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皮膚リーシュマニア症の治療
—Promastigote型原虫による温熱療法の基礎実験—

矢後　文子

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緒　言

サウジアラビアより帰国した，皮膚リーシュマニア症患者を治療する機会を得た。薬物療法が終了し，原虫採取を試みた患部の皮膚からは原虫が検出されなくなっても，経過が緩慢なので皮膚所見から，皮内の原虫がすべて死滅したかどうか判定するのは困難であった。そこで，皮内に残っているかも知れない原虫を，薬物療法以外で治療する方法を検討した。皮膚リーシュマニア症を発症させる例えば，*Leishmania tropica* は内蔵型リーシュマニア症を発症させる *L. donovani* より低温を好み，ヒトの体内部の温度では長期間生存できないと考えられている（Berman and Neva, 1981)。しかし，治療と関連づけて温度による殺原虫効果を検討した研究や，実際に治療を試みた患者数は少なく，その方法の詳細もはっきりしていない（Peters and Killick-Kendrick, 1987)。そこで，皮膚の表面より低温火傷を生じない温度と時間の範囲で皮内の原虫に温熱を加え，弱化または死滅させる条件を *in vitro* で検討した。ヒトの皮内には Amastigote 型原虫が寄生しているが，現在実験に供する程多数の Amastigote 型原虫を得るのは困難なので，Promastigote 型原虫を用いて温熱を加え，その後の原虫の動きと増殖状態を観察し，若干の知見を得たので報告する。

材料および方法

1）*Leishmania* 原虫

原虫は，サウジアラビアのブレイダ市に1987年6月より同年12月まで滞在し，虫刺を受けた51歳の日本人男性の右大腿伸側の皮疹より，1988年3月31日分離し（KN株とす），NNN培地（Leventhal and Chadle, 1989）で継代培養中の Promastigote 型で，新培地に植え継いで14日以内の原虫を実験に供した。

2）温度負荷試験

固形斜面のNNN培地1.3 m*l* に生理的食塩水（以下生食と略記す）1.0 m*l* を入れた小試験管（内径1 cm×高さ10 cm）内で原虫を培養し（以下培養小試と略記す)，恒温室内または恒温水槽に一定時間静置後，速やかに至適培養温度である25℃に戻し，原虫の自発的な動きの有無，および増殖状態を，経日的に少なくも14日間観察した。恒温水槽での保温は，水槽の液面と培養小試内の生食液面を同一位にし，固形斜面が1.8 cm 液面上に出ている同一水位法（表2の図参照）と，水槽の液面を培養小試の生食液面より2.5 cm 高くし，NNN培地の固形斜面をすべて水槽の液面下に沈めた高水位法（表5の図参照）の2方法で行った。同一水位法または高水位法での実験終了後，引き続き高水位法で実験を行う場合には，実験終了後の培養小試内の液体0.2 m*l* を新しい生食0.8 m*l* を入れたNNN培地にいれ，再び保温してその後の動きを観察した。

3）原虫の記録法

培養小試内の液体を攪拌後スライドに1滴採り，400倍率の顕微鏡下で5視野観察し，原虫に自発的な動きが観察された場合を生虫と判定し，得られ

東京女子医科大学寄生虫学教室

た生虫の最大数を次の基準で記録した。

＋　　生虫の最大数が10未満/視野
＋＋　生虫の最大数が10以上100未満/視野
＋＋＋　生虫の最大数が100以上/視野
－　　鞭毛にも虫体部にも自発的な動きをもつ原虫が検出されない

成　績

1）治療に応用可能な温度の検索

皮膚を傷害せず，原虫を弱化または死滅させる温度を検索する目的で，56℃，入浴湯温である42℃，ヒトの体内深部温である37℃，*L. tropica* の培養至適温度である25℃，そして冷蔵室内温度の7℃を用い，それぞれの恒温室内に培養小試を24時間静置後25℃でさらに4日間培養し，表1を得た。

56℃と42℃の培養小試には，24時間恒温室で静置直後に観察しても，その後25℃で培養した4日間にも，動きを示す原虫は観察されなかった。37℃では，恒温室より取り出した直後には原虫は不動であったが，25℃で培養すると1日目から生虫が認められ，その後3日間は生存した。7℃では，恒温室より取り出した直後には原虫が活発に動いていたので，そのまま7℃に10日間保ったところ生存し続けた。対照として行った25℃では，5日間原虫は生存した。

すなわち，原虫は7℃のような低温には抵抗性を持ち，42℃や56℃に24時間静置すると動きが認められなくなった。皮膚を外部から暖める治療に応用するのには，56℃では熱すぎるので42℃を選び，以下の実験を行った。

2）42℃，同一水位法による保温

a）30分より90分までの継続保温

患部を保温するには，小面積で短時間の方が患者の負担が少ないので，まず皮疹部のみを温めるモデル実験として，同一水位法で30分より90分まで10分間隔で7群に分けて保温後25℃に戻し，その後の原虫の動きと増殖状態を観察し，表2を得た。

培養小試を恒温槽より取り出した直後に観察すると，30分の保温ですでに原虫は自発的な動きを示さなくなり，90分までの試みたどの培養小試の原虫にも自発的な動きは認められなかった。しか

Table 1　The effects on the movement and multiplication of promastigotes of maintenance at five different temperatures for 24 hr

	24 hr*	Days kept at 25℃				
		1	2	3	4	
56℃	－**	－	•	－	－	
42℃	－	－	•	－	－	
37℃	－	＋	•	＋＋	＋＋	
25℃	＋＋	＋＋	•	＋＋	＋	
		Days kept at 7℃				
		1	2	3	4	10
7℃	＋＋＋	＋＋	•	＋	＋	＋＋

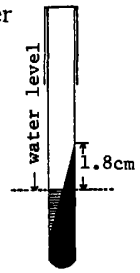
* Test tubes contained NNN medium, saline and promastigotes were kept in each rooms maintained at the temperatures indicated.

** Observations were made in 5 visual fields under a microscope, at a magnification of 400, and the movement and multiplication of promastigotes were recorded as follows: －, moving promastigote were not observed; ＋, maximum number of moving promastigotes per field was 9 or less; ＋＋, the number of moving promastigotes was between 10 and 99; ＋＋＋, the number was greater than 100; •, not tested.

Table 2 The effects on the movement and multiplication of promastigotes of keeping them for 30 to 90 min at 42°C when the water levels were the same*

Day †	Minutes kept at 42°C							
	0	30	40	50	60	70	80	90
0 ‡	++§	−	−	−	−	−	−	−
1	+++	−	+	−	−	−	−	−
2	+	−	−	−	−	−	−	−
3	++	++	++	+	−	−	−	−
5	++	+	+	+	+	+	−	−
8	++	+	+	+	++	++	++	++
15	+++	++	+++	+++	+++	+++	++	+

* The level of liquid of medium in test tubes and the level of the water in the water bath at 42°C were the same.
† Days at 25°C after heating at 42°C.
‡ Observations were made just after the heating at 42°C
§ Refer to the explanations in Table 1.



し，その後25°C に戻して観察を続けると，30分より50分までの保温では 1 または 3 日目には，60分および70分の保温では 5 日目には，80分および90分の保温では 8 日目にはそれぞれ生虫が認められ，観察した15日目まで生存した。

すなわち，42°C，同一水位法での90分までの保温では原虫は死滅できなかった。

b）1時間から 4 時間までの継続保温

90分までの保温では，原虫を死滅させ得なかったので，保温時間を 1 時間より 4 時間まで延長して実験を行い，表 3 を得た。

1 時間の保温では25°C に戻して 2 日目から，2 時間および 3 時間の保温では 7 日目には生虫が観察された。4 時間の保温では25°C に戻しても観察した14日間には，鞭毛や虫体部に自発的な動きを示す原虫は認められなかった。

すなわち，同一水位法では原虫の動きを 2 週間止めるのに 4 時間の継続保温が必要と考えられた。

c）繰り返し保温

実際に治療を行う場合，4 時間継続保温するのでは患者の負担が大きすぎるので，毎日一定時間繰り返して保温し，1 回の保温時間を短縮できないか検討した。1 日 1 回，1 時間より 4 時間までの保温を，1 日より 3 日間まで連続して行い，その後の原虫の動きと増殖状態を観察し，表 4 を得

Table 3 The effects on the movement and multiplication of promastigotes when water levels were the same* after continuous heating at 42°C

Day*	Hours kept at 42°C				
	0	1	2	3	4
1	++*	−	−	−	−
2	++	++	−	−	−
3	•	•	•	•	•
4	+++	++	−	−	−
5	++	+++	−	−	−
6	•	•	•	•	•
7	++	+++	+	+	−
8	•	•	•	•	•
9	+++	+++	++	++	−
10	•	•	•	•	•
11	++	++	+++	+++	−
12	++	+++	++	+++	−
13	+	++	++	++	−
14	++	++	+++	++	−

* Refer to the explanations in Tables 1 and 2.

Table 4 The effects on the movements and multiplication of promastigotes after they were kept at 42°C for 1 to 4 hr on consecutive days when the water levels were the same*

No**	1	2	3	4	5	6	7	8	9	10	11	12	13	14
						Hours kept at 42°C								
Day*	0	0	1	1	1	2	2	2	3	3	3	4	4	4
1	++*	++	−	|1	1		|2	2	−	|3	3	−	|4	4
2	+	++	−	−	|1	−	−	|2	−	−	|3	−	−	|4
3	++	++	−	−	−	−	−	−	−	−	−	−	−	−
4	++	++	+	−	−	−	−	−	−	−	−	−	−	−
5	+	+	+	−	−	−	−	−	−	−	−	−	−	−
6	•	•	•	•	•	•	•	•	•	•	•	•	•	•
7	+	+	++	−	−	−	−	−	−	−	−	−	−	−
8	++	+	+	−	−	−	−	−	−	++	−	−	−	−
9	+	++	++	−	−	−	−	−	−	++	−	−	−	−
10	+	+	+	+	−	−	−	−	−	+++	−	−	−	−
11	+	++	+	+	−	−	+	−	−	++	−	−	−	−
12	+	+	++	+	−	−	−	−	−	−	−	−	−	−
13	•	•	•	•	•	•	•	•	•	•	•	•	•	•
14	+	+	+	+	−	−	−	−	−	+	−	−	−	−
15	+	++	+	+	−	−	−	−	−	++	−	−	−	−
16	+	+	+	+	−	−	−	−	−	+++	−	−	−	−

* Refer to the explanations in Table 1 or 2.

** The assigned number of the test tube, as reforred to in the text.

た。

1日1時間の保温を1回行った表4の試験管番号（以下試番と略記す）3では，25°Cに戻して4日目より，1時間2日間の保温では（試番4）では25°Cに戻して9日目より生虫が認められたが，1時間3日間（試番5）では観察した14日間運動性を持つ原虫は認められなかった。2時間群（試番6より8）では，2日間保温した試番7の25°Cに戻して10日目に生虫が1回観察されたが，他には原虫の動きは認められなかった。ところが，3時間2日保温（試番10）に，25°Cに戻して7日目より多数の生虫が観察された。なお4時間保温群（試番12より14）では，動きを示した原虫は認められなかった。

試番10で観察された原虫が，42°Cに抵抗性を持っていたのか，または恒温水槽の水面上に出ていたNNN培地の斜面や試験管内壁が42°Cに保温されていなかったために，その部分に付着していた原虫が生き残り，再び増殖してきたのかを調べるために，実験終了後高水位法を用いて再保温した。表4の25°Cに戻して15日目の試番10の培養小試より高水位法用に培養小試を4本用意し，再保温した。保温は3時間1日のみと3時間2日間をそれぞれ2本ずつの培養小試で行ったが，自発的な動きを示した原虫は，25°Cに戻したその後15日間には観察されなかった。

すなわち，同一水位法では1時間以上3日間繰り返し保温するか，4時間継続保温を1回行えば，原虫は自発的な動きを示さなくなった。3時間以内2日間までの保温では再増殖することがあるが，42°Cに抵抗性ではなかった。

3）42°C，高水位法による保温

a）1時間より8時間までの継続保温

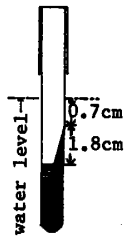
表2，3および4に示したように，42°C，同一水位法での3時間までの保温では，25°Cに戻すと再び原虫は動きを取り戻したり，増殖することがあった。そこで，恒温槽の水面上に出ている培地の斜面や，試験管内壁も充分保温するために，高水位法を用いて保温し，その後の原虫の動きと増殖状態を観察し表5を得た。

1時間の保温では，25°Cに戻して4または5日目に生虫が認められたが，2時間以上の継続保温

Table 5 The effects on the movement and multiplication of promastigotes of continuous heating at 42°C in the deep water*

	Hours kept at 42°C										
	0	0	1	1	2	3	4	5	6	7	8
Day†	a	b	a	b	‡	‡	‡	‡	‡	‡	‡
1	+†	+	−	−	−	−	−	−	−	−	−
2	•	•	•	•	•	•	•	•	•	•	•
3	•	•	•	•	•	•	•	•	•	•	•
4	++	++	+	−	−	−	−	−	−	−	−
5	+	+	+	+	−	−	−	−	−	−	−
6	•	•	•	•	•	•	•	•	•	•	•
7	+	++	+	++	−	−	−	−	−	−	−
8	+	++	++	++	−	−	−	−	−	−	−
9	•	•	•	•	•	•	•	•	•	•	•
10	++	++	++	++	−	−	−	−	−	−	−
11	+	++	++	++	−	−	−	−	−	−	−
12	++	++	++	++	−	−	−	−	−	−	−
13	+	++	++	++	−	−	−	−	−	−	−
14	•	•	•	•	•	•	•	•	•	•	•
15	++	++	++	++	−	−	−	−	−	−	−

* The level of water in the water bath was 0.7 mm above the top of the solid slant of NNN medium and also 2.5 cm above the surface of the liquid of the medium.
† Refer to the explanations in Tables 1 and 2.
‡ Each group was examined in duplicates, a and b, but the results were same in each case.



では，25°Cに戻しても観察した15日間には，自発的な動きを示す虫体は認められなかった。

すなわち，同一水位法の4時間の継続保温に比べて，より短い2時間の保温で，原虫はその動きを少なくとも15日間は停止した。

b）繰り返し保温

同一水位法で，1日より3日間まで繰り返し保温する（表4の試番3より5）と，必ずしも4時間のような長時間の保温をしなくとも同じ効果が得られたので，高水位法でも時間を短縮して1回20分から120分までの保温を1日より3日間まで繰り返し，その後の原虫の動きと増殖状態を観察し，表6を得た。

20分保温群（試番3より5）と40分保温群（試番6より8）では，保温終了翌日または2日目から生虫が認められた。60分および80分と保温時間が長くなると，動きを持つ虫体が観察され始める日が，2日目より9日目と遅れてきた。100分保温群では，1日保温（試番15）では7日目以後生虫が観察されたが，2日（試番16）または3日（試番17）と繰り返し保温すると25°Cに戻しても，その後14日間は動きを示す原虫は認められなかった。120分保温群では，1日の保温（試番18）で，原虫はその後14日間は動きを示さなかった。

不動の原虫が死滅したかどうか調べるために，実験終了後の各培養小試（試番16より20）から原虫を含んだ液体を，引き続き新たなNNN培地に移し，さらに培養を続けると，100分2日保温(試番16）では再培養7日目（通算24日目）以後は生虫の増殖が観察されたが，100分3日保温(試番17)および120分保温群（試番18より20）では，再培養14日目（通算31日目）まで生虫は観察されなかっ

Table 6 The effects on the movement and multiplication of promastigotes of keeping them at 42°C for 20 to 120 min on consecutive days in the deep water*

No*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Day*	Minutes kept at 42°C																			
0	0	0	20	20	20	40	40	40	60	60	60	80	80	80	100	100	100	120	120	120
1	+*	++	+	20	20	+	40	40	−	60	60	−	80	80	−	100	100	−	120	120
2	+	++	++	+	20	+	+	40	+	−	60	−	−	80	−	−	100	−	−	120
3	++	+	+	++	++	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−
4	+++	++	++	++	+	+	++	+	++	−	−	−	−	−	−	−	−	−	−	−
5	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
6	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
7	+	++	+	++	+	++	++	+	+	+	+	++	−	−	++	−	−	−	−	−
8	+	++	+	+	++	++	++	+	++	++	+	+	−	+	+	−	−	−	−	−
9	+	+	+	+	++	+	++	++	+++	++	+	++	−	++	+	−	−	−	−	−
10	++	+	++	++	++	++	++	++	++	++	+	+	+	++	+++	−	−	−	−	−
11	+	+	++	++	+	+	++	++	++	++	+	++	+	+	++	−	−	−	−	−
12	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
13	++	++	+	+	+	++	++	+	++	++	+	++	++	++	+	−	−	−	−	−
14	++	+	+	+	+	+	++	++	++	++	+	+	++	++	++	−	−	−	−	−
15	+	+	+	+	+	+	++	+	++	++	++	++	++	++	++	−	−	−	−	−
16	++	+	+	+	+	++	+	++	++	++	+	++	++	++	++	−	−	−	−	−
24**	++	++	·	·	·	·	·	·	·	·	·	·	·	·	·	++	−	−	−	−
31	++	++	·	·	·	·	·	·	·	·	·	·	·	·	·	++	−	−	−	−

* Refer to the explanations in Tables 1, 2, 4 and 5. **Refer to in the text.

