MODIFIED COURSE OF *T. CONGOLENSIS* INFECTION IN MICE GIVEN DIETS WITH MILK CASEIN*

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In February, 1952, mice which had been fed on diets with different amounts of milk casein as protein were inoculated with *Trypanosoma congolense* (Busimbi strain). Contrary to expectation, blood films from the animals failed to reveal trypanosomes and the mice did not die. This phenomenon was so unexpected that it was further studied with a range of diets as described in this paper. The results of two further experiments confirmed the original observation and showed that the unexpected result might be attributed to a change of diet.

In the absence of fuller investigation the results were not published. However, since they show a close analogy with the findings of Maegraith, Deegan, and Jones (1952) of the suppression of *Plasmodium berghei* infection in rats fed on milk diets, it now seems desirable to give a brief account of the experiments with trypanosomes.

Five diets were tested: (1) Rowett Research Institute Stock Diet No. 86 (Howie, 1951). This is a cubed diet of a mixture of natural foods giving good growth and breeding performance. (2) Diet containing 30% casein. (3) Diet containing 20% casein. (4) Diet containing 10% casein. (5) Stock diet of bread soaked in milk daily, with a handful of oats weekly. The percentage composition of diets 1-4 is given in Table I.

*This work was done with a grant from the Medical Research Council.

TABLE I.—Composition (g. per 100 g.) of Diets Tested

Diet 1	Diets 2, 3, and 4				
		2	3	4	
Wheat, whole ground ..	50	Casein (lactic, un- extracted; Glaxo Labs.) ..	30	20	10
Barley, whole ground ..	25	Maize starch ..	61	71	81
White-fish meal ..	7	Margarine* ..		3.0	
Meat-and-bone meal ..	6	Cod-liver oil ..		0.5	
Dried brewers' yeast ..	5	Salt mixture† ..		4.0	
Dried grass meal ..	5	Dried yeast powder ..		1.5	
Cod-liver oil ..	1				
Common salt (sodium chloride) ..	1				
Total ..	100	Totals ..	100	100	100

* Ministry of Food special margarine containing 450-550 I.U. vitamin A per oz. and 90 I.U. vitamin D per oz.

† McCollum's salt mixture No. 185 with iron and potassium iodide (0.221 g. per kg.).

Experiment I

Young white Swiss female mice of about 6 weeks of age of the laboratory inbred strain, which had been bred and reared on diet 1, were allocated to one or other of diets 1 to 4, on which they were fed for three weeks before inoculation, and thereafter throughout the course of the experiment. Twenty mice were allocated to each group. The mice were kept in individual glass jars with sawdust litter, which was changed weekly, and with individual water-bottles. The "casein" diets were made up in bulk and kept in the refrigerator. Each mouse was given 5 g. of diet daily.

The inoculum was a 10% mixture in saline of blood at "acme" (see below) from four mice infected with *T. congolense* (Busimbi); 0.5 ml. was injected into each mouse subcutaneously. It may be explained that for over five years in this laboratory *T. congolense* (Busimbi strain) remained fully virulent for mice. The strain was maintained by passage to fresh mice whenever the number of trypanosomes in the blood of an infected mouse first became uncountable. This phase is known as "acme" and the strain derived thereby as an acme strain (Browning, Calver, and Adamson, 1948). The usual course of infection in a previously uninoculated animal is that small numbers of trypanosomes appear in the blood in three to six days, and increase rapidly until acme is reached (five to nine days). Parasitaemia persists up to a week thereafter (acute phase); then

TABLE II.—Diet 1 (Stock Diet 86, Rowett Research Institute). Results of Infecting Mice with *Trypanosomes*

