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Further Observations on Chinese Kala Azar.

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[PLATES 19-20.]

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General Account.

The results of our experiments at Tsinan, in 1926, on the development of flagellates in sandflies fed on infected hamsters, and also on human cases of Chinese Kala Azar (Patton and Hindle, 1927), indicate that both *Phlebotomus major* var. *chinensis* and *P. sergenti*, but especially the former, are favourable hosts for *Leishmania*.

During the summer of 1927 these experiments were continued, employing the same methods as those described in the previous reports of our Commission. Unfortunately, owing to political disturbances, the laboratory had to be moved to Tsingtao, on the coast, where the only sandflies we succeeded in finding were *Phlebotomus taianensis* (Patton and Hindle, 1928), and in one

house odd specimens of *P. major* var. *chinensis*. Consequently, it was necessary to establish a collecting station in some region where sandflies could be obtained in greater numbers, and eventually Wei-hsien, about 100 miles from the coast, was selected as the most convenient place.*

The sandflies were bought from the local inhabitants and were at once sorted into breeding pots, or feeding boxes, according to whether they contained undigested blood or not. Each day the sandflies were sent to Tsingtao by train, and this method worked fairly satisfactorily so long as the package was not overheated, as, for example, by being exposed to the sun, as under these conditions the flies very soon died. Unfortunately, a local rebellion interrupted communications between Wei-hsien and Tsingtao for a fortnight at the height of the *P. major* season, and curtailed the number of experiments we were able to make with this species.

The flies were fed in the manner we have previously described, with the exception that all the hamsters used for feeding experiments were given an injection of urethane previous to the operation. This preliminary narcotisation was found to produce more satisfactory results, as by its use the flies when feeding were not disturbed by any movements of the animal.

The results of our experiments in 1927 confirm the observations on the type of development of Chinese *Leishmania* in the two Chinese species of *Phlebotomus* that feed on man. In the case of *P. major* var. *chinensis*, the flagellates become attached to the wall of the mid-gut and usually within three or four days of the infective feed may be seen lining the narrow anterior region adjoining the œsophagus. In a certain proportion of sandflies, the flagellates pass forward through the œsophagus and then multiply in the pharynx and extend down the proboscis. Invasion of the pharynx, when present, usually takes six days from the infective feed, although occasionally it was observed after five days. The persistence of flagellates in the gut of *P. major* does not require the presence of undigested blood, as in many instances flies which had completely digested their last meal contained large numbers of flagellates attached to the lining of the mid-gut.

P. sergenti seems to be an equally favourable host for the early development of Chinese *Leishmania*, but in this species the flagellates remain confined to the broad posterior region of the mid-gut and do not become attached to the lining of the alimentary canal. Also invasion of the œsophagus or pharynx

* We should like to express our thanks to Dr. Ewers, of the American Presbyterian Mission, for much valuable assistance in the establishment of this station and help in obtaining cases of kala azar.

has never been observed. The persistence of flagellates in the mid-gut seems to be dependent on the presence of undigested food material, unlike *P. major*, and consequently when the alimentary canal does not contain any food material the flagellates disappear. Many examples are given below in which batches of *P. sergenti* were fed simultaneously on an infective animal, and when dissected after four days' incubation period all flies that still contained undigested blood in the mid-gut were positive, and those which had completely digested the meal were negative.

The examination of between three and four thousand sandflies has shown that the above types of development are very constant, and although in one or two instances flagellates were seen in the hind-gut and once in the diverticulum, these were such obvious exceptions that their presence was probably the result of accidents. Although carefully looked for, no trace of intracellular development in the wall of the alimentary canal was ever observed, nor any invasion of the salivary gland. In *P. sergenti* the infection remains confined to the mid-gut, whilst in *P. major* var. *chinensis* the flagellates, in addition, extend forward and may invade the pharynx and proboscis.

Feeding Experiments on Human Patients.

During 1927 only four patients were used for feeding experiments, as partly owing to the widespread disturbances and also to the general distrust of new treatment, it was almost impossible to persuade anyone to come to the laboratory.

Kala Azar has not yet been recorded from Tsingtao, and although a tour of the neighbouring villages was made, no cases were found in the immediate vicinity. About 60 miles to the north and north-west, however, many villages were found to be infected, and in particular a town, Nan-Tsun, where, in a brief survey, numerous cases were observed. Also this place had a record of numerous deaths from Kala Azar, and the local official was very desirous of our opening a treatment centre in the town. As this was not possible, with great difficulty we managed to persuade four patients to come into Tsingtao for treatment. The results of feeding sandflies on these patients, who all had well-marked symptoms of the disease, are given below, from which it will be seen that two gave positive results and two negative.

Case 1.—This patient, a woman, stated that she had had the disease for 24 years, but recently the symptoms had become worse. Treatment with intravenous injections of "Stibosan" for a period of 5 weeks produced an interrupted recovery. A total of 44 *P. major* var. *chinensis* and 160 *P. sergenti*

were fed on this patient on four separate occasions, and dissected after suitable intervals with entirely negative results.

Case 2.—This patient, a boy about 11 years old, first showed signs of the disease in April, 1927, and came to the laboratory on July 1. Sandflies were fed on six occasions with the following results :—

July 1.—Fed 49 *P. sergenti* and 12 *P. major*, which were dissected after intervals of 4 and 6 days, respectively. One *P. major* showed a very slight infection of the mid-gut, but all the other sandflies were negative.

July 2.—14 *P. major* fed and dissected after intervals of 4, 5 and 8 days. One individual, dissected after 5 days, showed a few flagellates in the mid-gut, which were inoculated into a hamster with negative results. The other flies were all negative.

July 4.—11 *P. major* fed and dissected after intervals of 2, 5 and 6 days. One individual, dissected after 6 days, was positive in the mid-gut; the others were all negative.

July 16.—11 *P. sergenti* fed and dissected after 4 days were all negative.

July 18.—8 *P. sergenti* fed and dissected after 3 days interval. Six flies were negative and two positive in the mid-gut. The contents of these flies were inoculated into a giant hamster, with negative results.

July 19.—21 *P. sergenti* fed and dissected after 3 days' interval. All negative.

It will be seen that a total of 89 *P. sergenti* and 37 *P. major* were fed on this patient, and in only two of the former and three of the latter species was there any subsequent development of flagellates.