た。

すなわち，高水位法では100分3日間または2時間保温では，再培養しても動きを示す原虫は認められなかった。

c）継続保温と分断保温

1回の継続保温時間は，少なくも2時間は必要と考えられたが，継続せず途中に休息を入れたモデル，すなわち42°C高水位法での保温の途中，一定時間培養小試を25°Cに戻す実験を行い，表7を得た。保温時間の合計は表5および6の結果より，2，3および4時間を用い，42°C 1時間の保温を1単位とした。継続保温した試番3，4，9，10，15および16と，1単位保温後15分25°Cに戻し再び1単位保温した試番5および6，同様にして1時間25°Cに戻した試番7および8，これらのことを繰り返し保温時間の合計を3時間，および4時間にした実験も行い，継続保温と分断保温の差を検討した。

合計保温時間が2時間では，継続および15分休息群で，25°Cに戻して14日目にそれぞれ2本ずつ行った培養小試の1本に生虫が観察された（試番4と6）。しかし，途中1時間25°Cに戻した試番7では11日目に，試番8では4日目に生虫が観察された。3時間保温の試番9より14では，自発的な動きを持つ虫体は，14日間観察されなかった。ところが4時間保温しても，継続または15分休息群には，自発的な動きを持つ虫体は観察されなかったが，1時間保温し1時間25°Cに戻すことを4回繰り返した試番20に，12日目より生虫が観察された。

すなわち，42°C 1時間を1単位として保温した場合，25°Cに戻しておける時間は15分以内が望ましく，保温時間の合計は，3時間以上が必要と考えられた。

考　察

皮膚リーシュマニア症の治療には，主にアンチモン剤，例えば sodium stibogluconate や meglumine antimoniate (Peters and Killick-Ken-

Table 7 The effects on the movement and multiplication of promastigotes of discontinuous heating at 42°C in the deep water*

	Total hours at 42°C																			
	0		2						3						4					
			continuous		kept at 25°C for 15m×1 1h×1**				continuous		kept at 25°C for 15m×2 1h×2				continuous		kept at 25°C for 15m×3 1h×3			
No*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Day*																				
1	++*	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
3	+	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	++	++	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
5	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
6	+	+++	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+++	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
8	+	+	−	−	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−
9	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
10	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
11	++	+++	−	−	−	−	+	++	−	−	−	−	−	−	−	−	−	−	−	−
12	+	+	−	−	−	−	+	++	−	−	−	−	−	−	−	−	−	−	−	+
13	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
14	++	++	−	+	−	+	++	++	−	−	−	−	−	−	−	−	−	−	−	+

* Refer to the explanations in Tables 1, 2, 4 and 5.

** Heating was performed for 1 hr at a time and interrupted by keeping samples at 25°C for 15 min or 1 hr between each heating.

drick 1987) などが多く使われている。しかし，アンチモン剤には副作用もあり，他に有効で安全性の高い治療法が望まれている。今回は，患部が大腿伸側の皮膚という，患者自身が自由に処置できる部位を利用して，皮膚表面からの温度負荷による治療を行う場合の基礎条件を検討した。

皮膚リーシュマニア症を発症させる原虫には，数種の亜種（*L. tropica complex*）が知られている(大鶴ら，1988)。主な亜種には，農村型または湿潤型（wet type）で四肢に潰瘍を生じる *L. tropica major* と，都市型または乾燥型（dry type）の主に顔に結節や潰瘍を生ずる *L. tropica minor* がある。実験に用いた原虫は，大腿部に皮疹を生じ，その中央より血性浸出液が漏出していた状態，および推定感染他がサウジアラビアのブレイダ市中心部より約30 km 離れた砂漠地帯であるという地理的条件より，*L. tropica major* と考えているが，正確な同定には免疫学的検索や，DNA分析などが必要である。

表1より原虫は7°Cのような低温には強く，42°Cや56°Cのような体温より高い温度にさらされると自発的な動きが停止し，顕微鏡下で観察すると原虫の内部の透明感が薄れ，粒状物質が観察され始めるなどの変化が認められたので，皮膚は冷却するより，暖めるほうが治療には有効と考えた。実際に赤外線ランプや，ビニール袋にいれた温湯で大腿伸側を加温すると，皮膚表面温を44°C以上にして温め続けるのは熱すぎて実施が困難であったので，入浴湯温である42°Cが適当と考えた。

原虫は，400倍率の顕微鏡下で5視野観察して，虫体部にも鞭毛にも自発的な動きが2週間連続して認められなかった場合に，弱化と判定した。原虫が不動であることと死滅とは同一ではなく，一時的な活動停止状態という可能性は否定できなく，実際，高水位法で100分ずつ2日間繰り返し保温した表6の試番16では，15日間自発的な動きが認められなかったにもかかわらず，新培地に移して7日目には生虫が観察された。100分3日間保温した表6の試番17，および120分保温した群（試番18, 19, 20）には，自発的な動きが少なくも14日間は認められず，その後新培地に移しても，なお14日

間動きを持つ原虫は観察されなかったので，この場合は死滅と考えた。保温実験後の原虫を，感受性を持つマウス例えばBALB/cに感染させ，皮疹発症の有無，さらに皮疹より採取した試料をNNN培地で培養し，Promastigote型原虫の増殖の有無を観察すればより確実であるが，今回は試みていない。また，治癒の判定のために，皮膚リーシュマニア症患者の皮疹より採取した組織や分泌物をNNN培地で培養した場合，約1週間前後よりPromastigote型原虫が多数検出され，2週間経っても原虫が観察されなかった場合には，その後さらに2週間観察を続けても原虫は培養されなかった経験，およびNNN培地で系統維持のため継代培養する場合，1週間前後より多数の原虫が容易に検出され始め，2週間後までには原虫や培地の状態の悪化を防ぐために，新しい培地へ植え継ぐのが一般的であることから，培地を取り替える2週間を目安に今回は観察を打ち切り，自発的な動きが2週間認められなかった時に弱化と考えた。

皮膚に寄生している原虫は，Promastigote型ではなくAmastigote型なので，同様な温熱療法に対する基礎実験を，Amastigote型原虫を用いて行う必要がある。ヒトのマクロファージ内のAmastigote型原虫の増殖を，*in vitro*で調べたBerman and Neva (1981) によれば，37°Cより35°Cの方が2倍増殖し，39°Cではほぼ完全にマクロファージ内から原虫が消失した。同時に行った内蔵型原虫の*L. donovani*では，35°Cでも37°Cでもほぼ等しく増殖し，39°Cでも40%減少したのみであった。このように*L. tropica*のAmastigote型原虫でも，39°Cより35°Cのような体内部温より低い温度で増殖し，Promastigote型と同様な傾向が認められている。

同一水位法で，42°C 3時間の継続保温を2日間繰り返した表4の試番10で，25°Cに移して7日目より多数の生虫が観察されたのは，高水位法の表5，および6に示したように2時間以上の継続保温で原虫が，弱化おそらくは死滅することから，同一水位法では，試験管内壁や恒温槽の水面上に出ていたNNN培地の固定斜面では，原虫の保温が42°C以下であったための再増殖と推定された。そこで，臨床的には皮疹としては認められなくとも，皮疹周囲にも原虫が散在している可能性があり，また皮疹の温度も一定に保ちやすいため，実際の治療には皮疹部の周囲をも含め，広範囲に保温する必要があると考えられた。

表4の試番3より5に示したように，同一水位法で1時間ずつ毎日保温を繰り返すと，1日保温より2日保温と生虫が観察され始める日が遅れてきて，3日間の保温では生虫は14日間観察されなかった。しかし，表6の試番11に示したように，高水位法で1時間ずつ3日間保温したにもかかわらず生虫が観察された。表6の試番15と16からも，1時間40分までの保温では原虫を確実に死滅させるのには不充分で，また表7の結果からも3時間の継続保温が必要と考えられた。

途中に休息を入れたモデル実験の結果（表7）からは，42°C，1時間の保温を1単位とした場合，25°Cに戻しておける時間は1時間では長すぎ，15分以内が適当と考えられた。実験では42°Cの恒温水槽より取り出した培養小試は，水道水で急速に25°Cまで冷却したが，実際の治療では保温終了後に患部は冷却せず，また皮膚温も外気温や衣服などにもよるが，25°Cより高いのでモデル実験とは条件が異なる。しかし，保温を中断する時間は15分以内で，できるだけ短時間が良いと推定された。

皮膚は外部より保温しても，血液の還流により絶えず体内部の温度に近づくように熱が奪われる。皮膚表面を一定温にした場合，その皮下の温度はどのくらい低下するかを，剃毛した家兎の皮膚を懐炉を使って暖めて検討した実験（宗，1975）では，皮表を43°Cより43.3°Cに保った時，深さ2.5 mmでは41.7°C，深さ7.0 mmでは39.8°Cであった。懐炉貼付前の皮表の温度は31.6°Cで，深さ2.5 mmでは33.7°C，7.0 mmでは36.3°Cであった。家兎の体内部の温度は，直腸温で38.0より40.9°C（前島ら，1986）でヒトの深部温の37°Cより高く，外気温や皮膚および皮下組織の状態によっても異なるが，試みた患者の大腿部表面を43°Cに保温した場合の，皮内温の目安として大変興味深い。現在，患者の皮表を43°Cおよび42°Cに保った時の皮内温の測定を試みている。

長時間の保温で生ずる低温火傷は，温度の高さと作用時間，および熱源の圧力が関係していると考えられる。剃毛したモルモットに，150 g の電気ゴテを圧着した実験（大島ら，1973）では，45°C で20分，または50°C で 7 分では紅斑のみで潰瘍形成はなく，50°C 10分以上で 2 度と 3 度の境界の熱傷を生じた。また，Moritz and Henriques (1947) によるヒトの前胸部や前腕を使っての実験では，43°C 以下で皮膚に熱傷を生ずるには約 6 時間必要であった。*L. tropica* の実験に用いた42°C は，実際に長く保温してみると少しぬるく感じるので，43°C を目標に，試みに51歳日本人男性の大腿伸側で 5 時間の継続保温を行ってみたが，低温熱傷は生じなかった。

化学療法にあまり反応しない皮膚リーシュマニア症の患者に温熱療法を試みた Neva ら (1984) によれば，39°C より41°C で数日にわたって少なくとも20時間以上保温した結果，大変有効な 3 例があったが，無効であった例もあった。Neva ら (1984) が用いた温度は，実験で使用した42°C より低く，表 4 の同一水位法の試番10で観察された，充分42°C に保温されていなかった原虫と同じ状態とも考えられる。しかし Neva ら (1984) は，無効であった症例は原虫の種が異なっている可能性を考察している。

以上のことから，実際に温熱療法を行う場合には，皮膚の表面温度は43°C を目標に，少なくとも 1 時間は継続保温し，中断しても15分位までとし，3 時間の継続保温を 1 クールとし，毎日繰り返して保温するのが有効と考えられ，現在治療に応用している。患者が自宅でいつでも実行でき，特殊な治療器具も必要とせず，温度管理に注意すれば大きな副作用も生じない温熱療法は，単独でもまた薬物療法と併用しても，試みられるべき治療法と考えられた。

結　論

サウジアラビアで感染した皮膚リーシュマニア症患者より原虫を分離し，治療に応用する目的で温度による弱化，または死滅効果を Promastigote 型原虫を用いて *in vitro* で検討し，次の結論を得た。

1．原虫を56°C および42°C で24時間保温すると，その後25°C で観察した 4 日間には，自発的な動きを示す虫体は認められなかったが，37°C，25°C および 7 °C では生存した。

2．42°C，同一水位法による 4 時間の継続保温では，25°C に戻しても観察した14日間には，生虫は認められなかった。

3．42°C，高水位法での 3 時間以上の継続保温では観察した14日間には，動きを示す虫体は認められなかった。

4．42°C 1 時間保温後，25°C に戻すことを繰り返した実験では，保温時間の合計が 3 時間以上で分断時間が15分では，運動性を示す虫体は観察されなかった。

5．42°C，同一水位法では25°C に戻すと再増殖することがあるが，高水位法では再増殖は認められなかった。

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THERAPY FOR CUTANEOUS LEISHMANIASIS —THE EFFECT OF TEMPERATURE ON THE MOVEMENT AND MULTIPLICATION OF *LEISHMANIA* PROMASTIGOTES AS A BASIS FOR LOCAL HEAT THERAPY—

AYAKO YAGO

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Leishmania was isolated from the skin of a Japanese man who had spent time in Saudi Arabia, and the movement and multiplication of promastigotes in NNN medium was observed after heating.

1) Promastigotes became immobile after 24 hr of heating at 56°C and 42°C, but they survived at 37°C, 25°C and 7°C.

2) When the level of water in the water bath at 42°C and of liquid of NNN medium in test tubes were the same, promastigotes lost their motility after continous heating for 4 hr and did not regain it until the 14th day at 25°C.

3) When the level of the water in the water bath at 42°C was 0.7 mm above the top of a solid slope of NNN medium in test tubes and 2.5 cm above the liquid surface(deeper water), promastigotes lost their motility after continous heating for 3 hr and did not regain it until the 14th day at 25°C.

4) Promastigotes lost their motility after heating 3 times for 1 hr at 42°C in the deeper water with intervals of cooling for 15 min at 25°C.

5) In the case when water levels were the same, promastigotes sometimes multiplied again, but this did not reoccur in the deeper water.

Department of Parasitology, Tokyo Women's Medical College

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THE EFFECT OF A SINGLE LARGE DOSE OF CHLOROQUINE ON THE INFECTIVITY OF *PLASMODIUM YOELII NIGERIENSIS* TO *ANOPHELES STEPHENSI*

KAZUYO ICHIMORI

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Abstract: The mean number of oocysts per mosquito fed on mice 12 hr after treatment with 100 mg chloroquine/kg was the same level as that in mosquitoes fed on control (untreated) mice despite the fact that no gametocytes were seen in an examination of 10,000 red blood cells in thin blood films. Though no gametocytes were seen, calculations based on 95% confidence limits from the binomial distribution indicate the possibility of up to 3 gametocytes in a sample of 10,000 red blood cells. This is equivalent to over 7,000 gametocytes per blood meal which would appear to be enough to saturate the stomach wall with oocysts.

INTRODUCTION

Chloroquine resistant malaria is a major problem in many tropical countries. In areas where resistant parasite strains have developed, high doses of chloroquine are often used to treat patients; alternatively a different antimalarial drug may be used. Chloroquine kills the asexual erythrocytic stages of malaria but has no effect on the infectivity of mature gametocytes of *P. falciparum* (Jeffery *et al.*, 1956). Young developing gametocytes, however, may be damaged by chloroquine (Smalley, 1977). In this study I investigated the effect of a single large dose of chloroquine on the infectivity of chloroquine resistant *P. yoelii* malaria.

MATERIALS AND METHODS

A sample of the N67 strain of *P.y. nigeriensis* which had been stored in liquid nitrogen was grown up in a TO (Theiler's original) mouse. Blood from the donor mouse with 5 to 20% parasitaemia was mixed with heparinized PBS to give 10^7 parasitized red cells in 0.2 m*l*. This volume was inoculated intra-peritoneally into each of 8 experimental mice. The mice were randomly assigned to 4 groups on day 3 after inoculation. The groups were inoculated intra-venously with 100, 10, 1 or 0 (control) mg chloroquine/kg body weight. Twelve hours after treatment mosquitoes were allowed to feed to repletion on these mice. Parasitaemia and gametocytaemia at the time of treatment and feeding were counted by an examination of 10,000 red blood cells in blood films from each mouse. The mice were killed immediately after feeding and the haematocrit was measured. The mosquitoes used were from a labora-

Department of Medical Zoology, Teikyo University School of Medicine, Kaga 2-11-1, Itabashi, Tokyo 173, Japan

tory colony of the BEECH strain of *Anopheles stephensi*. They were infected when 2-5 days old. On the day before feeding, 50 female mosquitoes were placed in a 300 m*l* paper cup covered with a mesh screen. The glucose feeder which was usually kept on the top of the cups was removed 6-12 hr before the feed. This made the mosquitoes hungry and also ensured that the abdomen was clear of glucose allowing complete engorgement to take place. The mice were anaesthetized and placed on top of cups containing the mosquitoes, which fed readily within 10 min.

