limited available space for radioactivity experiments. We are now in the process of renovating a two room structure which will greatly add to our existing facilities.

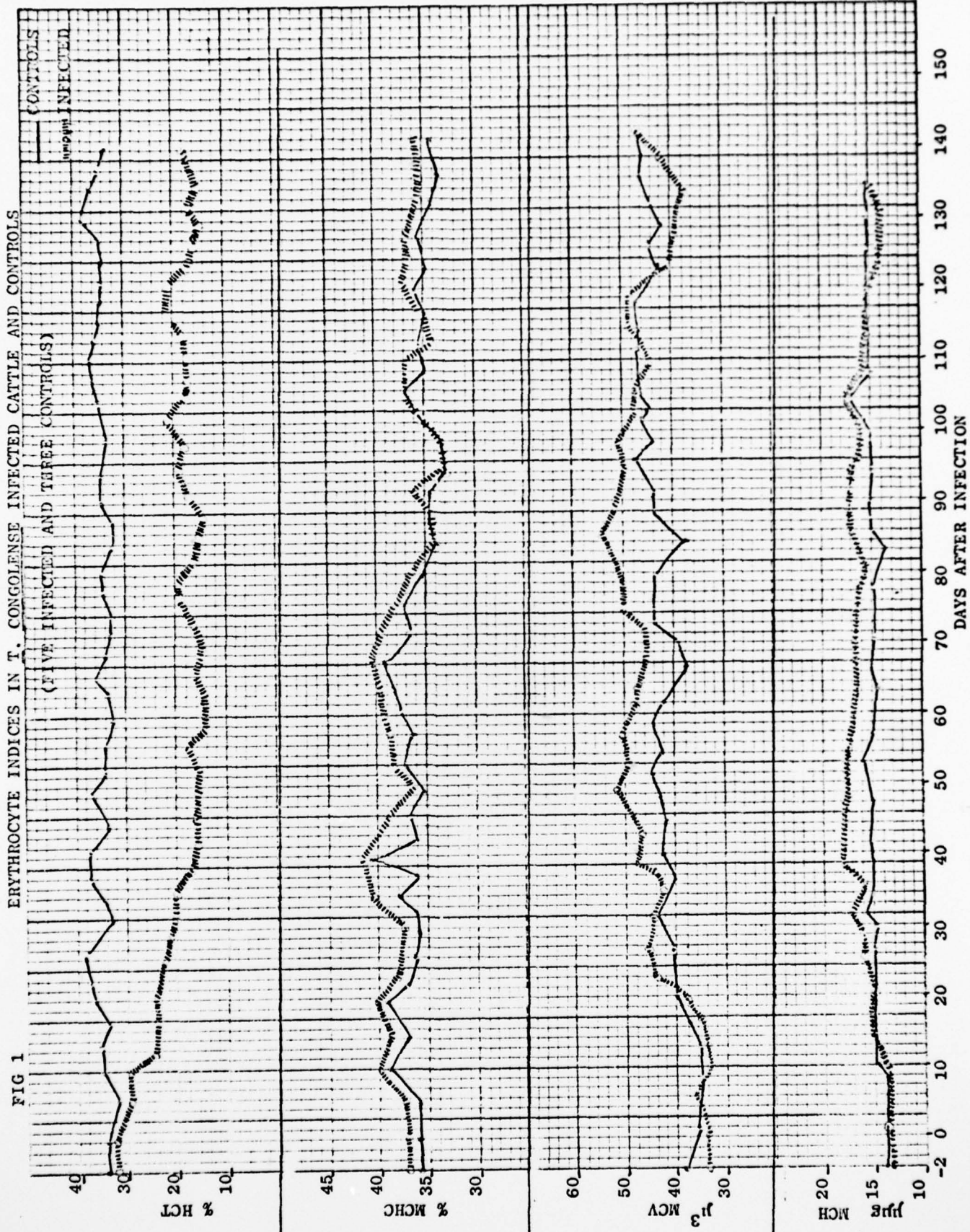


FIG 2
TYPICAL COURSE OF TRYPANOSOMA CONGOLENSIS INFECTION IN CATTLE

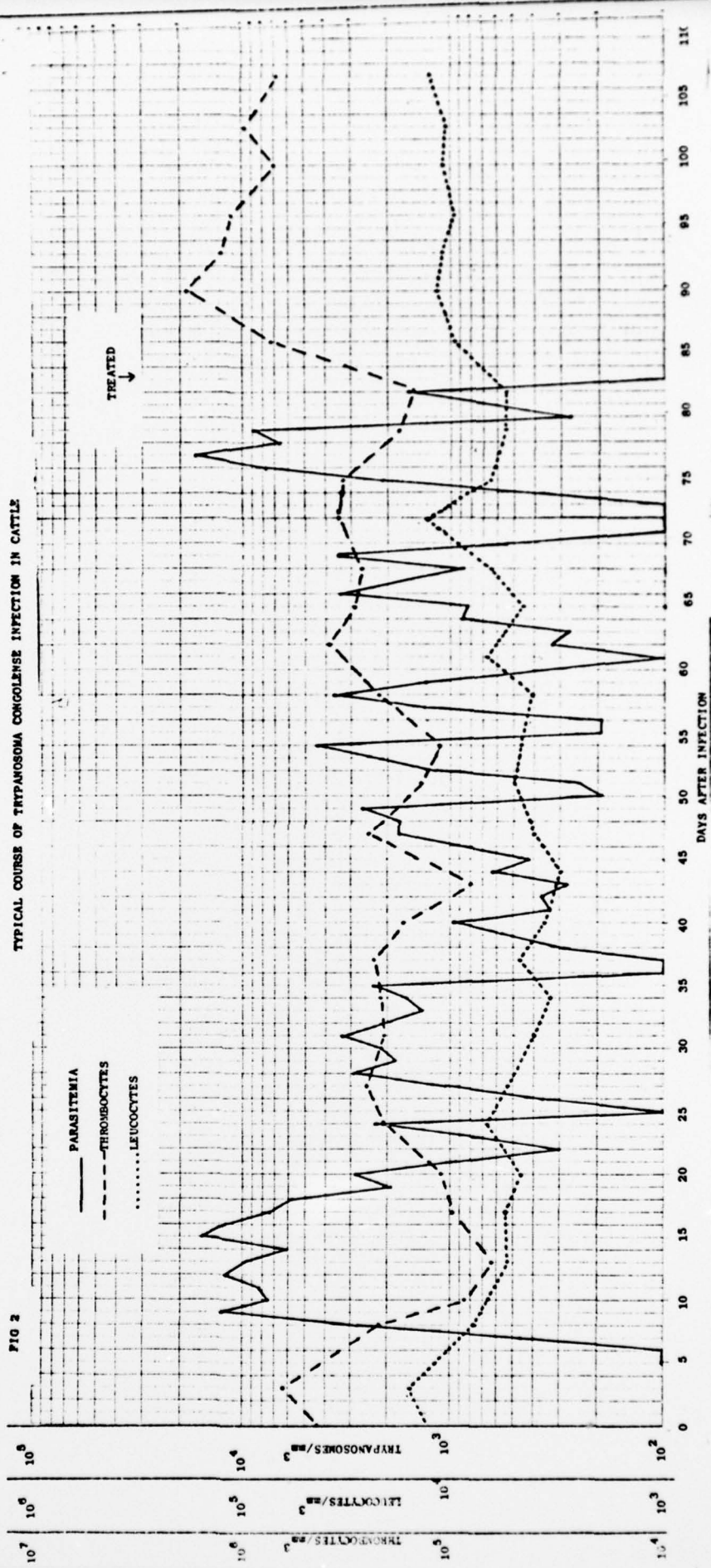


FIG 3 RECOVERY OF PACKED CELL VOLUME AFTER CURATIVE TREATMENT