Case 3.—A woman about 40 years of age, in a very advanced stage of the disease. Treatment with "Stibosan" was begun on July 5. Sandflies were fed on six occasions as follows :—

July 2.—Fed 37 *P. major* and 24 *P. sergenti*. The latter were all dissected after 4 days' interval and one was positive in the mid-gut; the others were negative. The *P. major* were examined after intervals of 4 days, 10 negative; 5 days, 12 dissected of which 3 positive in mid-gut and 9 negative; 6 days, 3 dissected, of which 1 positive and 2 negative; 7 days, 12 dissected, of which 3 positive and 9 negative. The contents of the positive flies were inoculated intraperitoneally into three hamsters with negative results.

July 4.—30 *P. sergenti* fed and dissected after an interval of 4 days. All negative.

July 16.—2 *P. sergenti* fed and dissected after 3 days' interval. Both negative.

July 17.—13 *P. sergenti* fed and dissected after 3 days' interval. All negative.

July 19.—17 *P. sergenti* fed and dissected after 3 days' interval. 16 were negative and 1 positive in the mid-gut, which was inoculated into a hamster with negative results.

August 1.—29 *P. sergenti* fed and dissected after 3 days' interval. All negative.

A total of 37 *P. major* were fed on this patient, of which 7 subsequently showed development of flagellates in the mid-gut; and 115 *P. sergenti*, of

which only 2 were positive, but many of these flies were fed after the treatment had begun.

Case 4.—A total of 80 *P. sergenti* were fed on this patient, a woman, on three separate occasions, and in each case dissected after an interval of 3 days. All the flies were negative.

If we compare the results of feeding sandflies on patients infected with the Chinese strain of Kala Azar with those infected with the Indian strain, a great difference is observed in the number of flies which subsequently show development of flagellates. Young and Hertig (1926) in their experiments obtained entirely negative results when *P. major* were fed on human cases, and Young has used these negative results as an argument against the view that sandflies are responsible for the spread of the disease in China.

Although we obtained positive results in both 1926 and 1927 by feeding sandflies on human patients infected with Kala Azar, only 4 out of 14 patients were infective to these insects, and even in these 4 the proportion of sandflies that showed any subsequent development of flagellates was only about 5 per cent. It is evident, therefore, that the Chinese strain of *Leishmania* is less infective to *Phlebotomus* than the Indian strain. This difference seems to be correlated with a lower degree of virulence, for in human beings the Chinese strain produces a more chronic type of disease than the Indian strain of Kala Azar.

Feeding Experiments with Hamsters infected with Chinese Kala Azar.

The results of feeding sandflies on human patients, all in advanced stages of the disease, gave such a large number of negative results that in 1927 especial attention was directed towards the influence, if any, of the nature of the infection in the vertebrate host. Consequently the infected hamsters were not specially selected as in the previous year, as we wished to find out what factors influenced the development of flagellates in sandflies that had ingested the blood of animals suffering from leishmaniasis.

The results of our experiments with both *P. major* and *P. sergenti* are summarised in Table I, which gives the number of each hamster, the size of the spleen, the number of parasites in the liver, spleen and bone marrow respectively, and the duration of the infection from the date of inoculation to the date of the first feeding experiment. In the case of *P. major*, records are given of the number showing infection of the pharynx. *P. sergenti*, which was the only species available for the later experiments, is not such a good test for infectivity as *P. major*, since the flagellates die out as soon as the

Table I.

Number of hamster.	Duration of infection (in months).	Number of parasites in organs of hamster.			<i>P. major</i> .		<i>P. sergenti</i> .		Remarks.
		Liver.	Spleen.	Bone-marrow.	Total number fed.	Number infected.	Total number fed (less empty ones).	Number infected.	
126	15	++	++	++	23	7	12 (-3)	7	Six <i>P. major</i> positive in pharynx. Three inoculation experiments positive.
140	15	+++	+++	+++	31	17	13 (-4)	7	
141	15	+++	+++	+++	84	81	44 (-13)	27	
146	15	++	++	+	5	0	11	0	Seven <i>P. major</i> positive in pharynx. Positive in pharynx.
155	15	Neg.	+	Neg.	2	0	22	0	
158	15	++	++	+	69	66	133 (-37)	96	
162	15	++	++	++	—	1	14	0	Positive in pharynx.
173	15	+++	+++	+++	1	1	—	—	
260	14	+++	+++	+++	2	1	95 (-53)	41	
303	14	+++	+++	+++	—	1	19 (-13)	2	Positive in pharynx. Details of this experiment are given below.
323	13	+++	+++	+++	2	1	—	—	
331	13	+++	+++	+++	100	96	84 (-23)	59	
382	13	+++	+++	+++	47	38	40 (-18)	22	Four <i>P. major</i> positive in pharynx. One inoculation positive.
644	8	+	++	++	14	0	6	0	
653	8	+++	+++	+++	10	0	9	1	
655	8	+++	+++	+++	2	2	40 (-25)	14	One <i>P. major</i> positive in pharynx.
658	8	+++	+++	+++	2	0	24 (-18)	5	
672	8	+++	+++	+++	9	4	70 (-21)	34	
673	8	+	++	++	8	1	2	0	Seven <i>P. major</i> positive in pharynx. Two inoculations positive.
690	8	+	Not examined.	+	22	9	10	2	
693	8	+	+++	++	2	1	9	3	
712	8	Neg.	Neg.	Neg.	—	—	23 (-8)	0	Seven <i>P. major</i> positive in pharynx. Two <i>P. major</i> positive in pharynx. Two inoculations positive.
737	8	+++	+++	+++	23	21	44 (-7)	37	
738	8	+++	+++	+++	32	19	46 (-12)	26	
753	6	+++	+++	+++	17	9	25 (-2)	23	Skin positive.
756	6	+++	+++	+++	7	0	36 (-25)	11	
758	16	+++	+++	+++	—	—	12	6	
759	6	+++	+++	+++	5	2	31	1	
760	6	+	+	+	10	2	12	4	
800	6	+	Neg.	+	—	—	26 (-13)	5	

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blood is digested. Consequently, it is necessary to strike a mean between the most favourable period for the development of the flagellates, and the time taken to digest the blood. In practice, the flies were left for 3 or 4 days and when dissected any that contained no trace of food material (which were invariably negative) were deducted from the total number. In Table I the number of these empty flies is indicated in brackets after the total number dissected.

Hamster No. 331.