After the blood meal, the mosquitoes were released into a 20 cm cube cage and only fully fed females were removed into new paper cups. Mosquitoes were then maintained at 24±1°C. Humidity was maintained with a humidifier or by covering the cages with damp lint. On day 7 after feeding, 20 mosquitoes from each cup were dissected in PBS and examined under a microscope at low power (×100). Oocysts on both sides of the stomach wall were counted by focusing up and down. In order to obtain a better representation of the results, William's geometric means of the oocyst numbers from each treatment group were calculated (William, 1937).

Results and Discussion

The parasitaemia and gametocytaemia of the mice given the higher doses of chloroquine decreased in the 12 hr after treatment, whereas they increased in the mice given the lowest dose and the untreated mice (Figure 1). No gametocytes were observed in blood films from the mice 12 hr after injection with 100 mg chloroquine/kg. However, oocysts were found in the mosquitoes fed on these mice and the William's mean number of oocysts was 327.8 which was almost the same as that in mosquitoes fed on untreated, control, mice (287.4). The maximum number of oocysts among the mosquitoes fed on mice 12 hr after 100 mg chloroquine/kg treatment was 651, which was enough to cover the whole stomach wall.

Obviously, it is necessary that there are gametocytes to produce oocysts. The apparent contradiction between the absence of gametocytes observed in blood films from mice on high doses of chloroquine and the presence of oocysts in mosquitoes fed on them may be explained by considering the sampling errors involved when only 10,000 red blood cells were counted in each blood film. Although no gametocytes were seen, the 95% confidence limits from the binomial distribution (Stevens, 1941) include the possibility of up to 3 gametocytes in a sample of 10,000 red blood cells. This is equivalent to over 7,000 gametocytes per blood meal, which would be enough to saturate the stomach wall with oocysts (Table 1).

Chloroquine has a schizontocidal effect, but there are some reports showing a gametocytocidal effect as well. Immature gametocytes (stage I-III) of *P. falciparum* are readily killed by chloroquine (Smalley, 1977) and the morphological effects of this drug at the ultrastractural level on the gametocytes appear very similar to those on the asexual parasites (Sinden, 1982). The infectivity of gametocytes of a chloroquine resistant line of *P. berghei* to *An. stephensi* is enhanced by chloroquine (Ramkaran and Peters, 1969). Recent results of experiments by Ichimori (1990) used *P.y. nigeriensis* N67 strain suggested that the enhancement of infectivity is a response to chloroquine stimulation shown only by certain of the genotypes within the heterogeneous strain. Treatment with chloroquine may eliminate many gametocytes of poor fitness and low infectivity thereby leaving a reduced number of very infective gametocytes in the blood.

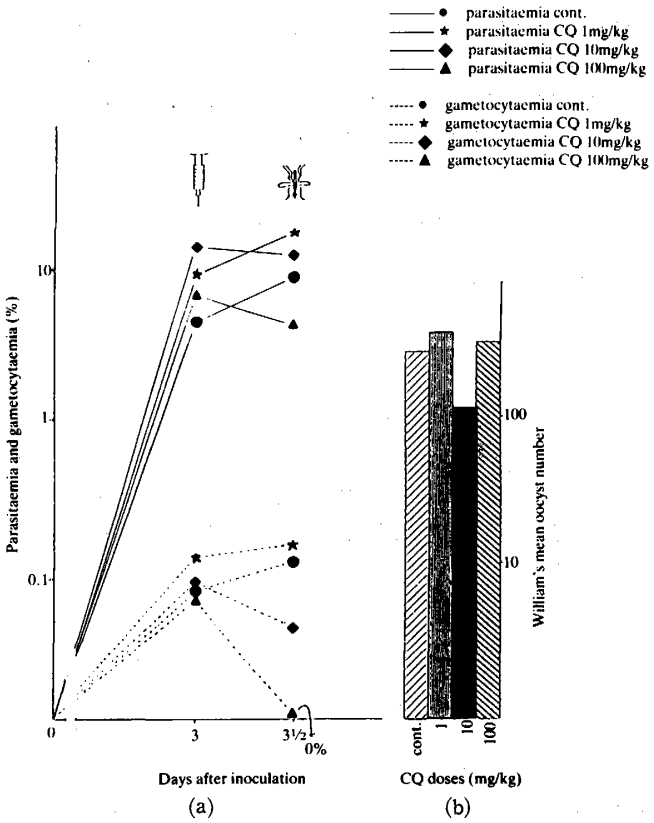

Figure 1 (a) Changes in parasitaemia and gametocytaemia in four groups on mice treated with different amounts of chloroquine; (b) the number of oocysts following feeding mosquitoes on day 3.5 (12 hr after chloroquine treatment).
Each point represents the average parasitaemia or gametocytaemia in the 2 mice in each treatment group. Each bar represents the William's mean oocyst number of 40 mosquitoes fed on 2 mice in each group.

Table 1 Calculation of the possible number of gametocytes taken up by each mosquito and relation of this to number of oocyst produced

CQ	A	B	C	D	E	F
doses mg/kg	no. of gametocytes/ 10^4RBC (95% confidence limit*)	haematocrit (%)	no. of RBC/μl blood meal (B×10^7/0.5†)	no. of RBC/ blood meal (C×3.3‡)	no. of gametocytes/ blood meal (A×D) (95% confidence limit)	William's mean number of oocysts
cont.	11 (6.17-18.21)	41.5	83×10^5	273.9×10^5	30,129 (16,899-49,877)	287.4
1	14 (8.46-21.89)	41	82×10^5	270.6×10^5	37,884 (22,893-59,234)	371.4
10	4 (1.37-9.15)	36.5	73×10^5	240.9×10^5	9,636 (3,300-22,042)	110.2
100	0 (0-3.00)	37	74×10^5	244.2×10^5	0 (0-7,326)	327.8

* : from bi-nominal distribution
† : normal mouse RBCs=$10^7/\mu l$, normal mouse haematocrit=50%
‡ : mosquito blood meal size=3.3 μl (average of 54 females)

Whatever the exact interpretation of the high oocyst production even when the gametocytaemia had been reduced to an undetactable level, if the present results can be extrapolated to human malaria, they suggest that chemotherapy with a single large dose of chloroquine would not reduce malaria transmission.

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クロロキン投与後のネズミマラリア原虫の蚊への感染性

一盛　和世

ネズミマラリア原虫（*Plasmodium yoelii nigeriensis* N67系）の感染したマウスにクロロキンを投与し，その12時間後に蚊（*Anopheles stephensi* BEECH系）に吸血させる。7日後に解剖し，蚊の中腸壁外のオーシスト数を数えることによって原虫の感染性を調べた。

クロロキン100 mg/kgを投与した場合，マウス血中にガメトサイトが観察されなくなった。しかし，その血液を吸血した蚊には，クロロキンを与えていない血液を吸血した対照蚊とほぼ同等の多数のオーシストが数えられた。

クロロキン1回投与による，原虫の蚊に対する感染性への影響は見られなかった。また赤血球10,000中にガメトサイト0の場合でも蚊への伝播が起こり得ることを示した。

帝京大学医学部医動物学教室
東京都板橋区加賀2－11－1

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SCHISTOSOME INFECTIONS AMONG JAPANESE DURING LONG STAYS IN ENDEMIC AREAS -EVALUATION OF SEROLOGICAL DIAGNOSIS, EOSINOPHILIA AND TREATMENT WITH PRAZIQUANTEL

HIROSHI OHARA[1], HAJIME MATSUDA[2], KOICHIRO FUJITA[3],
HIROSHI NARUTO[4] AND HIROSHI TANAKA[5]
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Abstract: Serological diagnosis using micro-ELISA was conducted for 152 Japanese who stayed for a long period in areas where schistosomiasis was endemic, and 7 cases were detected. Including these 7 cases, 9 cases were determined as schistosomiasis (3 of schistosomiasis mansoni, 2 of schistosomiasis haematobia and 4 of schistosomiasis mansoni or haematobia) and all of them were treated (7 with praziquantel, 1 with metrifonate and 1 with niridazole). By the observation of antibody titers of the cases after treatment confirmed that the antibody gradually declined and that all the cases examined became negative by 20 months after treatment. In 152 subjects examined, there were 24 cases with eosinophilia. Out of 24, 7 were cases of schistosomiasis. Among these 7 cases, 2 had noticeable eosinophilia at more than 30% of total leukocyte count. After treatment, the eosinophil count decreased and was normalized earlier than serum antibody titer. After people have stayed in endemic area for a long period, examinations for schistosome is necessary. For early diagnosis, the serological diagnosis using micro-ELISA is effective, and treatment with praziquantel at an early stage is recommended. It was suggested that results of micro-ELISA and eosinophil count were healpful in evaluating therapeutic effect.

INTRODUCTION

Schistosomiasis is widely distributed and prevalent in tropical and subtropical areas, where control of the disease has become a serious health problem for inhabitants (W.H.O., 1985; Chickwen and Alaka, 1987). With recent expansion of the role played by Japanese international cooperation, more Japanese tend to stay abroad, and in parallel with the increased number of overseas travellers and stayers, the risk of acquiring diseases is increas-

1 Department of International Cooperation, National Medical Center, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162, Japan
2 Department of Parasitology, Institute of Medical Science, the University of Tokyo, 4-6-1, Shiroganedai, Minato-ku, Tokyo 108, Japan
3 Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113, Japan
4 Japan Overseas Cooperation Volunteers, Japan International Cooperaton Agency, 4-2-24, Hiroo, Shibuya-ku, Tokyo 150, Japan
5 Public Health Laboratory of Chiba Prefecture, 666-2, Nitona-cho, Chiba 280, Japan

ing in those areas. Schistosomiasis is one of the important diseases as a subject of health management of people staying in endemic areas, and some Japanese cases of schistosomiasis mansoni infected in Africa have been reported so far (Namba *et al.*, 1979; Hayashi *et al.*, 1980).

Althogh the most reliable way to diagnose schistosomiasis is to detect eggs in stool or urine, this is difficult in many cases because of light infections. Recently, serological diagnosis has been tried (Buck *et al.*, 1964; Yokogawa *et al.*, 1967; Warren *et al.*, 1973; Wilson *et al.*, 1977), and especially enzyme-linked immunosorbent assay (ELISA) is expected to be a useful tool for early diagnosis (Hillyer and Rios, 1979; McLaren and Lillywhite, 1981; Matsuda *et al.*, 1982).

Serological diagnosis using micro-ELISA was conducted in Japanese who had been living in endemic areas and possibly exposed to schistosomiasis infection. Nine cases of schistosomiasis which were infected during their stays in East Africa were experienced including these serologically diagnosed. Treatment was undertaken in all the 9 cases, and the subsequent changes in antibody titers were followed up. The present paper deals with the studies in serological diagnosis, treatment with praziquantel and follow-up study after treatment.

Materials and Methods

Subjects of survey: The subjects of the present survey were Japanese who had stayed for a long period in East Africa, West Africa, Southeast Asia, and Central and South Americas, who were suspected to have chances of acquiring schistosomiasis. The duration of their stay was 1-3 years and the present survey was conducted in 2 years from April 1985 to March 1987 followed by follow-up study thereafter. The subjects were people who returned to Japan and those who had been diagnosed as schistosomiasis at local hospitals.

Method of examinations: The number of subjects was 152 who were examined for the serological examination as well as stool, urine, blood and liver function tests upon their arrival at Japan. In the serological examination, the micro-ELISA was employed, and in the positive cases, circumoval precipitin test (COPT) was also performed. The methods of micro-ELISA (Matsuda *et al.*, 1984b) and COPT (Tanaka, *et al.*, 1975; Matsuda *et al.*, 1977) are followed to the previous descriptions. For micro-ELISA used, the antigens were the extracts of *Schistosoma japonicum* and *Schistosoma mansoni* eggs, and ABTS, 2-2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) was used as substrate of peroxidase. When an absorbance (optical density, OD) exceeded 0.2, the case was determined to be positive. The positive degree of COPT are classified into type 1-3 (Matsuda *et al.*, 1977). Stool examinations were performed by a combination of the following 3 methods; formalin-ether sedimentation method, zinc sulfate centrifugation-flotation method and Harada & Mori's test-tube cultivation method. And Heidenhain's iron-hematoxylin staining was made for identifying Rizopoda. The methods of these stool examinations are followed to the previous descriptions (Yamaura *et al.*, 1983). Those who showed positive serological reactions, were further examined for stool using AMS-III method.

In addition to those 152 Japanese, 2 subjects who were diagnosed as schistosomiasis and treated at local hospitals, were also studied. Various examinations were also conducted to those 2 cases as well as the above 152 subjects after returning to Japan.

Treatment: Six of the 9 cases who were diagnosed as schistosomiasis were treated with praziquantel (Biltricide®, Bayer), 1 with niridazole (Ambilhar®, Ciba-Geigy) and 1 with metrifonate (Bilarcil®, Bayer). Praziquantel was administered at doses of 60 mg/kg divided in 3 in 1 day, niridazole at 25 mg/kg daily divided in 3 doses for 7 days and metrifonate at 3 single doses of 7.5 mg/kg given 14 days apart.

Observation after treatment: Adverse reactions to anthelmintics were observed after administration of these drugs. Blood examinations including eosinophil count, stool and urine examinations were performed more than 1 month after treatment. The antibody titers could be followed-up in 7 cases (5 of schistosomiasis mansoni and 2 of schistosomiasis haematobia) out of the 9 treated. After treatment, the serum was collected from each case 1 to 4 times, and changes in the antibody titers at time course were observed. Since sera were collected from each subject before departure to the assigned areas and preserved, the change of antibody titers was determined in comparisom with the preserved sera.

Eosinophil count and parasitic infection: Based upon the findings of the blood, stool and urine examinations performed when 152 subjects returned to Japan, a survey of the relationship between eosinophil count and parasitic infection was made. Cases with eosinophil count exceeding 8.0% of total leukocytes were regarded as the eosinophilia.

RESULTS

Serological examinations: All 7 schistosomiasis cases out of the 152 subjects had returned to Japan after stays in East Africa. There were no positive cases found among people who stayed in other areas. Out of a total of 66 people returning from East Africa, 7 were positive (10.6%). All 7 subjects positive for the micro-ELISA with *S. mansoni* egg antigen were examined for COPT with *S. mansoni* eggs (Table 1). Besides those 7 subjects, there were 2 others who were diagnosed as schistosomiasis haematobia and treated during their stays in East Africa. Results of their serological examinations conducted 15 and 16 months, respectively, after treatment were negative.

Detection of schistosomiasis: The list of cases in which diagnosis was made as schis-

Table 1 Results of serological examination (micro-ELISA) for schistosomiasis

Region sojourned	No. tested	Positive cases		Positive rate (%)
		S.m.†	S.j.‡	
East Africa	66*	7	0	10.6
West Africa	25	0	0	0
Southeast Asia	35	0	0	0
South America	26	0	0	0
Total	152	7	0	4.6

* Two cases which were diagnosed as schistosomiasis haematobia during their stay in East Africa are not included

† Positive for *Schistosoma mansoni* egg antigen

‡ Positive for *Schistosoma japonicum* egg antigen

Table 2 Cases of schistosomiasis

Case	Age	Sex	Country infected	Diagnosis*	Serological examination	Eggs		Eosinophilia (%)	Treatment
						Stool	Urine		
1.	28	M	Malawi	s.h.	Neg†	−	+	− (2)	metrifonate
2.	30	M	Zambia	s.m.	+	+	−	+ (10)	praziquantel
3.	27	M	Zambia	s.h.	Neg‡	−	+	− (5)	niridazole
4.	26	M	Malawi	s.m.	+	+	−	+ (30)	praziquantel
5.	24	M	Tanzania	s.m. or s.h.	+	−	−	+ (23)	praziquantel
6.	24	M	Tanzania	s.m.	+	+	−	+ (32)	praziquantel
7.	29	M	Tanzania	s.m. or s.h.	+	−	−	+ (8)	praziquantel
8.	31	M	Kenya	s.m. or s.h.	+	−	−	+ (18)	praziquantel
9.	28	M	Tanzania	s.m. or s.h.	+	−	−	+ (19)	praziquantel

* s.m.: schistosomiasis mansoni s.h.: schistosomiasis haematobia
† negative when conducted 16 months after treatment
‡ negative when conducted 15 months after treatment

tosomiasis is shown in Table 2. There were 3 cases of schistosomiasis mansoni, 2 cases of schistosomiasis haematobia and 4 suspected cases (schistosomiasis mansoni or haematobia). Of these 9 cases, eggs of schistosome were detected in stool in 3 cases (Cases 2, 4 and 6) and in urine in 2 (Cases 1 and 3). There were 4 cases (Cases 5, 7, 8 and 9) in whom eggs were not detected, although all of them showed positive serological reactions and eosinophilia. In 6 of the cases of schistosomiasis (Cases 4, 5, 6, 7, 8 and 9), the presence of eggs was not detected at examinations when they arrived at Japan. In these cases, schistosomiasis was first suspected by serological examination.