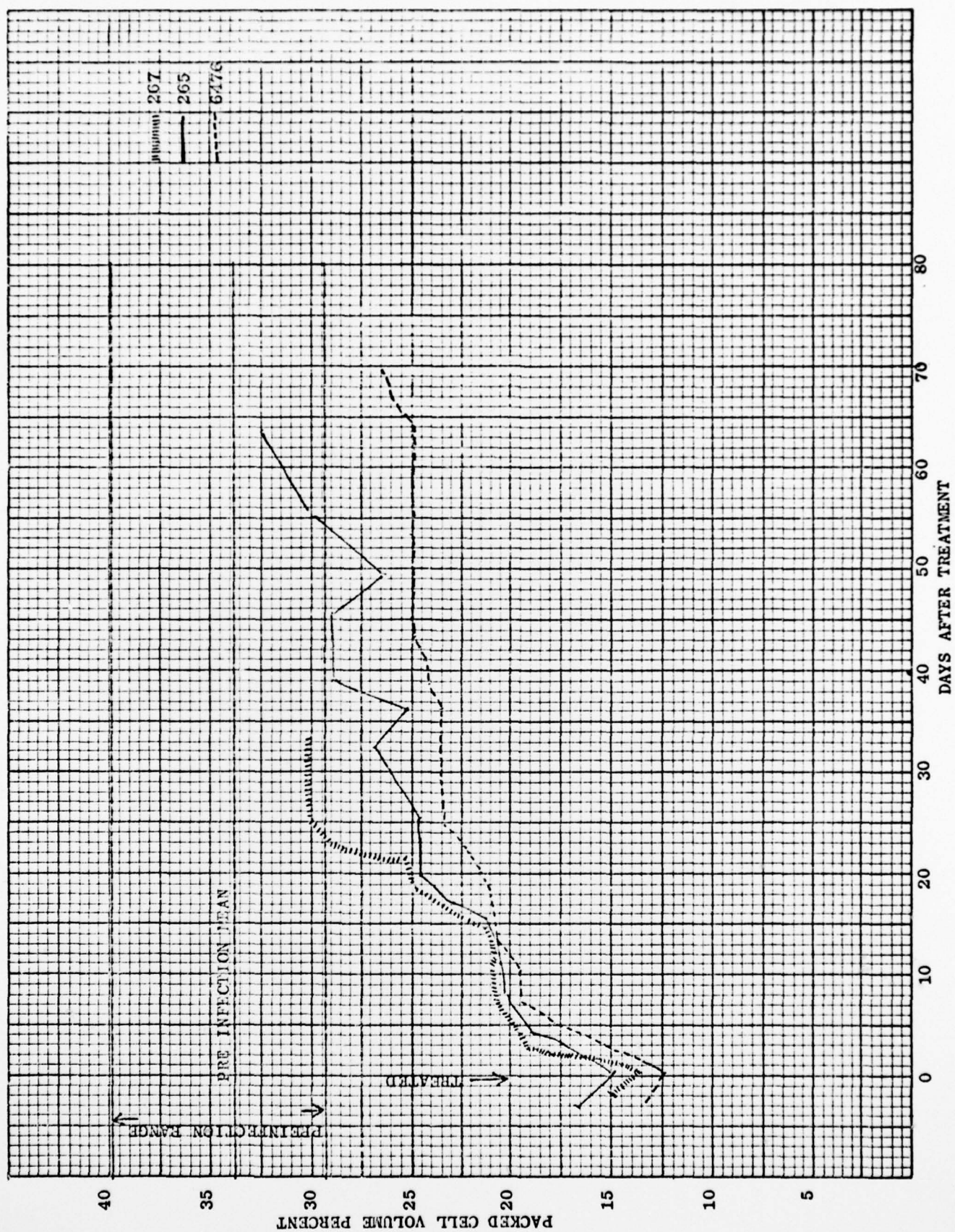


FIG 4

BLOOD DISAPPEARANCE IN ONE EXPERIMENTAL (o-o-o) AND ONE CONTROL (---) CALF
INFECTED WITH T. CONGOLENSE

