STUDIES WITH *BRUGIA PAHANGI*
11. MEASUREMENT OF LYMPH FLOW IN INFECTED CATS

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INTRODUCTION

Many cases of lymphoedema and elephantiasis in man are thought to be due to functional changes in the lymphatics secondary to the damage produced by filarial worms. Although histopathological and anatomical studies of the lymphatics of various animals infected with either *Brugia malayi* or *Brugia pahangi* have revealed extensive damage, in only a few cases have there been clinical manifestations, such as lymphoedema (Schacher *et al.*, 1969; Gooneratne *et al.*, 1971; Ewert *et al.*, 1972; Schacher *et al.*, 1973; Rogers and Denham, 1974).

Sage and Gozun (1958a, b) and Sage *et al.*, (1964) used radio-active colloidal gold to measure lymph flow and functional patterns of lymphatics and lymph nodes in the extremities of human patients. Threefoot *et al.*, (1965) used radio-iodinated albumin and ethiodol to detect lymphatic-venous connections in dogs. Hollander *et al.*, (1961) studied lymph flow in human patients by measuring the disappearance of ^{131}I-labelled albumin from subcutaneous tissue. Jasani and Lewis (1971) measured the rate of lymph flow during healing and rejection of rabbit skin grafts by inserting cannulae into the lymphatics and collecting the lymph over a measured period.

None of these techniques have been previously used in studies of filariasis. We have used the colloidal gold ^{198}Au method of Sage *et al.*, (1964) to study a series of cats infected with *B. pahangi* to determine whether lymph flow decreased during filarial infection and after the development of lymphoedema.

MATERIALS AND METHODS

The basic parasitological techniques used to maintain infection, inoculate cats and determine microfilarial levels were described by Denham *et al.*, (1972). Infective larvae of *B. pahangi* were injected subcutaneously into the dorsal surface of one or both hind feet of the cats. If only one leg was inoculated, it was invariably the left hind leg.

The colloidal gold was obtained as a sterile suspension of particles 5-20 nm in diameter, stabilized with gelatin and glucose, from the Radiochemical Centre, Amersham (Item GSC IP). The half life of ^{198}Au is 2.7 days and the concentration of the colloidal suspension was 0.376-0.500 mg Au per ml (1 mCi/ml).

For the study of gold disappearance, 0.05 ml of a 1 in 10 dilution of the suspension of colloidal gold in sterile saline was injected into the dorsum of the hind feet. The amount of radio-active gold at the site of inoculation at different times after its injection was measured by placing the foot of the cat on a glass-topped table over a shield-headed unit containing a scintillation counter 663 (Isotope Development Ltd.) and measuring the number of counts by means of an IDL Computer Scaler 6000. Counts were made for 5 or 10 second periods every 30 or 60 minutes after inoculation of the gold. Correction factors were applied to the counts obtained to account for background radiation and

radioactive decay of the gold. The clearance of the gold and, therefore, the activity of the lymphatic system was gauged in three ways:

(1) The rate of disappearance of the gold from the feet was demonstrated graphically by plotting the number of counts obtained in 5 or 10 seconds against the time from injection of the gold.

(2) The percentage difference between the count obtained immediately after injection and the count at 300 minutes was used to gauge the long term rate of removal of gold from the foot, i.e.

$$\% \text{ gold remove} = \frac{\text{initial count} - \text{count at 300 mins}}{\text{initial count}} \times 100$$

(3) A comparison between the activity of the infected and normal legs was obtained by calculating the ratio:-

$$\frac{\% \text{ gold disappearance from infected leg in 300 mins}}{\% \text{ gold disappearance from normal leg in 300 mins}}$$

Thus the smaller the figure produced by this calculation the more inefficient in comparative terms were the lymphatics of the infected leg.

Observations were made on three group of cats.

(1) Uninfected cats.

(2) Cats infected for various periods after primary inoculations of *B. pahangi* larvae into one or both hind feet.

(3) Cats given a primary challenge and then repeatedly challenged with *B. pahangi* larvae.