Eggs were detected by the AMS-III method in 2 cases (Case 4 and 6) although they were not by the routine method. In one case (Case 2) with *S. mansoni,* eggs were detected by routine stool examination upon arrival at Japan. In 2 cases (Case 1 and 3) with schistosomiasis haematobia, eggs were confirmed by urine examination at hospitals where they stayed. Except in 1 case (Case 3) with *Schistosoma haematobium,* there were no subjective symptoms, 2 cases (Cases 5 and 9) were showing slight anemia, and none of the cases had abnormal hepatic functions, nor abnormal renal functions.

Personal histories of schistosomiasis cases: An outline of each case will be described below.

Case 1: A 28-year-old man who had stayed in Malawi since August 1984, and had taught science and mathematics at a junior high school. He went swimming several times in lakes. In April 1985, the periodic physical examination performed at a local hospital detected eggs of the *S. haematobium* in his urine (Photo. 1), and he was treated with metrifonate. He showed no subjective symptoms. He returned to Japan 16 months after treatment. The results of urine and serological examinations conducted on his return were both negative.

Case 2: A 30-year-old man. From August 1983 to August 1985, he stayed in Zambia where he worked as a veterinarian. He often swam in a nearby river. During the physical examination on his return to Japan, in August 1985, eggs of *S. mansoni* were detected in stool by routine stool examination, and he was treated with praziquantel.

Case 3: A 27-year-old man. In August 1984 he went to Zambia as a technical instructor for rice plantation. He lived in a farming village, and often entered paddy fields as his job.

Beginning in November 1985, he complained of lumbago and hematuria and visited a nearby hospital for examination. By the complete medical examination, there found eggs of *S. haematobium* in his urine (Photo. 2). After treatment with niridazole, the symptoms disappeared and eggs were no longer found in his urine by the subsequent examinations. The result of the serological examination performed 15 months later on his return to Japan was negative.

Case 4: A 26-year-old man. He stayed in Malawi as a technical instructor of navigation since March 1985. He often went into Lake Malawi. In August 1986, a physical examination made during his temporary return to Japan detected noticeable eosinophilia (30% of total leukocyte count). The result of a serological test for schistosomiasis was positive (OD 0.666 by ELISA, COPT type 2). After he returned to his job in Malawi, a stool examination at a hospital detected eggs of *S. mansoni* in stool by AMS-III method, and treatment was immediately undertaken with praziquantel (September 1986). One month later, another examination showed that the eosinophil count was normal and eggs were not detected.

Case 5: A 24-year-old man. From March 1984 to February 1986, he stayed in Tanzania as a civil engineering instructor. The result of serological examination using eggs of *S. mansoni* as the antigen was positive (OD 0.245 by ELISA, COPT type 2), strongly suggesting schistosomiasis mansoni infection. Eosinophilia (23%) was also noted although no eggs of the parasites were detected. The case was treated with praziquantel in September 1986. The result of a serological examination conducted 3 months later turned to negative.

Case 6: A 24-year-old man. In October 1983, he went to Tanzania as an instructor of agricultural civil engineering. He lived in a farming village on the coast of Lake Victoria in the northern part of the country, where he used water from Lake Victoria for his daily bathing and laundry, and swam in the lake often. A periodical physical examination in July 1985 identified noticeable eosinophilia (32%). Although a parasitic infection was suspected, no parasites were detected. He returned to Japan in October 1985. A physical examination performed on his arrival detected eosinophilia, but there was no abnormality in the stool examination. A serological test for schistosome showed a high titer (OD 1.067 by ELISA, COPT type 2), and eggs of *S. mansoni* were detected by AMS-III in his stool. In October 1986, he was treated with praziquantel, and stool and urine examinations one month later did not show eggs of the parasite. (Photos 3 and 4)

Case 7: A 29-year-old man, who had stayed in Tanzania since July 1983, and worked as a veterinarian in a local livestock breeding farm. He occasionally walked into a river with bare foot. In August 1986, he returned to Japan. The serological result using eggs of *S. mansoni* as antigen showed a very high titer (OD 1.576 by ELISA, COPT type 3) on his arrival at Japan, and infection with schistosome was strongly suspected. Eggs were not detected although low grade eosinophilia was recognized. In October 1987, he was treated with praziquantel.

Case 8: A 31-year-old man. From October 1985 to April 1987, he stayed in Kenya as a technical adviser for rice cultivation. He often went into the paddy fields. In the physical examination conducted when he returned to Japan, the serological result using eggs of *S. mansoni* was positive (OD 0.745 by ELISA, COPT type 2) and infection with schistosome was strongly suspected. Eosinophilia (18%) was recognized, although no eggs of the parasites were detected. He was treated with praziquantel in October 1988.

Case 9: A 28-year-old man. Since July 1984, he had stayed in Tanzania in a farming

village by the Lake Victoria where he worked as a technical instructor for rice planting. He swam in the lake and went into the paddy fields with bare foot. A serological examination was conducted when he temporarily returned to Japan in August 1986, and the result was positive (OD 1.134 by ELISA, COPT type 2). Eosinophilia (19%) was found, but eggs were not detected. He later resumed his work and returned to Japan again in October 1988. The titer of a serological examination by ELISA on his arrival at Japan was still high (OD 1.040), and infection with schistosome was strongly suspected. He was treated with praziquantel in November 1989.

Adverse reaction of anthelmintics: Out of 7 cases of schistosomiasis mansoni treated with praziquantel 4 (Cases 2, 4, 6, 7) complained of transient nausea and discomfort in the upper abdomen, but all of them subsided without any treatment within 4-5 hours. In the cases treated with meriforate (Case 1) and niridazole (Case 3), reactions including headache, cough, nausea, abdominal pain, poor appetite and lasitude in the former, and vomiting, nausea, lower abdominal pain, diarrhea, and fever in the latter were observed. These symptoms continued for several days and symptomatic treatments against adverse reactions were required.

Progressive changes of serum antibody titer: The result of our study on the changes in antibody titers following time course for 7 cases with schistosomiasis is shown in Fig. 1. Antibody changes could be followed most closely in Case 6, in whom there was a gradual decline in antibody titers after treatment. Although results of tests made 12 months after treatment were positive, they were negative 19 months after treatment. In all cases of schistosomiasis that could be tested (Cases 4, 5, 6, 7 and 8), there were gradual declines in antibody titers after treatment, and in 3 of these cases (Cases 5, 6 and 7), antibody titers had become negative by 20 months after treatment. In 2 cases (Cases 4 and 8) antibody titers

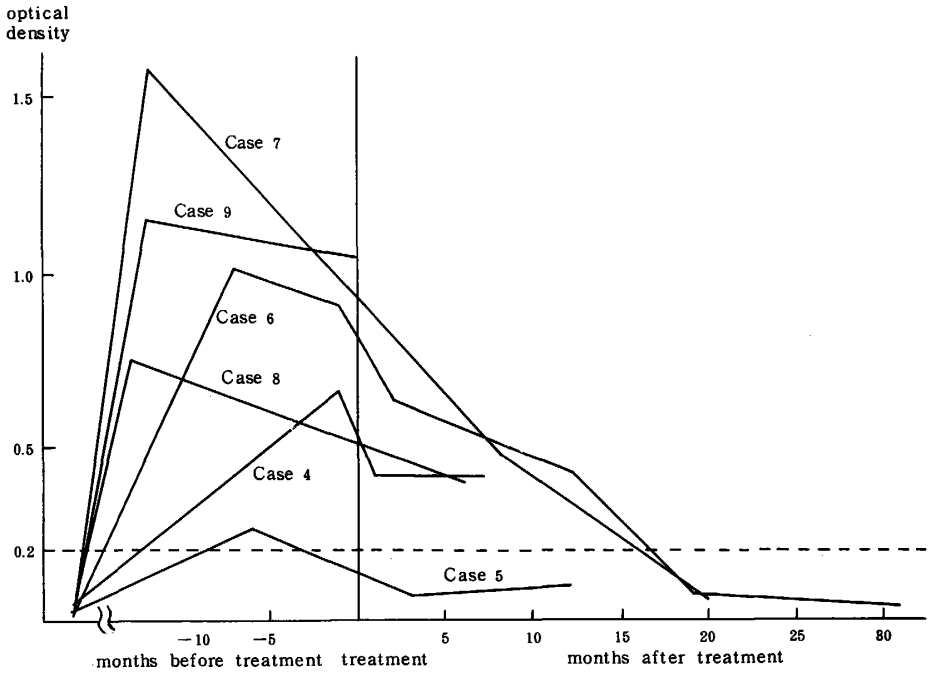

Figure 1 Progressive changes of *S. mansoni* specific antibody titer to eggs in human cases before and after treatment with praziquantel.

were still positive, because only 7 and 8 months have passed, respectively, after treatment. In these cases titers are decreasing and it is expected to become negative in the near future. Of 2 patients with schistosomiasis haematobia (Cases 1 and 3) the antibody titers 16 and 15 months after treatment were negative (OD 0.065, 0.071 by ELISA, respectively). And they were also negative by the subsequent examinations. All the serum collected from those persons before departure from Japan showed negative antibody titer against eggs of *S. mansoni* and *S. japonicum.*

Eosinophil count and infection by parasites: Of the 9 cases of schistosomiasis, eosinophilia was recognized in 7. Of these 7, there were 2 cases with noticeable eosinophilia at higher than 30% of total leukocyte count. All the cases with *S. mansoni* showed eosinophilia, and the two cases of schistosomiasis haematobia had normal eosinophil counts. All of the cases of eosinophilia became normal after treatment of schistosomiasis (Fig. 2).

Of the total of 152 subjects there were 24 with eosinophilia in which 7 were cases of schistosomiasis. In 18 subjects including the 7 above mentioned, there were infections with parasites. This suggested a relationship between eosinophilia and infections with parasite. There was, however, no infection with parasites in the remaining 6 subjects. The eosinophil count of cases with eosinophilia were all in the normal range prior to their departure from Japan (Table 3).

DISCUSSION

A previous study employing micro-ELISA which uses *S. japonicum* crude antigen showed that 171 out of 177 egg positives showed positive reactions (96.6%), while none of the non-

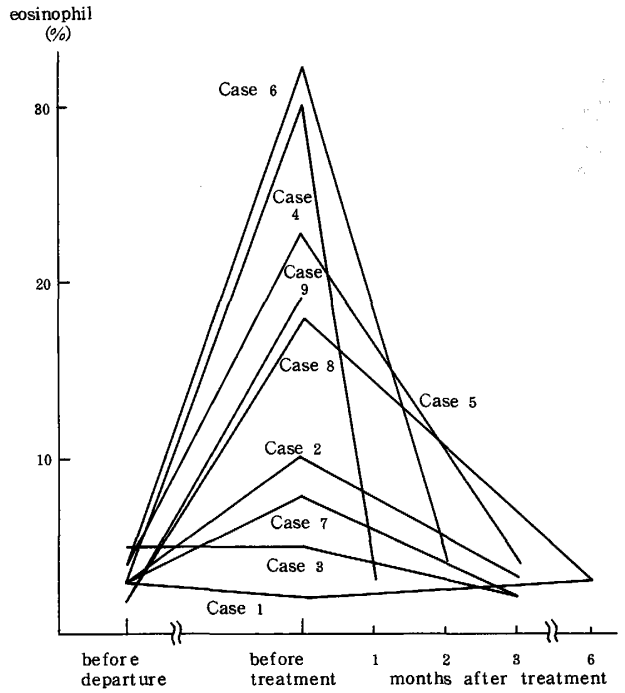

Figure 2 Eosinophil ratio to the total leukocyte count. A comparison before and after treatment.

Table 3 Subjects of eosinophilia, relationship between eosinophilia and parasitic infections

Subject No.	Eosinophil (%)	Total leukocyte count	Parasites
1.	32	6,400	*Schistosoma mansoni* (Case 6)
2.	30	14,400	*Schistosoma mansoni* (Case 4)
3.	23	7,300	schistosome (Case 5)
4.	18	5,800	schistosome (Case 8)
5.	19	9,600	schistosome (Case 9) *Entamoeba histolytica* *Trichuris trichiura*
6.	10	6,700	*Schistosoma mansoni* (Case 2)
7.	8	6,000	schistosome (Case 7)
8.	13	7,700	*Giardia lamblia*
9.	12	7,900	*Giardia lamblia*
10.	12	8,200	*Ascaris lumbricoides*
11.	11	6,900	*Trichuris trichiura*
12.	10	8,100	—
13.	10	7,400	—
14.	10	8,100	*Trichuris trichiura* *Entamoeba coli*
15.	9	5,600	—
16.	9	5,600	*Ancylostoma duodenale*
17.	8	4,900	*Giardia lamblia*
18.	8	6,200	*Giardia lamblia* *Entamoeba coli*
19.	8	6,900	—
20.	8	6,000	*Entamoeba histolytica*
21.	8	7,900	*Trichuris trichiura*
22.	8	8,300	—
23.	8	9,400	—
24.	8	7,700	*Giardia lamblia*

infected control group was positive (Tanaka *et al.*, 1983). In a study testing humans and rodents, the absorbance was less than 0.08 in 99% of the negative cases (Matsuda *et al.*, 1984b). And it is known that in this method cross reaction between other helminthes was very rare (Ogunba *et al.*, 1982). Moreover, it was reported that ABTS was most sensitive, stable and the best in visuality by its bluish-green colour among substances (Matsuda *et al.*, 1984b). From the results of these studies, the reliability of serological examination by the micro-ELISA is considered to be extremely high.

In the present survey, there were 4 subjects who showed positive with the micro-ELISA, although eggs of the parasites were not detected. Of the above 4, 3 subjects (Cases 5, 7 and 8) are strongly suspected to be infected with *S. mansoni* or *S. haematobium* for the following reasons; Decline in antibody titers after extermination of parasites; an increase in eosinophil count followed by decline after treatment; and no findings endorsing infection with other parasites. Another case (Case 9) is also suspected to be infected with schistosome although this case was not yet examined serologically after treatment.

Observation of changes in antibody titers after treatment revealed that those of cases of schistosomiasis mansoni gradually declined after treatment, and that 20 months later, all re-examined cases which could be observed showed negative. In cases of schistosomiasis haematobia, it was supposed that they had become to be normal range, earlier than the case of schistosomiasis mansoni, since the antibody titer against *S. haematobium* was measured by eggs of *S. mansoni* in the present survey. However, it was noticed that for measuring antibody titer against *S. haematobium,* the extent of difference of antigenicity between eggs of *S. haematobium* and *S. mansoni* was not so remarkable as in other reports (Farag *et al.,* 1979; Hirata *et al.,* 1986).

Investigation of changes in antibody titers in rabbits infected with *S. japonicum* showed that titers declined gradually after treatment with praziquantel and it took 38 weeks until reactions became negative with adult worm antigen while low antibody titers maintained against egg antigen longer than 38 weeks (Matsuda *et al.,* 1984a). The present survey showed the similar results. Further follow-up studies on antibody titers in our cases will be important for evaluating real therapeutic effects.

There are many reports suggesting the efficacy of praziquantel against schistosomiasis (Davis *et al.,* 1979; Katz *et al.,* 1979; Santos *et al.,* 1979; Matsuda *et al.,* 1983). In all the cases in which stool examination, antibody examination by micro-ELISA and calculation of eosinophil count could be performed after treatment with praziquantel, they were normal. Therefore the treatment was regarded as successful, and the efficacy and safety of praziquantel were reconfirmed.

In making a diagnosis of schistosomiasis, attention should be paid, besides the stool and urine examination, the history of patient's ways of living in the endemic area, his symptoms, and eosinophil count (Arafa *et al.,* 1985; Hagan *et al.,* 1985). Especially for persons who returned from endemic areas and show an eosinophil increase with unknown causes, an examination for schistosomiasis is necessary. Since this disease progresses chronically, and results in serious conditions such as liver cirrhosis, cystitis and obstructive uropathy, it is important to find this disease as early as possible. However an attention should be paied to the fact that it is often impossible to find and identify it through the routine methods.