No. of Days After Inoculation	Mouse Numbers																			
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
4	—	—	—	+vl	+vl	—	—	—	—	—	—	—	+vl	—	—	—	—	+vl	—	—
5	+	+	—	++	++	+vl	+	+vl	+vl	+	+	+1	+	+	+vl	+vl	+	+	+	+
6	+++	+++	+	+++	+++	+	+++	+++	+++	+++	+	+	+++	+++	+++	+++	+++	+++	+++	+++
7	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
10	+++	+++	+++	+++	+++	+++	+++	+++	Dead	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
11	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
12	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
13	Dead	—	+++	—	—	+++	+++	—	+++	+++	+++	+++	Dead	+++	—	—	—	—	—	—
14	—	+++	—	—	+	+++	+++	—	—	—	+	+	+++	+++	—	—	—	—	—	—
16	+	+	+++	—	+++	+++	+++	—	—	+	+	+	—	+++	—	+	+	+	+	+1
17	+++	+	+++	+++	+++	—	+	+++	—	+	+++	+++	—	—	+++	+++	+++	+++	+	+++
18	+++	+++	+++	+++	+++	—	+	+vl	+	+	+++	+++	+	+++	+++	+++	+++	+++	+	+++
19	+++	+++	+++	+++	+++	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
20	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
21	+++	+++	Dead	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Dead	+++	+++	+++	+++	+++
23	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+1	+++	+++	+++	+++
24	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
25	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
26	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+
27	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
29	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
30	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
31	+++	+	+++	+++	Dead	+++	+++	+++	+++	+++	+++	+++	+++	+++	+1	+++	+	+++	+++	+++
32	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	—	+++	+	+++
33	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
34	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+vl	+++	+++	+++
35	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
37	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
38	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
40	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	Dead	+++	+++	+++

— to +++ = range of numbers of trypanosomes in blood (see text, p. 854). 1 = little; vl = very little.

trypanosomes disappear for about seven days, but reappear in the blood in large numbers and persist as a rule until the death of the animal (chronic phase). Death may take place in the acute phase or in the chronic phase. Animals may survive up to about four months after inoculation, but usually die much before that time.

In the present experiments the blood of each mouse was examined daily by pressing out a drop of blood between a cover-slip and slide and examining a wet film under the $\frac{1}{2}$ -in. (4-mm.) lens of the microscope. In recording the results of blood examination, the numbers of trypanosomes per microscope field were indicated in the following way :

— =No trypanosomes seen after three minutes' search
+vl =1 trypanosome in over 2 fields
+l =1 trypanosome in 1-2 fields
+ =1-3 trypanosomes per field
++ =3-15 " " "
+++ =Over 15 " " "

Results

The results of daily blood examinations are given in Tables II to V. All the mice fed on the four different diets ate their food well and showed no signs of ill-health, having smooth sleek coats and gaining weight throughout the experiment until such time as those which died of the infections were actually dying. The weights of uninfected mice given diets 1 to 4 are shown in Table X.

Diet 1 (see Table II).—Trypanosomes first appeared in the blood (+vl, +l, +) of the animals of this dietary group four to six days after inoculation. They reached acme (+++) in six to nine days and persisted in numbers till 11 to 17 days after infection. Trypanosomes were absent from the blood for two to six days. Thereafter the infection, now chronic, continued with more or less constant numbers of trypanosomes in the blood until death or termination of

