CHEMOTHERAPEUTIC STUDIES ON EXPERIMENTAL T. CONGOLENSE INFECTIONS.

THESIS

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INTRODUCTION

Introduction

The name nagana is applied to a serious disease of cattle and other domestic animals in Africa — tsetse-fly disease — which is characterised especially by wasting and anaemia and is now known to be due to infection with trypanosomes. The pioneer work of Bruce and his associates pointed to the causal agent being the trypanosome known as T. brucei; but later investigations have shown that so far as cattle are concerned T. congolense is probably the most important of the parasites responsible for nagana. It resembles T. brucei and its allies, T. rhodesiense and T. gambiense, and also the T. evansi group in that mice and certain other laboratory animals are susceptible to experimental infection; thus it lends itself fairly readily to exact study. On the other hand, T. congolense differs from all the above-named trypanosomes morphologically, since it has very little in the nature of a free flagellum; and also, infections due to it are poorly influenced by the chemotherapeutic agents which act vigorously on the other trypanosomes mentioned above, e.g. trypanblue, trypaflavin, arsenicals, suramin (Bayer 205, or germanin), styryl-314, etc. The chlorinated parafuchsin dye, tryparosan, and bismuth salts have some therapeutic action, which, however, does not seem to be capable of practical application.

The problem of the cure of cattle infected with T. congolense is exceedingly important economically and many attempts have been made to discover efficient drugs. In the field the first compound with considerable effect was found to be tartar emetic; Hornby (1919) reported favourably on its use and Richardson (1928) by means of this drug controlled several natural outbreaks, affecting large numbers of

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of animals. However, owing to the extremely irritant nature of the solution tartar emetic must be injected intravenously, which presents considerable difficulties under field conditions, also the therapeutic dose approximates to the toxic limit. A more easily administered and better tolerated compound, antimosan, a complex of antimony and pyrocatechin, was next extensively tested; success with repeated dosage was reported by Parkin (1930), who observed no objectionable sequelae, by Evans (1936) and du Toit (1936).

An organic compound, Surfen C, like antimosan of German origin, stated to be an amino-quinoline derivative, was claimed to be curative in a single intramuscular dose (Iensch 1937). Van Rensburg (1938) from his survey of the published work on treatment with this drug, concluded that results, in respect both of efficiency and local reaction, were conflicting. He obtained unsatisfactory results in cattle, using several samples of the drug, since it had been claimed that the efficiency of different batches varied. Evans (1936) on the other hand, considered that a single dose was superior to a course of antimosan. It appears, however, that none of the above drugs is highly effective in cattle.

Browning, Morgan, Robb and Walls (1938) in the course of investigations on compounds of the phenanthridinium series synthesised by Morgan and Walls, showed that 7 amino-9 (p-aminophenyl)-10-methyl-phenanthridinium chloride (No. 897) — 23 of Morgan and Walls, 1938 — exerted a definite therapeutic effect on T. congolense in mice; but in their original experiments the curative dose approximated to the maximum tolerated. Further investigations showed, however, that much more favourable results might be obtained (Browning, Browning and Robb, 1940); and an investigation of the reason for the discrepancy between the /

the earlier and the later results, in which the present author took part, has yielded a satisfactory explanation for the difference; and the conditions have been defined under which experimental infections with T. congolense in mice may be cured with a small fraction of the tolerated dose of the drug, see section II — for reference to field trials see section VII. This opened the way for an investigation of the immunological properties of T. congolense, which have turned out to differ markedly from those of the T. brucei group which had hitherto been examined from this point of view by various investigators (see section VI). A series of substances related chemically to No. 897 has also been studied (section III). A part of the results has been published in summary form (Browning and Calver, 1943), but the following account embodies the experimental work actually carried out by the author. Subsequently certain amidine compounds have been found active against T. congolense (section III).

SECTION I

EXPERIMENTAL STRAINS OF T. CONGOLENSE.

Experimental Strains of T. Congolense.

Two laboratory strains of T. congolense have been used throughout the experiments; the origin, characters of the infection and methods of maintaining one strain (Strain I), have been fully described (Browning, Cappell and Gulbransen 1934), but they are now summarised.

Strain I

A rat infected with this strain was received through Dr. C.M. Wenyon, Wellcome Bureau of Scientific Research, London, from Mr. H.E. Hornby, Director of Veterinary Services, Tanganyika Territory. Prior to passage through rats the strain had been passed through a rabbit. Continuous passage through mice has been maintained since December 1929. In order to transmit the strain, fresh mice are inoculated subcutaneously with a suspension in saline of infected blood; the inoculum for each mouse consisting of 6 to 8 drops of blood taken from the tip of the tail of an infected mouse in which the parasites are abundant, in 0.5 c.c. normal saline. Scanty trypanosomes can usually first be demonstrated in a fresh film of the blood with the 1/6" objective on the 4th or 5th day after inoculation; the following day the numbers have increased to 3 to 12 per field and by the 3rd day they are abundant, i.e. at the "fastigium". The table below gives symbols used to indicate the progress of the infection.

-	=	no parasites
+vl	=	very scanty parasites
+l	=	1 parasite in every 2nd or 3rd field
+	=	1 to 3 parasites per field
++	=	4 to 12 parasites per field
+++	=	an uncountable number of parasites per field (fastigium).

The /

The stage of fastigium may be said to have been reached on the first occasion on which an uncountable number of trypanosomes are observed following a progressive increase in their numbers, e.g. in mouse 1784H the infection had reached the fastigium on the 8th of March (see appended table).

Table - Mouse No. 1784H.

1.3.44.	inoculated
6.3	+vl
7.3	+
8.3	+++ (fastigium)
9.3	+++
10.3	+++
13.3	-
15.3	++
17.3	+++
20.3	+++
22.3	+++

Course of the infection. Three courses of infection subsequent to the fastigium were originally described, viz. acute (in which death occurs in a few days with the trypanosomes persisting in large numbers), relapsing and chronic. The relapsing-chronic course is still the most common, with the first negative period occurring from 2 to 5 days after the fastigium. The trypanosomes may never disappear completely from the blood; or again, they may persist in considerable numbers, although fluctuating for days or weeks. During the first 50 passages of the strain, before it became accommodated, about 1 mouse in 7 failed to become infected with the usual inoculum; since then, however, animals resistant to infection although still met with occasionally, are very exceptional. A slight decrease in the incubation period, which is described as the interval in days between the subcutaneous inoculation and the first appearance of parasites in the blood, was observed in the course of early passages; the

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the period being 5 to 10 days for the majority of animals of the first 50 passages, 4 to 9 days for the next 50 and 4 to 8 days for passages 100 to 150. After continuous transmission through mice for over 14 years no further noteworthy alteration has taken place in the incubation period. This is shown in Table I.

Maintenance of the strain. The strain is maintained by passage to a fresh animal immediately the parasites become abundant; this is designated as a fastigially propagated strain. The stock strain which has been propagated in this way now for many hundred passages is referred to as the acme strain of T. congolense I; and in this connection the fastigium is termed the acme. In the light of the present work, it is considered highly important to transfer the trypanosomes to a fresh host immediately the fastigium (acme) is reached in order that their immunological characters may remain unchanged. When the infection becomes relapsing-chronic and the parasites after diminishing in the blood or disappearing, again become numerous, the event is spoken of as a relapse fastigium.

Strain II.

Strain II mentioned by P. Browning (1938) was received in a guinea-pig from Mr. H.E. Hornby; the previous history of this strain is unknown. Immediately on arrival at this laboratory on December 9th, 1933, blood from the infected pig was passed to mice and since then the strain has been continuously propagated through mice in the form of an acme strain by the methods described. Table II shows the percentages of mice with the same incubation periods and total numbers of mice in groups at first of 10 or 20 passages and later of 100. Only a slight shortening of the incubation period was observed as the strain became accommodated to /

to mice; this corresponds with observations with Strain I. By the 80th to 100th passages the incubation period was 3 days for over 50 per cent. of the animals and 4 days for most of the rest; after passage for 11 years the incubation period is now slightly longer, being 4 or 5 days for about 70 per cent. (Table II).

Unlike the early passages of Strain I, in the first 50 passages of Strain II only 1 mouse failed to become infected with the ordinary inoculum; a reinoculation was successful, there being no difference in incubation period from that of a fresh mouse similarly inoculated. Thus appreciable natural resistance to this strain was not evident even in early passages.

The parasites, as with Strain I, after appearing in the blood, increase rapidly in numbers, becoming abundant 1 or 2 days later, i.e. the acme stage is commonly reached on the 5th or 6th day. In the early passages, however, the strain took several days longer than at present to reach the acme stage. The course of the infection in 12 animals of the first 100 passages was exceptional, in that the parasites after appearing in the blood in scanty numbers, increased to + or ++ but diminished again before finally reaching the +++ stage, which was not attained until from 9 to 20 days after inoculation; e.g.

mouse 38A	30.7.34	inoculated
	3. 8.	+vl
	4. 8.	+l
	5.8.	++
	6.8.	+l
	7.8.	+l
	8.8.	+
	9.8.	+
	10.8.	+
	11.8.	+
	12.8.	++
	13.8.	+++
	14.8.	+++
	15.8.	+++

No /

No abnormalities of this nature have been noted in later passages up to date — 550th to almost the 700th.

The numbers of trypanosomes in the blood in Strain II infections may fluctuate subsequent to the acme stage, but only in very exceptional cases do they disappear completely — twice in many hundreds — or even fall to small numbers. Death of the host may occur from 2 days to several weeks after the acme stage. Thus, in mice, Strain II is more virulent than Strain I; but neither attains the virulence of T. brucei for those animals. Considerable enlargement of the spleen is a feature of chronic infections with both strains.

Treatment and Methods of Estimating its Effects.

In the treatment of infected mice the drug is given subcutaneously in a single dose in the proportion of 1 c.c. of aqueous solution containing the amount of drug stated per 20 g. body weight; e.g. a dose of 1/1,000 g. means that a mouse weighing 20 g. would receive 1/1,000 g. dissolved in 1 c.c. of water. Mice weighing between 18 and 25 g. were usually selected for the tests. After treatment, a small drop of blood from the tail is examined daily or every 2nd day for a month and frequently, at least weekly, during the next month, then at monthly intervals for some time — 4 to 6 months — before cure is reported. Permanent disappearance of the parasites from the blood, and sterilisation of the infection, i.e. cure, results from treatment with an effective trypanocidal agent. Table III shows representative details for each strain of the examinations of a cured infection, a relapse and an infection uninfluenced by the drug. The time taken for the trypanosomes to disappear varies with the drug and the strain of trypanosome. On the other hand, if the drug has only a marked /

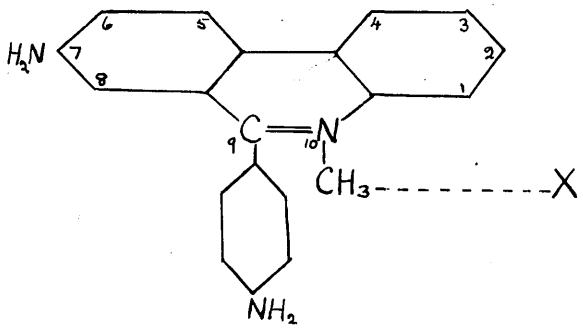
marked trypanocidal action but is not capable of effecting cure, or if a subcurative dose of an effective one is administered, the trypanosomes will disappear from the blood for a time, only to reappear later and increase in numbers, as in the untreated animal. This reappearance of the trypanosomes is termed a relapse. The course followed by the infection after treatment with a substance devoid of trypanocidal properties is identical with that in untreated animals.

SECTION II

TREATMENT OF EXPERIMENTAL T. CONGOLENSE INFECTION IN MICE WITH NO.897.

Treatment of Experimental T. Congolense Infection in Mice with 7 amino-9 (p-amino-phenyl) 10-methyl-phenanthridinium chloride — No.897.

The drug used throughout this investigation, unless otherwise specified, is 7 amino-9 (p-amino-phenyl) 10-methyl-phenanthridinium chloride, No. 897 — 23 (Morgan and Walls — 1938), prepared at the Chemical Research Laboratory, Teddington, Middlesex. The drug is a dark red crystalline compound which dissolves in hot water to give a stable 1 per cent. solution of a clear orange-red colour and of pH just above 7.0. The solution may be boiled. Its constitution may be represented graphically as follows:-



Toxicity.

A dose of 1/1,000 g. subcutaneously is well tolerated both generally and locally, while 1/750 g. kills almost 30 per cent. of mice — usually within 24 hours — and causes hair to disappear from areas of the back at the site of the injection in most of the survivors and necrosis of the subcutaneous tissues in some of them; 1/500 g. kills about 75 per cent. acutely and larger doses are always fatal. The table below gives details of mice used in toxicity experiments.

Dose of drug.	No. of survivals.	Total No. treated.
1/300 g.	0	2
1/400 g.	0	8
1/500 g.	3	12
1/750 g.	30	42

Acute Infections — Strains I and II.

Effect of size of dose. In the present work, the satisfactory results of Browning, Browning and Robb (1940) have been confirmed and extended and investigations on the curability of both strains at various stages of infection have been carried out. Table IV shows the dose of drug, stage of infection at treatment and results for both strains when treatment was given at the early (+vl) or the acme (+++) stage. It is seen that Strain I responds better to treatment than Strain II. Thus of 41 mice infected with the former 25 (almost 61 per cent.) were cured at the acme stage by a dose of 1/75,000 g. to 1/100,000 g., while 1/10,000 g. to 1/15,000 g. cured 26 out of 62 (almost 42 per cent.) infected with the latter at the same stage. With each strain doses smaller than those mentioned failed to effect cure.

Effect of stage of infection at the time of treatment. With both strains the stage of infection at the time of treatment influences the curability of the disease, the drug being more effective when given at the acme than at the early stage when parasites are scanty in the blood. Thus with Strain I, a dose of 1/75,000 g. or 1/100,000 g., which cured 61 per cent. at acme, when given at the early stage cured only 2 out of 32 mice (6 per cent.); and with Strain II a dose of 1/10,000 g. or 1/15,000 g., which cured 42 per cent. at acme, yielded only 1 cure out of 15 at the early stage (7 per cent.). Both these results are highly significant statistically[1]. This indicates that immunity reactions probably play a considerable part in helping to effect cure. After /

[1] All estimations of statistical significance are made according to a modification of Fisher's factorial method (Loewenthal and Wilson, 1939).

After administration of the drug in a curative dose, trypanosomes seldom disappear from the blood within 24 to 48 hours; as a rule, they persist for 2 to 3 days, sometimes increasing in numbers, especially when treatment is given at the early stage. These facts also support the view that the intervention of antibodies assists the action of the drug. The observation that more favourable results are obtained by treating T. congolense infections in mice at the acme rather than at an earlier stage, is novel and is in contrast to the treatment of the acute type of infection, e.g. with T. brucei. With the latter species it is generally agreed that as the infection progresses the more difficult it becomes to effect cure.

From Table IV it can be seen that the action of the drug shows considerable regularity in relation to the dose. Occasional failures, however, may result from treatment with an amount of the drug which is within the curative range. This is seen in Strain I, where in a single case there was failure with the relatively large dose of 1/15,000 g. given at acme, in spite of almost 54 per cent. of those treated at the same stage with 1/100,000 g. being cured. Failure in such cases is, of course, most probably due to variation in the individual animal.

Relapses — Time Interval after Treatment.

Strain I. The majority of relapses occur between the 9th and 14th days after non-curative treatment which temporarily clears the blood of parasites. Analysis of the results recorded in Table IV has been carried out in order to determine if (a) the dose of the drug or (b) the stage of the infection at the time of treatment influences the length of the period following treatment during which the blood was free from trypanosomes.

(a) /

(a) The relapses following relatively large doses, 1/15,000 g. and 1/25,000 g., occurred on the 13th and 8th days respectively after treatment, while in the 5 mice which received the small dose of 1/200,000 g., they were observed on the 8th, 13th, 14th, 17th and 19th days. (b) The stage of infection at the time of treatment does not appear to influence the duration of the free period; of the 34 mice which relapsed after treatment with doses varying from 1/30,000 g. to 1/100,000 g. at an early stage (Table IV), 2 did not respond to the drug, but in 29 (90 per cent.) of the remainder the relapse occurred between the 9th and 14th days. Of the 25 animals which relapsed following treatment at the acme stage with doses varying from 1/15,000 g. to 1/200,000 g., 17 (68 per cent.) occurred during the same period. The only exceptionally late relapse observed was in a mouse (No. 1672D) treated at the acme stage with 1/100,000 g. Numerous examinations of the blood of this animal during the first 2 months were all negative, but a further examination made 3 weeks later showed abundant trypanosomes; therefore the relapse probably took place at least 2 months after treatment, certainly not during the first 6 weeks when examinations were frequent. Details of this mouse and of a few others which relapsed, selected at random, are given in Table V.