The results of feeding experiments with this hamster are given in detail as it represents one showing a very high degree of infectivity to sandflies. The animal was inoculated intraperitoneally on May 10, 1926, with flagellates from a 4-day old culture. Eight months later parasites were found on liver puncture, and on May 20, 1927, parasites were also found in the peripheral blood. Subsequent examinations of the blood in June were sometimes negative and sometimes positive.

Phlebotomus major were fed on this animal on various dates, with the following results :—

June 10.—7 fed and 1 dissected after 2 days and the others after 6 days. All heavily infected and the 6 individuals dissected after 6 days all showed anterior growth of the flagellates. One fly showed a heavy infection in the pharynx extending to the tip of the proboscis. The contents were inoculated into 3 hamsters with negative results.

June 15.—19 fed and dissected after intervals of 3, 5, 6, 7 and 8 days, respectively. With one doubtful exception all the flies were heavily infected with flagellates, and 15 dissected on the seventh and eighth days all showed infection of the pharynx and proboscis. The contents of these flies were inoculated into 5 hamsters with negative results.

June 21.—9 fed and dissected after intervals of 5, 6 and 7 days, respectively. All heavily infected up to the œsophagus but not in the pharynx. Contents inoculated into 3 hamsters with negative results.

June 25.—16 fed and dissected after intervals of 5 and 6 days. Fourteen were heavily infected, 4 in the pharynx, and 2 were negative. The contents of these flies were inoculated into 3 hamsters and one of these, inoculated intraperitoneally with flagellates from 4 infected flies fed 6 days previously, became infected with *Leishmania*. The other two were negative.

July 1.—30 fed and dissected at intervals of 3 to 9 days. Omitting obviously degenerate examples all were heavily infected, and from the seventh day onwards 3 out of 16 were positive in the proboscis. Their contents were inoculated into 5 hamsters and the flies re-fed on normal animals with negative results.

July 8.—6 fed and dissected after intervals of 4, 5 and 6 days were all positive. The contents of one were inoculated into a hamster with negative results.

July 16.—3 fed and dissected after 5 days' interval. All positive, one of them showing flagellates in the pharynx. These were inoculated into a giant hamster with negative results.

July 23.—5 fed and dissected after 3 and 4 days' interval. All positive, but not in pharynx. The contents were inoculated into 2 hamsters with negative results.

July 27.—2 fed and dissected after 3 and 5 days' interval, respectively. The latter was heavily infected in the œsophagus; the contents were inoculated into 2 hamsters with negative results.

August 1.—3 fed and dissected after intervals of 3, 4 and 7 days. Two positive and one negative, but degenerated. The contents were inoculated into two hamsters also the flies re-fed on a normal hamster with negative results.

Summarising the results of these experiments it will be seen that 100 *P. major* were fed on this hamster, all of which, with one or two doubtful exceptions, showed a heavy infection with flagellates in the mid-gut. In every case the flagellates had grown forward to the anterior end of the mid-gut, and out of 57 flies that were dissected after an interval of 6 or more days from the infective feed, 15 of them, approximately one quarter, showed flagellates in the pharynx and proboscis. Although no hamsters were infected by the bites of these flies when re-fed, in one instance a typical infection with *Leishmania* was produced by the intraperitoneal inoculation of the contents of four infected flies.

Phlebotomus sergenti were fed on this hamster on four occasions as follows :—

June 10.—10 fed and dissected after 2 and 3 days were all positive in the mid-gut. The contents of 8 flies dissected on the third day were inoculated intraperitoneally into a hamster which became infected and was heavily infected when examined 6 months later.

June 15.—39 fed and dissected at intervals of 3 and 4 days. Eleven flies were empty, and consequently negative, but the remaining 28 all contained numerous flagellates. The contents of 27 positive flies, fed 3 days previously, were divided into two portions and one inoculated intracutaneously, and the other intraperitoneally into two hamsters. The hamsters which received the intracutaneous inoculation remained uninfected, whilst the other animal, inoculated intraperitoneally, showed a heavy *Leishmania* infection when examined after an interval of six months.

August 8.—9 fed and dissected after 3 days. Seven positive and two empty ones negative. The contents of one of the infected flies were inoculated into a hamster with negative results.

August 14.—14 fed and dissected after 3 days' interval. Seven positive and 7 empty ones negative.

It will be seen that 72 *P. sergenti* were fed on this animal and, with the exception of 20 empty flies, every individual when dissected showed flagellates in the mid-gut, and in two instances infections were produced in hamsters by the intraperitoneal inoculation of the contents of infected sandflies.

Correlation between Skin Infection and Infectivity to Sandflies.

The certainty with which sandflies became infected when fed on the above described hamster was so remarkable that a careful examination was made of this animal in order to see if any explanation could be found of this high degree of infectivity. The most obvious explanation seemed to be the presence of parasites in the peripheral circulation, and therefore films were made of the blood at the same time as the sandflies were fed. No correlation could be found between the presence of parasites in the blood and the development of flagellates in the sandflies that fed on the hamster. The blood films were negative for *Leishmania* on three occasions, when all the sandflies became infected, and although the blood was positive on four other occasions, the parasites were so rare that their numbers were insufficient to explain the development of such large numbers of flagellates as were present in the insects after only 3 days. Consequently, when the hamster died all its organs were carefully examined and it was found that the sub-dermal layer contained incredibly large numbers of *Leishmania*, in certain regions the infection being so intense as to produce nodules closely resembling those of Oriental Sore (see figs. 1 and 2). A section of a typical part of the skin of this hamster is shown in figs. 3 and 4. The parasitised endothelial cells, clasmotocytes, are seen to have migrated from the peripheral blood vessels into the surrounding tissue and in fig. 3 may be seen arranged concentrically around one of the vessels. It is evident that in feeding, the proboscis of the sandfly must pierce some of these infected cells and the contained parasites be ingested.

In addition to this hamster No. 331, several other animals were examined to see whether there was any correlation between the presence of *Leishmania* in the skin and the number of sandflies that became infected when fed on these hosts. The results clearly indicate that there is such a correlation for, with one exception, parasites were found in the sub-dermal tissues of all animals that were infective to sandflies, whilst conversely, no matter however intense the infection of the internal viscera, unless parasites were present in the skin, insects fed on such hamsters remained negative. Hamster No. 800 is an example of an animal with a very slight infection of the liver and bone-marrow, and the spleen was negative, yet 5 out of 13 *P. sergenti* that were fed on it showed development of flagellates. Blood films made immediately after the feeding experiment were negative, but when sections of the skin were examined a moderate infection was found to be present.