For early diagnosis, serological diagnosis using micro-ELISA is extremely useful. It is also recommended to administer praziquantel at an early stage to all cases with micro-ELISA positives for schistosomiasis, even though eggs are not detected. It is suggested from our cases that the results of micro-ELISA and eosinophil count are good parameters in evaluating the therapeutic effect.

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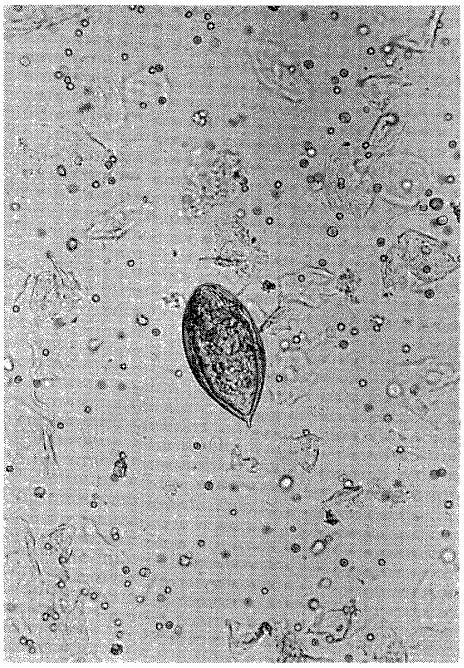

Photo. 1

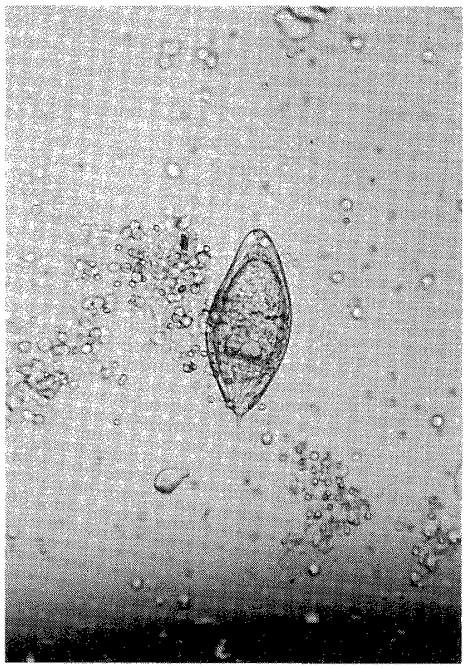

Photo. 2

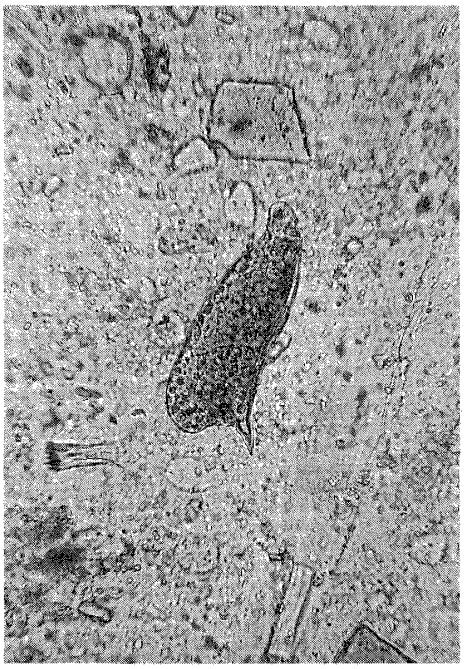

Photo. 3

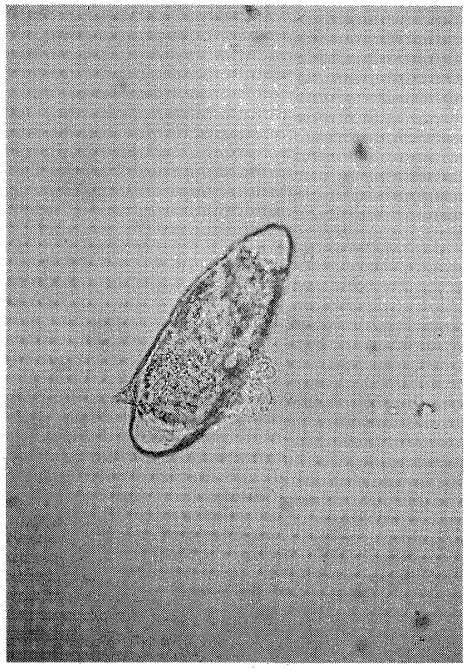

Photo. 4

Photo. 1 An egg of *S. haematobium* in urine (Case 1) *
Photo. 2 An egg of *S. haematobium* in urine (Case 3) *
Photo. 3 An egg of *S. mansoni* in stool (Case 6)
Photo. 4 COPT type 2 (Case 6)
* These photos were taken at a hospital in Malawi

流行地長期滞在日本人の住血吸虫症例に関する研究
—血清診断，好酸球増多およびプラジカンテルによる治療の評価

小原　博[1]・松田　肇[2]・藤田紘一郎[3]・
鳴戸　弘[4]・田中　寛[5]

住血吸虫の流行地に長期間滞在していた日本人152名を対象に，micro-ELISA 法による住血吸虫の血清診断を実施した。血清診断による陽性者 7 例を含め，9 例の住血吸虫症例（マンソン住血吸虫症 3 例，ビルハルツ住血吸虫症 2 例，マンソンまたはビルハルツ住血吸虫症例 4 例）を経験し，全例に対し治療を行った(このうち 7 例に対しプラジカンテルを用いた)。さらに治療後における抗体価の推移を観察した結果，抗体価は徐々に低下して20カ月後までには全例陰性化していることが確認された。調査対象者152名中24例に好酸球増多症が認められ，そのうち 7 例は住血吸虫症であった。7 例中 2 例は，30%以上の著明な好酸球増多を呈していた。治療薬を投与後，好酸球数は抗体価よりもすみやかに低下し，比較的早期に正常化した。流行地に長期間滞在した際には，住血吸虫の検査も必要であると思われる。早期診断上 micro-ELISA 法による血清診断は有力であり，陽性例には早期にプラジカンテルによる治療が勧められる。治療後，micro-ELISA 法と好酸球数を測定することにより，治癒の判定に役立つことが示唆された。

1　国立病院医療センター国際医療協力部
2　東京大学医科学研究所寄生虫研究部
3　東京医科歯科大学医学部医動物学教室
4　国際協力事業団青年海外協力隊事務局
5　千葉県衛生研究所

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SEASONAL ABUNDANCE OF DENGUE VECTORS IN RELATION TO RAINFALL AND PREVALENCE OF BREEDING CONTAINERS IN FIJI, 1981

MASAHIRO TAKAGI[1], ISMAIL M. RAKAI[2], DEO NARAYAN[2],
RAMESH RAM[2] AND GYAN PRAKASH[2]
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Abstract: A larval monitoring of dengue vectors, *Aedes aegypti* and *Ae. pseudoscutellaris,* was carried out as regularly as possible in several cities of Viti Levu, the main land of Fiji in 1981. Larval indices of both the two vector species were apparently precipitation dependent, and it was due to abundant available breeding containers. Seasonal fluctuation of those indices was more drastic in *Ae. pseudoscutellaris* than in *Ae. aegypti.* More breeding containers were found in rural area than in urban area. Performance of regular garbage collection service in urban area could reduce breeding containers for the species effectively. The variety and the density of potential containers were different in rural and urban areas. These differences were also found among industrial, commercial and residential blocks in a city, and were considered to affect species composition of the vectors.

INTRODUCTION

It has been reported by some workers that the abundance of *Aedes aegypti* is influenced by rainfall (Gould *et al.,* 1970; Chan, 1973, 1985; Mogi *et al.,* 1988), and in Fiji Goettel *et al.* (1980) also suggested the influence of rainfall from their results obtained by larval monitoring in Suva, the capital of this country. It has been also indicated that *Ae. aegypti* is endophilic and prefers to breed in artificial and rather large containers (Gould *et al.,* 1980; Chan, 1973, 1985; Sunarto *et al.,* 1979; Nelson *et al.,* 1984; Knudsen, 1983). These characters of the species show a good contrast with those of *Ae. albopictus,* another important vector of dengue/dengue haemorrhagic fever especially in southeast Asian countries, and result in the difference in incrimination of these species as vectors in those countries (Gould *et al.,* 1970; Chan, 1973, 1985; Sunarto *et al.,* 1979).

In Fiji, as in many other countries, *Ae. aegypti* has been considered as the most incriminatory vector mosquito for dengue/dengue haemorrhagic fever (Reed *et al.,* 1977; Self, 1979; Miles and Mataika, 1980). Furthermore, in place of *Ae. albopictus* in southeast Asian countries, *Ae. pseudoscutellaris,* a local species, is also common in this country. This species has been suspected to play the same role as *Ae. albopictus* of southeast Asian countries.

1 Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University, Sakamoto-machi 12-4, Nagasaki 852, Japan
2 Vector Control Unit, Ministry of Health, Fiji Government, P.O. Box 30, Suva, Fiji
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However, in Fiji there had been few information from temporal monitoring in some limited areas to evaluate the vector competence of the two species until 1980. Considering the above situation of the country the first large scale ecological and systematic survey of the vectors was conducted to clarify the status of infestation and roles of these species for the disease from 1980 to 1981. We report here the seasonal abundance of the two vector species in relation to rainfall, and the prevalence of potential and available breeding sources in several areas with different environmental conditions.

Study Areas and Methods

The data obtained by a monthly monitoring about the infestation of vectors through the house to house inspection in urban and rural areas of Suva from October 1980 to September 1981, and those obtained in two areas of Lautoka were analyzed. In the inspection, the number and type of potential and available breeding containers (available breeding containers=water holding containers among potential breeding containers) in and around more than fifty premises were recorded in each area, and the presence of vector larvae and/or pupae in the available breeding containers were examined. At least ten old larvae of the vectors were sampled by pipetting from every containers. Larval samples were identified in the laboratory, and the larval indices (container index (CI) =number of vector larvae and/or pupae positive containers $\times 100$/number of available containers inspected; premise index (PI) =number of those positive premises $\times 100$/number of premises inspected; and Breteau index (BI) =number of those positive containers $\times 100$/number of premises inspected) were calculated.

Suva is the biggest city and the capital of Fiji, where approximately 150 thousands people were populated. It is located in the southeast side of Viti Levu, the main island of the country, and 178° 26'E and 18° 08'S. On the other hand Lautoka is the second biggest city, where about 75 thousands people were populated. It is located in the western side of the same island as Suva, and 177° 27'E and 17° 37'S.

Monthly precipitation records during the investigation were collected from the Central Meteorological Office for Suva, and from Nadi International Airport for Lautoka. The airport was situated approximately 14 km south of Lautoka.

Results

Seasonal change of larval indices of both *Ae. aegypti* and *Ae. pseudoscutellaris* were illustrated in Table 1 and Fig. 1. It is clear that all larval indices of the two aedine vectors were higher during rainy season (October-April) than those during dry season (May-September) irrespective of areas, and that the seasonal trends of the indices of both species well corresponded to the trend of monthly precipitation in both areas. Comparing with two districts, the indices except CI were higher in Suva than in Lautoka. Comparing with two species, change of the infestation between two seasons was more drastic in *Ae. pseudoscutellaris* (Table 1 and Fig. 1).

To examine the relation between the prevalence of both of two vectors and precipitation in detail, monthly Breteau index of two districts was plotted to monthly precipitation in equivalent areas (Fig. 2). Linear relations were confirmed between them. Especially a close

Table 1 Seasonal change of larval indices (container index, CI; premise index, PI; and Breteau index, BI) of *Aedes aegypti* and *Aedes pseudoscutellaris* in Suva and Lautoka, Fiji

Place	Suva						Lautoka					
Species	*Ae. aegypti*			*Ae. pseudoscutellaris*			*Ae. aegypti*			*Ae. pseudoscutellaris*		
Month\Indices	CI	PI	BI	CI	PI	BI	CI	PI	BI	CI	PI	BI
Oct. '80	34	50	105	45	75	122	—	—	—	—	—	—
Nov.	31	40	85	36	70	103	43	48	89	15	17	23
Dec.	17	48	90	18	44	89	40	29	56	14	13	16
Jan. '81	20	39	81	16	46	74	58	28	70	26	35	50
Feb.	14	39	73	13	29	50	37	46	67	24	29	40
Mar.	15	31	71	8	15	38	32	33	30	25	24	29
Apr.	17	35	92	12	36	53	22	19	23	48	26	49
May	19	29	84	10	22	40	19	29	34	17	18	20
Jun.	28	27	68	8	20	26	20	24	40	2	5	4
Jul.	23	27	40	10	16	22	14	21	32	14	8	4
Aug.	17	29	49	7	12	47	22	26	45	19	21	20
Sep.	17	35	47	8	12	43	7	12	28	4	12	16
Average												
Oct.-Apr.	21.1	40.3	85.3	21.1	45.0	75.6	38.7	33.8	55.8	25.3	24.0	34.5
May-Sep.	20.8	29.4	57.6	8.6	16.4	35.6	16.4	22.4	35.8	11.2	12.8	12.8
Allover	21.0	35.8	73.8	15.9	33.1	58.9	28.5	28.6	46.7	18.9	18.9	24.6

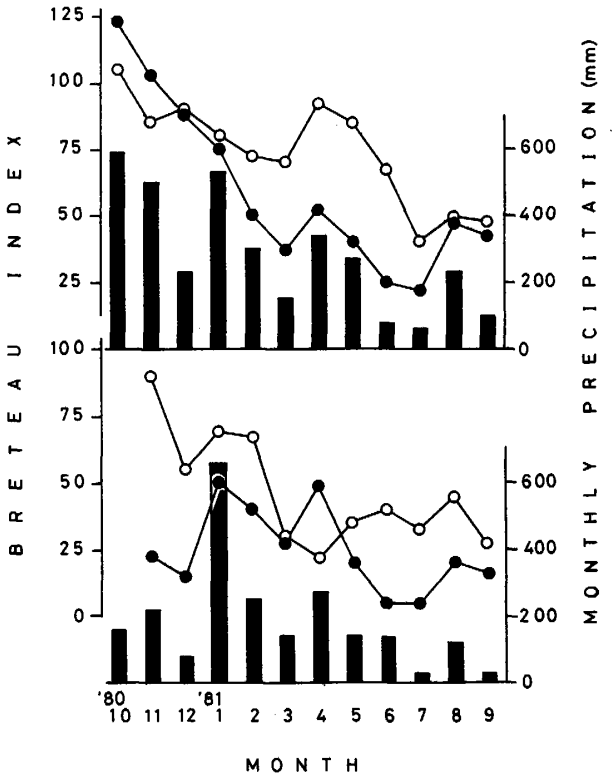

Figure 1 Seasonal change of Breteau index of *Ae. aegypti* (open circle) and *Ae. pseudoscutellaris* (closed circle), and monthly precipitation (histogram) in Suva (upper) and in Lautoka (lower), Fiji.

relation was found between the prevalence of *Ae. pseudoscutellaris* and precipitation. They fit following equations (Y is Breteau index and X is precipitation in mm): for *Ae. aegypti,*
$Y=0.082X+50.651$ ($r=0.743$) in Suva
$Y=0.058X+35.789$ ($r=0.481$) in Lautoka,
and for *Ae. pseudoscutellaris*
$Y=0.146X+17.571$ ($r=0.845$) in Suva
$Y=0.071X+11.263$ ($r=0.778$) in Lautoka.

It was assumed that, if precipitation was more, the available breeding containers became more. A clear linear relation was also confirmed between the average number of available containers per premise (Y) and the monthly precipitation (X): $Y=0.006X+1.610$, $r=0.852$.