This striking regularity of the duration of the free period is further illustrated by analysis of the relapses following treatment at the acme stage with other phenanthridinium drugs, related in chemical structure to No. 897. Here again, after administration of 30 different compounds, relapses occurred in 64 mice, of which 45 (70 per cent.) took place between the 9th and 14th days after treatment. Table VI shows /

shows the duration of the free period in days after different doses of No. 897 administered to Strain I infections at an early stage, at acme, and also after other phenanthridinium drugs. From this table the remarkable regularity of the relapses appears clearly, and is seen to be independent of the particular drug or of a considerable range of dosage or stage of the infection at which the drug was administered. Also the few exceptions are shown in which relapses were observed after a longer time.

Strain II. Relapses occurred mostly 8 to 11 days after treatment, irrespective of the dose of drug administered within wide limits and the stage of infection at the time of treatment. In 4 mice — 2 treated with 1/20,000 g. a relatively large dose, and 2 with the small dose of 1/100,000 g. — the relapses of each pair occurred on the 9th and 10th days respectively. Table IV shows that 26 and 52 relapses followed treatment at the early and the acme stages respectively; in 21 (81 per cent.) of the former and 38 (73 per cent.) of the latter the relapses were observed between the 8th and 11th days. Exceptionally the free period was much longer — 2 relapses occurring on the 21st, 2 on the 24th, and 1 on the 50th day after treatment. Similar details to those given for Strain I are given for Strain II in Tables V and VI.

From the foregoing data it may be deduced that after administration of the drug in an amount which is not curative, an immunity develops which breaks down after a period, which is relatively constant for each strain, constituting a relapse.

Treatment /

Treatment of Chronic Infections — Strains I and II.

In view of the difference in curability of the infection when treated at the early and acme stages, it appeared important to delay treatment until the chronic stage, this being arbitrarily fixed at 3 to 6 weeks after the acme for Strain I; thus time was given either for a relapse to occur or for parasites to have persisted in the blood for a considerable period. In the case of Strain II, the chronic stage was reckoned as having been reached a few days after the acme, since by then abundant parasites had been present continuously in the blood for some time and the host would probably die if left untreated. Such delays caused still further difficulty in effecting cure; the results are given for both strains in Table VII under the heading "chronic in the original mouse". It is of interest to note that with Strain II, 3 to 4 days after the acme stage the curability of the strain has altered considerably. Thus it can be seen that by witholding treatment until the chronic stage, in the case of Strain I a dose of 1/10,000 g. cured 12 mice out of 29; similarly with Strain II a dose of 1/2,000 g. to 1/3,000 g. cured only 6 out of 40 animals.

The contradictory results reported in the two early publications of Browning et al. referred to in the introduction (p.2), are readily explained as a result of the above investigations on the curability of the strains at varying stages of infection. Examination of the records has shown that the original trials of the drug on Strain I infections were all carried out by treatment at the chronic stage; thus, Browning, Morgan, Robb and Walls (1938) found that cures were obtained with doses approximating to the maximum tolerated. On the other hand, the more favourable results obtained by Browning, Browning and Robb (1940) were all

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all in animals with acute infections (either early or acme).

Infections with a Chronic Strain — Strains I and II.

When the inoculum is derived from a mouse in which the infection has become chronic, the resulting infection due to this "chronic strain" is more difficult to cure than one from the customary acme infection (p.6). Again, the chronic stage has been arbitrarily fixed as 3 to 6 weeks after the acme for Strain I and a few days after the acme for Strain II. Details for both chronic strains are given in Table VII; thus 1/10,000 g. cured 13 out of 17 mice with chronic Strain I infections, while 1/2,000 g. to 1/3,000 g. cured 14 out of 22 mice with chronic Strain II.

Failure to develop infection when the inoculum is derived from a chronically infected animal is common with Strain I, e.g. out of 149 inoculated mice, 41 (almost 28 per cent.) failed to become infected; but all inoculations with chronic Strain II trypanosomes were successful. The failures with chronic Strain I inoculations are most probably due to resistance of the individual host, since in most of the series of inoculations at least one animal became infected. This variation in susceptibility is similar to that found by Binns (1938) in individual rats and rabbits to inoculation with 7 strains of T. congolense from infected cattle.

Summary. By comparing Tables IV and VII it can be seen that the order of curability of the various stages of the infection of both strains, starting with the most readily cured, is as follows:- (a) acme, (b) early — +vl, (c) a chronic infection and (d) the chronic stage in the original mouse. Thus it has been found that in mice the curative dose for a chronic infection may be at least 5 to 10 times /

times that required at acme. These observations on the experimental treatment of chronic infections probably have an important bearing on the results under field conditions.

Prophylactic Action.

No. 897 in a dose of 1/1,000 g. administered 2 days before the inoculation, protected 2 mice against Strain I. Doses given 4 to 6 days before inoculation did not exert any prophylactic action, since in each instance the trypanosomes appeared in the animals under test at the same time as in the untreated controls, see table below.

No.	Dose of drug.	Time before inoculation.	Result.
1663A	1/1,000 g.	2 days	complete protection
1663B	1/1,000 g.	2 days	" "
1664D	1/2,000 g.	4 days	as control
1673A	1/1,000 g.	5 days	" "
1673B	1/1,000 g.	5 days	" "
1664A	1/1,000 g.	6 days	" "
1664C	1/1,000 g.	6 days	" "

Thus only a slight prophylactic action is evident; these results are in contrast to the prolonged protective effect of Bayer 205 and certain benzoylamino quinoline styryl compounds against T. brucei (Browning and Gulbransen, 1934). In field trials Carmichael and Bell (1944[1]) found that No. 897 protected cattle against T. congolense infection up to 48 hours after administration (section VII).

Oral Administration of No. 897.

Daily feeding with bread soaked with 1 c.c. of 1/1,000 solution of No. 897 was given in place of normal diet to 6 mice for 11 days. On the 11th day the mice were infected with Strain I, and feeding with the drug was continued for a further 7 days, during which time the trypanosomes /

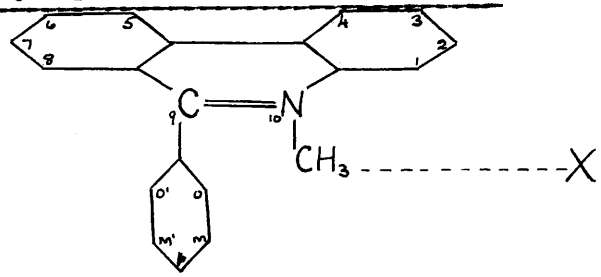
trypanosomes developed as in the controls. By this time the infection was at the acme stage; a subcutaneous dose of 1/30,000 g. was given to each and feeding with the drug discontinued; 4 out of the 6 mice were cured. Drug feeding as above was instituted in 6 mice infected with Strain I when trypanosomes were in scanty numbers in the blood and continued for 7 days, during which time the course of the trypanosome infection progressed through the acme to the relapse stage as in the controls. Thus there is no evidence that absorption of the drug in an active form takes place from the alimentary tract, since No. 897 on oral administration exerts neither prophylactic nor curative action.

SECTION III

TREATMENT WITH OTHER DRUGS.

Phenanthridinium Series.

TABLE OF PHENANTHRIDINIUM COMPOUNDS.



9.....phenyl-10-methyl-phenanthridinium compounds : X = acid radicle.

Serial No. for table.	No. of Compound.	Substituents in phenanthrene nucleus	Substituents in benzene ring.	X	Max. Tol. Dose in gms/20 gm. mouse.	Therapeutic action in Mice infected with T. congolense Strain I at acme.
1.	896	7 acetamido	p-acetamido	chloride	1/800	1/2,000 R
2.	897	7 amino	p-amino	chloride	1/1,000	1/50,000 - 1/100,000(C)
3.	1551	"	"	bromide	1/600	1/75,000 C 1/100,000 R
4.	1552	"	"	iodide	1/500	1/50,000 C 1/75,000(C)
5.	1570	"	"	methane sulphate or X	1/750	1/100,000(C)
6.	1543	7 carbethoxyamido	p-carbethoxyamido	chloride	1/700	1/700 C 1/1,500 R
7.	1160	7 benzamido	p-benzamido	acetate	1/100	1/500 R
8.	1583	7 carbamido	p-carbamido	chloride	1/300	1/500 R
9.	1507	7 acetamido	m-acetamido	chloride	1/200	1/200 (C)
10.	1508	7 amino	m-amino	chloride	1/600	1/200,000(C) 1/300,000 R

Serial No. for table.	No. of Compound.	Substituents in phenanthrene nucleus	Substituents in benzene ring.	X	Max. Tol. Dose in gms/20 gm. mouse.	Therapeutic action in Mice infected with T. congolense Strain I at acme.	
11.	1568	7 amino	m-amino	iodide	1/600	1/50,000 C 1/100,000(C)	
12.	1569	7 carbethoxyamido	m-carbethoxyamido	chloride	1/400	1/400 C	1/1,000 R
13.	1579	"	o-amino	chloride	1/500	1/10,000 C	1/30,000 R
14.	1504	7 acetamido		chloride	1/100	1/100 C	1/400 R
15.	1505	7 amino		chloride	1/2,000	1/10,000 C	1/60,000 R
16.	1506	"		iodide	1/3,000	1/20,000 C	1/60,000 R
17.	1554	2:7 diacetamido		chloride	1/2,000		1/2,000 R
18.	1555	"		sulphate	1/500		1/1,000 R
19.	1565	2:7 diamino		chloride	1/1,000	1/200,000 C 1/300,000(C)	
20.	1553	"		bromide	1/1,000	1/200,000 C 1/400,000(C)	
21.	1566	2:7 dicarbethoxy-amido		chloride	1/1,000	1/1,000 C	1/5,000 R
22.	810		p-acetamido	chloride	1/450		1/1,500 R
23.	208		p-amino	chloride	1/4,500		1/20,000 R
24.	621		"	iodide	1/4,500	1/9,000 C	1/18,000 R
25.	812		p-dimethylamino	chloride	1/40,000		1/100,000 R
26.	620		"	iodide	1/10,000		1/40,000 R
27.	1053		m m'diacetamido	chloride	1/500		1/2,000 R
28.	1052		m m'-diamino	chloride	1/500		1/2,000 R
29.	1061		m m'-dinitro	chloride	1/500	1/2,000 (C)	

Serial No. for table.	No. of Compound.	Substituents in phenanthrene nucleus	benzene ring.	X	Max. Tol. Dose in gms/20 gm. mouse.	Therapeutic action in Mice infected with T. congolense Strain I at acme.	
30.	207		m-amino	chloride	1/3,000		1/3,000 R
31.	1060		m-nitro	chloride	1/250	1/1,000 C	
32.	206		o-amino	chloride	1/2,500		1/10,000 R
33.	1162	3:7 diacetamido		chloride	1/500		1/500 R
34.	1542	3:7 diamino		chloride	1/1,500	1/100,000 C	1/200,000 R
35.	1567	3:7 dicarbethoxyamido		chloride	1/100	1/100 C	1/500 R
36.	893	3 acetamido	p-acetamido	chloride	1/100	1/400 (C)	
37.	1576	"	"	methane sulphonate	1/250	1/500 (C)	
38.	894	3 amino	p-amino	chloride	1/2,500	1/50,000 C	1/100,000 R
39.	1544	3 carbethoxyamido	p-carbethoxyamido	chloride	1/350	1/700 C	1/2,000 R
40.	1577	"	"	methane sulphonate	1/400	1/1,600 C	1/10,000 R
41.	1584	3 carbamido	p-carbamido	chloride	1/200	1/300 C	1/600 R
42.	1574	3 acetamido	m-acetamido	chloride	1/250		1/400 R
43.	1573	3 amino	m-amino	chloride	1/1,500	1/3,000 C 1/100,000(C)	
44.	1575	3 carbethoxyamido	m-carbethoxyamido	chloride	1/1,000		1/1,000 R
45.	1142	3 bromo 7 acetamido	p-acetamido	chloride	1/500		1/2,000 R
46.	1141	3 bromo 7 amino	p-amino	chloride	1/1,000	1/20,000 C	1/50,000 R

C = cure — the lowest curative dose is shown.

(C) = cure in a proportion of animals.

R = relapse.

Phenanthridinium Series.

The foregoing table gives a list of the compounds and the results obtained. It can be seen that the greatest therapeutic activity against T. congolense Strain I infections is exerted by compounds with 2 amino substituents, situated either both in the phenanthrene nucleus or one in the latter and the other in the benzene nucleus, e.g. compounds 1565 (19)*; 1553 (20): 1542 (45): 897 (2); 1551 (3); 1552 (4); 1570 (5): 1508 (10); 1568 (11): 894 (38) and 1573 (43). Compound 1141 (46) which has a bromine atom in the 3 place, with amino groups as in 897, is inferior to the latter. All these compounds are of considerable therapeutic activity. Accordingly, a variety of positions of the amino groups is apparently a matter of comparative indifference so far as therapeutic action is concerned. Two amino groups both in the benzene ring in the m positions are not associated with activity (No. 1052 (28)). A single amino group situated in either nucleus increases the toxicity and reduces considerably or destroys the therapeutic action, e.g. Nos. 206 (32); 207 (30); 208 (23); 621 (24); 1505 (15) and 1506 (16). Much larger doses of the acetylated derivatives than of the corresponding amino compounds are as a rule well tolerated, but little or no activity against the infection is manifested, e.g. 896 (1); 897 (2); 1507 (9); 1508 (10); 1504 (14); 1505 (15); 1555 (18); 1554 (17); 1565 (19): 1162 (33); 1542 (34); 893 (36); 894 (38): 1574 (42); 1573 (43); 1142 (45) and 1141 (46).

The irritating effect of the drug on the tissues at the site of injection is an important property. No. 1553 is outstanding on account of its relative lack of irritating action in comparison with its therapeutic efficiency.

* (The numbers in brackets are the serial numbers in the table).

Phenanthridine compounds. The phenanthridine compound No. 892 (maximum tolerated dose 1/250 g.), corresponding to No. 894 (38); and No. 895 (maximum tolerated dose 1/2,500 g.), corresponding to No. 897 (2), are both without therapeutic action on acme infections with Strain I.

Diamidine Series.

The compound 4:4'-diamidino stilbene was shown by Lourie and Yorke (1939) to be curative in maximum tolerated doses against T. congolense infections in mice. A compound of this class, 4:4'-diamidino dimethyl stilbene, more effective against the same parasite was described by Fulton and Yorke (1942). In 1943 the same authors compared the latter compound with No. 897, both being given subcutaneously, and considered it to be "at least as active curatively against our strain of T. congolense infections in mice". The present author working with laboratory Strain I infections at acme in mice, according to the methods described in the text, has found that 4:4'-diamidino dimethyl stilbene in a dose of 1/5,000 g. cured 21 out of 24 (87.5 per cent.), while under the same conditions No. 897 in a dose of 1/75,000 g. cured 11 out of 15 (73.3 per cent.) and 1/100,000 g. cured 14 out of 26 (54 per cent.); the maximum tolerated doses are 1/500 g. and 1/1,000 g. respectively. Accordingly, the statement of Fulton and Yorke does not hold under these conditions, since 1/75 of the tolerated dose of No. 897 has about the same curative action as 1/10 of the tolerated dose of the dimethyl stilbene drug. In chronic infections, however, the dimethyl stilbene compound appears to be almost as effective as at acme. Therefore it is possible that their observations were made on such infections.

Quindoline Methochloride (I.C.I.).[1]

This compound in doses approaching the maximum tolerated (1/750 g.) cured 9 out of 10 mice. The very severe local necrotic action at the site of subcutaneous injection almost precludes its use.

The corresponding hydrochloride (I.C.I.), (maximum tolerated dose 1/200 g.) is without therapeutic action in a dose of 1/250 g.

[1] I am indebted to Imperial Chemical Industries, Manchester, for the supply of this drug and others marked I.C.I.

SECTION IV.

TREATMENT OF RELAPSES.

Strain II Relapses.