RESULTS

The graphs of counts per 10 seconds against time in minutes after the injection of gold for several of the cats studied are shown in Figs. 1-3. Tables 1-3 show the site and

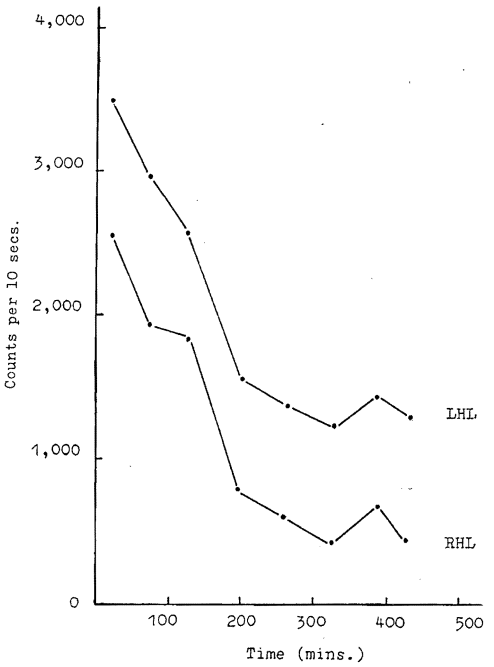

Fig. 1—Graph showing the rate of disappearance of colloidal gold (^{198}Au) from the hind feet of an uninfected cat.
LHL = left hind leg; RHL = right hind leg.

duration of infection, the number of challenge infections, the percentage disappearance of colloidal gold from the feet of infected (usually left hind leg) and uninfected (usually right hind leg) of the cats and the gold disappearance ratio (left hind leg divided by right hind leg).

Control cats (Fig. 1 and Table 1): In uninfected cats, the rate of disappearance of colloidal gold from the feet was usually similar for both hind legs in a particular animal. The disappearance ratio ranged from 0.62-1.33 with a mean of 0.94 (calculated from the gold disappearance ratios) and of 0.86 (calculated from the means of percentage disappearance at 300 minutes). The percentage disappearance of gold in 300 minutes varied considerably between the various animals. This may be due to individual differences in physiological efficiency or to

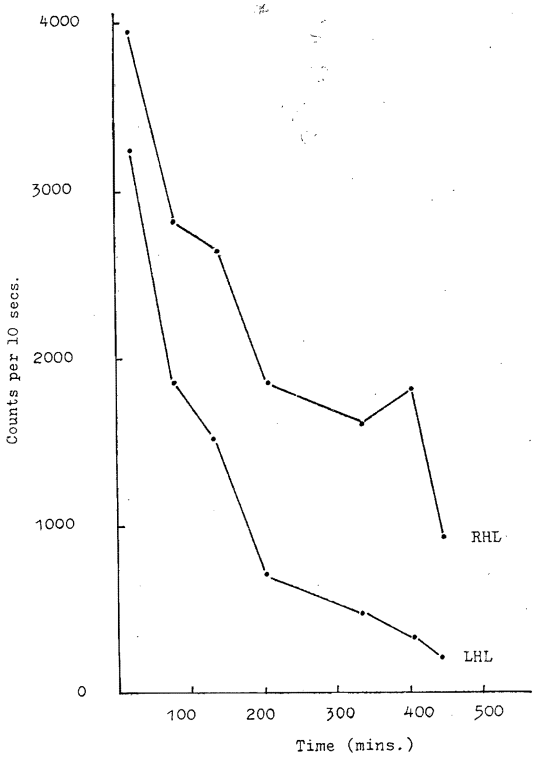

Fig. 2—Graph showing the rate of disappearance of colloidal gold (^{198}Au) from the infected and uninfected hind limbs of a cat with an 8 month infection with *B. pahangi*.
LHL = infected hind leg; RHL = uninfected hind leg.

the amount of activity of the cats during the intervals between measuring the radioactive counts. In all these cats at least 19% of the gold was removed from the feet in 300 minutes and the maximum clearance was 97%.

Cats with single infections (Fig. 2 and Table 2): Two different groups of cats were studied in this section. The first group received a primary infection in the left hind leg only; the second group received an infection in each hind leg on the same day. Both groups were studied at various intervals after infection. As in the normal cats, both infected groups showed great variation in the amount of gold removed from the feet. In the group

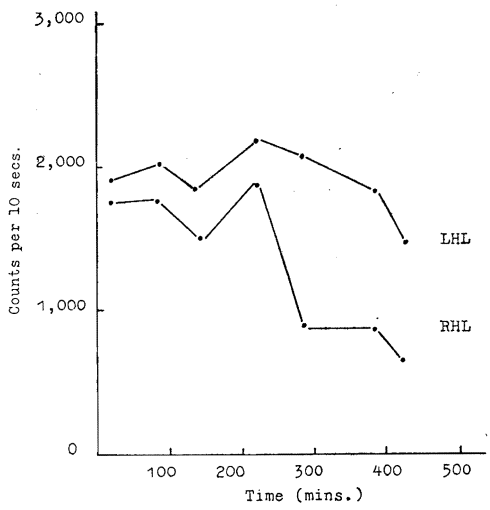

Fig. 3—Graph showing the rate of disappearance of colloidal gold (^{198}Au) from the hind feet of the cat which had been rechallenged 16 times during 24 months infection with *B. pahangi*. The infected foot had become oedematous and showed early stages of elephantiasis.
LHL = infected hind leg; RHL = uninfected hind leg.