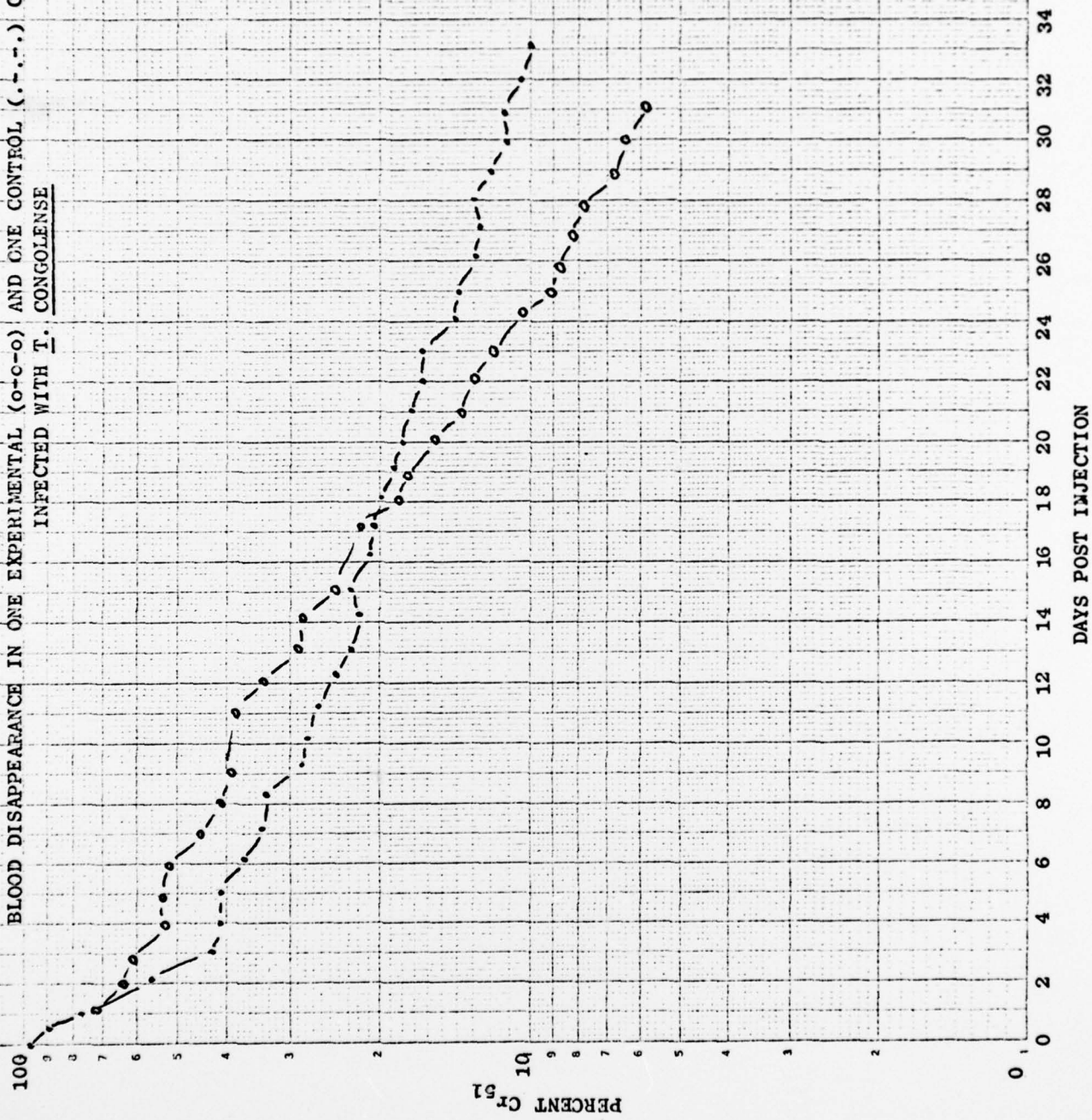


FIG 5 ERYTHROCYTE DISAPPEARANCE IN ONE EXPERIMENTAL (o-o-o-o) AND ONE CONTROL (.-.-.-) CALF
INFECTED WITH T. CONGOLENSE

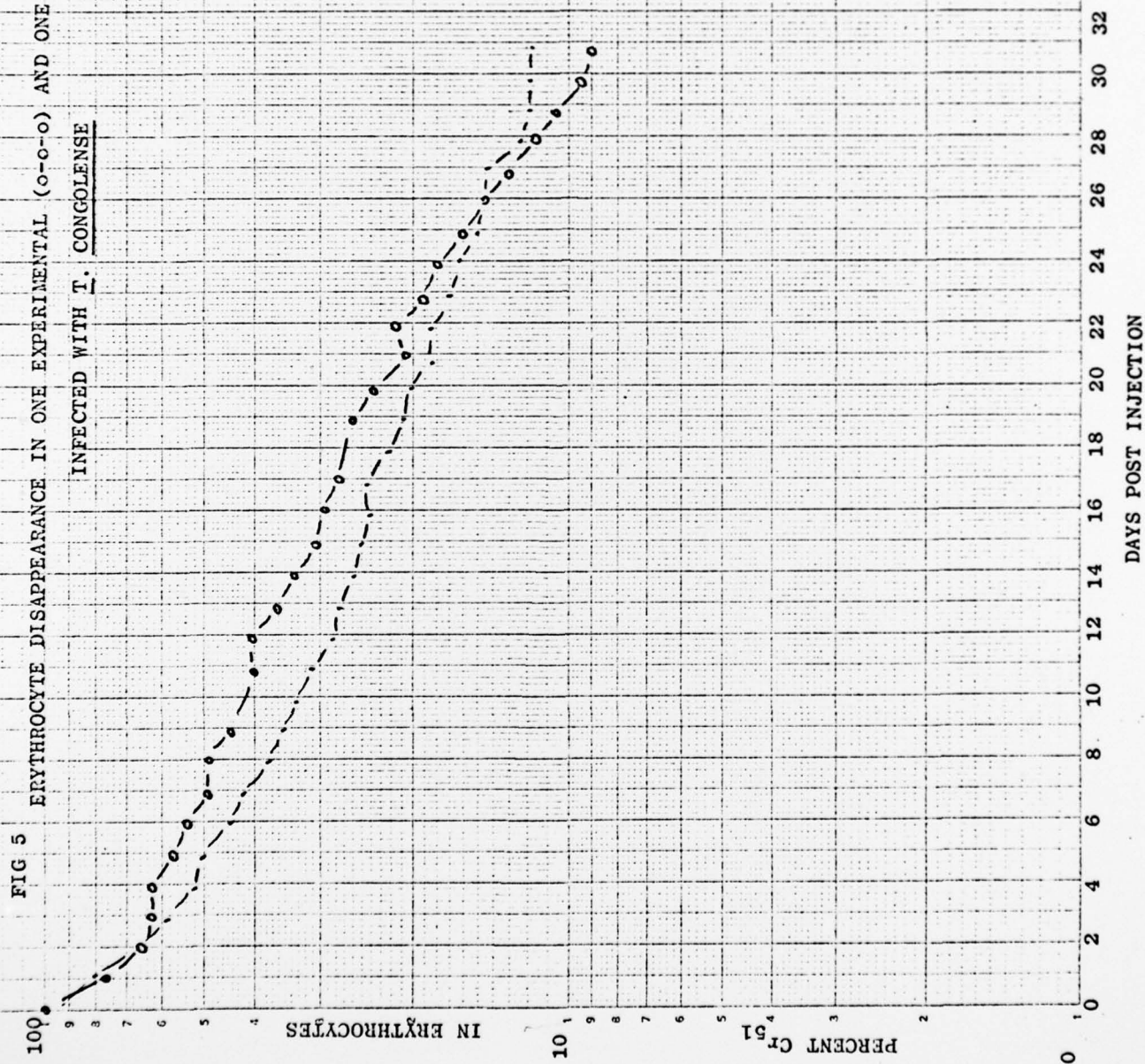