It has been noted that a marked therapeutic response occurred to sub-curative doses of No. 897, and that this was within wide limits independent of the amount of the drug and of the degree of infection as measured by the number of parasites in the blood at the time of treatment (see Tables IV and VI). Accordingly, it was decided to treat all relapses of Strain II infections with a single dose of 1/15,000 g. This amount cures 38 per cent. of the infections at acme, but few if any at an earlier stage (see Table IV). With this dose in animals which had relapsed for the first time, a very definite therapeutic effect was observed, the blood invariably becoming free of trypanosomes for a period, while in a small proportion of cases complete sterilisation resulted. Therefore, it appeared desirable to treat successive relapses, each with a single dose of 1/15,000 g., irrespective of the initial dosage. Almost all the relapses were treated immediately the trypanosomes became abundant, but occasionally the drug was administered at an earlier stage of the relapse. A feature of the experiment was that most of the mice kept in good condition throughout; only occasionally was there a slight loss of hair from the back at the site of injection of the drug, but there was never any necrosis. Owing to the repeated tail-examinations, 3 mice (758B, 765B and 758A) had to be chloroformed after the 11th, 13th and 14th relapses respectively, because necrosis of the tail made further examinations impossible. These animals were exceptional, as the tails of all the others withstood the repeated handling, e.g. the tail blood of mouse 761A was examined 187 times in almost 13½ months.

Cure /

Cure frequently resulted from repeated treatments, and usually within 6 retreatments; but one animal (760A) was cured after the 21st and 2 (590A definitely and 593B probably) were cured after 20 relapses — 12, 9½ and 7 months respectively after the original treatment. In mouse 761A response to treatment of 29 relapses was obtained over a period of 1 year, the greatest number treated; but cure was not effected. Table VIII summarises the treatments of relapses and results. Of the 56 relapsed animals, some treated initially with doses greater than and some with doses less than 1/15,000 g., 23 were cured after a number of relapses varying from 1 to 21; of the rest 20 died intercurrently and the remaining 13 were chloroformed for various reasons. The stage of infection at the initial treatment or the dose of drug given at that time do not seem to influence the results of retreatments. It is interesting to note that in 2 instances the 1st relapse was cured by a smaller dose than failed initially, e.g. 586B and 589B, which received originally 1/5,000 g. and 1/10,000 g. respectively.

It is a striking observation that with Strain II the chance of cure under repeated treatment of relapses is practically the same as the chance of cure of the original infection, since with an initial dose of 1/15,000 g. or over at the acme stage 23 out of 60 (38 per cent.) were cured, while 14 (11 definitely and 3 most probably) out of 37 (38 per cent.) were cured after a number of retreatments. This is contrary to the widely accepted belief based on observations, with other species of trypanosomes, that infections are most readily cured by vigorous initial treatment, while the cure of relapses presents especial difficulties.

One /

One of the salient features of the treatment of relapses in Strain II with a dose of 1/15,000 g. is the regularity with which successive relapses occurred and the immediate response to the drug at each treatment. As has been shown previously (p. 14), almost all the first relapses occurred from the 8th to the 11th day after treatment; and subsequent relapses usually took place at regular intervals of from 10 to 16 days after the previous treatment, irrespective of whether or not cure ultimately resulted; these are considered normal relapses. The interval between relapses may be conveniently reckoned as the number of days between successive treatments. Mouse 590A after initial treatment with 1/15,000 g. No. 897 before the acme stage, was cured after treatment of the 20th relapse; the intervals between relapses were as follows:- 9, 12, 11, 19, 16, 14, 17, 14, 14, 16, 12, 16, 15, 13, 15, 13, 12, 14, 11, and 14 days.

A longer interval between relapses was observed in 13 of the mice; details are given in Table IX. Mouse 700A is noteworthy in that 6 of the 12 relapses were late. With mouse 761B the first relapse occurred 50 days after the initial treatment; it was retreated 6 days later, although the numbers of trypanosomes in the blood had not increased beyond +. Details of the examination of the blood have already been given (Table V). Four further normal relapses occurred; and as no parasites were detected in examinations of the blood in the 6 weeks following treatment of the 5th relapse, the mouse was presumed to be cured. But the 6th relapse was noted in the course of routine monthly examinations; accordingly, this relapse must have occurred more than 89 days after the previous retreatment. In 3 mice (683A, 738A and 613B) trypanosomes appeared in small numbers in the blood at the 5th, 10th and /

and 15th relapses respectively, and then disappeared spontaneously several times without becoming abundant. This very protracted type of relapse was considered exceptional and no attempts were made to treat it.

Repeated treatment with sub-curative doses of drug is one method of producing acquired drug-resistance (see section V); thus, in these experiments evidence of this condition might have been expected. Only in 2 mice out of the 47 which received 3 or more retreatments were there any indications of a slight resistance to the drug, in so far as relapses failed to respond to further treatment. The details are as follows:- mouse 761A after 29 normal relapses, did not respond to the 31st treatment — 3 more doses were then given in the course of the next few days, but without result. Further work on the behaviour of the parasites from this mouse is given later (see section V and Table XV). Also, resistance to the drug appeared in the case of mouse 685A; after normal responses to retreatments the infection failed to respond to the treatment of the 14th relapse.

Treatment of Relapses with Doses Other Than 1/15,000 g.

Failure to cure infections with Strain II at the acme stage by doses of the drug greater than 1/10,000 g. are unusual (see Table IV). Two such exceptional relapses in 745A and 795A, following 1/3,000 g. and 1/5,000 g. respectively, were both cured after 3 relapses which had been treated by administering on each occasion the same doses as initially when parasites were abundant in the blood. In spite of the relatively large doses, the relapsing infections in these two mice ran a course very similar to those in the animals described in the previous section, as the intervals between the relapses were 13, 18, and 31 days in the former and 13, 23 and 14 in the latter. Another mouse (784A) was /

was treated at the acme stage originally and for the 1st 9 relapses with a dose of 1/100,000 g. throughout, then for the 10th to 13th relapses with 1/50,000 g.; and was finally cured after treating the 14th relapse with 1/3,000 g.

Therefore, as far as Strain II is concerned, there is no reason to despair of curing an infected animal when the initial dose has failed; success may result after a number of retreatments. These experiments are similar to those carried out by Browning and Gulbransen (1928) with T. brucei in mice, in which sterilisation was obtained after varying numbers of retreatments, with a dose of a styryl compound which had failed to cure the original infection and earlier relapses.

Strain I Relapses.

Similar treatment of relapses in Strain I infections was carried out. Doses of 1/75,000 g. to 1/100,000 g. for this strain were considered to correspond in therapeutic efficacy with a dose of 1/15,000 g. for Strain II (Table IV). Accordingly, relapsed mice which had been treated at the acme stage, one with 1/75,000 g. and 5 with 1/100,000 g., were treated immediately the parasites became abundant, the same dose of No. 897 being used as that given initially; in each case there was a definite therapeutic effect. Similar treatment was given for the 2nd relapse; only in 4 of the 6 animals was there a definite response, 1 being cured; of the remaining 2 animals one did not respond to the drug and the other only very slightly. Table X gives details. Where only a slight response to the drug, or none, occurred, the next treatment was given within a week.

Definite /

Definite indications of resistance to treatment were noted in 4 mice, from 3 weeks to 2 months after the initial treatment — in 2 animals after the 3rd treatment and in the others after the 4th and 6th treatments respectively; these are underlined in the table. The dose of the drug for each treatment in mice 1561A and 1561B was increased to 1/3,000 g. at the 6th and 8th treatments respectively; in the former the 6th to the 11th treatments only produced a slight response, but in the latter considerable action was observed following the 8th to the 11th treatments; however, there was no response to the 12th treatment in either animal or to subsequent treatments in 1561A (owing to its condition 1561B could not be treated further). Infections derived from these 2 mice were treated in fresh animals; Table XI gives details. The blood used for infecting the fresh animals was withdrawn immediately before the infection was treated in the parent mouse, consequently all the drug had been eliminated. The parasites showed more response to the drug in fresh hosts than in the original, but in most the therapeutic action disappeared after a few retreatments. Occasionally, after only slight responses to several consecutive treatments, subsequent treatments produced a definite response (e.g. mouse 1623B). It might appear from the above observations that in this strain drug-resistance to No. 897 could be readily developed; this, however, proved to be extremely difficult (section V).

The experiments on repeated treatment of relapses in Strain I infections were discontinued, as the results recorded above were not encouraging <u>qua</u> ultimate curability. Thus 4 out of the 6 animals failed to respond after a few retreatments, while this only occurred in 2 out /

out of 56 animals with Strain II, the earlier being after 14 relapses.

Thus it appears that once a degree of resistance to treatment with the drug has been manifested, no greater response will result from further repeated treatment even in doses approximating to the maximum tolerated. Moreover, on passage of the trypanosomes to a fresh host this resistance is maintained on about the same level; but it is not increased by further treatments and passages; consequently a typical drug-resistant strain has not been produced. These findings are most probably due to the chronic character of the infection, which is similar to a condition encountered in field work in the treatment of relapses in cattle infected with T. congolense (section VII).

Discussion

There appears to be no simple explanation of the above observations on the treatment of relapses, since variable factors on the part both of the host and the trypanosomes may be involved; such are (1) the power of the host to produce antibody, (2) the ability of the parasites to adapt themselves to the serum antibodies, (3) the curability of relapse (chronic) strains and (4) the development of drug-resistant strains. It is remarkable that relapsed infections with Strain II should respond better to treatment than those with Strain I in view of 2 facts, viz. (a) Strain I trypanosomes are more readily influenced by the drug than those of Strain II both at acme and at the chronic (relapse) stage and (b) the immunity following cure of Strain I infections is much more "solid" than is the case for Strain II. As is shown later (section VI), following the cure of a Strain I infection at the acme stage the host invariably resists further inoculation with the homologous organisms at the same stage, presumably

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presumably owing to the development of antibodies, while only about 50 per cent. of the cured mice originally infected with Strain II manifest immunity to the homologous strain. On the other hand, since the relapse trypanosomes of Strain I develop in the presence of blood and body fluids which have most probably a relatively effective antibody content, it appears likely that the parasites of each relapse may be less readily influenced by the drug than those of a previous relapse owing to the parasites being "serum-fast", i.e. resistant to the antibodies, whereas the relapse parasites in the case of treated Strain II infections develop in a serum which does not contain such effective antibodies, thus each relapse is influenced by further treatment to about the same degree as the previous one.

In the following section it will be shown that in spite of resistance to retreatment of relapses noted in the case of certain animals with Strain II and commonly in the case of Strain I, difficulty was experienced with both strains in attempts to produce drug-resistant organisms.

SECTION V.

ATTEMPTS TO DEVELOP STRAINS OF T. CONGOLENSE RESISTANT TO DRUG NO. 897.

Attempts to Develop Strains of T. Congolense Resistant to Drug No. 897.

Experimental investigations on the development of drug-resistant strains of T. congolense have not hitherto been possible, as no efficient drug was available to sterilise infections with this species of trypanosome. However, in the course of examining the biological properties of compound No. 897 attempts have been made to develop strains in mice of T. congolense resistant to the drug.

Since Franke and Roehl (see Ehrlich 1907) first recorded their observations on acquired drug-resistance, strains of T. brucei and T. rhodesiense resistant to almost every type of trypanocidal agent have been described. The original method was to treat infected mice by feeding with the drug (parafuchsin) in doses which banished the parasites from the blood for a time. The trypanosomes of the relapse were treated similarly; this procedure was repeated until no therapeutic response resulted from feeding with the drug. The trypanosomes were then transferred to a fresh host and if the drug exerted a therapeutic effect in the new mouse the treatments were continued. In this way after treatment in successive passages a drug-resistant strain resulted, which was also a relapse strain as regards its serological and immunological properties. The same result might be got by administration of the drug subcutaneously. Resistant strains serologically identical with the parent strains are obtained by treating inoculated mice at a time when the infection is developing and the trypanosomes are scanty in the blood, with a dose of the drug which does not affect the course of the infection; the parasites are then transferred at the acme stage to a fresh host. In successive passages the dose of the drug which /

which fails to produce a therapeutic effect, is gradually increased until maximum resistance is obtained. Yorke et al. (1931) have described another method of obtaining a resistant strain, which they claim gives rapid results. The trypanosomes after exposure in vitro to the active drug, e.g. reduced tryparsamide, are washed and injected into mice; when the infection develops this procedure is repeated in a series of passages with increasing concentrations of the drug, until maximum resistance is obtained.

Strain I.

The first attempt to develop resistance to No. 897 followed the method of treating relapses. Evidence of slightly increased resistance appeared in a mouse (No. 1528A) infected with Strain I, which relapsed after treatment with 1/75,000 g. given at acme. The same dose was repeated when the trypanosomes of the 1st and 2nd relapses became abundant. The 3rd treatment had no therapeutic effect, neither had a 4th and a 5th. After the 5th treatment the trypanosomes were transferred to fresh mice which were treated similarly. Details of the treatments and passages are given in Table XII; all initial treatments were given at the acme stage, and the repeated doses when the parasites were abundant in the blood. Fresh animals were inoculated when the parasites were abundant in spite of such treatment. During a period of 6 months the dose of the drug was increased from 1/75,000 g. to 1/10,000 g. in 20 treatments over 12 passages. In passage 13 however, the strain was lost, as one mouse died intercurrently and the infection failed to develop in the second. It does not appear that any great degree of resistance was acquired, especially in view of the fact that /

that in the course of the treatments a relapse (chronic) strain was developed, which according to my observations (p. 16) would not be markedly influenced in vivo by a dose of 1/10,000 g. (Table VII).

Another unsuccessful attempt was made to develop a strain of T. congolense I resistant to No. 897, this time with the serological and immunological properties of the parent strain, by treating the infected mice as soon as the parasites were present in the blood in scanty numbers, with doses which did not influence the course of the infection. Then when the acme stage was reached, the infection was transferred to fresh hosts. This procedure was followed in each passage; the amount of drug was gradually increased in successive passages, care being taken to adjust the dosage so that no diminution in the numbers of trypanosomes resulted in the treated animals, as compared with the numbers in the untreated controls; e.g. in passage 3, a dose of 1/150,000 g. led only to a very slow increase in the numbers of parasites, while in a second animal of the same passage after a dose of 1/200,000 g. the trypanosomes increased to the acme as rapidly as in the untreated control; therefore the passage was made from the second animal. In 16 passages over a period of 4 months the drug was increased from 1/200,000 g. to 1/50,000 g., see Table XIII. Unfortunately, none of the 3 mice of passage 17 became infected; however, the result so far as it went was disappointing qua the development of drug-resistance.

Strain II.

The method of gradually increasing doses in each passage was also followed with Strain II, the doses used being those which just failed to slow down the course of the infection. Over a period of 8 months the

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the strain was passed 34 times and the dose of the drug increased from 1/750,000 g. to 1/60,000 g., see Table XIV. At the 31st passage the strain was tested in parallel with the original strain against a dose of No. 897 which has a distinct therapeutic effect on the parent strain; two mice infected with the supposedly resistant strain and two infected with the parent strain each received at acme 1/100,000 g. No. 897. A therapeutic effect was observed, the blood from all four being free of trypanosomes 3 days after treatment. No significant difference in the sensitiveness of the two strains was detected, as relapses occurred on the 6th and 8th days after treatment in the mice infected with the supposedly resistant strain, and both on the 8th day in those infected with the parent strain. In passage 34 one of the mice did not become infected; the second showed parasites in the blood after an exceptionally long incubation period and treatment then produced a definite response. No further passages were made, as a relapse strain most probably had been developed.

In view of the failure described above, it was decided to continue the treatment of the exceptional infection in mouse 761A, which appeared resistant to the drug after the 31st treatment (p. 28). Three further treatments with 1/15,000 g. were given at intervals of a few days, but again without therapeutic effect. Fresh animals were infected with the parasites from this mouse and the strain was maintained in further passages, each of which was treated (see Table XV for details). Two of the fresh mice, 836D and 837E, infected from 761A after the 33rd and 34th treatments respectively were each treated with several doses of 1/15,000 g. of No. 897 with little or no effect. In all, 11 mice were inoculated in successive passages, but one failed to become infected.

This /

This somewhat intractable strain is, in general, more readily influenced by the drug in a fresh host than in the original mouse, e.g. a fresh mouse 851D was infected from 851C 4 days after its 5th treatment. A slight therapeutic effect was observed in this fresh mouse to treatment with 1/3,000 g. at the acme stage, while in mouse 851C the 4th, 5th and 6th treatments with 1/3,000 g. did not influence the infection. This observation is, of course, paralleled by the results of treatment of a fresh infection from chronic (relapse) parasites, as compared with the treatment of the infection in the original mouse, the former being more readily cured than the latter (see Table VII). Table XV shows that none of the 6 mice repeatedly treated with the relatively large dose of 1/3,000 g., or the 2 repeatedly treated with 1/15,000 g. was cured. Actually, with the exception of 1 animal (852D), little or no response resulted from the majority of treatments, which were given at intervals of approximately 7 days. The variation in individual animals is demonstrated by the retreatments of mice Nos. 852C and 852D infected with the same inoculum. In the former all 5 treatments had no effect; in the latter there was a slight response to the first 4 treatments and the 5th to 8th treatments produced a definite therapeutic effect, but the 9th and 10th treatments did not reduce the number of parasites present in the blood.