Hamsters Nos. 856, 860 and 883 are good examples of animals in which the

liver, spleen and bone-marrow were very heavily infected, but which gave negative results when sandflies were fed on them. The examination of the skin in these cases, and also in all others in which the hamster was negative to sandflies, has never resulted in the discovery of *Leishmania* in the subdermal tissues. One individual, hamster No. 922, which had been inoculated only one month previously, gave positive results when sandflies were fed on it, but no parasites could be found in the portion of skin examined by us. The examination of the blood, however, was negative and one is compelled to assume that in this individual the skin infection was very localised and the flies fed on a particularly favourable region.

In view of the undoubted fact that parasites may sometimes be present in the peripheral blood, although in extremely small numbers, the sandflies may occasionally derive their infections from this source, but there is such a marked correlation between the number of parasites in the skin and the proportion of flies becoming infected that in the majority of cases the infection would seem to be derived from the skin.

Existence of Strains of Varying Degrees of Virulence.

The extremely variable results obtained by feeding sandflies on hamsters inoculated at the same time with similar doses of infective material is shown in the above table, and clearly indicates the importance of the vertebrate host in producing variations in infectivity towards the insect host. It seemed of interest, therefore, to see if there was any evidence of the existence in nature of human strains of varying degrees of virulence. With this object a number of hamsters were infected by inoculating them with the material obtained by liver puncture of patients suffering from Kala Azar. Five strains were thus isolated, subinoculated into other hamsters, and then tested as regards their infectivity to sandflies.

Human Strain A.—On May 19, 1926, a hamster was inoculated intraperitoneally with liver puncture material from case No. F 1584*, a typical case of Kala Azar in a Chinese patient. Ten months later this hamster was killed and examined for *Leishmania*. The spleen was only moderately enlarged, but the liver, spleen and bone-marrow contained large numbers of parasites. A saline suspension of the liver and spleen of this animal was subinoculated into 12 hamsters, care being taken to give each animal the same dose.

* The case numbers are those of the Hospital of the Shantung Christian University, Tsinan.

The following table indicates the results of the inoculations, and also the percentage of sandflies that became infected when fed on these animals :—

Table II.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen.	Number of parasites in—			
			Liver.	Spleen.	Bone-marrow.	
A 1	5 months	Much enlarged	+	+	+++	60 per cent.
A 2	1 day	—	—	—	—	—
A 3	10 days	Normal	+	+	Neg.	—
A 4	5 months	Enlarged	+	+	+	None.
A 5	15 days	Normal	Neg.	Neg.	Neg.	—
A 6	5 months	Much enlarged	++	++	++	None.
A 7	5 months	Enlarged	++	++	+++	31·3 per cent.
A 8	5 months	Much enlarged	+++++	+++++	+++++	87 per cent.
A 9	5 months	Much enlarged	+++++	+++++	+++++	33 per cent.
A 10	4 months	Enlarged	+	+	Neg.	9 per cent.
A 11	2 months	Enlarged	+	+	+	—
A 12	5 months	Much enlarged	++	++	++	22 per cent.

Human Strain B.—On May 1, 1926, hamster No. 305 was inoculated intraperitoneally with liver puncture material from case No. F 1488, and killed on March 14, 1927. The spleen of this animal was very much enlarged and the liver and spleen contained enormous numbers of parasites. The bone-marrow was only moderately infected. A liver and spleen suspension was inoculated intraperitoneally into 12 hamsters, each receiving an equal quantity of infective material.

The results are indicated in the following table :—

Table III.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen.	Number of parasites in—			
			Liver.	Spleen.	Bone- marrow.	
B 1	5 months	Slightly enlarged	Neg.	Neg.	Neg.	None.
B 2	5 months	Enlarged	++++	++++	++	8.7 per cent.
B 3	5 months	Enlarged	++	+	+	None.
B 4	7 days	Normal	++	++	Neg.	—
B 5	5 months	Enlarged	++++	++++	++++	33.3 per cent.
B 6	2 months	Enlarged	++	++++	++++	—
B 7	5 months	Normal	++	++	+	10 per cent.
B 8	2 days	—	—	—	—	—
B 9	5 months	Enlarged	+++	+++	+	20 per cent.
B 10	2 months	Enlarged	+	++	Neg.	—
B 11	3½ months	Enlarged	Degenerated.			—
B 12	5 months	Enlarged	++++	++++	++++	50 per cent.

Human Strain C.—On June 18, 1926, hamster No. 429 was inoculated intra-peritoneally with liver puncture material from case No. F 1734, and killed on March 15, 1927. The spleen of the animal was much enlarged and both liver and spleen contained very large numbers of *Leishmania*.

Twelve hamsters were inoculated from this animal and the results are indicated in the following table :—

Table IV.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen.	Number of parasites in—			
			Liver.	Spleen.	Bone-marrow.	
C 1	3½ months....	Enlarged	+++	+++++	+++++	None.
C 2	2 months	Normal	Neg.	Neg.	Neg.	—
C 3	5 months	Enlarged	+	+	Neg.	None.
C 4	9 days	Normal	+	Neg.	Neg.	—
C 5	3½ months....	Much enlarged	+	++	++	1 out of 6.
C 6	5 weeks	Enlarged	++	++	Neg.	—
C 7	5 months	Much enlarged	+++++	+++++	+++	None.
C 8	5 months	Much enlarged	++	+	+	None.
C 9	5 months	Slightly enlarged ..	+	Neg.	Neg.	None.
C 10	3½ months....	Enlarged	+++++	+++++	+++++	None.
C 11	5 months	Enlarged	++	++	+++	None.
C 12	5 months	Slightly enlarged ..	Neg.	+	Neg.	None.

Human Strain D.—On July 5, 1926, hamster No. 468 was inoculated intra-peritoneally with liver puncture material from case No. F 1836, and killed on March 15, 1927. The spleen of this animal was enlarged, and the liver, spleen and bone-marrow all contained very large numbers of parasites.

Twelve hamsters were inoculated from this animal and the results are indicated in the following table :—

Table V.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen.	Number of parasites in—			
			Liver.	Spleen.	Bone- marrow.	
D 1	5 months	Slightly enlarged	+++	+++	+++	None.
D 2	5 months	Much enlarged	+++++	+++++	+++	None.
D 3	5 months	Slightly enlarged	+	+	Neg.	None.
D 4	2 months	Enlarged	+	+	Neg.	—
D 5	5 months	Enlarged	+++++	+++++	+++++	None.
D 6	5 months	Enlarged	+++++	+++++	++++	None.
D 7	5 months	Much enlarged	+	+	+	None.
D 8	3½ months....	Slightly enlarged	+	Degen erated		None.
D 9	5 months	Much enlarged	++	++	++	None.
D 10	3½ months....	Slightly enlarged	+	+++	+	2 out of 19 (-11).
D 11	5 months	Much enlarged	+++++	+++	+++++	1 out of 18 (-12).
D 12	5 months	Much enlarged	++	++	++	None.