TABLE III.—*Diet 2 (30% Casein). Results of Infecting Mice with Trypanosomes*

No. of Days After Inoculation	Mouse Numbers																		
	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	+vl	—	—	—	—	+vl	—	—	—	—	—	—	+vl	—	—	—	—
6	—	—	+l	—	—	—	—	+	—	—	—	—	—	—	+l	—	—	—	—
7	—	—	+	—	—	—	—	+l	—	—	—	—	+vl	—	+	—	—	—	—
9	—	—	+++	—	—	—	—	+	—	—	—	—	+++	—	—	+++	—	—	—
10	—	—	+++	—	—	—	—	+	—	—	—	+	+++	—	—	+++	—	—	—
11	—	—	+	—	—	—	—	—	—	—	—	—	+++	—	—	+++	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Dead	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	+++	—	+	++	—	—	++	—	—	—	+	—	—	—	—	—	—
17	—	—	+++	—	+++	+++	—	—	+++	—	—	—	+++	—	—	—	—	—	—
18	—	—	+++	—	+++	+++	—	—	+++	+l	+	—	+++	—	—	—	—	—	—
19	—	—	+++	—	+++	+++	—	+++	+++	—	—	+	+++	—	+vl	—	—	—	—
20	—	—	+++	—	+++	+++	—	+++	Dead	—	—	—	+++	—	—	—	—	—	—
21	—	—	Dead	—	—	—	—	+++	—	—	—	—	+++	—	—	—	—	—	—
23	—	—	—	—	—	—	—	+++	—	—	—	—	Dead	—	—	—	—	—	—
24	—	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	—	—	—	—
25	—	—	—	—	+	+vl	—	+++	—	+vl	+	+	+	—	—	—	—	+	—
26	—	—	—	—	++	+	—	+++	—	+++	+l	+	+	—	—	—	—	—	+
27	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	—	—
29	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	—	—
30	—	—	—	—	Dead	+++	—	+++	—	+++	+++	+++	+	+vl	—	—	—	+l	—
31	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	—	—
32	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	—	—
33	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	+++	—
34	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	—	—	—	+++	—
35	—	—	—	—	+++	+++	—	+++	—	+++	+++	+++	+	—	+++	—	—	+++	—
37	—	—	—	—	Dead	+++	—	+++	—	+++	+++	+++	+	—	+++	—	—	+++	+l
38	—	—	—	—	—	—	—	+++	—	+++	+++	+++	+	+	+++	—	—	+++	+++
40	—	—	—	—	—	—	—	+++	—	Dead	—	—	+++	+++	+++	—	—	+++	+++

TABLE IV.—*Diet 3 (20% Casein). Results of Infecting Mice with Trypanosomes*

No. of Days After Inoculation	Mouse Numbers																		
	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Dead	—
5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—
7	+	—	—	—	—	—	—	—	+++	—	—	+++	—	—	—	—	—	—	—
9	—	—	—	—	—	+l	—	—	Dead	—	—	+++	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—	—	—	—	Dead	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17	+l	—	—	—	—	—	—	+	—	—	+	+	—	—	—	—	—	+	—
18	+++	—	—	—	—	+vl	—	—	—	+++	+++	+++	—	—	—	—	—	+++	—
19	+++	—	—	—	—	+	+vl	—	—	+++	+++	+++	—	—	—	—	—	+++	+vl
20	—	—	—	—	—	+++	—	—	—	—	—	+++	—	—	—	—	—	+++	—
21	—	—	—	—	—	+++	—	—	—	—	—	+++	—	—	—	—	—	+++	—
23	—	—	—	—	—	+++	—	+l	—	—	—	+++	—	—	—	—	—	+++	—
24	—	—	—	+++	—	+++	—	—	—	—	—	+++	—	—	—	—	—	Dead	—
25	—	—	—	+++	—	+++	—	—	—	—	+vl	+++	—	—	—	—	—	+++	—
26	+vl	—	—	+++	—	+++	—	—	—	—	+++	+++	—	—	—	—	—	+++	—
27	+l	—	—	+++	—	+++	—	—	—	—	+++	+++	—	—	—	—	—	+++	—
29	+	—	—	+++	—	+++	—	—	+++	—	+++	Dead	—	—	—	—	—	+++	—
30	++	—	+vl	Dead	—	Dead	—	—	+++	—	+++	—	—	—	—	—	—	+++	—
31	+l	—	—	—	—	—	—	Dead	—	—	+++	—	—	—	—	—	—	+++	—
32	++	+vl	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
33	+++	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
34	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—
40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	—

— to +++ = range of numbers of trypanosomes in blood (see text, above). l = little; vl = very little.