From early passages of this strain, 836D, 852A and 852C, it might be concluded that drug-fast parasites had been produced. However, therapeutic responses were observed in subsequent passages. In the series of passages from 761A, which was resistant to treatment with No. 897, ending in 863B which failed to become infected, the parasites

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parasites were passed through 7 mice whose treatments totalled 39 with 1/15,000 g. and 26 with 1/3,000 g., and yet true drug-resistance was not produced; this confirms the difficulty of developing an 897-fast strain of T. congolense. The series of passages is indicated by the arrows in Table XV.

An unsuccessful attempt was made to produce resistant parasites by subjecting the trypanosomes of Strain II to the drug in saline solution for 15 minutes and then infecting animals with the treated trypanosomes. This was carried out by adding 0.5 c.c. of the normal inoculum to an equal volume of 1/250,000 dilution of the drug in normal saline, another 0.5 c.c. of the suspension of trypanosomes to 1/400,000 g. of the drug and a 3rd to 0.5 c.c. of saline alone. Thus the parasites were actually in contact with 1/500,000 and 1/800,000 dilutions of the drug respectively. After 15 minutes motile trypanosomes were observed in each solution. Mice which were infected with the treated trypanosomes had longer incubation periods (10 days) than the untreated control. When the infection in the mouse which received the trypanosomes treated with the weaker solution of the drug was at the acme stage, inocula were prepared from its blood. These were treated with the same doses of the drug as above. In this, the 2nd, passage through mice one animal failed to become infected and in the other the infection had an incubation period of 26 days, while that of the untreated control was 6 days. Further passages were not made, as a relapse strain had probably developed.

Summary /

Summary. These experiments show that with T. congolense drug-resistance, which is frequently assumed to be the cause of failures to cure in the field (see section VII), is not so easily developed in the laboratory by means of No. 897 as in the case of many drugs with T. brucei. In this connection it must be remembered, however, that it is difficult to obtain drug-resistant strains in the usual way with Bayer-205 (suramin). There are also indications that with T. congolense in mice loss of virulence of the trypanosomes results from attempts to develop resistance by the usual methods.

In 1936 du Toit reported 2 attempts to develop resistance to antimosan in guinea-pigs infected with T. congolense; firstly by treating with non-sterilising doses of the drug and infecting a fresh host with the parasites of the relapse; and secondly by treatment of successive relapses in the original animal and only infecting a fresh host when in danger of losing an animal. No indications of resistance were observed from either method, the former being carried over 32 generations and the latter for 54 treatments in 10 animals.

SECTION VI.

IMMUNITY RESPONSES AFTER CURE.

IMMUNITY RESPONSES AFTER CURE.

In general, trypanosomes are antigens which stimulate the production of antibodies in the serum of the host. But without the co-operation of a chemotherapeutic agent the natural defences of most animals are incapable of disposing effectively of the pathogenic species. However, spontaneous recovery from T. congolense infection occurs in sheep and goats (Laveran et al., 1902; Laveran, 1911) and young calves (van Saceghem, 1938), conferring on them acquired immunity to natural infection and, in the case of the calves, to experimental reinoculation.

The question of the immunity phenomena following cure of T. congolense infections in mice with drug No. 897 has been thoroughly investigated. There are no published data on this subject, since hitherto a curative agent was not available.

Results with other species of trypanosomes have shown that different strains may vary in their immunising power. With some complete resistance to reinoculation with the homologous strain may be produced, while with other strains there is merely prolongation of the incubation period. Further, such immunity is most pronounced when the reinoculation is made with the same strain, whereas there may be little or no immunity toward other strains. In this connection also, it must be borne in mind that when a relapse occurs the strain as a rule undergoes a change in its immunological characters; thus a multiplicity of serological races may develop in repeated relapses. This work is summarised by Browning, (1931) and Culbertson, (1941). The only certain means for preserving the immunological characters of a strain of trypanosomes is to pass it continuously at the height of infection (acme) by inoculating fresh /

fresh animals of the same highly susceptible species, in most cases mice or rats. In an animal such as the rabbit in which untreated trypanosome infections, e.g. with T. brucei, usually pursue a relapsing course, a succession of serological strains tends to develop. It is of interest, however, that the "solid" immunity to reinfection which is acquired after cure of such a relapsing infection, while protecting against the same strain which has been passed continuously through mice at acme, does not protect against another strain which has been passed similarly (Browning, Cosgrave and Leckie, 1939).

The present work has been carried out in the light of the knowledge summarised above and certain novel observations have been made.

Methods of Demonstrating Immunity.

The method used to demonstrate active immunity is that of Ehrlich and Shiga, (1904); the infected mouse after an interval of a week or longer following treatment with a curative dose of the drug, was reinoculated with trypanosomes. The interval must, of course, be sufficient for all the drug to be excreted. The blood was then examined for trypanosomes as in a therapeutic experiment. If no parasites appeared within a month, reinfection was concluded not to have occurred. Two apparent exceptions are discussed later.

Treatment after reinfection. Each cured animal in which reinoculation was successful in producing infection, was treated with 1/3,000 g. No. 897 immediately the parasites became abundant in the blood. Before a further reinoculation was carried out, at least one month was allowed to elapse, during which time the blood was examined every 2nd

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2nd day in order to establish cure.

Other tests confirming that sterilisation had been effected. The possibility had to be considered that in spite of frequent negative blood examinations, the parasites were persisting in very scanty numbers either owing to the original infection (or a reinfection) not being cured or to persistence of the trypanosomes introduced at the reinoculation. To confirm that sterilisation had occurred 2 further methods were used (a) the inoculation of an animal with the whole blood of the mouse which is presumed to be free from trypanosomes and (b) observation of the size of the spleens of the experimental animals.

(a) 12 mice presumably free from trypanosomes according to the results of blood examinations, were selected at random; these had mostly been repeatedly reinoculated. They were chloroformed and one fresh mouse was injected subcutaneously with the total volume of blood obtained from each heart ($\frac{1}{2}$ to $\frac{3}{4}$ c.c.). Their blood was examined every 2nd day for one month, and in the case of 10, the examinations were continued at longer intervals for a period up to 4 months. None of these animals became infected. Two were reinoculated with the normal inoculum of acme parasites at the end of one month; in each the organisms appeared as in the untreated control, showing that these mice were susceptible to infection and so had not acquired immunity owing to a previous latent infection with trypanosome-containing blood. The animal inoculation test is extremely sensitive, although exceptionally failures may result owing to the virulence of the parasites being highly attenuated (Browning and Gulbransen, 1935).

(b) The spleens of animals which have been infected with T. congolense for weeks or months always show considerable enlargement

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enlargement (Browning, Cappell and Gulbransen, 1934). Accordingly, the spleens of 14 supposedly cured mice were examined, in most cases after at least one successful reinoculation which had been treated; 12 were normal in size, while the remaining 2 were only slightly enlarged.

From the above observations it may be deduced that in the circumstances the microscopic examination of the blood was adequate for the purpose of detecting trypanosomes and that the quantity of drug administered effected cure of the reinfections.

Application of the Rieckenberg adhesion phenomenon. Attempts to apply the adhesion phenomenon of Rieckenberg, (1917) with a view to detecting immunological differences or identities among the various strains proved a failure. The method of the test is to mix on a slide a drop each of (a) blood from a cured mouse, containing the antibody, (b) citrate broth and (c) blood containing the trypanosomes, i.e. the antigen. A positive reaction, shown by coating of the trypanosomes with blood platelets, proves that the antigen and antibody are homologous. A number of workers have confirmed the value of this reaction; thus Leupold, (1928) working with T. brucei claimed agreement between reinfection experiments and the adhesion reaction. Duke and Wallace, (1930), however, were unable to distinguish between passage and relapse strains of T. rhodesiense by their red cell adhesion test which is a modification of the platelet reaction.

STRAIN I.

Cured Acme Infections Reinoculated with Acme Parasites.

The results with Strain I infections cured at acme and reinoculated with the homologous trypanosomes at the same stage (acme), usually about 1 month after the administration of the drug, give convincing evidence of a solid immunity which lasts for at least 13 months — the longest interval tested. 7 animals — 1559B, 1560A, 1562A, 1570A, 1581B, 1588B and 1591A — were immune to reinoculation from 11 months to 13 months after initial cure. The size of the inoculum does not affect the result, as 4 animals — 1581B, 1588B, 1591A and 1633B — manifested complete resistance when each was reinoculated with the whole heart blood of a heavily infected mouse. Two mice — 1529A and 1529B — cured at the acme stage with No. 897, resisted 20 reinoculations with acme trypanosomes at approximately fortnightly intervals. 4 and 6 weeks respectively after the 20th reinoculation, the mice were chloroformed and the heart blood transferred to fresh animals, which did not become infected; this is strong evidence that no living trypanosomes had been harboured (p. 42). The spleens of 1529A and 1529B were normal in size, whereas chronically infected animals show marked splenic enlargement (p.42).

The actual dose of No. 897 administered, which varied from 1/3,000 g. to 1/100,000 g. does not affect the immunity response provided cure is effected. The chemical nature of the curative drug does not appear to influence the result of reinoculation with the homologous organisms. In 2 instances the drug was quindoline methochloride (I.C.I.), in 2 a substituted quindoline methochloride (I.C.I.) and in 28 others phenanthridinium derivatives related to No. 897 (Nos. 621, 893, 894, 1060, 1504, 1505, 1506, 1507, 1508, 1542, 1551, 1565, 1566, 1568, 1569, and /

and 1573). In all of the 32 mice there was complete resistance to reinoculation with the homologous parasites.

A total of 55 cured mice reinoculated with the same strain of acme parasites from 1 week to 13 months after the drug had been administered all resisted reinoculation.

Cured Acme Infections Reinoculated with Chronic Parasites.

On the other hand, there is practically no immunity when animals cured at acme are reinoculated with blood containing parasites derived from a mouse in which the infection has become chronic, i.e. with "chronic" parasites. Of 55 mice cured at the acme stage, not one resisted reinoculation with a random chronic inoculum. Also, 11 mice cured at acme which had resisted reinoculation with acme parasites, were reinoculated with random chronic parasites; 10 became infected and only one proved refractory. In extension of this line of investigation it was found that a mouse inoculated with the acme strain and cured at acme, although immunised to trypanosomes derived from any animal at acme, promptly became infected when reinoculated 2 to 4 weeks later from the same mouse, whose infection by this time had become chronic — this was noted with 6 animals (1415B, 1425B, 1426B, 1432B, 1503A and 1544A).

Cured Chronic Infections Reinoculated with Acme Parasites.

When the mice had been infected originally with a chronic strain and cured, they then resisted reinoculation with acme parasites in the same way as those cured of acme infection. Out of 31 animals there was only one exception (mouse 1495A, discussed later). It is noteworthy that the immunity to acme parasites should be so pronounced after the cure of a chronic infection; 2 mice (1552A and 1552B)

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1552B) originally infected with chronic parasites and then cured with No. 897, each resisted 13 reinoculations with acme parasites at approximately fortnightly intervals. 4 and 6 weeks respectively after the 13th reinoculation the animals were chloroformed and the whole heart blood injected into fresh mice, which did not become infected. The spleen of 1552B was slightly enlarged, while that of the other was normal. Also, as in the case of cured acme infections, the resistance to acme parasites following cure of a chronic infection appears to persist for a long time; thus 2 mice (1600C and 1639A) resisted reinfection with acme parasites 12½ months after the administration of the drug.

Cured Chronic Infections Reinoculated with Chronic Parasites.

When the original infection and the infective blood used for reinoculation were both derived from chronically infected mice, there was very little evidence of immunity to the reinoculation; only 1 out of 15 mice of this class resisted reinoculation with chronic trypanosomes.

Thus it appears (Table XVI) that following the cure of either acme or chronic Strain I infections, the animals are almost without exception resistant to reinoculation with acme trypanosomes, but usually become infected after reinoculation with chronic parasites. Similar responses are noted to the 2nd and subsequent reinoculations. Successful reinoculations with chronic trypanosomes were, of course, treated in the manner described (p.41); but occasionally this treatment failed to effect cure. In all, the results of 229 reinoculations with acme parasites were observed on 118 cured mice, of which only 1 produced infection (a possible explanation in this case is given /

given later). Whereas 210 out of a total of 231 reinoculations with chronic parasites tested on 103 cured mice, were successful in producing infection. By way of illustration representative details of the reinoculations of 4 mice are given in Table XVII.

Isolated Instances of Immunity to Reinoculation with Chronic Trypanosomes.

Of the 103 mice (mentioned in the preceding paragraph) which received random reinoculations with chronic trypanosomes, 4 proved genuinely immune on one occasion and another on 2 occasions (the resistance of the other refractory animals might be explicable on other grounds (see below)). These immune mice were reinoculated at different times. 2 (1480B and 1500A) had been infected originally with chronic parasites and after cure had been reinfected once and twice respectively with random chronic trypanosomes before proving immune to a further chronic reinoculation. The others (1516A and 1525B) were infected originally with acme trypanosomes and after reinfection with chronic parasites on 1 and 2 occasions respectively completely resisted the next chronic reinoculum. 2 of those 4 died intercurrently, and the other 2 were subsequently reinfected with chronic parasites. The fifth mouse (1534A) after cure of the original infection with chronic parasites and 2 reinfections with chronic trypanosomes, was immune to a 3rd and 4th random reinoculation with chronic trypanosomes, but became reinfected by the 5th.

It is possible that in all these cured mice the antibodies corresponded with the receptors of the chronic trypanosomes of the inoculum to which they proved resistant. On the other hand, some of these mice may have had an increased natural resistance to the chronic parasites, which would account for their withstanding the reinoculation.

Increased Susceptibility to Reinoculation with Chronic Trypanosomes Shown by Cured Mice.

In experiments on the reinoculation of cured animals with inocula derived from chronically infected mice, an interesting observation on the duration of the incubation period has been made.

The incubation periods for infections of normal mice with an inoculum of chronic parasites are longer than the corresponding periods with acme infections (see p. 6) — usually 9 to 13 days in the former case. If, however, the same chronic inoculum is used to infect fresh /

fresh mice and also to reinoculate cured mice — irrespective of whether the original infection was acme or chronic — the incubation periods of the reinfections, contrary to what would be expected, are usually a few days shorter than those of the untreated animals (see infections from mice 1524A, 1549B and 1619A (series 1, 2 and 3 Table XVIII)). Thus in the case of 80 cured mice which received 150 reinoculations with chronic parasites, the incubation periods were shorter on 97 occasions than the incubation periods of the corresponding control animals, while on 36 occasions they were longer — it should be added that the latter figure includes 12 reinoculations which failed to produce infection. The incubation periods of the remaining 17 reinfections were of the same duration as those of the controls. These results are statistically highly significant.

This result was unexpected, since some degree of immunity to such a reinoculation following the cure of a chronic infection, manifested possibly by a few days' protraction of the incubation period, was anticipated, similar to that observed with certain strains of T. brucei (Browning, 1927).

Results of reinoculation of (a) mice infected with the acme strain and cured after a relapse following insufficient treatment at acme; (b) animals inoculated from such a relapse then cured; and (c) mice in which the infection had become chronic before curative treatment.

The study of the immunological properties of Strain I infections was completed by the following 3 series. (a) The relapses in 4 mice (1525B, 1527A, 1527B and 1528B) following treatment with 1/75,000 g. were cured with 1/3,000 g. No. 897. (b) 4 mice (1577A, 1578A, 1579B and 1580B) were inoculated from animals which had relapsed after treatment with 1/100,000 g.; the infection which developed was then cured with 1/3,000 g.

1/3,000 g. No. 897. (c) 7 mice (1625A, 1625B, 1627B, 1628A, 1637A, 1638A and 1638B) in which the infection had become chronic, i.e. 3 to 6 weeks after the acme were cured with a dose of 1/5,000 g. or 1/10,000 g. No. 897. All these 15 cured mice behaved on reinoculation with acme or random chronic parasites in the same way as those cured of infections with acme or chronic parasites, i.e. they resisted the former and became infected with the latter.

Anomalous Responses to Reinoculation shown by Mice Cured of an Infection with Chronic Parasites.

Of the 176 mice reinoculated with Strain I trypanosomes derived from various stages, 2 animals (1495A and 1532A) are exceptional (Table XIX). In all the others the reaction to the reinoculation was absolutely definite, either immunity or prompt reinfection.