FIG 6

PLASMA IRON DISAPPEARANCE IN ONE EXPERIMENTAL (o-o-o) AND ONE CONTROL (.-.-.) CALF

INFECTED WITH T. CONGOLENSE

INFECTED $T_{1/2} = 65 \text{ min}$

CONTROL $T_{1/2} = 119 \text{ min}$

MINUTES AFTER INJECTION

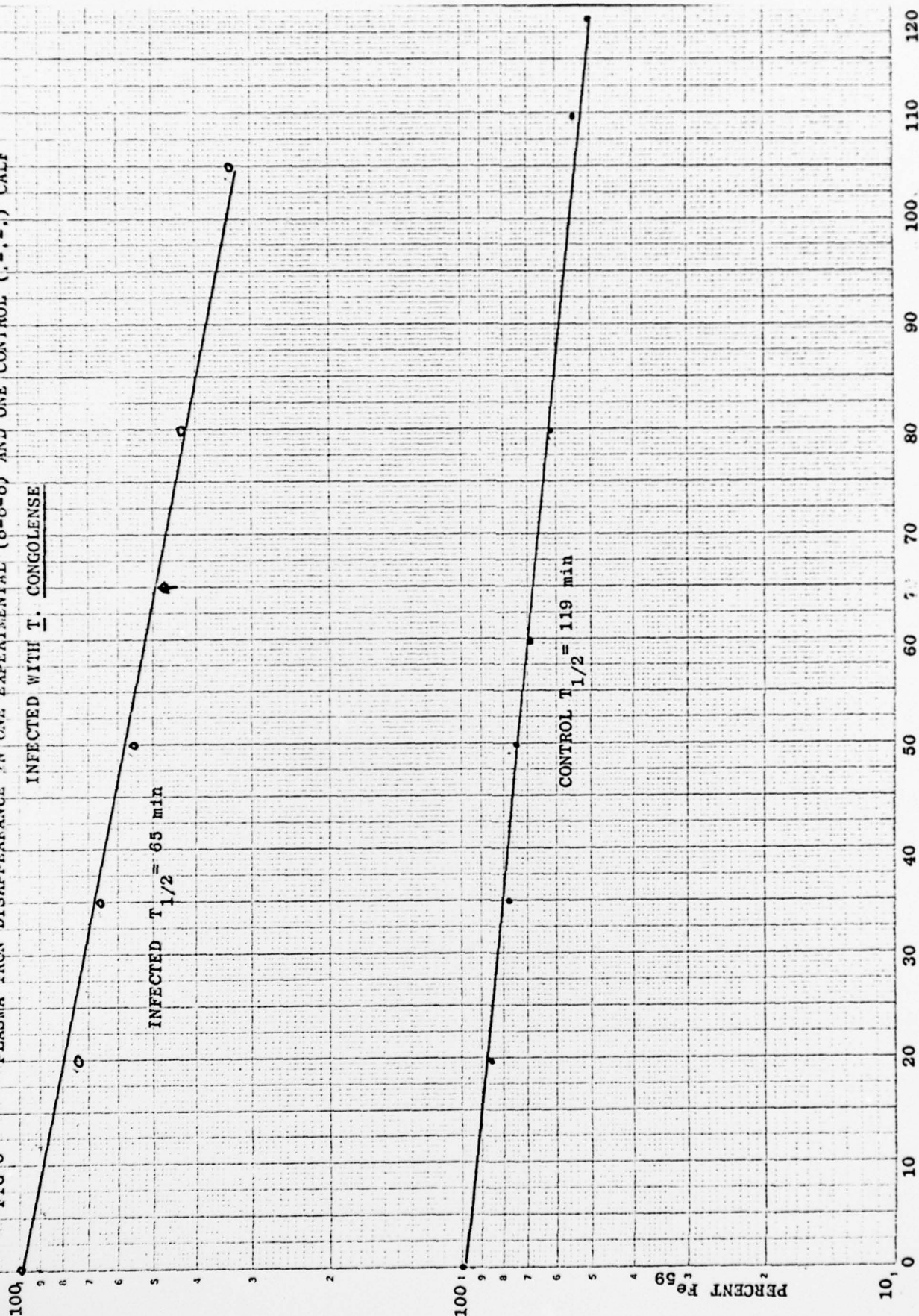


TABLE I

RESULTS OF THE IN VIVO COUNTING (COUNTS PER MINUTE) OVER SELECTED BODY
SITES (DAY 32)