Table 1

Control cats-lymph drainage measurement using ^{198}Au.

% disappearance of gold from feet in 300 mins.		Gold disappearance ratio
LHL	RHL	LHL/RHL
28	26	1.08
23	37	0.62
38	42	0.90
33	31	1.06
38	42	0.90
21	19	1.10
50	97	0.52
68	51	1.33
		$\bar{x}$ 0.94
$\bar{x}_L$ 37	$\bar{x}_R$ 43	$\frac{\bar{x}_L}{\bar{x}_R}$ = 0.86

LHL = left hind leg; RHL = right hind leg.

Table 2

Lymph drainage measurements in cats with single *B. pahangi* infections, using ^{198}Au.

Infection	Number of cats	Duration of infection (days or months)	Average percentage disappearance of gold from feet in 300 mins.		Average gold disappearance ratio
			LHL	RHL	LHL/RHL
Single infection LHL	5	8 d	33	40	0.83
	5	20-30 d	37	23	1.61
	1	2 m	33	42	0.78
	3	7-8 m	66	56	1.18
	4	10-14 m	51	49	1.04
Single infection EHL	4	10-20 d	30	36	0.83
	3	1-3 m	45	28	1.61
	2	6-7 m	59	66	0.89
	4	10-14 m	25	23	1.09

LHL = left hind leg; RHL = right hind leg; EHL = each hind leg.

Table 3a

Lymph drainage measurements in cats repeatedly infected with, *B. pahangi*, using ^{198}Au.

Infection	No. of cats	No. of challenges	Duration of infections (months)	Average % disappearance of gold from feet in 300 mins.		Gold disappearance ratio
				LHL	RHL	LHL/RHL
Repeat infections LHL	2	4	14, 15	37	43	0.86
	4	5	6, 8, 8, 14½	28	36	0.78
	5	7	6, 8, 9, 10, 10	62	64	0.97
	2	8	7, 10	15	51	0.29
	1	9	18	61	38	1.61
	3	10	12, 12, 18	27	23	1.17
	1	10	19	−5	14	−0.36
	3	11	12, 14, 16	7	33	0.21
	1	12	21	15	15	1.00
	1	18	19	16	2	8.00
	2	19	19, 21	11	15	0.73
	1	27	21	19	17	1.12
	1	46	20	9	24	0.38
	1	62	37	12	31	0.38
	1	66	33	57	62	0.92
	1	68	35	28	31	0.90
Repeat infections —initially EHL— challenged LHL	1	19	19	14	15	0.93
	1	28	16	21	32	0.66
	1	35	16	14	29	0.48
	1	38	14	65	63	1.03
Repeat infections which became microfilaria negative	1	1	7 (neg. 3 m.b.s.)	85	99	0.86
	1	1	10 (neg. 6 m.b.s.)	60	26	2.31
	1	2	36 (neg. 7 d.b.s.)	56	51	1.10
	1	8	18 (neg. 14 m.b.s.)	36	13	2.77
Cat with oedematous foot	1	11	19 (neg. 2 m.b.s.)	8	8	1.00
	1	16	24 (neg. 7 m.b.s.)	−11	19	−0.58
	1	19	20 (neg. 16 m.b.s.)	2	38	0.05
	1	31	28 (neg. 7 d.b.s.)	30	19	1.58
	1	32	14 (neg. 4 m.b.s.)	75	78	0.96
	1	48	14 (neg. 3 m.b.s.)	41	84	0.49

d.b.s. = days before study of lymph flow; m.b.s. = months before study of lymph flow; neg. = microfilaria negative.

Table 3b

Mean gold disappearance ratios for repeatedly infected cats.

Infection	No. of challenges	$\bar{x}$-gold disappearance ratio
Repeat infections LHL	1-10	0.76
	11-20	2.49
	21-30	1.12
	41-50	0.38
	61-70	0.73
1° infection EHL—rechallenged LHL	11-20	0.93
	21-30	0.66
	31-40	0.76
Microfilaria negative repeat infections	1-10	1.76
	11-20	0.16
	31-40	1.27
	41-50	0.49

infected in one leg, 6 of 18 cats had a lower percentage gold disappearance from the infected than from the uninfected hind leg. In the group infected in each hind leg, 6 of 13 also had disappearance ratios less than 1. We concluded from this that there is no significant difference between infected and uninfected hind limbs in their lymph drainage capacity (see Fig. 2). Lymph flow was not noticeably diminished in any of these animals up to 14 months after infection compared with control cats.