Mouse 1495A infected originally with chronic parasites and treated with 1/3,000 g. No. 897, was reinoculated 63 days later with acme trypanosomes; 22 days afterwards scanty parasites were observed in the blood for the first time; they increased to ++ on the 23rd day and diminished slightly on the 24th day, but disappeared on the 25th day. On the 34th day scanty parasites were again present in the blood; they now increased normally to the abundant stage, at which the animal was treated with 1/3,000 g. No. 897. Three further reinoculations with acme parasites were completely resisted. Here, the possibility must be considered that the original infection was not cured, but that an extremely late relapse occurred. On the other hand, it cannot be excluded that the long incubation period followed by the spontaneous disappearance of the trypanosomes indicated a high degree of immunity to the acme parasites, just short of complete refractoriness.

Mouse 1532A, originally infected with chronic parasites, received a dose of 1/3,000 g. No. 897; 17 days later it was reinoculated with chronic parasites; and as trypanosomes appeared in the blood after 9 days, the same dose of the drug was injected. 31 days later a 2nd reinoculation with chronic parasites was carried out; as no trypanosomes were detected in the blood (examined every 2nd day), an inoculum of acme parasites was injected 32 days after the previous reinoculation. Scanty trypanosomes were observed in the blood 9 days later; although fluctuation in numbers occurred over the next 3 weeks, they never became abundant. Here it seems most probable that the 2nd reinoculation with a chronic inoculum had led to infection after an exceptionally long incubation period (41 days).

Resistance /

Resistance of Normal and Cured Mice to an Inoculum of Chronic Trypanosomes.

It has been seen that almost 28 per cent. of fresh mice inoculated with chronic parasites, fail to become infected (p. 16); thus it may be inferred that the trypanosomes at that stage of the infection are not so virulent as at the acme. Nevertheless, it appears that fewer cured mice resist infection with a chronic inoculum than do fresh animals. Thus of 231 chronic reinoculations tested on cured mice, only 21 (9 per cent.) were resisted. These results are statistically highly significant. Further, out of 55 inocula of chronic trypanosomes used to reinoculate groups of from 2 to 6 mice, 4 failed to produce infection in the control animals, while reinfection occurred in all the cured mice of these sets. In a large number of the remaining sets of mice, the incubation periods of the reinoculated mice were shorter by a few days than those in the corresponding control animals (p.48). It is not determined whether this increased susceptibility is due to allergy or whether it results from a decrease in natural resistance caused by the effects of previous infection or of the drug.

Does Inoculation with Chronic Trypanosomes of Low Virulence which Fails to Cause Obvious Infection, Produce Immunity to Subsequent Reinoculation with Chronic Trypanosomes?

As mentioned, van Saceghem, (1938) claimed that immunity to both natural infection and experimental reinfection follows the spontaneous cure of a benign infection in young calves. It appears from the present work that mice cured either at acme or when chronically infected may occasionally acquire immunity to reinfection with a chronic strain as a result of a preceding inoculation with chronic trypanosomes of low virulence which fails to set up obvious infection. The evidence is as follows. Inocula derived from 4 chronic animals appeared to be of very low virulence, since none of the 4 cured mice inoculated from 2 of them (1514A and 1524D) became infected, while 6 out of 9 cured mice failed to become infected with inocula from the other 2 (1602C and 1602D) — series 4,5,8 and 9, Table XVIII. These 10 refractory mice received a further chronic inoculum, which 4 (1498A, 1515B, 1516B and 1567A) resisted in so far that number 1567A showed a considerable protraction of the incubation period, while number 1498A manifested parasites in the blood transiently 10 days after the reinoculation and the fastigium was not attained until 13 days later; both were cured by the drug. The other 2 mice never showed trypanosomes in their blood. However, on the following reinoculation with random chronic trypanosomes 3 of the 4 became reinfected promptly and the 4th after a protracted incubation period. Accordingly, any immunity following inoculation with chronic trypanosomes of diminished virulence, was of low degree and evanescent.

Do the Antigens of the Trypanosomes Alter Progressively in the Course of a Chronic Infection?

The fact has already been noted (p. 45) that a mouse cured at acme, although immune to reinoculation with trypanosomes from another animal at acme, did not resist infection from the latter after it had reached the chronic stage. The problem now remained whether the immunological change in the trypanosomes of a chronically infected animal was constant or progressive. Accordingly, the experiments detailed in Tables XX and XXI were carried out. Two groups of animals were studied:-

(a) in which an animal inoculated originally from a chronic infection received a curative dose of the drug and 11 to 34 days later was reinoculated from the same mouse. None of the 9 mice showed immunity to the reinoculation — details are given in Table XX.

(b) From the experiments described above and on p. 45, it appeared that a mouse cured either of the acme infection or a chronic infection would in all likelihood prove not to be immune on reinoculation with chronic trypanosomes from a random source, since the immunological characters of a strain underwent progressive change in the course of a chronic infection in each animal. To test this point further, mice originally infected with acme trypanosomes and cured, were in the course of a series of reinoculations reinfected with random chronic parasites and again cured, reinfection and cure being effected several times in some of the animals. Finally after cure of a reinfection with chronic trypanosomes, each mouse was reinoculated after an interval of 10 to 17 days — once after 57 days — from the same chronic mouse as on the immediately preceding occasion. The result was that in 6 out of 8 animals there was no immunity, whereas in the other 2 (1432A and /

and 1540B) the repeated inoculation did not lead to infection (see Table XXI). It might be argued that in contrast with what is found after cure of an acme infection, the immunity following cure of infection derived from a chronic inoculum was of short duration, as is usually the case with T. brucei infections (Browning and Gulbransen, 1936). Accordingly, the reinoculations in these experiments were chiefly carried out 10 to 20 days after the administration of the drug. The results, however, do not afford evidence that there is an immunity response which reaches a height shortly after cure and then rapidly falls off.

Fixation of the Immunological Characters of a Chronic Infection by Passage.

It has been seen that when a mouse infected with T. congolense Strain I survives the acme of infection the trypanosomes undergo changes in their immunological characters; it may be said that they are chronic strains. Further, these changes appear to be progressive. This progressive alteration can be prevented by fastigial propagation of a chronic strain, i.e. by inoculating fresh mice with the chronic trypanosomes and passing the infection to further mice as soon as parasites become abundant in the blood, — referred to as a fixed chronic strain. In the course of repeated passages the immunological character of the trypanosomes tends to revert to the acute acme type; but this does not occur until after at least one passage and frequently requires a considerable number of passages.

Accordingly, one can obtain for a time a strain with fixed chronic immunological characters apparently identical with those in the original chronic mouse. The evidence for this is as follows. A series of /

of mice are inoculated from a chronically infected animal; several of these are used for propagating the strain fastigially, and the rest are cured by treatment with 1/3,000 g. No. 897 at the fastigium. These cured animals now resist reinoculation with the homologous fixed chronic strain. On the other hand, neither mice infected from another chronic animal and cured (p. 46), nor cured acme mice manifest immunity to this fixed chronic strain (Table XXII). Further details concerning the effects of continued passages on chronic trypanosomes are given in the next paragraph.

The Immunological Characters of Chronic Trypanosomes as Affected by Continued Passage.

As mentioned above, the immunological characters have been investigated in the case of several strains of chronic trypanosomes which have been fastigially propagated — referred to hereafter as passaged chronic trypanosomes. (It has been shown already that the immunological characters of such a strain remain fixed for one passage at least). These passaged chronic trypanosomes were used in successive passages to reinoculate mice of the original acme strain which had been cured at acme. If infection occurred it could be inferred that the chronic characters had persisted; if, on the other hand, immunity was manifested it appeared that the trypanosomes had reverted to the characters of the acme strain. Nine separate chronic strains were passaged and tested in this way (see Table XXIII); it has been found that the acme characters may be regained after a variable number of passages, e.g. 3 to 9, while in some strains the chronic characters persisted until the 9th to the 12th passage, the latest tested. Where the acme characters had been regained, this was always confirmed by repeating the test for immunity with later passages.

On account of the tendency for mice not to become infected with chronic parasites, attempts to establish passages of several other chronic strains failed.

It is not clear from the results whether the interval (14 to 37 days) after the acme stage before passages were started, is significant in regard to the number of passages required to re-establish the acme characters. Actually, those strains derived from mice 14 to 24 days after the acme stage, preserved their chronic characters over a greater number of passages than those derived after a slightly longer interval, but this may be fortuitous.

In strains A and B the chronic character altered in the course of not more than 3 or 4 passages. This may also have been the case with strain D, which was tested for the first time in the 6th passage.

Summary and Conclusions.

It is well established that a remarkably solid immunity to reinoculation with acme trypanosomes develops in mice infected with T. congolense Strain I and cured with a chemotherapeutic agent either at acme or in the chronic stage. This immunity lasts for at least 13 months after cure. (No. 897 has been used chiefly as the therapeutic agent; other drugs have been used with a similar result, but only acme infections have been treated with the latter). This immunity is genuine and is not accounted for by prophylactic action of the drug, which only lasts for less than 4 days after the administration of a large dose — 1/1,000 g.

Under similar conditions, practically no immunity is evident to reinoculation with random strains of chronic trypanosomes, irrespective of the nature of the original infection of the host, whether acme or /

or chronic. This holds even when reinoculation is carried out a few days after treatment; and even if the mouse is reinoculated from the same animal as yielded the original infection. After cure of repeated reinfections with chronic trypanosomes further inoculations with the acme strain were also resisted, while similar reinoculations with chronic parasites almost always caused infection.

Exceptions in the sense of immunity to random strains of chronic trypanosomes, are very rare in spite of the large numbers of reinoculations carried out. The following possibilities to account for these exceptions may be considered. (a) The natural resistance of mice to reinoculation with chronic trypanosomes, which are generally reduced in virulence. Actually, animals cured of a chronic infection are more susceptible to reinfection with a random chronic strain than are normal mice. (b) Immunity may be conferred on mice by previous inoculation with chronic trypanosomes of low virulence which failed to produce obvious reinfection. Such immunity, however, has been shown to be transient. (c) Occasionally it may happen that the combination of receptors in the trypanosomes used for the reinoculation corresponds with those to which antibodies have been developed in the cured animal. This explanation is supported by the fact that the immunity was not effective against subsequent inoculation with other chronic trypanosomes.

The immunological character of the infection undergoes progressive change in each chronically infected animal. However, by transfer of a chronic infection to fresh mice and passage thereafter as soon as the number of parasites in the blood becomes abundant, i.e. at fastigium, the immunological characters of a strain may be preserved unaltered /

unaltered for one or several passages, i.e. a fixed chronic strain may be established. But repeated passages at fastigium tend eventually to produce trypanosomes with the immunological character of the acme strain. The number of passages required before reversion to the acme strain is very variable, e.g. within 3 to over 12.

Ehrlich's receptor theory and the above data. The findings may be satisfactorily explained on the basis of the receptor theory of Ehrlich. It is clear that the acme strain of T. congolense I possesses constant immunological characters — or antigenic receptors (A receptors) — since a mouse cured at acme has a lasting solid immunity to trypanosomes derived from any other mouse infected with the same acme strain. When, as is frequent, an untreated infected animal survives the acme stage or when an insufficiently treated acme infection relapses, the immunological characters of the trypanosomes alter. This is shown by the fact that on inoculation into mice cured of an acme infection they readily produce infection. So far the phenomena resemble those met with in relapse strains of T. brucei; but here the resemblance ceases, because a mouse cured of a chronic infection with T. congolense or of a relapse, is still insusceptible to reinfection with the acme strain.

Chronic strains are immunologically heterogeneous, because cure of a chronic infection does not as a rule confer immunity against random reinoculation with trypanosomes from other chronically infected mice; and even when a mouse cured of infection derived from a chronic animal is again reinoculated from the same animal infection follows, which shows that the trypanosomes have altered immunologically in the chronically infected mouse in the interval between the 2 inocula. On the other hand, there are the further facts — (1) the immunological

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immunological characters of a chronic strain may be temporarily fixed by fastigial passage and (2) on repeated fastigial passages there is a tendency to reversion to the acme type eventually.

Accordingly, when the infection becomes chronic additional receptors develop in response to the action on the trypanosomes exerted by the defence mechanisms of the host, but they do not replace the original A type. Thus the receptors of trypanosomes in a chronically infected animal may be regarded as becoming progressively A+B, A+B+C, and so on, which on cure give rise to antibodies with the characters anti-$\overline{A+B}$, anti-$\overline{A+B+C}$ etc. respectively. Thus, a mouse whose immunity depended on anti-$\overline{A+B}$ would resist reinoculation with trypanosomes possessing receptors A or A+B (or B), but not with those which contained additional receptors, e.g. C as in A+B+C, etc. The effect of continued fastigial passages is to prevent development and accumulation of additional receptors and eventually to lead to their disappearance.

STRAIN II

Cured Acme Infections Reinoculated with Acme Parasites.

In the case of the second strain of T. congolense (II), in contrast to Strain I, when mice cured at acme were reinoculated with the homologous trypanosomes taken from an infected mouse at acme, solid immunity was not manifested in 100 per cent. It appeared rather that the chances of immunity and reinfection are equal almost, (Table XXIV — result of first reinoculation), 22 mice out of 40 resisting reinoculation.

The amount of No. 897 administered to cure the initial infection varied between 1/3,000 g. and 1/15,000 g. Analysis of the responses to subsequent reinoculations showed that the dose of drug does not affect the result. Only one mouse was reinoculated which had been treated with a drug other than No. 897; viz. No. 715A, which was not immune to reinoculation with the acme strain 94 days after treatment with a substituted quindoline methochloride (I.C.I.).

The interval between the administration of the drug and the reinoculation did not affect the result, as can be seen from the following table, which shows that immunity to a 1st reinoculation may or may not be present soon after the treatment and also that either result may follow reinoculation about 8 months after cure.

No. of Mouse.	Interval in days between treatment of the original infection and the 1st reinoculation.	Result.
688B	9	Immune
689A	9	
615A	208	
603A	257	
689B	7	Not immune
688A	9	
597A	240	
591A	274	

Results of Repeated Reinoculations and Retreatments.

A dose of 1/3,000 g. No. 897 was given immediately the parasites of a reinfection became abundant in the blood and in most cases an interval was allowed of at least 1 month afterwards before a further inoculation; provided the blood examinations carried out every 2nd day during that time were uniformly negative, cure was considered to have been effected. This dose failed to cure 9 out of 27 reinfected mice, while with the original infection at acme there was 1 failure out of 13 similarly treated (Table IV). These figures are statistically insignificant.

The 2nd and subsequent reinoculations, irrespective of whether the 1st reinoculation was resisted or succeeded in producing infection, again led to infection in approximately 50 per cent. of the cases; but there seemed to be a tendency to a higher proportion of immunes as the number of reinoculations increased (Table XXV — mice 701A, 738B, 760B and 792A). However, mouse 754B was reinfected by the 6th reinoculation after displaying immunity to the 1st, 3rd, 4th and 5th;

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5th; and all 4 reinoculations took in mouse 726B. In all, out of 114 reinoculations 66 were unsuccessful in producing infection and 48 successful (Table XXIVa).

3 mice — 701A, 760B and 792A — which had resisted 4 to 6 consecutive reinoculations with acme parasites were reinoculated with approximately twice the usual number of organisms; 1 became infected, the 2nd was immune and probably also the 3rd. Number 701A was exceptionally resistant to reinoculation, as a 2nd double strength inoculum and also reinoculation with the whole heart blood of a heavily infected mouse at acme, failed to produce infection.

Incubation Period of Reinfected Mice Compared with That of Controls.

Of the 57 inocula which were used to reinoculate sets of from 2 to 5 cured mice, 26 failed completely to produce infection, while all the corresponding control animals — 1 and occasionally 2 for each set — became infected. In the remaining 31 sets of cured mice, some or all became reinfected; the incubation period, except on 3 occasions in which they corresponded, being on the average 4 days longer than that of the untreated controls. This is interpreted as indicating a slight degree of resistance to reinoculation with the homologous strain. Two mice (738B and 726B — Table XV), reinoculated at different times, were the only animals whose incubation periods were protracted beyond those of the controls by more than 7 days — 11 and 17 days till scanty parasites appeared.

Cured /

Cured Acme Infections Reinoculated with Chronic Trypanosomes.

As with Strain I, mice cured of an acme infection showed no immunity to reinoculation with random chronic trypanosomes — thus 9 mice which had proved immune to reinoculation with acme trypanosomes on 1 or more occasions all became infected promptly when reinoculated with chronic parasites of Strain II. A significant result.

Cured Chronic Infections Reinoculated with Acme Trypanosomes.

Only 6 animals cured of a chronic infection were tested for immunity responses owing to the difficulty of effecting cure in such cases. As with cured acme mice, half proved resistant to reinoculation with the acme parasites. All however, became infected after either the 2nd or the 3rd reinoculation (details are given in Table XXVI). All which became reinfected received a dose of 1/3,000 g. No. 897; this failed to cure 4 animals — 3 after the 1st reinfection and 1 after the 2nd — consequently no further reinoculations could be tested in these animals. A tendency to immunity after several reinoculations was observed in mouse 696A, which became reinfected after the 1st and 2nd reinoculations, but proved immune to the 3rd, 4th, 5th and 6th reinoculations (Table XXV).