The number of available containers is not solely decided by precipitation. Artificial factors such as life style of people and conditions of sanitation should be also causative to the difference in density and diversity of breeding containers. The frequency distribution of six representative containers per premise was investigated in urban area of Suva S (U), rural area of Suva S (R), urban area of Lautoka L (U), and rural area of Lautoka L (R). It was summarized in Fig. 3. Total number of available containers per premise was 6.3 in S (R), 3.9 in S (U), 6.4 in L (R), and 2.2 in L (U). An apparent difference was distinguished between urban area and rural area. As to the diversity of available containers, rural areas were

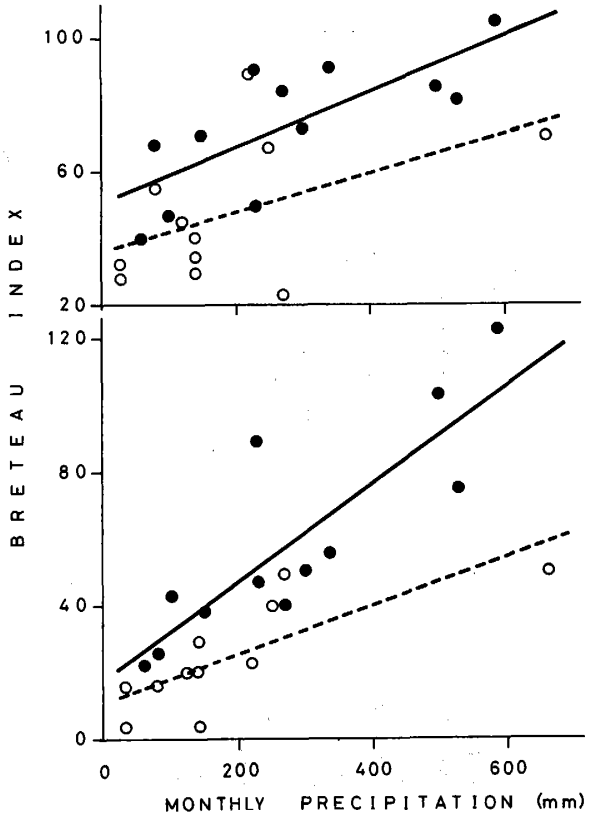

Figure 2 Relation between monthly precipitation and monthly Breteau index of *Ae. aegypti* (upper) and *Ae. pseudoscutellaris* (lower) in Suva (closed circles and solid lines) and Lautoka (open circles and broken lines), Fiji.

characterized by higher percentage of small containers (tin cans, empty bottles, and iron parts etc.) and drums. Little difference was found in the density of tire between two areas.

Difference in the density of available containers in the areas might cause the difference in predominance and prevalence of the two target species reflecting their oviposition preference. Table 2 shows the container index of the two species for six types of breeding containers. Actual preference for breeding containers was apparently different in the two species. As shown in Table 2, *Ae. aegypti* selectively bred into drums and tires, which were big in size, and water was not just temporal. On the other hand, *Ae. pseudoscutellaris* rather preferred to breed in small containers, but did not show strong preference for any particular types of containers.

Generally an urbanized area is composed by a variety of blocks with different socio-economic functions, such as the industrial block, residential block, commercial block, and so on. We examined the vector status in four functionally different blocks in Suva. Results were summarized in Table 3 and Fig. 4. As shown in Table 3, annual average Breteau index for *Ae. aegypti* was undoubtedly high in an industrial block located near a harbor. It was

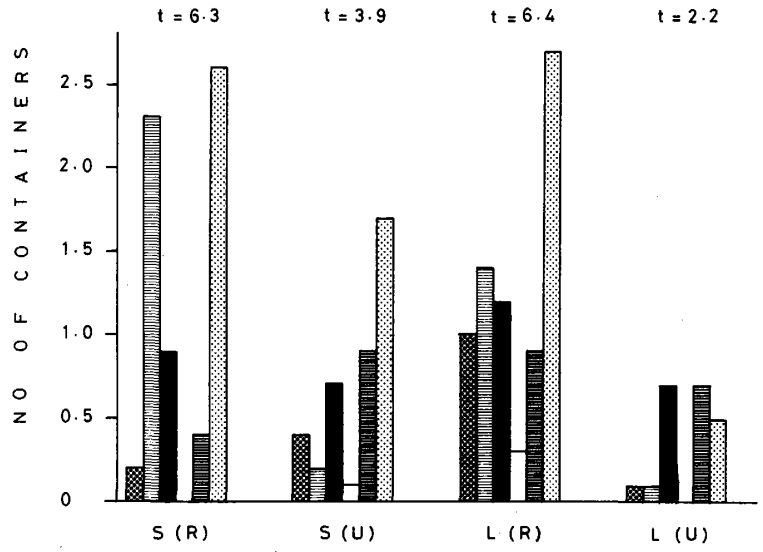

Figure 3 Average number of six types of available breeding containers per premise in rural area of Suva (S(R)), urban area of Suva (S(U)), rural area of Lautoka (L(R)) and urban area of Lautoka (L(U)), Fiji. "t" shows a total number of those containers per premise. Histograms are flower bases, drums, tires, roof gutters, planters and other small containers such as tin cans, empty bottles, sea shells and iron parts, etc. in order from the left in each area.

Table 2 Container index of *Ae. aegypti* and *Ae. pseudoscutellaris* for six types of breeding containers

Species \ Containers	Flower bases	Drums	Tires	Roof gutters	Planters	Other small containers*
Ae. aegypti	6.3	25.3	36.8	1.2	6.5	9.5
Ae. pseudoscutellaris	1.3	11.6	14.8	0.0	16.5	21.8

* Small containers include tin cans, empty bottles, sea shells and iron parts, etc.

followed by a residential block 'L', which was composed of two apartments of five stories, and the block was mainly occupied by residents with low income. A commercial block came to the third. In a residential block of the high income 'H', where premises were separated each other with a good marginal garden though almost all of them were rather older than the apartments of 'L', the index was extremely low. On the other hand, the index for *Ae. pseudoscutellaris* was nearly same in both of the residential blocks and the commercial block irrespective of housing conditions. Only in the industrial block, Breteau index was apparently low. The average number of six types of available breeding containers among four blocks was shows in Fig. 4. It was found by Fig. 4 that the number of available tires plus drums, which were most preferable breeding containers for *Ae. aegypti,* was evidently large in the industrial block, and the order among four blocks was just the same as for Breteaux index in Table 3. The number of containers other than tires and drums was not so different from each other except the industrial area.

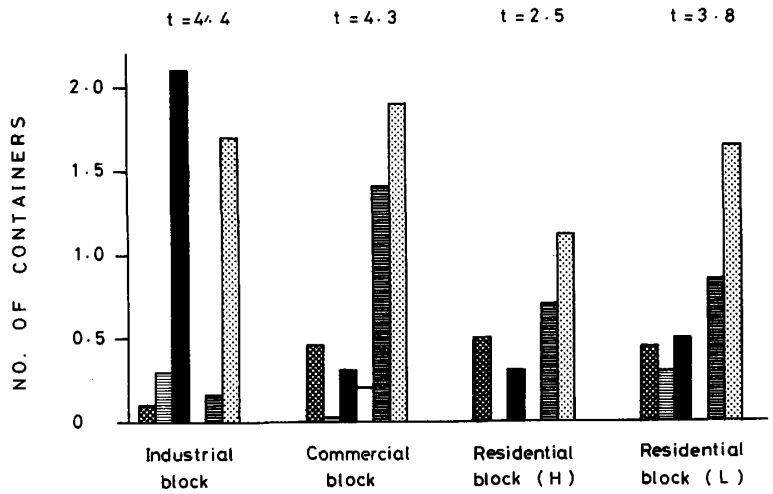

Figure 4 Average number of six types of available breeding containers per premise in industrial block, commercial block and two types of residential blocks in Suva, Fiji. "t" shows a total number of those containers per premise. Histograms are flower bases, drums, tires, roof gutters, planters and other small containers such as tin cans, empty bottles, sea shells and iron parts, etc. in order from the left in each area.

Table 3 Breteau index of *Ae. aegypti* and *Ae. pseudoscutellaris* in four town blocks with different socio-economic functions in Suva, Fiji

Species \ Type of block	Industrial block	Commercial block	Residential block (H)	Residential block (L)
Ae. aegypti	168	42	6	60
Ae. pseudoscutellaris	22	58	63	67

Discussion

The difference of average larval indices between rainy season and dry season in Table 1, and the positive regression between monthly total of precipitation and the monthly Breteau index in Fig. 2 clearly indicated that the amount of precipitation affects the incidence and abundance of *Ae. aegypti* and *Ae. pseudoscutellaris* in Fiji, as in the case of *Ae. aegypti* and/or *Ae. albopictus* in southeast Asian countries reported by Gould *et al.* (1970), Chan (1973, 1985), and Mogi *et al.* (1988). Severer infestation by the vectors in Suva, which is in 'the rainy side' as called by local people, is reasonable because the annual amount of precipitation in 1981 in Suva (3,320 mm) was nearly twice that in surroundings of Lautoka (1,685 mm), which is in 'the dry side'. It was also confirmed that the number of available breeding containers actually depends on the amount of precipitation.

But the number of available containers is not only decided by precipitation but also by the artificial factors in communities reflecting the life style, condition of the sanitation, and so on. Through this survey we could draw out the vector situation in rural and urban areas in the country as such that the number of available containers, especially small containers and drums, was prominently larger in rural area. The smaller number of containers in urban area was due to the establishment of a regular garbage collection service twice a week. This service greatly contributed to reduce the number of small containers such as tin cans, empty bottles, iron parts, etc. Another feature in respect of breeding containers in rural area is abundant drums, which were usual water storage tank in the country, owing to the lacking of piping water supply system in the area. It was interesting that the frequency of tires was similar irrespective of area. These differences in the density and the diversity of containers should have caused the differences in predominance and prevalence of the two target species in the areas, as their oviposition preference was different.

The container index for six types of breeding containers was different in the two species as shown in Table 2. *Ae. aegypti* apparently preferred to breed into not small and not just temporal containers such as drums and tires. On the other hand, the index for *Ae. pseudoscutellaris* was rather high for small containers, which were abundant in rural area. The closer relation between Breteau index and the amount of precipitation in *Ae. pseudoscutellaris* shown in Fig. 2, may be attributed to its oviposition preference. Similar difference was observed between *Ae. aegypti* and *Ae. albopictus* by Sunarto (1979) in Indonesia.

The vector situation was different in several urban blocks with different socio-economic functions but the same amount of precipitation. The high infestation of *Ae. aegypti* and the high density of tires were ascertained in the industrial block.

Considering the nature of two vector species and the status of available breeding containers in the country, improvement of the environmental sanitation such as regular garbage collection and piped water supply, would be the most effective larval control measures. Among a variety of breeding containers, tires are the most incriminatory to the infestation of the vectors as they distribute to all types of area in the country with high frequency. Any counter measures to this container especially in industrial areas should be given the first priority in this country expecting effective control of *Ae. aegypti*.

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フィジーにおけるデング熱媒介蚊の季節的消長と，降雨および幼虫発生容器の多寡の関係（1981年の調査結果）

高木　正洋[1]・I. M. Rakai[2]・D. Narayan[2]・
R. Ram[2]・G. Prakash[2]

1980年から1981年にかけて，フィジーの *Aedes aegypti* と *Ae. pseudoscutellaris* の発生状況と発生原因調査を，毎月定期的に実施した。多雨地域に位置する首都スバ市域と周辺の村落部，少雨地域の都市ラウトカの市域とその周辺の村落部の幼虫発生指数を検討した。発生は利用可能な容器の密度に依存すること，容器の密度は降雨量に依存することが確かめられた。降雨への依存程度は，*Ae. pseudoscutellaris* の方が高かった。

発生容器の多寡と多様性は，地域固有の社会経済的機能，住民の生活様式，公共の公衆衛生的サービスの質に左右された。降雨量の多少に拘らず，村落部では空き缶等の小容器とドラム缶の密度が高かった。これはゴミの定期的収拾と，パイプ給水の未実施と関係がある。古タイヤは工場地帯を除けば，地域を問わずまんべんなく分布していた。自然的および人為的要因による幼虫発生容器の存在状況の違いは，両デング熱媒介蚊種が属性として示す産卵選好水域の違いを通して，両種の浸淫度の地域間差異に帰結する。ゴミを定期的に収拾する地域の拡大と古タイヤ対策が，同国におけるデング熱媒介蚊防除対策の要と考えた。

1　長崎大学熱帯医学研究所病害動物学部門
2　Vector Control Unit, Ministry of Health, Fiji

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DEVELOPMENT OF MICROFILARIAE OF TWO BOVINE *ONCHOCERCA* SPECIES IN BLACKFLIES IN JAPAN

HIROYUKI TAKAOKA

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Abstract: Injections of blackflies with two types of bovine *Onchocerca* microfilariae (so far regarded as different stages of *O. gutturosa*) obtained from engorged *Simulium* spp. and umbilical skins of a slaughtered cattle in Oita, Japan, were carried out to determine whether they can develop in blackflies to the third-stage larvae (L_3). As a result, one type of microfilariae (i.e., long, slender and with uncoiled tail) developed in *S. arakawae* to the short L_3 (478 μm long), while another type (short, thick and with a strongly coiled tail) developed to the elongate L_3 (1,100-1,230 μm long) in two other *Simulium* species. Morphometric analysis of these L_3 shows that the former type microfilariae are likely to include *O. lienalis*, whereas the latter represent an undescribed species, readily distinguishable from *O. gutturosa* and *O. gibsoni* so far reported from Japanese cattle.

INTRODUCTION

During the surveys on the causative agent for "Wahi" or "Kose", a skin disease of cattle in Japan, Niimi and Kono (1953) found two types of unsheathed microfilariae in the skins of cattle—one with coiled tail and another, somewhat longer and thinner than the former and with its tail not coiled. These microfilariae, designated as X and Y respectively, were regarded as those of different developmental stages of the same species. Sato *et al.* (1954) considered both types of microfilariae as those of *Onchocerca gutturosa* Neumann, 1910, when they reported for the first time this bovine filaria from Japan based on the adult specimens. No other *Onchocerca* species has been found in Japanese cattle except for *O. gibsoni* (Cleland et Johnston, 1910), adult worms of which were recorded from a cow in Tokyo (Itagaki, 1953).

Recently, three types of third-stage larvae (L_3) belonging to *Onchocerca* were found in blackflies collected at a cattle shed in Oita, southern Japan (Takaoka and Bain, 1990). Among these, type I is probably a new species, while types II and III are each suspected as the L_3 of *O. gutturosa* and *O. lienalis* Stiles, 1908. Two types of unsheathed microfilariae, which corresponded to those reported by Niimi and Kono (1953) as mentioned above, were also recognized in the blood-fed female blackflies captured at the same cattle shed.

The present experiment was attempted to determine whether both types of microfilariae would develop in blackflies and whether their L_3, if developed, are related to three types of L_3 already found in blackflies.

Division of Medical Zoology, Medical College of Oita, Hazama, Oita 879-56, Japan

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Materials and Methods

Experimental infections were performed by injecting blackflies with a single or two microfilariae, following the method used by Reid (1979).

Two types of microfilariae examined were obtained by dissecting the stomach of freshly blood-fed blackflies soon after being captured at a cattle shed in Shimo-Onozuru, Oita City, where about 18 Holstein cattle were bred. Unfed female blackflies collected at the same site were inoculated with a single X or Y type viable microfilaria (Figs. 1, 3 and 4) in TC-199

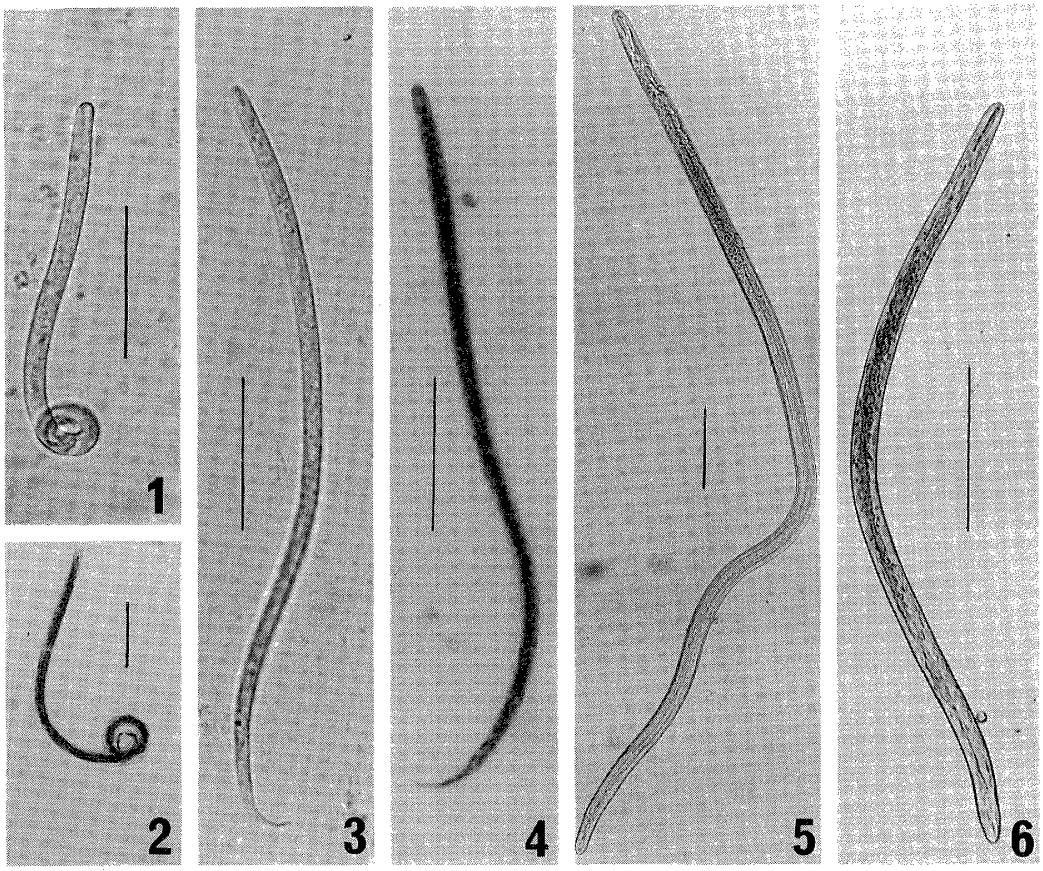

Figure 1 X type microfilaria found in the stomach of newly-blood fed blackfly, showing a strongly coiled tail (not stained). Scale, 50 μm.