TABLE V.—Diet 4 (10% Casein). Results of Infecting Mice with *Trypanosomes*

No. of Days After Inoculation	Mouse Numbers																		
	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179
4	—	—	—	—	—	—	+1	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	—	—	—	—	—
7	+	+1	—	+1	—	+1	+++	—	+1	+	+	—	—	+	—	—	—	+1	—
9	—	—	—	+++	—	—	Dead	—	—	+++	+vi	—	—	+++	—	—	—	—	—
10	—	—	—	Dead	—	—	—	—	—	+++	—	—	—	+++	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	+++	—	—	—	+++	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	+++	—	—	—	+++	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	+++	—	—	—	+++	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	+++	—	—	—	+++	—	—	—	—	—
16	+vi	+vi	—	—	—	—	—	—	—	—	—	—	—	Dead	—	—	—	—	—
17	+1	++	—	—	—	—	++	+1	—	—	—	—	—	—	—	—	—	+	++
18	—	+++	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	+++	+++
19	—	+++	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	+++	+++
20	—	+++	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	+++	+++
21	—	+++	—	—	—	—	++	—	—	—	—	—	—	—	—	—	—	+++	+++
23	—	Dead	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++
24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+++	+++
25	+vi	++	—	—	+vi	—	++	+++	—	—	—	—	—	Dead	—	—	—	+++	+++
26	++	—	—	—	++	+vi	++	+++	—	—	—	—	—	—	—	—	—	+++	+++
27	+++	—	—	—	+++	++	++	+++	—	—	—	—	—	—	—	—	—	+++	+++
29	+++	—	—	—	+++	++	++	+++	+vi	—	—	—	—	—	—	—	—	+++	+++
30	+++	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
31	+++	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
32	+++	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
33	+++	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
34	Dead	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
35	—	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
37	—	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
38	—	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++
40	—	—	—	—	+++	++	++	+++	++	—	—	—	—	—	—	—	—	+++	+++

— to +++ = range of numbers of trypanosomes in blood (see text, p. 854). 1 = little; vi = very little.

the experiment. All animals were successfully infected. During the 40-day period of observation three mice died at the acute stage and four at the chronic stage of the infection—that is, 7 out of 20 died.

Diet 2 (see Table III).—Trypanosomes first appeared in the blood of 5 of the 20 mice in five to seven days, and of these only three showed the normal course of increase to +++ with relapse or death at the acute stage. Of the remaining 15 mice, six did not at any time show evidence of infection in the blood, and in the other nine the first demonstrated appearance of trypanosomes was from 10 to 26 days after inoculation. In 7 of the 20 mice, trypanosomes in very small numbers appeared in the blood for a very short time, sometimes on more than one occasion, before they reached ++, after which the course of persistent chronic infection always followed till death. During the 40 days 10 of the 20 mice died of infection—only one of these died at the acute stage—and one mouse (No. 127) died of intercurrent infection. The five mice in which trypanosomes had not been demonstrated were reinoculated from a fresh mouse of the acme passage strain, and their blood was examined for a further 15 days, during which time no sign of infection was observed.

Diet 3 (see Table IV).—One mouse (No. 158) died of intercurrent infection at the beginning of the experiment. Five of the 19 mice showed trypanosomes in the blood from six to nine days after inoculation; of these only two increased to +++ at this stage. Of the remaining mice, eight showed trypanosomes in 16 to 32 days, but only four of the eight ever showed a +++ infection or reached the

chronic phase—that is, four mice showed trypanosomes but never at the level of +++. In 6 of the 19 mice, trypanosomes were not demonstrated. These animals also were reinoculated as described before, but no resulting infection was seen. Seven of the 19 mice died during the 40 days, two at the acute stage.