Summary.

In mice cured of an acme Strain II infection a solid immunity to reinoculation with acme trypanosomes was evident only in about 50 per cent. of the animals, whereas such immunity occurred in 100 per cent. of the Strain I infections under the same circumstances. The responses were independent of the interval between dosage with the drug and the reinoculation, and independent of the amount of drug given. Animals which proved immune were repeatedly reinoculated; both in these and in /

in those which were cured after successful reinoculations, the subsequent reinoculations again each produced infection in about 50 per cent., whereas cured mice originally infected with acme Strain I parasites withstood up to 20 reinoculations with acme trypanosomes. Those reinfections which developed with Strain II showed a slight protraction in the incubation period.

Cured chronically infected Strain II mice, on reinoculation with acme parasites give the same responses as cured acme animals, but no immunity was observed in cured acme infected mice to reinoculation with chronic parasites.

Discussion.

The outstanding feature of the results is that Strain II is much inferior to Strain I in immunising power as tested by the behaviour of animals cured at acme and reinoculated with the homologous acme strain. The fact that about 50 per cent. of such cured animals become reinfected after a slightly protracted incubation period, hinders satisfactory observations along the lines followed with Strain I.

Even when such reinfected animals are cured and again reinoculated only about 50 per cent. resist and this is the case after several subsequent reinfections and cures. Eventually, however, an increase in the immunity appears to occur.

So far as the limited number of observations permit a conclusion, it appears that when an infection becomes chronic, the trypanosomes undergo immunological changes similar to those of Strain I, i.e. there is an addition to the original receptors, and not a replacement of the latter.

CROSS IMMUNITY RESPONSES.

In order to ascertain whether cross immunity exists between Strains I and II, mice of each strain infected with acme parasites and cured at acme, were reinoculated with acme parasites of the other strain.

Mice Cured of Acme Strain I Infections and Reinoculated with Acme Strain II.

12 mice cured of Strain I infection (6 of which had resisted re-inoculation with acme Strain I parasites) promptly became infected when inoculated with trypanosomes of Strain II, and showed no protraction of the incubation period beyond that of the control. After this was cured in 10 animals a 2nd similar reinoculation with Strain II parasites caused infection in only 1; 6 of the 9 resistant mice received a further inoculum of Strain II parasites, which was resisted by 5 — Table XXVII gives details. These few experiments indicated that mice cured of acme Strain I infections did not possess immunity against acme Strain II parasites. But the further results suggested that when the latter infection was cured, immunity to subsequent reinoculations with Strain II parasites developed more readily than in animals which had never been infected with Strain I, the difference being significant although not highly so.

Effect of a Cured Strain I Infection on the Subsequent Development of Immunity after Inoculation with Strain II.

To test this point further, 8 mice were infected with an inoculum consisting of a mixture of both strains. The incubation periods of the controls inoculated with the separate strains and of the mice receiving the mixed inoculum were all of the same duration. After cure, the test animals were reinoculated repeatedly with Strain II parasites

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parasites (Table XXVIII). The first reinoculation with Strain II parasites in these mice produced about a 50 per cent. immunity response, just as in animals not infected with Strain I. The 3rd, 4th and 5th reinoculations with Strain II trypanosomes were completely resisted; this result is highly significant and supports the conclusion from the previous experiment (preceding paragraph) that greater immunity to Strain II parasites is produced in the presence of Strain I antibodies. One animal withstood up to 7 reinoculations, but a 2nd became infected by a 6th reinoculation.

Mice Cured of Acme Strain II Infections and Reinoculated with Acme Strain I.

8 animals cured of Strain II infection all became infected on reinoculation with Strain I parasites, after a normal incubation period.

Thus, there is no cross immunity between the 2 strains, i.e. animals cured at acme after infection with acme trypanosomes of Strain I became infected on reinoculation with acme parasites of Strain II and vice versa.

In spite of this, immunisation to Strain II appears to be facilitated by the occurrence of an immunity reaction to Strain I.

SECTION VII.

FIELD TRIALS OF NO. 897 AND NO. 1553 ON T. CONGOLENSE INFECTIONS.

FIELD TRIALS OF NO. 897 and NO. 1553 on T. CONGOLENSE INFECTIONS.

Compound No. 897.

T. congolense infections in cattle have been treated with this drug by Hornby, Evans and Wilde (1943) in Tanganyika, Carmichael and Bell (1944[1]) in Uganda and Marshall in Nigeria (preliminary communication to Department of Scientific and Industrial Research, 1943, of experiments interrupted by an intercurrent outbreak of pleuropneumonia). They report favourably on its action and state that from their experience it has a high place among the trypanocidal agents tested against this intractable infection, although du Toit (communication to the National Institute for Medical Research, dated September 1941) discontinued trials with the drug after failures in treating 3 sheep infected with T. congolense. Hornby et al. and Carmichael and Bell used 0.5 or 1.0 per cent. aqueous solution, while Marshall used a 0.1 per cent. solution; all administered the drug in the proportion of 1.5 or 2 mgm. per kilo of body weight. The low solubility of No. 897 is a disadvantage, as a large volume of solution is required for dosage. Hornby et al. observed severe local reactions after a dose of 2 mgm./kilo given subcutaneously. A local reaction which does not result in permanent lameness follows the administration of such doses intramuscularly.

Infections tested and stage of infection at time of treatment. Hornby et al. used zebus naturally infected with T. congolense, others experimentally infected which were treated from 2 days to 5 weeks after the first appearance of parasites in the blood, and also animals which had relapsed after treatment with No. 897 or other drugs. Cattle experimentally infected with 2 strains of T. congolense were treated by /

by Carmichael and Bell, treatment being delayed until the infection had become well established ("chronic"). The course of the infection in untreated hosts was not described by either group of workers. Marshall used a strain which had been recently transmitted by wild Glossina. In sheep it caused a chronic undulant type of infection, but it was much more virulent for cattle, killing most of them in 14 to 36 days after inoculation. In the first set of animals tested the drug was administered 3 to 8 days after the parasites appeared in the blood; in the second set after 10 to 17 days.

Criteria of cure. Hornby et al. considered cure had been effected if weekly examinations of the blood were all negative for 6 months, the general condition of the animal was good and also the blood haemoglobin value was "reasonably high". Carmichael and Bell reported cures after daily negative blood examinations for 4 months, the animals being well and subinoculations of the blood into susceptible cattle proving negative. Constant observation for a period of at least 6 months, including registration of any fluctuations in temperature, was considered by Marshall to be the minimum required to assess the value of the treatment.
The results of treatment are shown in Table XXIX.

Treatment of relapses. 44 days after the initial treatment the 27 relapsed animals were retreated by Carmichael and Bell with an intravenous dose at the rate of 2 mgm./kilo; 22 of them were cured. The 5 remaining animals received 2 further similar treatments, relapsing after each. Of the 21 infected beasts treated with 1.5 mgm./kilo No. 897 intramuscularly by Hornby et al., 13 relapsed; 4 of these were cured after a similar injection into another quarter. The /

The second relapse in the remaining 9 animals was treated by weekly intravenous dosage at the rate of 2 mgm./kilo; 6 animals were cured by this treatment — 2 requiring only 1 intravenous injection. Treatment with another drug was commenced in the 3 remaining animals, as the infecting parasites were considered to be fast to No. 897.

General scheme of treatment. Hornby et al. recommend one intramuscular injection of 1.5 mgms/kilo; this to be repeated into another quarter if a relapse occurs; and 3 intravenous doses of 2 mgm./kilo at weekly intervals if a further relapse is observed. Carmichael and Bell prefer to introduce the drug intravenously or to administer it simultaneously by the intravenous and intramuscular routes. Marshall advised the intramuscular method of administration rather than the subcutaneous.

Hornby et al. and Carmichael and Bell considered that trypanosomes fast to No. 897 were obtained when repeated treatment of relapses failed to produce cure. The former workers, however, were able to cure such infections by treatment with antimosan. Hornby et al. and Marshall found that so far as their strains of T. congolense were concerned, failures were more common in animals treated at an early stage of the infection than in those in which treatment was postponed until the infection was well established; accordingly, as Hornby et al. state, the consequent development of antibodies appeared to "more than compensate for the disadvantages of increased anaemia and weakness." It should be noted that the present author working with mice found that both strains of T. congolense respond better to treatment at the acme stage than at either the pre-acme or the chronic stages (Tables IV and VII).

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In addition to therapeutic trials, Carmichael and Bell carried out tests on the prophylactic action of No. 897. They found that the drug in the larger dose above-mentioned protected cattle against a heavy inoculation of T. congolense up to 48 hours after administration. This they considered might be of value for protecting animals during short journeys through fly belts.

Phenanthridinium compound No.1553.

Trials carried out by Carmichael and Bell (1944[2]) indicate that this drug is more suitable than No. 897 for use in the field. It can be administered subcutaneously without serious local reactions; single doses of 2 mgm./kilo of a 1 or 2 per cent. solution in most cases effected sterilisation.

Treatment of T.congolense Infections of Cattle by No.1553.

Mode of Administration.	Dose in mgm./kilo.	No.cured / No.treated
Intramuscular	2.0 (2%)	4/4
Intravenous	2.0 (2%)	4/4
Subcutaneous	2.0 (2%)	8/12
	2.0 (1%)	3/3
	1.5 (2%)	4/4
	1.5 (1%)	4/4
	1.0 (2%)	4/4
	1.0 (1%)	4/4

The figure in brackets after the dose gives percentage solution used.

Treatment of relapses. The 4 relapsed animals were retreated with the same dosage subcutaneously; all were cured. Since the compound is readily soluble in water, the volume of solution required is relatively small. In mice No. 1553 proved slightly more toxic than No. 897 but therapeutically it was somewhat more active than No. 897, since smaller

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smaller doses effected cure, i.e. the "therapeutic index" is higher. It had the added advantage of causing no induration in maximal doses.

Discussion of the Results of Field Trials in the Light of Experimental Work.

(1) It is gratifying that favourable reports on the action of drug No. 897 on T. congolense infections in cattle were received from the different regions, since some failures might have been expected. It has been shown that the 2 experimental strains of the trypanosome differ markedly in (a) virulence, (b) curability with a single treatment, (c) response to repeated treatment of relapses and (d) in the immunity response which seems to play an important part in conducing to cure. Accordingly, very considerable variation in the tractibility of infections in the field might be anticipated.

(2) The stage of the infection at the time of treatment of experimental infections should be noted, as it has been shown that in mice the curability of an infection due to either strain alters considerably as the infection progresses. With Strain II decrease in curability is manifested both before the acme stage and also 3 to 4 days after.

(3) The difficulty of producing a drug resistant strain under experimental conditions and also the fact that with one strain repeated treatment of relapses effects cure, indicate that No. 897 is a valuable drug for use in the field. The resistance to the drug shown by relapsed infections which has been noted by Hornby et al. and Carmichael and Bell, can most probably be attributed to the chronicity of the infection rather than to true acquired drug resistance. Thus it has been found in the present work that in mice the curative dose /

dose for a chronic infection may be at least 5 to 10 times that required at acme (Tables IV and VII).

(4) Immunity to the homologous trypanosomes following the cure of acme infections in one strain is absolutely solid, whereas only a 50 per cent. immunity is observed under the same conditions with the other strain. If such a solid immunity can be demonstrated in the field it may be possible to utilise it to protect animals passing through infected belts by previously infecting them and then producing cure with the drug. Also from experimental work it would appear that under field conditions, after cure of certain strains of T. congolense, reinfections would not occur, while under similar conditions with other strains reinfections might be expected.

(5) It has been shown experimentally that in mice a greater degree of immunity was manifested to laboratory Strain II parasites in the presence of antibodies to Strain I than in animals which had never been infected with the latter. Accordingly, it might be expected that a more solid immunity would follow cure of a mixed infection with several strains of T. congolense than would occur after infection with a single strain. This would apply particularly in the case of a strain with relatively poor immunising properties.

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APPENDIX.

TABLE I

Strain I.

Percentage of animals whose infection had the incubation period shown.

Incubation (days)	Passages	
	101 to 150	100 Recent about 700th
4	14.8	12.5
5	32.9	47.5
6	24.3	30.5
7	18.0	7.5
8	6.3	2.0
9	1.6	
10	1.6	
11	.5	
No. of animals	189	200

TABLE II

Strain II.

Percentage of animals whose infection had the incubation period shown.

Incubation (days)	Passages								
	1-10	11-20	21-30	31-40	41-60	61-80	81-100	101-200	591-690
2						1.2	2.5	5.2	.5
3	4	3.0	22.2	20.8	25	40.5	60	52.2	17
4	22	27.3	36.1	54.2	57.5	48.8	32.5	36.0	45.5
5	30	42.4	36.1	25	15	9.5	5	5.4	24.5
6	30	18.2	2.8		2.5			.4	9
7	6	9.1	2.8					.4	2.5
8	8								1
No. of animals	50	33	36	24	80	84	80	272	200

TABLE III

Results of Treatment on the number of Trypanosomes in the Blood.

Cures		Relapses		Ineffective Drug	
Strain I No. 1692B	Strain II No. 615A	Strain I No. 1731B	Strain II No. 604A	Strain I No. 1734D	Strain II No. 632A.
27.2.43 inoc.	21.1.41 inoc.	27.6.43 inoc.	3.12.40 inoc.	16.9.43 inoc.	10.2.41 inoc.
5.3 -	24.1 -	4.7 +	9.12 +++ * d	23.9 +vl	15.2 +vl * f
6.3 +vl	27.1 +++ * b	5.7 +++ * c	10.12 +++	24.9 +++ * e	16.2 +l
8.3 +++ * a	28.1 +++	6.7 +++	11.12 +++	26.9 to 29.9 4 exams. in 4 days all +++	17.2 ++
10.3 to 16.4 17 exams. in 38 days all -	29.1 +++	7.7 +++	12.12 to 16.12 4 exams. in 5 days all -	30.9 +vl	18.2 to 21.2 3 exams in 4 days all +++
26.4 to 3.6 5 exams. in 39 days all -	30.1 to 27.2 20 exams. in 29 days all -	8.7 to 13.7 5 exams. in 6 days all -	17.12 +l	2.10 -	
1.7 to 24.11 6 exams. in 5 months all -	3.3 to 25.3 5 exams. in 23 days all -	14.7 +vl	18.12 +	3.10 +++	
		15.7 +++	19.12 +	4.10 +++	
	17.4 to 26.5 4 exams. in 40 days all -		20.12 +++		
	16.6 to 23.8 4 exams. in 2½ months all -				
a 1/100,000g.No.897	b 1/15,000g.No. 897	c 1/2,000g.No.1053	d 1/15,000g.No.897	e 1/100,000g.No.812	f 1/18,000g.No. 621

* = drug administered.

TABLE IV

Showing Results of Treatment of Mice infected with T. congolense.

Dose in gm.	Strain I Stage of Infection at Treatment +vl No. cured	+vl No. treated	Acme No. cured	Acme No. treated	Dose in gm.	Strain II Stage of Infection at Treatment +vl No. cured	+vl No. treated	Acme No. cured	Acme No. treated
1/3,000			57	57	1/3,000			12	13
1/10,000			2	2	1/5,000	1	6	9	12
1/15,000			3	4	1/10,000	1	6	6	10
1/20,000			4	4	1/15,000	0	9	20	52
1/25,000			1	2	1/20,000			4	6
1/30,000	1	2	4	4	1/25,000	0	2	0	2
1/50,000			2	3	1/35,000	0	2		
1/60,000	0	3	3	4	1/50,000	0	2	0	2
1/75,000	1	11	11	15	1/75,000			0	2
1/100,000	1	21	14	26	1/100,000	0	1	0	4
1/200,000			0	5					

TABLE V

Examples of Relapses after Treatment of T. congolense infection with No. 897.