Human Strain E.—On June 18, 1926, hamster No. 430 was inoculated intra-peritoneally with liver puncture material from case No. F 1733 and killed on March 15, 1927. The spleen of this animal was considerably enlarged, but the liver and spleen contained only small numbers of *Leishmania* and the bone-marrow was negative. Twelve hamsters were inoculated from this animal with the following results :—

Table VI.

Number of hamster.	Interval between inoculation and death.	Result of post-mortem examination.				Percentage of sandflies showing flagellates when fed on hamster.
		Size of spleen.	Number of parasites in—			
			Liver.	Spleen.	Bone-marrow.	
E 1	6 weeks	Slightly enlarged	Neg.	Neg.	Neg.	—
E 2	5 months	Not enlarged.....	+	+	+	None.
E 3	5 months	Not enlarged.....	Neg.	Neg.	Neg.	None.
E 4	5 months	Not enlarged.....	Neg.	Neg.	Neg.	None.
E 5	5 months	Slightly enlarged	+	+	Neg.	None.
E 6	5 months	Slightly enlarged	+	+++	+	None.
E 7	5 months	Slightly enlarged	+	++	+	None.
E 8	5 months	Slightly enlarged	Neg.	Neg.	Neg.	None.
E 9	5 months	Enlarged	+	+	Neg.	None.
E 10	6 weeks	Normal	Neg.	Neg.	Neg.	—
E 11	5 months	Not enlarged.....	Neg.	Neg.	Neg.	None.
E 12	4 months	Slightly enlarged	Neg.	Neg.	Neg.	None.

A comparison of these five strains A to E shows what different degrees of virulence are met with in nature. All five patients were advanced cases of Kala Azar with no obvious differences in the clinical symptoms, yet by sub-inoculation into hamsters very different results were obtained as regards the infectivity of the parasite.

Strains A and B both possess a comparatively high degree of infectivity to hamsters, and also to sandflies fed upon them, for in strain A six out of eight animals were infective to sandflies, and in strain B five out of six. It is noteworthy, moreover, that in strain A a high degree of infectivity to sandflies might be associated with a mild infection in the hamster, as in experiment A 1. Strain C shows a high degree of infectivity in the vertebrate host, for all the hamsters inoculated except one became infected with *Leishmania* in varying degrees of intensity. With the exception of one insect, however, all sandflies fed on these animals remained negative, so in this strain a high degree of virulence in the vertebrate host is accompanied by an almost complete lack of infectivity towards the insect host. Strain D also shows the same high infectivity towards the vertebrate host, and low infectivity to the sandfly.

Strain E is an example of one with a very low degree of infectivity in the hamster, and also in the sandfly, for none of the insects fed on animals infected with this strain showed any development of flagellates.

The presence of such different strains, revealed by the detailed investigation of only five cases of the disease, suggests that in nature a similar, if not greater,

range of variation may be expected to occur. The different results obtained by feeding sandflies on patients, as described above (p. 601), also supports this view, and most of the Chinese cases that we have examined would seem to possess a comparatively low degree of infectivity to the insect host.

The occurrence of strains of such varying degrees of virulence doubtless explains the epidemiology of the disease, which, as is well known, is very unequally distributed in the endemic areas.

Other things being equal, when a strain with a high degree of infectivity is present, a large number of cases may be expected to develop in the neighbourhood, but when, as is the general rule in China, the strain has only a low degree of infectivity, very few cases will occur; so one finds occasional villages in which a considerable proportion of the inhabitants are infected, but in the great majority of the towns or villages only three or four cases will be present in each. It is suggestive of different degrees of virulence that in highly infected villages the disease is much more feared, and according to local reports seems to be more rapidly fatal, than in regions where only isolated cases occur.

Transmission Experiments in 1927.

The methods used in 1927 were identical with those employed by the Kala Azar Commission during the previous year and described by Hindle and Patton (1927).

Experiments with Phlebotomus major var. chinensis.

- (a) *Feeding Experiments.*—Twenty-six hamsters were exposed to the bites of sandflies which had fed on infected hamsters, respectively, 3, 4, 5, 6, 7 and 8 days previously. In every case the flies were proved to contain flagellates by subsequent dissection, and in one instance, S 151, these parasites produced infection when inoculated intraperitoneally into another animal. All the hamsters remained negative after intervals of approximately 6 months.
- (b) *Intracutaneous Inoculation.*—Five hamsters were inoculated intracutaneously with the contents of infected sandflies at various intervals after their infective feeds, but all the animals remained negative.
- (c) *Intraperitoneal Inoculation.*—A total of 124 hamsters were inoculated intraperitoneally with saline suspensions of the gut contents of one or more sandflies that had fed on an infected animal respectively 2 to 10 days previously, some of the flies having re-fed once or twice. Seven of these animals subsequently became infected with *Leishmania*, and the particulars of these positive experiments are as follows:—

Experiment S 92.—Hamster inoculated intraperitoneally with contents of seven infected sandflies fed 6 days previously on infected hamster 141 and kept at 25° C. Six months later this animal was killed and found to be heavily infected with *Leishmania*. Two other hamsters each inoculated with the contents of a single infected sandfly from the same batch remained uninfected.

Experiment S 104.—Hamster inoculated intraperitoneally with contents of five infected sandflies, fed 6 days previously on infected hamster 331, and kept at 25° C. Six-and-a-half months later the animal was killed and found to be well infected with *Leishmania*. Two other hamsters inoculated with the contents of sandflies from the same batch remained negative, although one of them received the contents of four, and the other of five, infected insects after 5 and 6 days' interval, respectively.

Experiment S 151.—Giant hamster, inoculated intraperitoneally with contents of 12 infected sandflies, fed 7 days previously on infected hamster 382, and refed 4 days previously on a normal animal; one fly was refed a second time the day before it was dissected. The flies were kept at 25° C. Nearly 7 months later the hamster was killed and found to be moderately well infected with *Leishmania*. Four hamsters that had been exposed to the bites of this batch of sandflies remained negative and also three other animals that received inoculations of the contents of infected flies.