Figure 2 X type microfilaria found in the umbilical skin of slaughtered cow (stained with Giemsa). Scale, 20 μm.

Figure 3 Y type microfilaria found in the stomach of newly-blood fed blackfly, showing uncoiled tail (not stained). Scale, 50 μm.

Figure 4 Y type microfilaria found in the stomach of newly-blood fed blackfly (stained with Giemsa). Scale, 50 μm.

Figure 5 Third-stage larva of an undescribed *Onchocerca* species recovered from *S. aokii* injected with X type microfilaria derived from an engorged fly. Scale, 100 μm.

Figure 6 Third-stage larva (probably *O. lienalis*) recovered from *S. arakawae* injected with Y type microfilaria derived from an engorged fly. Scale, 100 μm.

medium and maintained individually with sucrose solution at ca. 25°C. Seven to nine days after infection, all survived flies were dissected in saline and examined for filaria larvae. Parous flies were excluded to avoid possible natural infections. Larvae recovered were preserved in formalin-glycerol solution (Wharton, 1959) for morphometric observations.

In addition, X type microfilariae with coiled tail (Fig. 2) harvested from the umbilical skins of a cow slaughtered at a local abattoir were injected to newly emerged female blackflies (less than one day old).

RESULTS

Table 1 shows the results of experimental infections with microfilariae originated from the engorged flies. Two of the 36 injected flies died before examination, and seven survivors were parous females and thus excluded in this table. Among the 14 flies injected with X type microfilariae, three flies were positive for second-stage larvae (L_2) or L_3. Two L_2 were each found in the thorax of *Simulium bidentatum* (Shiraki), while one L_3 (Fig. 5) was recovered from the head of *S. aokii* (Takahasi). On the other hand, of 13 flies injected with Y type microfilariae, only one fly (*S. arakawae* Matsumura) had an L_3 (Fig. 6).

Table 2 reveals the results of infections of newly emerged *S. bidentatum* with dermal microfilariae of X type. Seventeen of the 18 flies injected survived until the eighth day after inoculation. Four of 17 flies dissected had L_3 in their thorax or abdomen. One fly harboured an L_2 in the thorax.

The measurements of all these larvae found are shown in Table 3.

DISCUSSION

The present data show that both X type microfilariae obtained from the engorged flies and the umbilical skins of a slaughtered cow could develop to the elongate L_3, as found in *S. aokii* and *S. bidentatum*. These L_3, as well as three L_2 found in *S. bidentatum*, probably belong to the same species, judging from the large body size and relative short oesophagus (less than

Table 1 Results of thoracic inoculations of blackflies with a single X or Y type microfilaria recovered from the stomach of blood-fed blackflies caught in cattle shed

Type of Mf.	Blackflies		No. flies dissected	No. flies infected	Stage* of larva
	Donor	Recipient			
X	*S. arakawae*	*S. bidentatum*	3	1	L_2
〃	*S. bidentatum*	*S. aokii*	3	1	L_3
〃	*S. bidentatum*	*S. arakawae*	1	0	
〃	*S. bidentatum*	*S. bidentatum*	7	1	L_2
Y	*S. aokii*	*S. arakawae*	3	0	
〃	*S. aokii*	*S. bidentatum*	1	0	
〃	*S. arakawae*	*S. aokii*	1	0	
〃	*S. arakawae*	*S. arakawae*	1	0	
〃	*S. bidentatum*	*S. aokii*	4	0	
〃	*S. bidentatum*	*S. arakawae*	3	1	L_3

* L_2, second stage; L_3, third stage

Table 2 Results of thoracic inoculations of *Simulium bidentatum* with type X microfilariae obtained from the umbilical skin of a slaughtered cattle

No. Mf. inoculated	No. flies dissected	No. flies infected	No. & stage* of larvae
1	9	2	$1L_3$, $1L_3$
2	8	3	$1L_2$, $2L_3$, $2L_3$

* L_2, second stage; L_3, third stage

Table 3 Measurements of *Onchocerca* larvae recovered from *Simulium* spp. dissected 7-9 days after injection of microfilariae

Source & type of Mf.		Landmarks*								Larval stage†	*Simulium*
		BL	BW	NR	OE	(GL)	OE/BL	TL	TW/TL		
Fly's stomach,	X	1,216	25.8	76	517	(407)	0.43	41.8	2.2	L_3	*S. aokii*
〃	〃	646	30.0	—	304	(—)	0.47	38.0	1.7	L_2	*S. bidentatum*
〃	〃	776	27.0	—	342	(—)	0.44	38.0	1.7	L_2	〃
〃	Y	478	18.8	53	304	(205)	0.64	30.1	2.0	L_3	*S. arakawae*
Cow's skin,	X	1,102	25.8	—	517	(384)	0.47	—	—	L_3	*S. bidentatum*
〃	〃	1,172	26.6	—	580	(410)	0.49	—	—	L_3	〃
〃	〃	1,231	26.2	—	600	(403)	0.49	41.8	2.1	L_3	〃
〃	〃	1,193	26.6	81	562	(433)	0.47	40.0	2.2	L_3	〃
〃	〃	1,140	26.6	91	502	(365)	0.44	41.8	1.9	L_3	〃
〃	〃	1,193	26.2	84	494	(357)	0.41	—	—	L_3	〃
〃	〃	532	28.5	—	209	(114)	0.39	—	—	L_2	〃

* BL: total body length; BW: maximum body width; NR: distance from head tip to nerve ring; OE: length of oesophagus; GL: length of glandular part of oesophagus; OE/BL: ratio of length of oesophagus/total body length; TL: tail length; TW/TL: ratio of tail width/tail length.

† L_2, second stage; L_3, third stage.

1/2 of the whole body length). These large L_3 conform well to those of type I *Onchocerca* which were reported from blackflies maintained alive for 6-9 days after collections at the same cowshed by Takaoka and Bain (1990). On the other hand, Y type microfilariae, though only in one case, developed to the short L_3 in *S. arakawae*. The measurement of this larva agrees with that of L_3 of type III *Onchocerca* found in *S. arakawae* which was suspected as *O. lienalis* (Takaoka and Bain, 1990).

In this experiment, no L_3 of *O. gibsoni* or *O. gutturosa* were attained. Biting midges are known as the vector of the former bovine filaria (Buckley, 1938; Ottley and Moorhouse, 1980) and of the latter (Bain, 1979; El Sinnary and Hussein, 1980; Davies *et al.*, 1989). Further, there were no reliable references reporting blackflies as the vector of these filariae, although several investigators (eg., Mwaiko, 1981) claimed so for *O. gutturosa*. The present data are not enough to conclude that microfilariae of *O. gibsoni* or *O. gutturosa* were present in the materials used but could not develop to L_3 in blackflies. Instead, it is considered that most of the Y type microfilariae examined were *O. lienalis* and a few, if present, were *O. gibsoni* or *O. gutturosa,* because an acid phosphatase activity pattern of Y type microfilariae observed (unpublished data) is consistent to that of *O. lienalis,* as reported by Trees *et al.* (1987). Further experiments are needed to determine the susceptibility of blackflies to *O. gutturosa*

and *O. gibsoni,* using identified dermal microfilariae of cattle, since L_3 of type II *Onchocerca,* indistinguishable from the former filaria, were already recovered from *S. bidentatum* (Takaoka and Bain, 1990).

In conclusion, X type microfilariae with coiled tail represent a hitherto undescribed species, distinct from *O. gutturosa* and *O. gibsoni,* while Y type microfilariae are likely to include *O. lienalis.* The present results indicate that there are at least four *Onchocerca* species in Japanese cattle (i.e., *O. gibsoni, O. gutturosa,* probably *O. lienalis* and an unnamed species). Two blackfly species, *S. arakawae* and *S. kyushuense* Takaoka, are naturally infected with L_3 indistinguishable from *O. lienalis* in Oita and Kumamoto, respectively. On the other hand, *S. bidentatum* serves as the natural vector of the fourth unnamed *Onchocerca* in both areas (unpublished data). More detailed data of seasonal transmission of these bovine *Onchocerca* spp. will be published in separate papers.

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我が国の牛に寄生するOnchocerca属仔虫二型のブユ体内での発育

高岡　宏行

我が国の牛に寄生する *Onchocerca gutturosa* の仔虫として報告されていた二型の仔虫(X, Y)を，牛舎で吸血した直後のブユの中腸と一部は直接牛の皮膚から得て，ブユの胸部へ実験的に感染させ，第三期幼虫への発育の有無を調べた。その結果，体長が短く，尾部を強く巻く特徴を有するX型仔虫は，キアシツメトゲブユとアオキツメトゲブユの胸筋で，体長が1,100-1,230μmと長い第三期幼虫へ発育した。一方，体長が細長く，尾部を巻いていないY型は，一例だけであるが，ヒメアシマダラブユで，体長が478μmの第三期幼虫へ発育した。第三期幼虫の形態観察から，前者は，既知のいずれの種とも異なる未記録種で，後者は *O. lienalis* と思われる。すなわち，今回の実験により，X型仔虫は，我が国の牛から既に報告されている *O. gutturosa* および *O. gibsoni* とは明らかに異なる未記録種の仔虫であることが示された。一方，Y型仔虫のなかには，*O. gutturosa* および *O. gibsoni* の存在を否定はできないが，*O. lienalis* が含まれていることが強く示唆された。

大分医科大学医動物学教室

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INVESTIGATIONS ON GUINEA WORM DISEASE IN ANAMBRA STATE, NIGERIA -TREATMENT OF PATIENTS, EXAMINATION OF DRINKING WATER AND INFLUENCE ON SCHOOL ATTENDANCE

HIROSHI OHARA[1] AND KOICHIRO FUJITA[2]

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Abstract: Investigation and treatment were performed on the cases with guinea worm disease (dracontiasis) in Abakariki district of Anambra State, Nigeria. In the classification of 63 patients under study by their professions, 76% were agricultural workers, 19% school boys and girls, and average age of the patients was 21 years old. For the treatment, albendazole was given to the patients, and the course up to the complete excretion of worm body was carefully observed. Whole worm body was excreted within 5 days in average, and the ulcerated region of the skin was healed thereafter. Bacterial colonies counts were examined on the drinking water by dividing water sources into five groups of ponds, shallow wells, deep wells, rivers and tap water. The results of the test revealed that the water in ponds, shallow wells and rivers was heavily contaminated and cyclops, the intermediary host of *Dracunculus medinesis,* was found in large quantity, suggesting frequent and easy infection of the disease. In elementary schools, many pupils (59.6% of all) were absent due to guinea worm disease, and this suggests that the disease exerts extensive social and economical influence such as the decrease of school attendance ratio or the decline of agricultural production. This disease is related with the hygienic standards of drinking water, and the prevention of the disease is extremely important. The construction of deep wells is urgently wanted, while it is also essential to provide good knowledge of hygiene to the inhabitants through education.

INTRODUCTION

Guinea worm disease is a parasitic diseases which is caused by infection of *Dracunculus medinensis,* and this disease is widely distributed on much of the Guinea coast of West Africa (Muller, 1971; Raffier, 1966; Onabamiro, 1952). Above all, Anambra State in eastern part of Nigeria is the heaviest endemic area (Nwosu *et al.,* 1982), and the majority of the inhabitants is infected in some districts. The disease exerts tremendous influence not only on health and hygiene but also on social and economical status of the country, and the preventive measures against this disease are very important. The source of infection is the drinking water containing cyclops (*Thermocyclops nigerianus* and/or *Mesocyclops leuckarti*) which have the

1 Department of International Cooperation, National Medical Center, 1-21-1, Toyama, Shinjuku-ku, Tokyo, 162 Japan
2 Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo, 113 Japan

infected larvae of *Dracunculus medinensis* (Onabamiro, 1952). For the prevention of the disease the supply of clean drinking water is considered as most essential (Brian, 1984).

Investigation was performed on the actual conditions of the guinea worm disease and the drinking water in this area, and conducted the treatment of the patients using albendazole. Further, the effective measure for the prevention of the disease was considered. The present paper deals with these results.

Materials and Methods

The investigations were performed for 28 days from early May, 1988 in agricultural area around Abakariki district of Anambra State in the eastern part of Nigeria. The investigation consisted of the following three items.

1. *Examination and treatment of the patients at the treatment center in the endemic area*: Sixty-three new patients with the disease were examined and treated. For the treatment, 400 mg of albendazole (Zentel®, Smith-Kline French) was given orally to most of the patients. The end of worm body was fastened with thread and slowly pulled out. The course from the administration of the drug to the complete excretion of worm body was carefully observed.

2. *Bacterial colonies counts of the drinking water and counting of cyclops contained in water*: These examinations were performed for 5 types of water: water in ponds (at 7 points), shallow wells (6), deep wells (5), river water (2) and tap water (4). Numbers of total bacterial colony and coliform group counts were measured by using URICULT set (Daiichi Kagaku Co.). Number of cyclops contained in 2 liters of water was measured. The method to count cyclops has been described in previous papers (Onabamiro, 1950; Onabamiro, 1954).

3. *Survey on the reasons of absence in elementary schools*: The ratio of the absent pupils due to guinea worm disease was investigated in 2 elementary schools (total number of pupils: 431). The survey was conducted on the materials kept in file at these schools on the absent pupils during the period from January to December, 1987.

Results

1. *Examination and treatment of the patients at the treatment center in the endemic area*: The patients complained of severe pain, and ulceration was seen on and around the site, from where worm body emerged. In most of the cases, the ulcerated lesions were affected by the secondary infection with bacteria, or the patients were incapacitated. After the drug was given, the pain was alleviated. Worm body was excreted completely after 5 days in average, and the skin lesion was healed. The age of 95% of the patients ranged from 5 to 40 years (21 years in average). In the classification by professions, 76% were agricultural workers, 19% school boys and girls and 5% others. The details of some of the cases are described below.

Case 1: 15-year-old junior high school boy. The patient lived in a village and has been drinking the water of a pond nearby. From May 4, 1988, a part of the skin on left dorsal region of foot was ulcerated, and worm body began to emerge. Similar symptom was seen two years ago, and the patient had undergone treatment. On May 9, the patient was examined at the treatment center and was diagnosed as guinea worm disease. Eosinophilia of mild degree (13%; WBC 8,300/mm^3) was noted. The ulcerated lesion was suppurated by

the secondary infection, and the patient complained of severe pain and suffered from dysbasia. 400 mg of albendazole was given, and the end of the worm was fastened with thread and slowly pulled out (Photo. 1). At the same time, antibiotic ointment was applied on the ulcerated lesion. After the onset of the treatment, pain was alleviated, and worm body was completely excreted until May 14. Thereafter, the ulcerated lesion began to heal.

Case 2: 18-year-old male farmer. The patient has been habitually drinking water of shallow well, however he was also drinking water of the ponds during his work on farm. From May 12, 1988, worm body began to appear from his left upper thigh. The patient wound up the worm body daily little by little by himself, and 22 cm of worm body came out after 10 days. He had suffered from guinea worm disease by 10 times in the past. On May 22, the patient was examined at the treatment center and was diagnosed as guinea worm disease (Photo. 2). 400 mg of albendazole was given. Eosinophilia (24%; WBC 7,700/mm^3) and mild degree of anemia (Hb 8.9 mg/d*l*; RBC 310×10^4/mm^3) were recognized. The pain was relatively mild. The worm body was completely excreted two days after the administration of albendazole.

2. *Bacterial colonies counts of the drinking water and counting of cyclops contained in water*: Contamination of water in deep wells and tap water was very little. Whereas the water in ponds, shallow wells and river was contaminated more in this order, and the water quality criteria of WHO were not met for the items. In all of the ponds and one-half of the shallow wells, innumerable bacterial colonies were found (Table 1). The number of cyclops in 2 liters of water was 0 in both tap water and deep wells, whereas it was 38, 10 and 120 in average in shallow wells, rivers and ponds respectively (Fig. 1).