Lymph flow studies on repeatedly infected cats (Tables 3a and b): Most of the cats in this section received a primary infection into the left hind leg and were then repeatedly challenged with 50 larvae per inoculation into the same leg at approximately 10 day intervals. A few cats were initially infected in both legs and then repeatedly challenged in the left hind leg only. Lymph flow studies were carried out on cats which had been rechallenged from 1-68 times after initial infection. Ten of the cats studied became microfilaria negative at different times before study (see Table 3a). In one of these, which will be described separately, there were clinical manifestations of filariasis.

There was great variation in the rate of disappearance of gold from the feet of these cats. In some cases disappearance from the infected leg was greater than from the normal leg, while in others there appeared to be a decrease in the rate of disappearance of gold in the repeatedly challenged leg.

Table 3b summarises the mean gold disappearance ratios for the repeatedly rechallenged cats. In cats with primary and repeat infections in the left hind leg, this leg showed lowered efficiency of gold removal after 1-10 and 40-70 challenges. All cats initially infected in each hind leg and challenged in the left hind leg showed lower gold disappearance rates from the left leg. Of the repeatedly challenged cats which were microfilaria negative, those challenged 11-20 and 41-50 times had low gold disappearance ratios.

It seems that variation in lymph flow occurs at different stages of infection but no regular pattern of lymph flow changes appeared in this series of cats.

Lymph flow in a cat showing clinical manifestations of filariasis (Fig. 3, Table 3a): Only one cat was studied when it had externally visible signs of filariasis. This cat had an intermittently oedematous, fibrous left foot. When first studied after 11 rechallenges (19 months infection), the cat had been microfilaria negative for 2 months. The left foot was normal in appearance and the gold disappearance ratio was 1.00. However, after 16 rechallenges (24 months infection), no gold disappeared from the foot of the oedematous infected leg and the gold disappearance ratio was –0.58.

DISCUSSION

Individual cats showed markedly different gold disappearance rates. These discrepancies may be due to differences in the efficiency or structure of the lymphatics of individual

cats, or, possibly, to the amount of movement of the limbs during the period over which the lymph flow measurements were made. This is a variable factor which made interpretation of the results difficult.

In most of the cats studied, there was no significant decrease in the speed with which gold disappeared from the infected limb compared with the control limb. Only 2 cats showed a marked decrease in the gold disappearance rate. One of these showed a gold disappearance ratio of –0.36 after 10 rechallenges (19 months infection). On two of three later occasions, however, gold was again being removed as rapidly from the left foot as from the uninfected foot. The other cat had developed clinical manifestations in the left hind leg resembling early elephantiasis in man with a very swollen, oedematous and palpably fibrosed foot. The gold disappearance rate was significantly diminished in the affected leg (disappearance ratio –0.58). The cat was killed soon after this. By this time, the original lymphatics had been severely damaged and were non-functional fibrotic cords with the lumens occluded by dead worms enclosed in lymphoid tissue. New, small lymphatics were developing.

Measurement of the rate of disappearance of radioactive colloidal gold from the feet of uninfected and infected cats proved to be difficult to interpret but may still be a useful method of investigating the functional efficiency of lymphatics. Perhaps the most significant finding is that the highly varicosed lymphatics seen with heavy infections usually function as well as do normal lymphatics.

Other possible methods of lymph flow measurement were considered. An attempt was made to measure flow rates by injecting a 1% solution of Evan's Blue in saline into the dorsum of the feet, collecting samples of blood from ear pricks at different times after injection and measuring the concentration of blue colouring in the serum collected. However, it proved difficult to collect enough serum by this method to make colour intensity measurement accurate and hence this method of lymph flow measurement was abandoned.

It was our hypothesis that, even when the lymphatics were damaged and not as efficient as before infection, lymph flow would decrease, but that the cat lymphatic system was so efficient that lymphoedema would not be produced. This was not correct. Clearly, even extensive damage to the lymphatics does not prevent their functioning as well as normal lymphatic in most cats. A few cats only showed transient lymphoedema, most so transient that it was possible to study lymph flow in one cat only.

SUMMARY

Rates of lymph flow in cats were measured by calculating the disappearance of radioactive colloidal gold (^{198}Au) from the feet of (1) uninfected cats, (2) cats infected for various periods after primary infection with *Brugia pahangi*, and (3) cats repeatedly challenged with *B. pahangi* infective larvae over long periods. The results of the study showed that (1) there is great variation in gold disappearance rates in different cats in all 3 groups above, (2) the cat lymphatic system is functionally highly efficient, and (3) in a cat with lymphoedema and early elephantiasis, there was a significant impairment of gold removal from the affected foot. The study proved useful in finding lymph drainage rates in the various animals but did not, as hoped, show any pattern of lymph flow decrease which might have enabled the use of the technique as a diagnostic tool for lymphatic pathology prior to the occurrence of external clinical manifestations of filariasis.

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