Experiment S 167.—Hamster, inoculated intraperitoneally with the contents of one infected sandfly fed 9 days previously on infected hamster 141 and re-fed 4 days later on a normal animal. The flies were kept at 25° C. Six months later the hamster was killed and found to have a very slight infection with *Leishmania*. Two other hamsters also inoculated with the contents of infected sandflies from the same batch remained uninfected, and also two other animals exposed to the bites of infected sandflies.

Experiment S 173.—Giant hamster, inoculated intraperitoneally with contents of six infected sandflies fed 6 days previously on infected hamster 141 and kept at 26° C. Six months later this giant hamster was killed and found to be moderately well infected with *Leishmania*. Another giant hamster inoculated with the contents of six flies from the same batch, which were dissected the previous day, remained uninfected.

Experiment S 184.—Giant hamster, inoculated intraperitoneally with contents of three infected sandflies fed 6 days previously on infected hamster 738 and kept at 27° C. Six months later this animal was killed and found to be heavily infected with *Leishmania*. Another giant hamster inoculated with the contents of three flies from the same batch 5 days after the infective feed remained negative, and also one exposed to the bites of these flies.

Experiment S 215.—Giant hamster, inoculated intraperitoneally with the contents of one infected sandfly fed 5 days previously on infected hamster 738 and kept at 28° C. The animal was killed nearly 6 months later and found to be heavily infected with *Leishmania*.

Experiments with P. sergenti.

- (a) *Intracutaneous Inoculation.*—Forty-one hamsters were inoculated intracutaneously with flagellates from the guts of sandflies previously fed at various intervals on infected hamsters. In every case the animals remained negative, although in one instance, when the same dose was inoculated intraperitoneally, the hamster became infected with *Leishmania*.
- (b) *Intraperitoneal Inoculation.*—Forty-eight hamsters were inoculated intraperitoneally with saline suspensions of the gut contents of one or more sandflies previously fed on infected hamsters at various intervals. Two animals

subsequently became infected with *Leishmania* and particulars of these experiments are given below :—

Experiment S. 15.—Hamster, inoculated intraperitoneally with contents of eight infected sandflies fed 3 days previously on infected hamster No. 331 (see above) and kept at 25° C. Six-and-a-half months later the hamster was killed and found to be heavily infected with *Leishmania*.

Experiment S. 43.—Hamster, inoculated with contents of 27 infected sandflies fed 4 days previously on hamster No. 331 (see above, p. 606) and kept at 25° C. Six-and-a-half months later this hamster was killed and found to be very heavily infected with *Leishmania*.

Discussion of Transmission Experiments.

The results of these experiments with both species of sandflies show that *Leishmania* in an infective state may develop in the alimentary canal of insects which have fed on animals suffering from this disease. The negative results of the feeding experiments agree with those obtained last year, but in no degree invalidate the author's view that in China *Phlebotomus major* var. *chinensis* is responsible for the transmission of *Leishmania*. Experimental difficulties prevented us from exposing hamsters to the bites of large numbers of infected sandflies at the same time, which must often occur in nature, when a person may be bitten by a hundred or more sandflies in the course of a single night. Moreover, the subcutaneous method of inoculation has been shown to be much less liable to produce infection than the intraperitoneal one, and in the case of hamsters inoculated intraperitoneally with the whole contents of varying numbers of infected *P. major* only 7 out of 124, or approximately 1 in 18, became infected.

Congenital Transmission.

The occurrence of Kala Azar in Chinese infants which, by reason of their age, could not possibly have been exposed to the bites of sandflies, shows that other methods of infection must take place occasionally. One of the most striking of these cases, for particulars of which I am indebted to Dr. Lei, of the London Mission Hospital, Tientsin, is recorded in detail.

The patient, a four months' old Chinese baby, was brought by its mother to the out-patient department of this hospital on March 19, 1926. On examination, the spleen was found to be greatly enlarged, extending half-way across the abdomen, and the child was very anæmic and emaciated. The mother stated that she first noticed a lump in the abdomen about two months previously, and the baby was then taken to a local Chinese practitioner who diagnosed the affection as "P'i Chi," or spleen disease. "In spite of taking a good deal of Chinese medicines, the baby showed no improvement, suffered from diarrhoea

and grew thinner." In view of the age of the child, Dr. Lei was doubtful as to its being a case of Kala Azar, and therefore made a spleen puncture and found abundant *Leishmania*.

The main interest of this case lies in the fact that it could not possibly have been exposed to the bites of sandflies, as their season ended approximately two months before the child was born. Although the mother showed no obvious signs of disease it is difficult of explanation except on the hypothesis of congenital transmission. Low and Cooke (1926) recorded a case of Indian Kala Azar in a child born in England, and there can be no doubt that in this patient the infection was derived from the mother who was also infected.

In the Tientsin case mentioned above, the early development of the disease, after only eight weeks, strongly supports the view that infection took place *in utero*, even though the mother was not shown to be infected. Dr. Marshall Hertig kindly informed me of a similar case at Hsü-Chowfu in which the patient, a five months old child, was successfully treated for Kala Azar at the local mission hospital. This infant also, from the date of its birth, could never have been exposed to the bites of sandflies. During 1927, enquiries were made in the neighbourhood of Wei-hsien where Kala Azar is endemic, and other cases discovered of infants with large spleens that could not have been bitten by sandflies. Although we were unable to obtain punctures of these cases, the clinical symptoms were characteristic of the disease. Moreover, the splenic enlargement in children was so well known in the neighbourhood as to have received a special name, Nai P'i, or milk spleen. Many of the inhabitants of endemic villages, including our assistant Mr. Fang Lan Chow, record having suffered from this splenic enlargement in infancy, and state that the disease is often fatal.

Although the evidence is incomplete, there seems to be no other obvious explanation of this splenomegaly except Kala Azar, and one is led to assume that not only may the disease be transmitted congenitally, but also a number of cases recover from the infection without treatment. Apart from the statements of persons who seem to have had the disease and recovered from it, we have met cases of undoubted Kala Azar in which the patients stated that they had suffered from enlarged spleen for many years—in one case as long as 25 years—and it is only a slight step from such a chronic infection to one which is no longer fatal.

Experiments with hamsters support the view that a certain number of cases recover without treatment, as a small proportion of these animals became negative after having been shown to be infected. Attempts to demonstrate

congenital transmission in these animals failed, as we were unable to get them to breed in captivity. A dog which was heavily infected with Chinese Kala Azar, the result of inoculation, had a litter of three pups which were stillborn, but the microscopic examination of smears from their organs, and also cultures, were negative.