Diet 4 (see Table V).—Ten of 20 mice showed trypanosomes in the blood in four to nine days, four reached the +++ level and died at the acute stage. Six further mice showed trypanosomes after 16 to 26 days, all of these mice eventually showing a +++ infection which became chronic. In four mice trypanosomes were not demonstrated, and these were reinoculated as before. Trypanosomes were not seen after reinoculation. In this group there were 14 deaths—four at the acute and 10 at the chronic stage of infection.

The results of experiment I are summarized in Table VI.

Experiment II

This experiment was done with diet 1, diet 3, and diet 5, which consisted of bread, milk, and oats. Diet 5 was that used for trypanosome-infected mice in this department for many years.

Details of inoculation and blood examination were similar to those in Experiment I. Male mice of the laboratory inbred strain 3–4 months of age were allocated to the three diets, on which they were fed for a period of two weeks before inoculation with *T. congolense* (Busimbi). The period of observation after inoculation was only 23 days in this experiment.

Results

Diet 1 (see Table VII).—Trypanosomes first appeared in the blood of infected animals in four to eight days after inoculation. Acme was reached at 6 to 10 days. The trypanosomes disappeared from the blood on the eleventh to the fifteenth day after inoculation and reappeared in four to nine days after disappearance. All the animals became infected. One mouse died at the acute stage of the infection, but 9 of 10 mice survived to the chronic stage, at which all remained well till the end of the experiment.

Diet 3 (see Table VIII).—In this group only 2 of 10 mice showed trypanosomes in blood films in six to seven days. Of these two, one had demonstrable numbers of

TABLE VI.—Summary of Data from Tables II–V

Dietary Group (20 Mice in Each Group)	No. of Mice Showing Trypano- somes in Blood Within 9 Days	+ No. of Mice Reaching + Within 9 Days	No. of Deaths			No. of Mice in Which Trypano- somes Were Not Demonstrated in the Blood	No. of Mice in Which Trypano- somes were Seen but did not Reach +++ Level
			At "Acute " Stage	At "Chronic " Stage (Up to 40 Days)	Total		
1	20	20	3	4	7	0	0
2	5	3	1	9	10	5	0
3	5	2	2	5	7	6	4
4	10	4	4	10	14	4	0

* One of the mice given diet 3 died of intercurrent infection at the start of the experiment.

trypanosomes on only one day of examination, while the other, in which trypanosomes persisted over three days, did not reach acme.

Diet 5 (see Table IX).—Trypanosomes first appeared in the blood of animals in this group five to seven days after infection. Acme was reached at seven to eight days. All the animals became infected. Trypanosomes persisted in large numbers in the blood for as long as 18 days after inoculation, when they disappeared for up to five days. Four mice died at the acute stage, but there were no deaths at the chronic stage in this period of observation.

Discussion

In Experiment I all the animals in the group fed on diet 1 followed the course of infection normally observed in this

laboratory with *T. congolense* (Busimbi) (see Table II). The results of the infection in mice given diets 2, 3, and 4, on the other hand, repeated the unexpected result referred to in the introduction of many failures to infect in the normal manner with this strain. Only 5 out of 20 mice on each of diets 2 and 3 and 10 of 20 mice on diet 4 showed trypanosomes in their blood in anything approaching the normal times; and of these numbers only three animals on diet 2, two on diet 3, and four on diet 4 showed the normal acme phase (Table VI). The remaining mice relapsed before the acute stage of infection had been reached, and did not show the usual chronic phase which normally persists for variable but substantial periods of time. In the other animals on diets 2, 3, and 4 trypanosomes were not demonstrated in the blood for up to as long as 26, 32, and 26 days (Tables III, IV, and V), and indeed were not demon-

TABLE VII.—Diet 1 (Stock Diet 86). Experiment II: Results of Infecting Mice with Trypanosomes