Animal	Group	Sternum	Heart	Liver	Spleen	Spleen/ Liver
5F	Control	3018	3498	1551	1515	0.98
6F	Control	3696	3013	3140	3067	0.98
7F	Infected	2248	3618	1666	4121	2.47
8F	Infected	2074	2517	1675	4607	2.75

THE FEASIBILITY OF ARTIFICIAL IMMUNIZATION BY THE USE OF IRRADIATED TRYPANOSOMES

Our initial results with T. rhodesiense showed that bovines could be immunized with irradiated trypanosomes (Wellde, et al 1973). If the immunizing inocula contained sufficient irradiated trypanosomes, the recipients became refractive to challenge with non-irradiated parasites of the same strain. Similar experiments with T. congolense (Wellde, et al 1974) did not yield as great an immunity as that produced by T. rhodesiense but prepatent periods of immunized animals were extended and antibodies developed after immunization (Lotzsch and Diendl 1974). Mice immunized with irradiated T. congolense were highly immune to a viable challenge of organisms (Duxbury et al 1973). Recently we have found that immunization with irradiated trypanosomes is specific for the antigenic type of the immunogen (Wellde et al 1975).

The antigenic typing of trypanosomes transmitted by tsetse flies has been little studied. Gray (1970) reported that antigenic variants of a given isolate return to a basic antigenic composition in the tsetse fly. Workers in Nigeria (personal communication) believe that the tsetse transmit the antigenic type which is ingested.

To study this problem (which seems to be of great importance in terms of practical immunization) we have proposed the establishment of a small colony of tsetse flies here in Kabete. Initially this was to have been undertaken during the early part of 1975 but budget limitations imposed by DA and USAMRDC have postponed the initiation. We are hopeful that some progress can be made during 1976.

Our experiments involving irradiated trypanosomes have also been hampered by the breakdown of the only Cobalt-60 source available to us. The maintenance is the responsibility of the Atomic Energy Commission and although they have been notified that it is out of order, repairs have not been made. The new International Laboratory for Research on Animal Diseases (ILRAD) will have a Cobalt source installed soon.

Other work involving immunity has yielded the following. Serum from cattle chronically infected with T. congolense conferred protection to mice infected with the homologous parasite. In a preliminary experiment 30 mice were injected with 2×10^6 T. congolense IP. Subsequently 10 of these mice (experimental group) received 1 ml of serum from a chronically infected animal. As controls 10 were injected with normal serum and 10 were not treated. Fifty percent of the mice receiving serum from the infected animal did not become patent and survived for at least 25 weeks after challenge whereas 100% of the control animals became

patent and 10% survived. An extension of the prepatent period was also observed in the experimental group. In a second experiment, globulins of both the immune and normal serum were collected by salt precipitation at 50% saturation, dialized, and reconstituted to 1/3 the original serum volume. These globulins were then tested in mice using the above procedure. Only 10% of the mice treated with immune globulins developed patent infection whereas all the controls became positive. Nine weeks after challenge 90% of the experimental group and 40% of the control group survived. Column fractionation of immune globulins on the Sephadex G-200 and subsequent testing in mice indicated that the protection activity is associated with the 7S gamma globulins.

Infection and chemotherapeutic cure as a method of immunization. Cattle which had been infected with T. congolense (Trans Mara) and cured with Berenil were rechallenged at various times after treatment with the homologous strain of parasite. Four animals undergoing infection of from 36 to 77 days did not show appreciable resistance when re-challenged with the stock strain. Prepatent periods for previously infected animals ranged from 6 to 8 days whereas five control steers were patent on the 5th day. Parasitemia levels appeared to be lower as the infection progressed although packed cell volumes in both groups were similar. One animal which had undergone two long periods of infection (total 10 months) remained refractive when challenged for the third time. These results indicate that infection and cure can be a method of immunizing cattle against T. congolense. It appears, however, that long term infections are needed in order to stimulate a significant degree of immunity.