STRAIN I

1424A.	1671B.	1525B.	1780B.	1672D.
22.1.41 inoc.	10.12.42 inoc.	19.1.42 inoc.	26.1.44 inoc.	8.1.43 inoc.
29.1 +++ treated 1/15,000g.	15.12 +vl treated 1/100,000g.	24.1. +vl treated 1/75,000g.	1.2 +++ treated 1/200,000g.	13.1 +
30.1 +++	16.12 +++	26.1 +	2.2 +++	14.1 +++ treated 1/100,000g.
31.1 to 10.2 9 exams. in 11 days all —	17.12 +++	27.1 +++	3.2 to 11.2 5 exams. in 9 days all —	15.1 +++
11.2 +vl	18.12 +++	28.1 to 2.2 5 exams. in 6 days all —	14.2 +vl	16.1 to 15.2 13 exams. in 31 days all —
12.2 +++	19.12 to 26.12 5 exams. in 8 days all —	3.2 +vl	16.2 +1	19.2 to 15.3 4 exams. in 24 days all —
	28.12 +1	4.2 +	17.2 +++	6.4 ++
	30.12 +++	5.2 +++		9.4 ++

STRAIN II

586B.	831A.	602A.	784A.	761B.
12.11.40 inoc.	18.2.43 inoc.	3.12.40 inoc.	14.5.42 inoc.	4.3.42 inoc.
19.11 +vl treated 1/5,000g.	24.2 +vl treated 1/10,000g.	9.12 +++ treated 1/5,000g.	19.5 +++ treated 1/100,000g.	10.3 +++ treated 1/15,000 g.
20.11 +	25.2 +	10.12 +++	20.5 +++	12.3 to 22.4 22 exams. in 41 days all —
21.11 to 28.11 8 exams.in 8 days all —	26.2 to 9.3 8 exams.in 12 days all —	11.12 +	21.5 +vl	29.4 +vl
29.11 +vl	11.3 +	12.12 to 31.12 16 exams.in 20 days all —	22.5 to 26.5 5 exams.in 5 days all —	1.5 +1
	12.3 +++	2.1.41 +vl	27.5 +1	2.5 +1
		4.1. +++	28.5 +	4.5 +
			29.5 +++	5.5 +

TABLE VI

Duration of Free Period following Treatment which led to Temporary Disappearance of Trypanosomes from the blood.

Drug Administered & Stage of Infection.	STRAIN	No. of animals showing the following free periods in days after treatment.					Total No. of Mice.
		8 or less	9 - 14	15 - 20	20 - 30	30 & over	
No. 897 early	I	1	29 (90%)	1	1	-	32
No. 897 at acme	I	2	17 (68%)	5	-	1	25
Other Phenanthridinium Compounds at acme	I	3	45 (70%)	9	5	2	64
		7 or less	8 - 11	12 - 20	20 - 30	30 & over	
No. 897 early	II	-	21 (81%)	5	-	-	26
No. 897 at acme	II	5	38 (73%)	4	4	1	52

TABLE VII

Results of Treatment with No. 897 of Mice Infected with a Chronic Strain and of Chronically Infected Mice.

	STRAIN I					STRAIN II			
Dose in gm.	Chronic strain treated at fastigium.		Chronic in the Original Mouse treated at +++		Dose in gm.	Chronic strain treated at fastigium.		Chronic in the Original Mouse treated at +++	
	No. cured	No. treated	No. cured	No. treated		No. cured	No. treated	No. cured	No. treated
1/3,000g.	57	62	8	9	1/1,750g.	3	5		
1/5,000g.			8	11	1/2,000g.	9	13	4	32
1/10,000g.	13	17	12	29	1/3,000g.	5	9	2	8
1/20,000g.	4	15			1/5,000g.			0	2

TABLE VIII

Results of Treatment of T. congolense II with special reference to relapses.

Stage of Infection at Initial Treatment.	Result of Treatment.	No. of Mice.	No. of Relapses.
at +vl	C	0	
	CR	9	1,3(2),5(3),6,14,20.
	D	8	4,6,9(3),14,16,19.
	K	1	15.
	Kp	1	14.
	Total	19	
	of which relapsed	19	
at acme	C	23	
	CR	14	1(3),2(2),3,4(2)[1],6,8[2],12,15,20[3],21.
	D	12	2(3),5,8,9,11(2),14(2),19,25.
	K	2	5,10.
	Kp	3	12,13,14.
	Kr	1	6.
	Kt	3	11,13,14.
	NA	2	14,30.
	Total	60	
	of which relapsed	37.	

C signifies mouse cured by initial treatment.
CR " " " after a number of relapses.
D " " died intercurrently.
K " " chloroformed in a protracted relapse.
Kp " " " because of poor general condition.
Kr " " " in an abnormally late relapse.
Kt " " " because of condition of tail.
NA " there was no response to treatment.

Figures in brackets indicate numbers of mice above 1.

1.2.3: in each of these cure is only presumptive in the case of 1 animal, death having occurred intercurrently 69, 55 and 52 days after the last treatment respectively.

TABLE IX

Mice Showing Unusually Late Relapses (Strain II).

No.	Total No. of Relapses.	Free Interval in days after last treatment; the figure in brackets indicates the numbers of the late relapses.	Result.
596A.	6	24 (6).	D
602A.	8	26 (1).	D
602B.	3	21 (1).	CR
613A.	14	47 (3), 28 (8 & 9), 35 (12), 31 (14).	D
613B.	15	79 (10), 32 (12), 36 (15).	K
683A.	5	21 (1), 30 (3), 40(4), 33 (5).	K
684A.	13	27 (12).	Kp
700A	12	38 (6), 26 (7), 34 (9), 69 (10), 29 (11), 21 (12).	CR
735A.	14	40 (14).	Kp
738A.	10	25 (1).	K
758B.	11	32 (11).	Kt
765B.	13	30 (13).	Kt
761B.	6	56 (1), 89 (6).	Kr

For code see Table VIII.

TABLE X

Repeated Treatment of Relapses in Strain I.

No. of Mouse.	Initial Treatment at acme.	Subsequent Treatments and Results.					
		2nd	3rd	4th	5th	6th	7th
1528A.	1/75,000 A	(14) 1/75,000 A.	(10) 1/75,000 N.A.	(6) 1/75,000 N.A.	(5) 1/75,000 N.A. used to start resistant strain.		
1560B.	1/100,000 A.	(15)1/100,000 A.	(12)1/100,000 A.	(12)1/100,000 A	(14)1/100,000 A.	(9)1/100,000 S.A. ✱ 4 days later.	
1561A.	1/100,000 A.	(16)1/100,000 A.	(12)1/100,000 S.A.	(6)1/100,000 N.A.	(5)1/100,000 N.A. dose increased		
1561B.	1/100,000 A.	(24)1/100,000 A.	(10)1/100,000 A.	(11)1/100,000 S.A.	(7)1/100,000 S.A.	(4)1/100,000 S.A.	(12)1/100,000 S.A. dose increased
1562B	1/100,000 A.	(16)1/100,000 A.	(21)1/100,000 C.				
1605A.	1/100,000 A.	(14)1/100,000 A.	(14)1/100,000 A.	(23)1/100,000 A.	Kt. at 4th relapse.	✱	

Figures in brackets represent numbers of days after previous treatments.

A = action. S.A. = slight action

C = cure. N.A. = no action.

Kt = chloroformed because of condition of tail.

✱ = dead.

TABLE XI

Treatments of Resistant Strain I Relapses.

Source of Inoculum. The figure in brackets shows the No. of previous treatments.	Mouse No.	Treatments each with 1/3,000 g. No. 897 and Results.
1561A (9)	1601B.	1 & 2 A: 3 - 5 S.A.: 6 - 10 A: 11 N.A.
1561A (13)	1622A.	1 - 4 A: 5 N.A.: 6 A: 7 N.A.
1561A (13)	1422B.	not infected:
1561A (14)	1622C.	1 - 3 A: 4 N.A.
1561A (14)	1622D.	1 - 3 A: 4 N.A.
1561B (12)	1623C.	1 & 2 A:
1601B (5)	1623B.	1 - 4 A: 5 - 18 S.A.: 19 - 21 A:
1623B (18)	1685A.	not infected.
1623B (18)	1685B.	1 A: 2 S.A.: 3 A: 4 S.A.: 5 - 16 A:

For code of results see Table X.

TABLE XII.

First Attempt to Develop Drug Resistance in Strain I.

No. of Passage.	Initial Treatment at acme.	Subsequent treatments given when abundant trypanosomes were present in the blood. Figures in brackets represent the No. of days after the previous treatment.
1		(14) 1/75,000 g. (10) 1/75,000 g. (6) 1/75,000 g. (5) 1/75,000 g.
2	1/75,000 g.	(2) 1/75,000 g.
3		
4	1/50,000 g.	(4) 1/50,000 g.
5	1/30,000 g.	
6		
7		(2) 1/15,000 g.
8	1/15,000 g.	
9		
10	1/12,000 g.	(3) 1/12,000 g.
11		
12	1/10,000 g.	

TABLE XIII

Second Attempt to Develop Drug Resistance in Strain I.

No. of Passage.	Dose of No. 897 injected when scanty trypanosomes were present in the blood.	No.	Dose.
1	1/200,000 g.	9 & 10	1/115,000 g.
2	no drug.	11	1/100,000 g.
3 & 4	1/200,000 g.	12 & 13	1/75,000 g.
5 & 6	1/175,000 g.	14	1/60,000 g.
7	1/150,000 g.	15 & 16	1/50,000 g.
8	1/130,000 g.		

TABLE XIV.

Attempt to Develop Drug Resistance in Strain II.

No. of Passage.	Dose of No. 897 injected when scanty trypanosomes were present in the blood.	No.	Dose.
1	1/750,000 g.	12	1/200,000 g.
2	1/500,000 g.	13 to 15	1/150,000 g.
3	1/750,000 g.	16 to 18	1/130,000 g.
4	1/650,000 g.	19 & 20	1/120,000 g.
5	1/450,000 g.	21	1/110,000 g.
6	1/300,000 g.	22 to 24	1/100,000 g.
7	1/450,000 g.	25 & 26	1/90,000 g.
8	1/300,000 g.	27	1/80,000 g.
9	1/250,000 g.	28 & 29	1/75,000 g.
10	1/200,000 g.	30 to 32	1/70,000 g.
11	1/300,000 g.	33 & 34	1/60,000 g.

TABLE XV

TREATMENTS OF RESISTANT STRAIN II RELAPSES.

Source of Inoculum. The figure in brackets shows the No. of previous treatments.	Mouse No.	Treatments each with 1/3,000 g. No. 897, except where the dose is mentioned, and Results.
761A (33)	836D.	1 - 6 (1/15,000g.) N.A.: 7 N.A.: 8 (1/2,000g.) N.A.:
836D (6)	852A.	1 N.A.
852A (1)	852C.	1 - 5 N.A.
852C (5)	855A.	1 & 2 S.A.: 3 - 5 N.A.
761A (34)	837E.	1 & 2 (1/15,000g.) S.A.: 3 - 5 (1/15,000g.) N.A.: 6 A: 7 N.A.
837E (5)	852B.	1 A.
852A (1)	852D.	1 - 4 S.A.: 5 - 8 A: 9 - 10 N.A.
852D (10)	851C.	1 - 3 S.A.: 4 - 6 N.A.: 7 A: 8 S.A.: 9 - 11 N.A.
851C (5)	851D.	1 - 5 S.A.
851C (10)	863A.	1 - 3 S.A.: 4 & 5 N.A.
863A (5)	863B.	Not infected.

For code of results see Table X.

TABLE XVI

STRAIN I

Summary of Reinoculations.

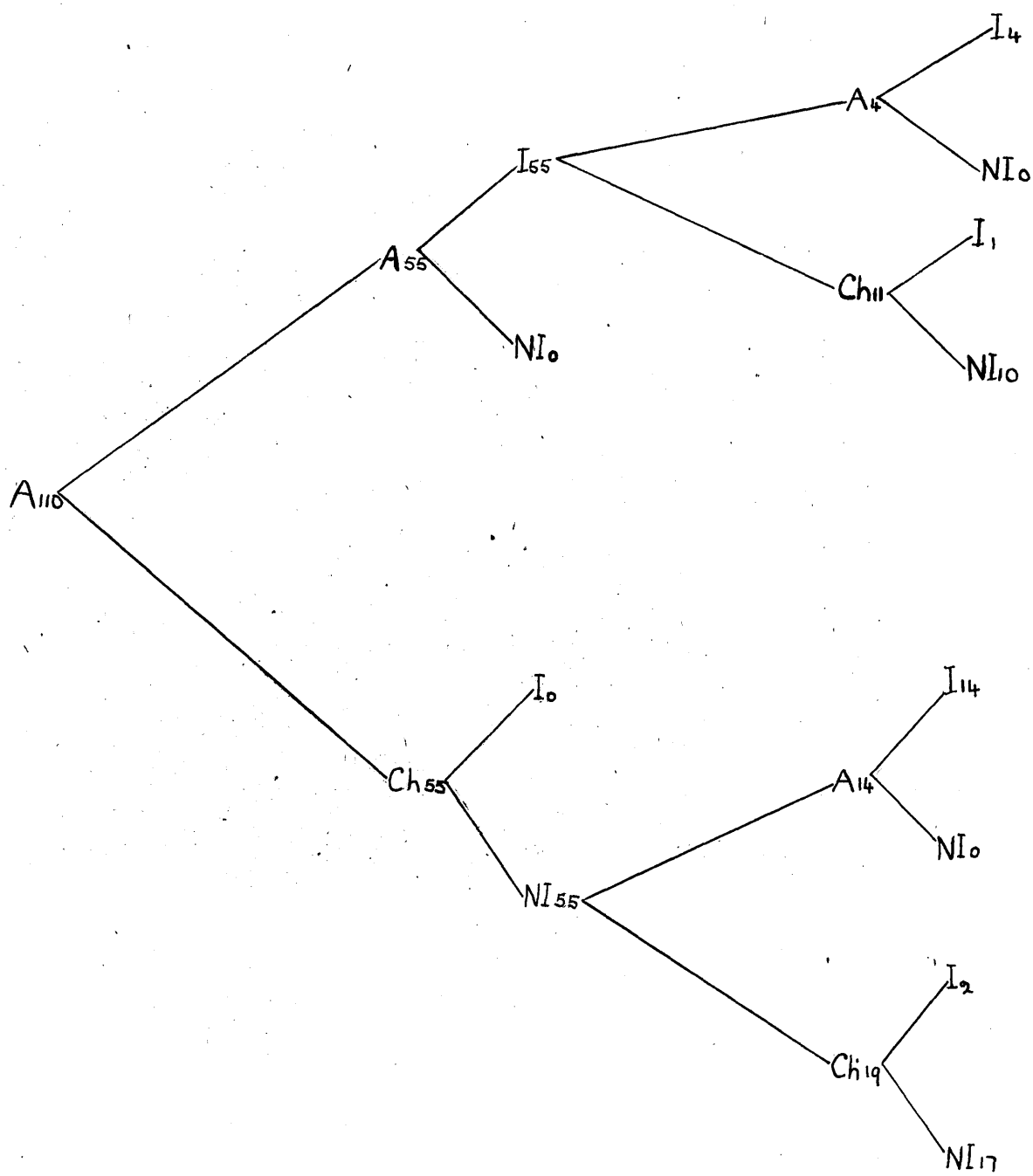
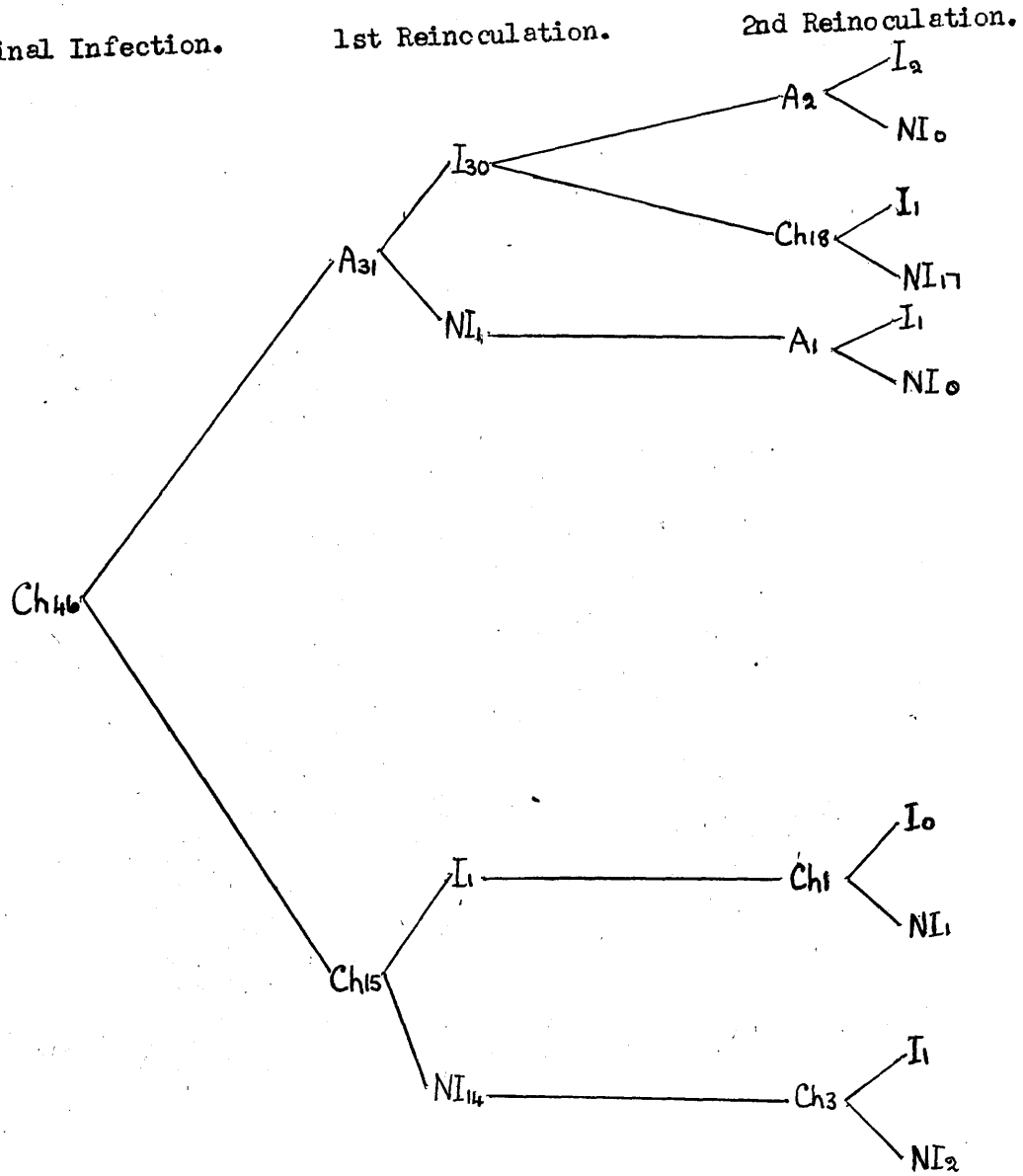


TABLE XVI (contd.).