Summary of Results.

1. *Phlebotomus major* var. *chinensis* is the most favourable species for the development of *Leishmania*, and in this insect the flagellates become attached to the lining of the mid-gut and grow forward until they reach the anterior part of the gut. Invasion of the pharynx usually takes 6 days and under favourable conditions about 25 per cent. of the flies show a proboscis infection.

2. *Phlebotomus sergenti* is an equally favourable host for the early development, but in this species the infection remains confined to the broad posterior regions of the mid-gut, does not become attached to the lining of the gut, and never invades the proboscis. The infection is dependent on the presence of undigested food material and soon disappears when the gut is empty.

3. Four out of 14 patients infected with Kala Azar gave positive results when sandflies were fed on them, but only about 5 per cent. of the flies became infected.

4. Experiments with a large series of hamsters infected with Chinese Kala Azar show that considerable variation may be observed in the proportion of sandflies that show development of flagellates after feeding on infected animals, ranging from completely negative up to practically 100 per cent.

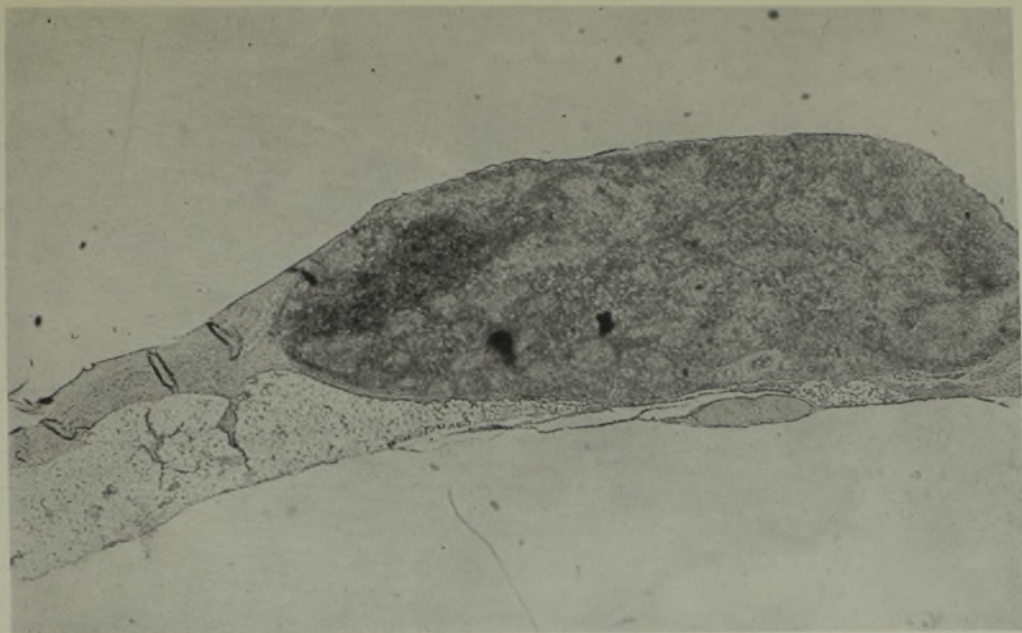
5. In hamsters there is a marked correlation between the number of parasites in the skin and the proportion of flies becoming infected.

6. No correlation could be found between the presence of parasites in the circulating blood and infectivity to sandflies.

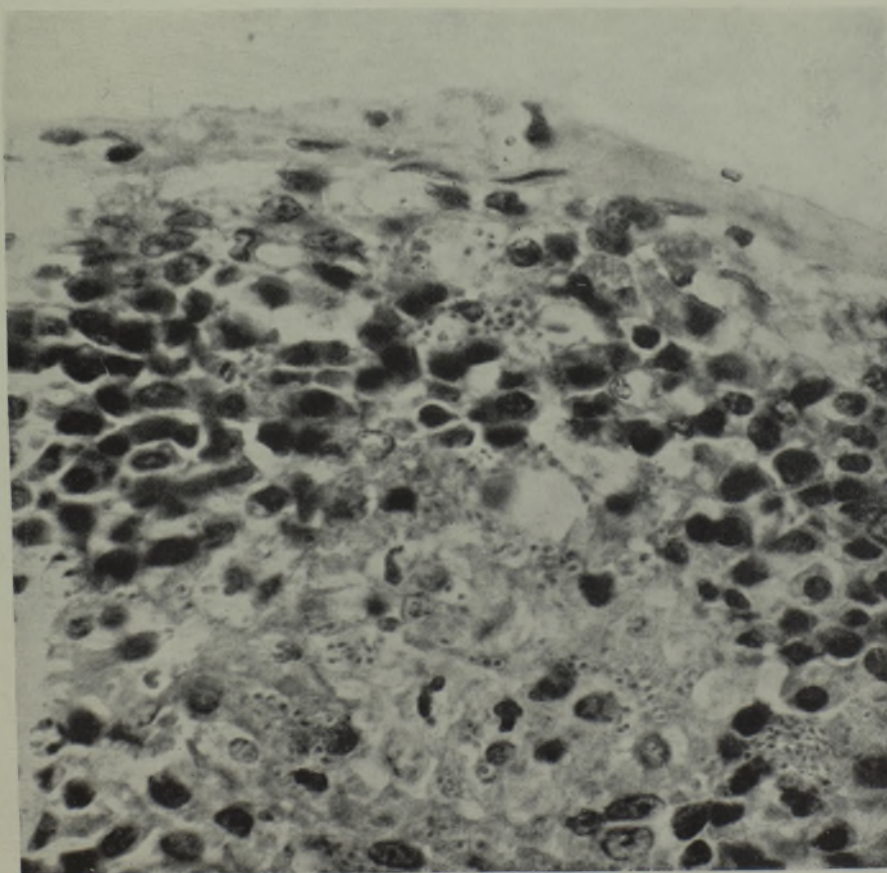
7. A study of five human strains in hamsters showed the existence of varying degrees of virulence, ranging from a strain which was completely non-infective to sandflies, up to one which had a high degree of infectivity both to hamsters and to sandflies.