LITERATURE CITED

Bruce, D., Harvey, D., Hamerton, A.E., and Lady Bruce. 1914. Trypanosome Disease of Domestic Animals in Nyasaland: Susceptibility of Animals to the Human Strain. Proc. Roy. Soc. 88:130-138.

Carmichael, J. 1934. Annual Report of the Veterinary Department of the Uganda Protectorate: 26-28.

Duke, H.L., 1928. Studies on the Bionomics of Polymorphic Trypanosomes of Man and Ruminants. Final Report of the League of Nations International Commission of Human Trypanosomiasis. 3:21-116.

Duxbury, R.E., Sadun, E.H., Anderson, J.S., Wellde, B.T., Muriithi, I.E., and Warui, G.M., 1973. Immunization of Rodents, Dogs, Cattle and Monkeys Against African Trypanosomiasis by the use of Irradiated Trypanosomes. Isotopes and Radiation in Parasit. III. International Atomic Energy Agency: 179-180.

Gray, A.R. 1970. A Study of the Antigenic Relationships of Isolates of Trypanosoma brucei Collected From a Herd of Cattle Kept in One Locality for Five Years. J. Gen. Microbiol. 62:301-313.

Kaliner, G. 1974. Trypanosoma congolense. II. Histologic Findings in Experimentally Infected Cattle. Exper. Parasitol. 36:20-26.

Kleine, von F.K. and Kunert, H., 1939. Das Verhalten des menschlichen Schafkrankheitserregers im Rand. Arch. Schiffs- u. Tropenhyg. 43:451-453.

Lötzsch, R. and Deindl, G., 1974. Trypanosoma congolense: Experimental Infections in Cattle. III. Serological Responses. Exper. Parasitol. 36:27-33

Onyango, R.S., Van Hove, K. and Raadt, P., 1966. The Epidemiology of Trypanosoma rhodesiense Sleeping Sickness in Alego Location, Central Nyanza, Kenya. Trans. Roy. Soc. Trop. Med. & Hyg., 60:175.

Seltys, M.A., 1957. Immunity in Trypanosomiasis. I. Neutralization Reaction. Parasitol. 47:375-389.

Wellde, B.T., Duxbury, R.E., Sadun, E.H., Langbehn, H.R., Löttsch, R., and Deindl, G., 1973. Immunization of Cattle by use of Gamma Irradiated Trypanosoma rhodesiense. Expr. Parasitol. 34:62-68.

Wellde, B.T., Löttsch, R., Deindl, G., Sadun, E.H., Williams, J.S., and Warui, G.M., 1974. Trypanosoma congolense: Experimental Infections in Cattle. I. Clinical Observations. Exper. Parasitol. 36:6-19.

Wellde, B.T., Schoenbechler, M.J., Diggs, C.L., Langbehn, H.R., and Sadun, E.H., 1975. Trypanosoma rhodesiense: Variant Specificity of Immunity Induced by Irradiated Parasites. *Exper. Parasitol.* 37:125-129.

Wilde, J.K.H., and French, M.H., 1945. Experimental Study of Trypanosoma rhodesiense Infection in Zebu Cattle. *J. Comp. Path.* 55:206-228.

DISTRIBUTION LIST

4 copies

HQDA (SGRD-AJ)
Washington DC 20314

12 copies

Defense Documentation Center (DDC)
ATTN: DDC-TCA
Cameron Station
Alexandria, Virginia 22314

1 copy

Superintendent
Academy of Health Sciences, US Army
ATTN: AHS-COM
Fort Sam Houston, Texas 78234

1 copy

Dean
School of Medicine
Uniformed Services University of the
Health Sciences
Office of the Secretary of Defense
6917 Arlington Road
Bethesda, Maryland 20014