STRAIN I

Summary of Reinoculations.



A = acme. Ch = chronic. I = immune. NI = not immune. The figure after I or NI represents the number of mice reacting in the way shown.

TABLE XVII

IMMUNITY EXPERIMENTS WITH STRAIN I.

No. of Mouse.	1544 A.	1535 B.	1533 B.	1551 A.
Original Infection	A	A	Ch	Ch
Initial Dose of No.897.	1/75,000 g.	1/3,000 g.	1/3,000 g.	1/3,000 g.
1st Reinoculation and Result.	A (52) I	Ch (17) N.I.	A (16) I	Ch (32) N.I.
2nd " " "	Ch (91) N.I.	Ch (67) N.I.	Ch (56) N.I.	A (85) I
3rd " " "	Ch (139) N.I.	A (109) I	Ch (92) N.I.	Ch (136) N.I.
4th " " "	A (180) I	Ch (142) N.I.	A (139) I	A (184) I
5th " " "	Ch (215) N.I.	A (200) I	Ch (191) I	
6th " " "			Ch (221) N.I.	

A = acme. I = immune.
Ch = chronic. N.I. = not immune.

The figure in brackets after the type of reinoculation represents the number of days after initial treatment.
When infection developed after reinoculation this was cured with a dose of 1/3,000 g. No. 897.

TABLE XVIII

SETS of MICE INOCULATED with the SAME CHRONIC STRAIN I TRYPANOSOMES.

Series No. of Set.	Source of Inoculum.	Incubation Period. Reinoculated Mice.	Controls.
1.	1524A.	1503B (7) 1530A (8) 1530B (26) 1535A (10) 1535B (17)	1541B (17)
2.	1549B.	1503A (8) 1532B (7) 1533B (8) 1534A (7)	1552A (13)
3.	1619A.	1499D (10) 1568A (12) 1576B (10) 1592B (10) 1593A (10)	1614B (12)
4.	1514A.	1515B ∝ 1516B ∝	no control.
5.	1524D.	1485A ∝ 1498A ∝ 1515B ∝	no control.
6.	1524D.	1498A (10) 1515B ∝ 1527A (10) 1528B (10) 1531B (10) 1535B (10)	1552B (7)
7.	1524A.	1500B (8) 1501B (8) 1516B ∝ 1522B (10)	no control.
8.	1602C.	1539B ∝ 1543B ∝ 1576A (12) 1598B (14) 1599B ∝	1582A (12)
9.	1602D.	1536B ∝ 1567A ∝ 1575A ∝ 1594A (9)	1585B (16)
10.	1619B.	1540B (9) 1547A (7) 1553B (7) 1567A (23) 1575A (9) 1621A (9)	1600C (9)
11.	1620H.	1567A (26) 1595B (7) 1582B (7) 1598A (7)	1640B (11)

The figure in brackets represents the duration in days of the incubation period.
Mice mentioned in text are underlined.

∝ = was not infected.

TABLE XIX

Details of Mice in which Anomalous Results to Reinoculation were Obtained.

No. of Mouse.	1495A.	1532A.
Original Infection cured by 1/3,000g.No.897.	Ch.	Ch.
1st Reinoculation & Result.	A (63) N.I. (37)?	Ch (17) N.I. (9)
2nd " " "	A (134) I	Ch (57) I ?
3rd " " "	A (192) I	A (89) N.I. (p)
4th " " "	A (250) I	

A = acme. I = immune.

Ch = chronic. N.I. = not immune.

(p) means that the infection ran a protracted course.

The figure in brackets after the type of reinoculation represents the number of days after the initial treatment.

The figure in brackets after N.I. represents the number of days between the inoculation and the appearance of an abundant number of trypanosomes in the blood.

When infection developed after reinoculation this was cured with a dose of 1/3,000 g. No. 897.

TABLE XX

T. Congolense I.

Response to Reinoculation in Cured Mice each of which had been Infected Originally with Chronic Trypanosomes and were Reinoculated from the Same Mouse.

No. of Mouse.	Original Infection and a Reinoculation each derived from a Chronic Infection of Mouse No.	Interval in days between cure of Original Infection and Reinoculation.	Result of Reinoculation
1480A.	1472B.	11	N.I. (9)
1480B.		11	N.I. (8)
1481A.		34	N.I. (11)
1481B.		34	N.I. (8)
1485A.	1473A.	12	N.I. (11)
1532A.	1514A.	17	N.I. (9)
1532B.		19	N.I. (9)
1534A.		19	N.I. (11)
1534B.		20	N.I. (8)

N.I. = not immune.

The figure in brackets after N.I. represents the number of days between the inoculation and the appearance of an abundant number of trypanosomes in the blood.

TABLE XXI

T. Congolense Strain I.

Mice cured of Acme Infection.	Intervening History of Reinoculations and Results.	Inoculations in each case from the same chronically infected mouse.			
		Result of Reinoculation.	Interval between treatment & reinoculation in days.	Result of Reinoculation.	
1425A.	A. I.	N.I. (9)	cured.	11	N.I. (14) cured.
1432A.	A. I.	N.I. (9)	cured.	11	I.
1425B.	A. I.	N.I. (10)	cured.	10.	N.I. (9) cured.
1426B.	A. I.	N.I. (9)	cured.	11.	N.I. (9) cured.
1479B.	—	N.I. (12)	cured.	58 31 days after treatment reinoculated with A. I. 27 days later 2nd Ch. inoculum injected.	N.I. (13)
1516B.	Ch. N.I. Ch. I. Ch. I.	N.I. (11)	cured.	17	N.I. (12) cured.
1527A.	Ch. N.I. Ch. N.I.	N.I. (19)	cured.	10	N.I. (12) cured.
1540B.	Ch. N.I.	N.I. (10)	cured.	12	I.

A = acme. I = immune.
Ch = chronic. N.I. = not immune.

The figure in brackets after N.I. represents the number of days between the inoculation and the appearance of an abundant number of trypanosomes in the blood.

TABLE XXII

Temporary Fixation of the Immunological Characters of Passaged Chronic Strains.

Strain No.	No. of Passage.	Result of Reinoculation of Cured Acme Mice with the Passaged Chronic Strain.	Results in Mice Infected with the Same Inoculum as the 1st Passage of the Corresponding Strain and, after Cure, Reinoculated from the Passage Shown.
E.	4	N.I.	Incubation period very protracted, viz. 40 - 49 days.
	5	-	I.
	6	N.I.	I.
	7	N.I.	I
F.	5	N.I.	I.

I = immune. N.I. = not immune.

TABLE XXIII

T. Congolense I.

The Effect of Repeated Passages on the Immunological Characters of Chronic Strains.

No. of Strain.	A	B	C	D	E	F	G	H	J
No. of days after acme stage at which 1st Passage was started.	37	36	28	36	32	34	20	14	24
Latest passage in which chronic characters were proved.	-	-	4	-	7	5 *	9 †	11	12
Earliest passage in which acme characters were proved.	3	4	5	6	9	-	-	-	-

* Both strain mice of passage 5 died during the incubation period.

† In passage 10, 1 mouse failed to become infected and the other died intercurrently before trypanosomes had time to appear in the blood.

TABLE XXIV

T. Congolense II. Acme Strain.

Results of Reinoculations of Mice Cured at Acme — the Reinoculum being the Homologous Strain at Acme.

No. of Mice cured of Original Infection and Reinoculated once.	Result of 1st Reinoculation.	No. of Mice Reinoculated twice.	Result of 2nd Reinoculation.
40	I_{22}	18	I_{10}
			NI_8
	NI_{18}	14	I_8
			NI_6

TABLE XXIVa

Summary of Results of All Reinoculations, leaving out of account, Previous Responses.

	1st Reinoc.	2nd Reinoc.	3rd Reinoc.	4th Reinoc.	5th Reinoc.	6th Reinoc.	Totals
Total	40	32	21	10	7	4	114
Immune	22	18	11	6	6	3	66
Not Immune	18	14	10	4	1	1	48

The numbers of mice receiving the successive reinoculations diminished in the course of the experiment, as some died intercurrently and in others it was impossible to cure the reinfection even with high dosage of No. 897 employed.

For code of results see Table XVI.

TABLE XXV

STRAIN II.

Reinoculations in Mice with Acme Trypanosomes.

No. of Mouse.	701A.	738B.	760B.	792A.	726B.	754B.	696A.
Original Infection cured with No. 897.	Acme	Acme	Acme	Acme	Acme	Acme.	Chronic.
1st Reinoculation	66 NI(10)	73 NI(17)	71 NI(7)	32 I	27 NI(8)	27 I	63 NI(13)
2nd "	101 I	132 I	116 I	75 I	62 NI(15)	68 NI(9)	115 NI(18)
3rd "	132 NI(11)	167 NI(9)	165 I	123 I	103 NI(24)	107 I	167 I
4th "	167 I	207 I	213 I	172 I	155 NI(11)	148 I	203 I
5th "	208 I	244 I	262 I	228[1] NI(10)		197 I	232 I
6th "	240 I	295 I	394[1] I[2]			255 NI(9)	264 I
7th "	275 I						
8th "	330 I						
9th "	384 I						
10th "	427[1] I						
11th "	540[1] I						
12th "	593[3] I						

I = immune. NI = not immune.

[1]Double strength inoculum. [2]Probably I; died intercurrently 28 days after reinoculation.

[3]Inoculum of whole heart blood.

The figure represents the number of days after initial treatment on which the reinoculation was carried out.

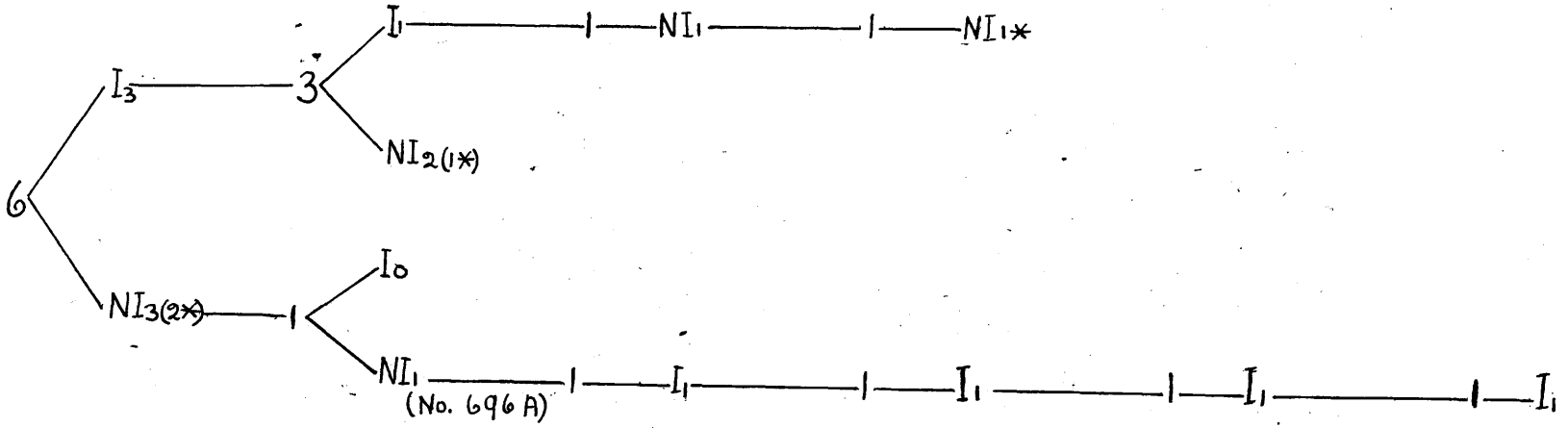
The figure in brackets after NI represents the number of days between the inoculation and the appearance of abundant trypanosomes in the blood.

TABLE XXVI

T. Congolense Strain II.

Results of Reinoculations of Chronically Infected Mice after Cure — the Reinoculations being with the Homologous Strain at acme.

1st Reinoculation. 2nd Reinoculation. 3rd Reinoculation. 4th Reinoculation. 5th Reinoculation. 6th Reinoculation.



* = failed to be cured by 1/3,000 g. No. 897.

For code of results see Table XVI.

TABLE XXVII

Immunity Responses of Cured Acme Strain I Mice to Reinoculation with Strain II Acme Parasites.

No. of Mice cured of Original Strain I Infection.	1st Reinoculation with Strain II Parasites.	2nd Reinoculation with Strain II Parasites.	3rd Reinoculation with Strain II Parasites.
12	I_0		
	NI_{12} x	10 mice cured of Strain II infection.	
		I_9	6 mice cured of Strain II infection.
			I_5
			NI_1
		NI_1	

I = immune.
NI = not immune.
x = 6 of these had been reinoculated once with acme Strain I trypanosomes and proved immune.

TABLE XXVIII

Responses in Mice cured of a Mixed Infection to Reinoculation with Strain II Parasites.

No. of Mice cured of original mixed infection.	1st Reinoculation and Result.	2nd Reinoculation and Result.	3rd Reinoculation and Result.	4th Reinoculation and Result.	5th Reinoculation and Result.	6th Reinoculation and Result.	7th Reinoculation and Result.
8	I_3	3 I_o	I_3	3 I_3	2 I_2	2 I_1	1 I_1
							NI_o
						NI_1	
					NI_o		
				NI_o			
			NI_o				
		NI_3	3 I_3	2 I_2	2 I_2	1 I_1	
							NI_o
					NI_o		
				NI_o			
			NI_o				
	NI_5 x	3 I_3	2 I_2	2 I_2	1 I_1		
							NI_o
					NI_o		
				NI_o			
			NI_o				
		NI_o					

I = immune.

NI = not immune.

x = 2 of these had been reinoculated once with acme Strain I parasites and proved immune.

TABLE XXIX

Treatment of T. Congolense Infections of Cattle by No. 897.

Dosage at the rate of 2 mgm./kilo body weight unless otherwise stated.

No. of animals cured / total No. treated.

Mode of Administration.	HORNBY et al.			CARMICHAEL and BELL			MARSHALL
	Initial Treatment	Relapses Treated No.		Initial Treatment	Relapses Treated. No.		
Subcutaneously	2/3			2/7			1/6 treated early. 6/14 treated late.
Intramuscularly	14/20 1/2 (2 doses i/m at interval of 1 week) 8/21[a] (1.5 mgm./kilo).	1st	4/13[a] (1.5 mgm./kilo).	1/8			From observations of an experiment spoilt by an outbreak of pleuro-pneumonia it appeared that several i/m injections were preferable to one sb.
Intravenously	0/1 2/3 (5 doses at weekly intervals)	2nd 2nd	2/9[a] 4/7[a] (3 doses i/v at weekly intervals)	7/11	2nd 3rd 4th	22/27 0/5 0/5	
Combined Methods				1/8 i/v and sb. simultaneously. 4/8 i/v and i/m simultaneously.			

sb = subcutaneously.
i/m = intramuscularly.
i/v = intravenously.

Index a shows that these animals were all of the same original series.
In Carmichael and Bell's series all animals which relapsed after initial treatment were retreated by i/v dosage.