No. of Days After Inoculation	Mouse Numbers									
	201	202	203	204	205	206	207	208	209	210
4	—	—	—	—	—	—	—	—	+vl	—
5	+l	—	+vl	—	—	—	—	+vl	+vl	+vl
6	+	+vl	+++	+vl	+vl	+vl	—	+++	+l	+
7	+++	+++	+++	+++	+++	+++	—	+++	+++	+++
8	+++	+++	+++	+++	+++	+++	+vl	+++	+++	+++
9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
11	+++	+++	—	+++	Dead	+++	+++	+++	—	+++
12	+++	+++	—	+++	—	—	+	—	—	+++
13	—	+++	—	—	—	—	—	—	—	—
14	—	+	+++	—	—	—	+	—	+++	—
16	—	—	+++	—	—	—	+	—	+++	—
18	+l	—	+++	+l	—	—	+++	+++	+++	+++
19	+++	—	+++	+++	—	—	+++	+++	+++	+++
20	+++	+	+++	+++	—	+	+++	+++	+++	+++
21	+++	+++	+++	+++	—	+++	+++	+++	+++	+++
22	+++	+++	+++	+++	—	+++	+++	+++	+++	+++
23	+++	+++	+++	+++	—	+++	+++	+++	+++	+++

TABLE VIII.—Diet 3 (20% Casein). Experiment II: Results of Infecting Mice with Trypanosomes

No. of Days After Inoculation	Mouse Numbers									
	211	212	213	214	215	216	217	218	219	220
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	+vl	—	—	—	—	—
7	+vl	—	—	—	—	—	—	—	—	—
8	—	—	—	—	+	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—

TABLE IX.—Diet 5 (Natural Diet of Bread, Milk, and Oats). Experiment II: Results of Infecting Mice with Trypanosomes

No. of Days After Inoculation	Mouse Numbers									
	221	222	223	224	225	226	227	228	229	230
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	+vl	—
6	—	+vl	—	+	++	—	+vl	++	++	++
7	+	+	+l	+++	+++	+	+++	+++	+++	+++
8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
11	+++	Dead	+++	Dead	—	+++	+++	+++	Dead	+++
12	+++	—	+++	—	—	+++	+++	+++	—	+++
13	+++	—	+++	—	—	+++	+++	+++	—	+++
14	+++	—	+	—	—	—	+++	—	—	+++
16	Dead	—	—	—	+	+	—	+	—	+++
18	—	—	+++	—	+++	+++	+++	+++	—	+++
19	—	—	+++	—	+++	+++	+++	+++	—	+vl
20	—	—	+++	—	+++	+++	+++	+++	—	—
21	—	—	+++	—	+++	+++	+++	+++	—	—
22	—	—	+++	—	+++	+++	+++	+++	—	—
23	—	—	+++	—	+++	+++	+++	+++	—	+++

— to +++ = range of numbers of trypanosomes in blood (see text, p. 854). l = little; vl = very little.

strated at all in five, six, and four animals of the respective dietary groups 2, 3, and 4, even after reinoculation with the blood of a fresh "acme" passage mouse. In mice given diet 2, 3, or 4 infection followed a much milder course than in those given diet 1. This was true in all mice not reaching or dying at the acute stage, as judged by the total numbers of trypanosomes in the blood of the animals over the period of the experiment. When the persistent chronic stage was reached, however, death was often rapid (cf. Table II and Tables III, IV, and V).

The results of Experiment I are supported by those of Experiment II. The mice fed on the casein diet 3 showed only the slightest evidence of blood-stream invasion by parasites during the 23-day period of observation. The infection followed an acute course in both the natural diets, Nos. 1 and 5, and this course was that normally recorded in this laboratory over many years.