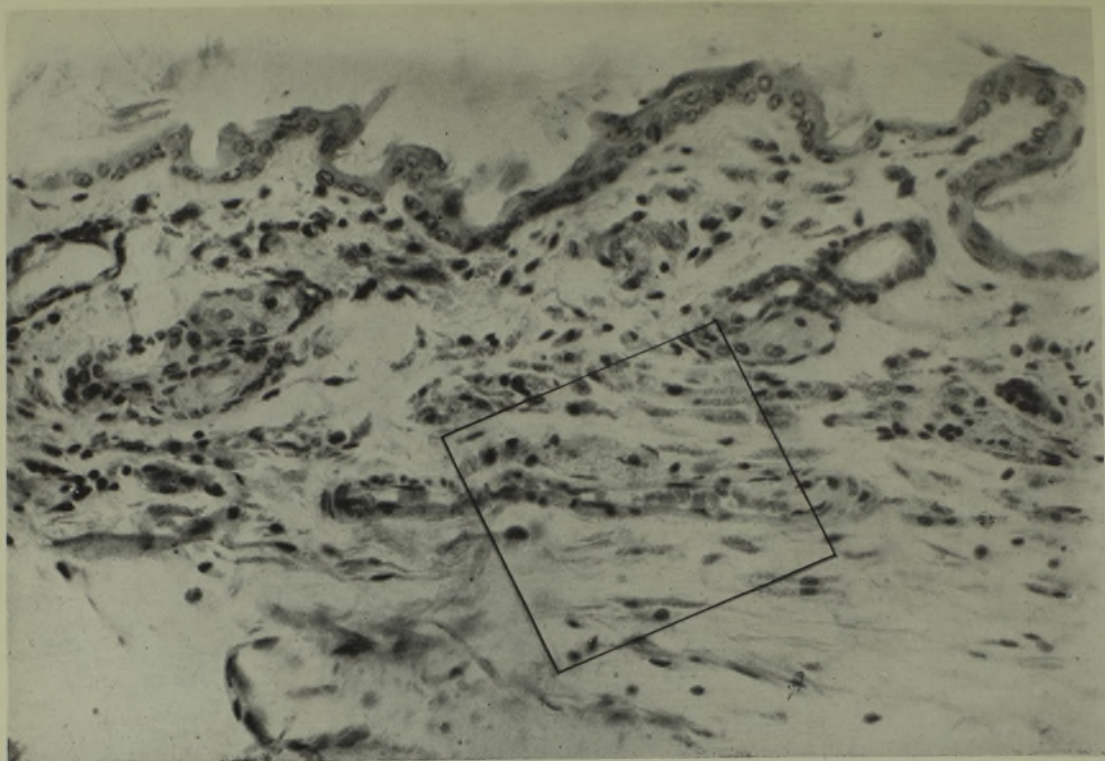
8. Both *P. major* var. *chinensis* and *P. sergenti*, infected with flagellates, gave negative results when fed on normal hamsters, and also when their contents were inoculated into the skin of hamsters. The intraperitoneal inoculation of the contents of one or more infected flies gave positive results in 7 out of 124 experiments with *P. major*, and 2 out of 41 with *P. sergenti*.

9. There is evidence in support of the view that congenital transmission

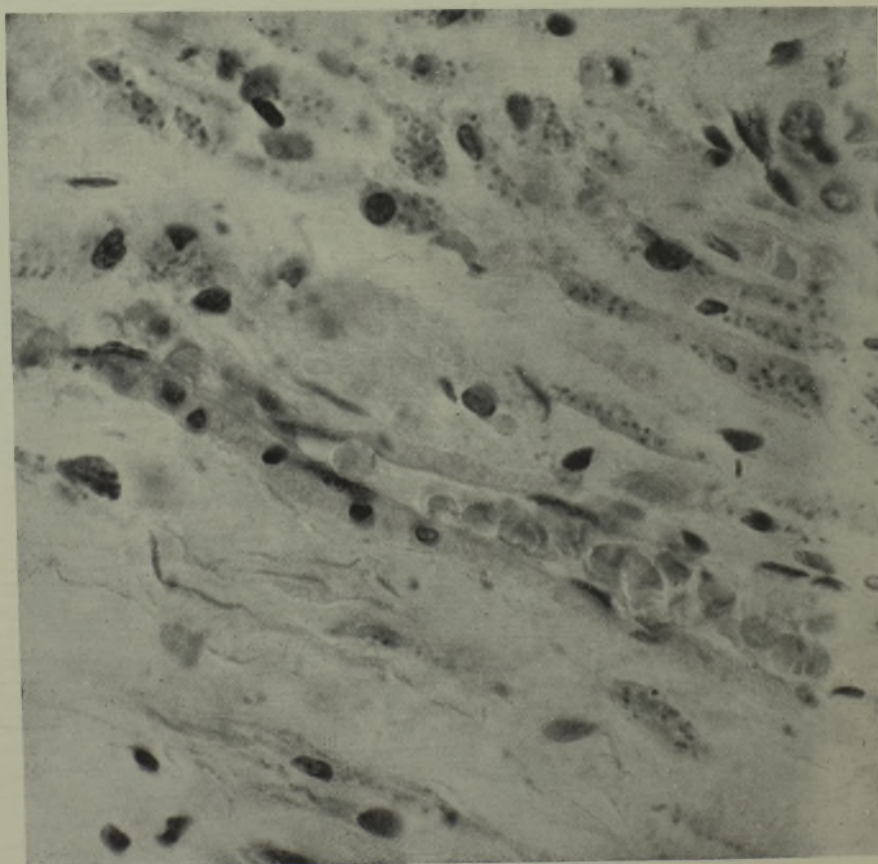


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occurs in Chinese Kala Azar, and also that a number of children recover from the disease without any treatment.

Finally, the author would like to express his heartiest thanks to Dr. Hosmer Johnson and Mr. Feng Lan Chow for their generous assistance and co-operation in the experiments recorded above.

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DESCRIPTION OF PLATES.

- FIG. 1.—Section of skin of hamster 331 ($\times 16$) infected with Chinese Kala Azar, showing presence of a nodule resembling the lesions found in Oriental Sore.
- FIG. 2.—More highly magnified portion of this nodule, showing the presence of numerous clasmatoocytes filled with *Leishmania* ($\times 950$).
- FIG. 3.—Section of skin of hamster 331 ($\times 180$), showing numerous parasitised clasmatoocytes scattered through the *cutis vera*.
- FIG. 4.—More highly magnified portion of the region outlined in fig. 3, showing the manner in which the parasitised clasmatoocytes are arranged parallel to the capillaries of the *cutis vera* ($\times 950$).