Macraith *et al.* (1952) concluded that milk probably contains something that can inhibit or restrict the development of the asexual phase of blood-transmitted *Plasmodium berghei* in the rat and the mouse. Another possibility is that milk may be deficient in certain nutrients required by the parasite (*British Medical Journal*, 1952). The second explanation would fit the results observed in the present experiments with *T. congolense* in mice given diets with milk casein as the source of protein. It is clear that parasitaemia developed much more rapidly and attained a higher level in mice given diets 1 and 5, which were nutritionally more complex than the casein diets. Moreover, since diet 5 also contained milk, inhibition of parasite growth from the absence of undefined nutrients rather than from an anti-parasitic effect of milk might appear to be the more tenable explanation. It may be of importance to note that the mice made good gains of weight on all diets, especially on diet 4, which contained a high proportion of carbohydrate (Table X).

TABLE X.—Weekly Mean Weights (g.) of Uninfected Mice Given Experimental Diets 1-4

Diet No.*	Age of Mice (Weeks)								
	4	5	6	7	8	9	10	12	17
1	19	24	29	30	31	32	34	36	39
2	17	25	30	30	32	32	33	35	35
3	20	24	30	33	33	35	37	37	37
4	18	25	32	34	36	37	39	41	44

* Each dietary group comprised 4 males and 4 females; litter mates were evenly distributed.

Summary

Mice fed on five different diets were experimentally infected with *T. congolense*.

Parasitaemia with acute followed by chronic infection developed as expected in mice fed on two of the diets, which were diets of the type normally employed in laboratories for rearing and maintenance of mice.

In mice fed on three of the diets in which milk casein was the source of protein the parasitaemia was either delayed or prevented.

Since one of the two "normal" diets contained milk but permitted normal development of the infection it might appear that failure of the incomplete diets to nourish the parasite is the explanation of the observed results, rather than that milk itself contains an anti-parasitic agent.

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HISTOPLASMOSIS IN GREAT BRITAIN

DESCRIPTION OF A SECOND CASE OF DISSEMINATED HISTOPLASMOSIS; TREATMENT BY ETHYL VANILLATE

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Work in the past few years on human infections by the *Histoplasma capsulatum* has emphasized certain features. Generalized dissemination (disseminated histoplasmosis) is common and usually fatal. Infection confined to the skin is frequent, though, unlike cutaneous blastomycosis, the skin lesion is usually within a short while followed by manifestations of dissemination (Curtis and Grekin, 1947) and is therefore often fatal. Infection can be confined to a single organ or viscus—usually the lung—with at no time evidence of dissemination and often no past history of even mild illness. These cases are identified usually after recovery by a positive intradermal histoplasmin test or by complement fixation.

Only one other case of disseminated histoplasmosis has been reported in Great Britain, and this occurred in 1940 (Derry, Card, Wilson, and Duncan, 1942). A regular soldier aged 30 developed his illness in France in 1940 and the diagnosis was eventually made at necropsy in Great Britain. He had been resident in India in 1932-6 and in the Sudan in 1936-7; this gives a possible incubation period of three years at least (assuming the disease is not indigenous to France).

In the second case, which we report below, the patient had not been out of England for seven years. Symptoms first appeared about a year before admission to hospital, and we must therefore assume either that the disease has an incubation period of at least six years or that he contracted his illness in England. We regard the first of these suggestions as the more probable.

Arblaster (1950) and Crofton (1950) report three cases of pulmonary calcification in England in which the diagnosis of "healed" histoplasmosis was suggested because of a positive histoplasmin intradermal test. All three patients had been resident in the U.S.A. or Canada. Duncan (1945, 1947) isolated an atypical organism from a skin lesion in a man from West Africa.

In 1951 Limerick described the development of a retrocaecal abscess occurring after appendicectomy in 1947 in a man aged 35. The abscess cleared up in three weeks with drainage and was said to be due to *H. capsulatum*. No further details are given.

The cases of Duncan, Arblaster, and Limerick, however, do not conform in many ways to the usually accepted criteria and must be regarded as very doubtful. Hutchison (1952) reported a localized laryngeal lesion due to *H. capsulatum* in a fit man of 72 who had lived