Table 1 Results of bacterial colonies counts on various water sources

Source		Temperature	pH	Total* colonies	Coliform* group
Pond	(1)	32	5.6	100**	80
	(2)	36	6.2	100**	40
	(3)	33	5.8	100**	120
	(4)	32	5.7	100**	110
	(5)	34	5.6	100**	110
	(6)	34	5.8	100**	100
	(7)	33	5.9	100**	80
Shallow well	(1)	29	5.6	100**	10
	(2)	28	6.6	100**	150
	(3)	28	6.4	100**	30
	(4)	27	6.4	100	20
	(5)	28	6.6	100	20
	(6)	29	6.5	100	10
River	(1)	27	6.4	80	20
	(2)	27	6.5	80	20
Deep well	(1)	31	7.6	4	0
	(2)	32	7.0	80	20
	(3)	28	6.8	0	0
	(4)	28	7.0	10	5
	(5)	29	7.1	10	0
Tap water	(1)	31	6.8	10	5
	(2)	29	6.7	0	0
	(3)	30	6.6	10	0
	(4)	31	6.8	5	0

* per 1 m*l* of water
** innumerable

3. *Survey on the reasons of absence in elementary schools*: Of the cumulative number of absent pupils (1,551) in a year (3.6 times in average per pupil), 924 pupils (59.6%) were absent due to guinea worm disease. The absence ratio due to guinea worm disease showed strong seasonal fluctuation, and it was high in March—September period, which agrees

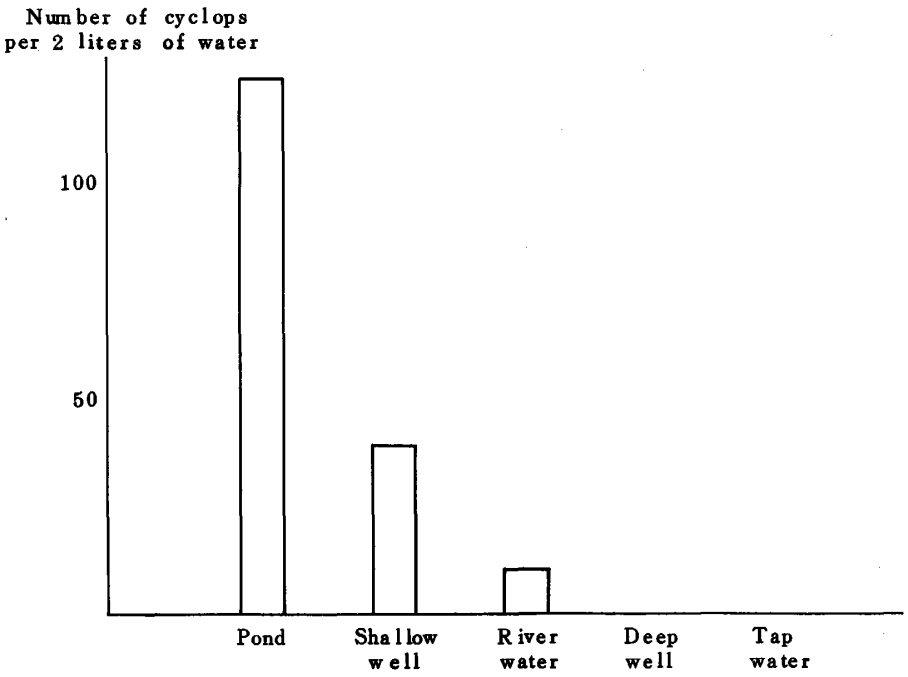

Figure 1 Total number of cyclops recoverd from various water sources.

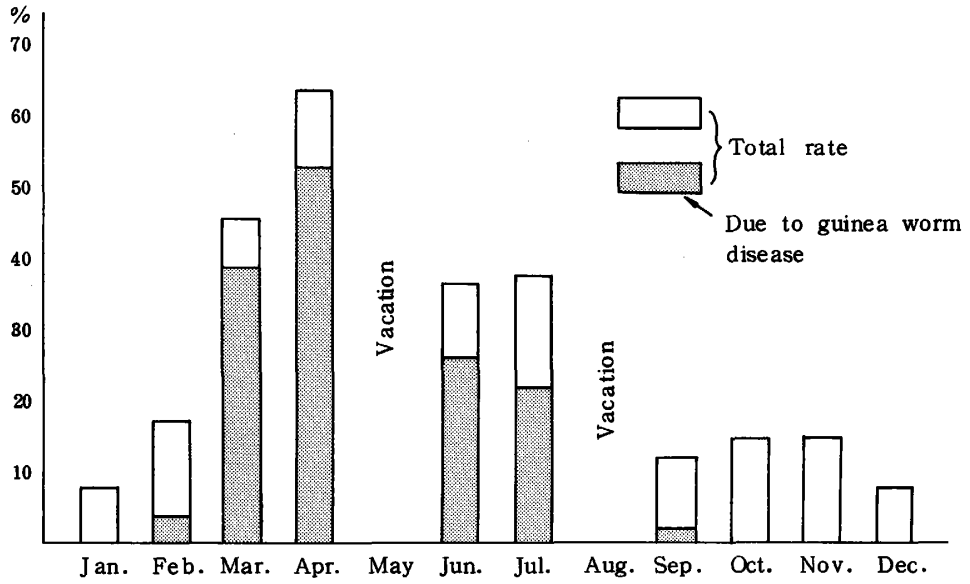

Figure 2 Percentage of absenteeism of school pupils by month.

well with the infection period of guinea worm season (Fig. 2).

Discussion

There have been several reports on the actual status of guinea worm disease in Nigeria (WHO, 1987; Kale, 1977; Abolarin, 1981). The infection ratio was particularly high in Abakariki district of Anambra State, and 50-75% of the inhabitants there are said to be infected (Nwosu *et al.*, 1982). The results of the present investigation revealed that guinea worm disease causes serious problems and that it is necessary to take effective measures promptly. The drug used for the treatment, albendazole, was effective to facilitate excretion of the worm and had little side effect. By giving this drug, strong antiparasitic effect is obtained not only on *Dracunculus medinensis* but also on *Ascalis lumbricoides, Anchylostoma duodenale, Enterobius vermicularis, Trichuris trichiura* (Rossignol and Mausonneuve, 1984) and hydatid disease (Suimot *et al.*, 1983). The treatment by this drug is recommended for the patients, however high price of the drug is the bottleneck.

The water which the inhabitants are usually drinking is contaminated with cyclops to considerable extent. Because about 10-25% of cyclops are considered as infected with *Dracunculus medinensis* (Nwosu *et al.*, 1982) the inhabitants become frequently infected with the disease when they drink the water. At the same time, total bacterial colonies and coli form colonies are innumerably found in water, and the inhabitants are easily infected with various types of water-borne infectious diseases. In fact, the incidence of typhoid fever, dysentery, diarrhea, etc. is high in this district (Ministry of Health, Nigeria, 1987). Relatively speaking, more patients of the disease are found in younger generation, and most of the patients are agricultural workers and school boys and girls. High incidence among the agricultural workers may be attributable to the fact that they become thirsty during work and drink water of the ponds very frequently.

Guinea worm disease not only causes much pain in the patients but results in the decline of agricultural production (Belcher *et al.*, 1975) or the decrease of school attendance ratio (Ilegbodu, 1986). Thus, the disease has tremendous social and economical influence. The supply of clean drinking water is the matter of primary importance. To prevent the disease, it is recommended to take such corrective measures as the propagation of water supply system, the construction of deep wells, the sprinkling of the pesticide against cyclops in the pond, the enlightenment campaign for the inhabitants to boil and filter the drinking water, etc. (Rao *et al.*, 1981; Hopkins and Foege, 1981).

Special emphasis is now given to the construction of deep wells, and the incidence of guinea worm disease and other water-borne infectious diseases has remarkably decreased in the areas where deep wells have been provided. In Abakariki district, the construction of deep wells is now under way under the assistance from Japan, and it is expected that this project will provide extensive effect for the prevention of guinea worm disease. However, there is the inveterate habit to drink the water of ponds among the inhabitants, and the elimination of such habit is not very easy. It is essential to propagate the good knowledge of hygiene through education among the inhabitants.

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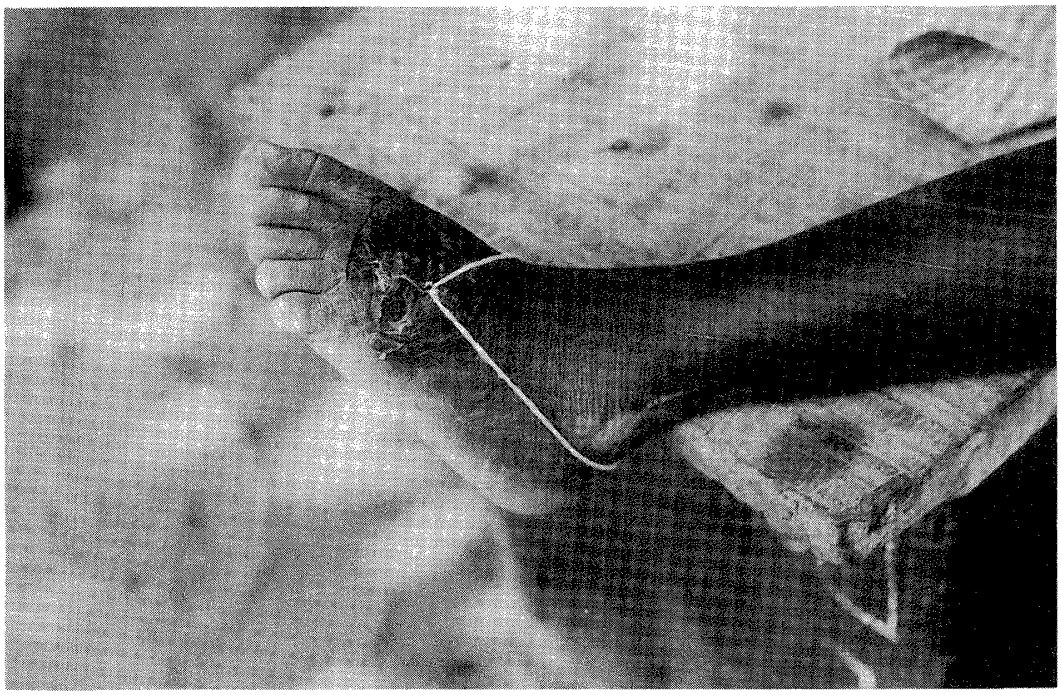

Photo. 1

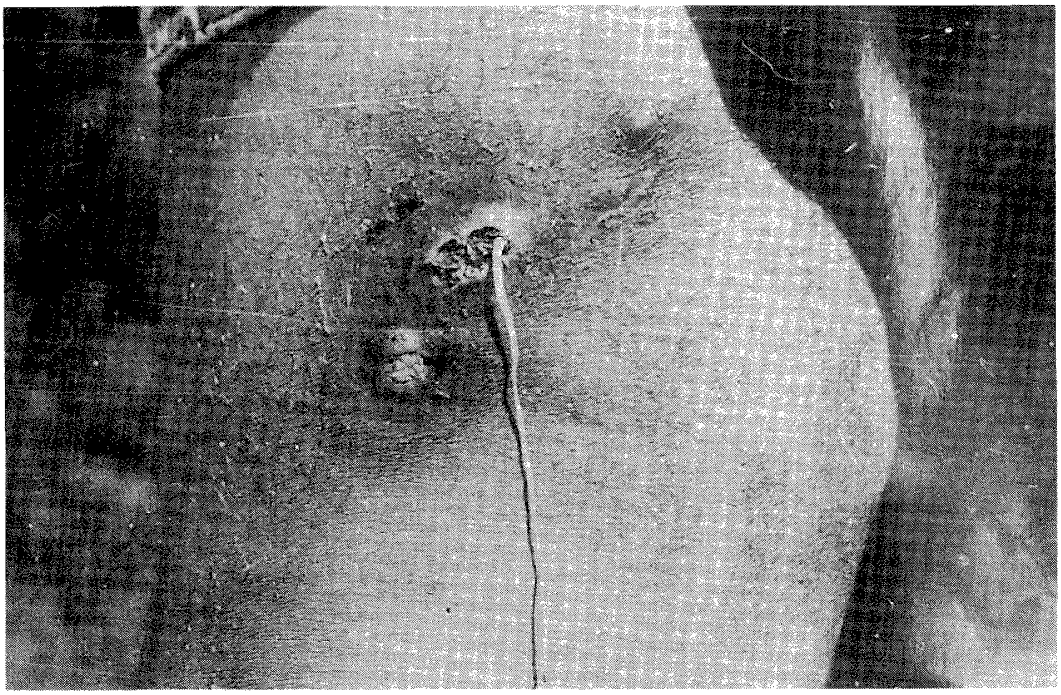

Photo. 2

ナイジェリア，アナンブラ州アバカリキ地区におけるギニアワーム症の調査
—治療，飲料水の検査および就学率に及ぼす影響

小原　博[1]・藤田紘一郎[2]

ナイジェリア，アナンブラ州アバカリキ地区において，ギニアワーム症の診療および調査を行った。63名の患者の職業別内訳は，農業が76%，学生が19%で，平均年齢は21歳であった。患者に対してはアルベンダゾール内服により治療を行い，虫体が排泄されるまで観察を続けた。虫体は平均5日で排泄され，その後皮膚の潰瘍部は治癒に向かった。溜め池，浅井戸，深井戸，河川水，水道水の5群に分けて飲料水の水質検査を行った結果では，溜め池，浅井戸，河川水の汚染がひどく，*Dracunculus medinensis* の中間宿主であるミジンコも多数認められ，容易に感染しやすいことが窺われた。小学校においてはギニアワーム症のために欠席する児童が多く（全体の59.6%），この疾患が就学率低下や農業生産低下など社会・経済的に及ぼす影響が大きいことが示唆された。この疾患は飲料水の衛生と密接に結びついたもので，その対策は極めて重要な課題である。現在，深井戸の建設が望まれているが，住民に対する衛生教育も同時に進めていく必要があると思われる。

1　国立病院医療センター国際医療協力部 東京都新宿区戸山1-21-1
2　東京医科歯科大学医学部医動物学 東京都文京区湯島1-5-45

症例報告

ヒトヒフバエ *Dermatobia hominis* による蝿症の1例

前田龍一郎[1]・牧田 絵美[1]・瀬川 満[2]・
澁谷 敏朗[1]・荻野 幹夫[2]

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はじめに

近年，海外との交流が盛んになり，種々の輸入症例に出会うことも稀ではなくなった。本報告で述べる，ヒトヒフバエ *Dermatobia hominis* は，中南米のメキシコからチリ，アルゼンチンに及ぶ地域に限局して分布している。その人体内寄生期間が2—3カ月と長いことからOldroyd and Smith (1973) が"Because of the long larval life, cases of infestation with *D. hominis* may appear in hospitals in any part of the world"と述べたとうり，すでに我が国でも5例 (Kagei *et al.*, 1974; 藤原ら, 1977; 大滝ら, 1978; 加藤ら, 1983; 滝ら, 1989) が記録されている。本症例も，患者が1988年12月中旬ブラジル滞在中に感染した後，来日して発症し受診したものと考えられる。

ヒトヒフバエは，成虫が主に昼間行動性の蚊などの吸血性昆虫を捕えて卵を産みつけ，その運搬者が吸血する時に，卵から孵った蛆が人に侵入し皮下に寄生する。したがって，その寄生部位は頭部，上腕部などが多い (Rook, 1986; Manson-Bahr and Bell, 1987)。これまでの我が国の5例でも，腰より上部がその寄生部位であった。James (1947) は，マダニが運搬者となりうると述べているが，本例はその発症部位が右足第1，2趾間であり，受診1カ月前に同部に小出血を認めたことから，運搬者としては虻またはダニが考えられる。

海外渡航者の増加とともに，今後このような症例が増加すると考えられるが，その発症部位にとらわれない対応が必要と考え，その1例として報告する。

症　例

患者：22歳女，10歳よりブラジルに在住。現在サンパウロ市から車で1時間のところに居住。

初診：1989年1月23日

主訴：右足第1，2趾間の発赤腫脹

家族歴，既往歴：特記すべきことはない。

現病歴：1988年12月中旬，右足第1，2趾間に吸血動物の刺咬によると思われる小出血を認め，その後しばらくして同部に希血性浸出液を含む瘻孔ができた。瘻孔を中心として，しだいに腫脹も出現してきた。1989年1月1日来日したが，1月23日に国立病院医療センター受診。受診時，右足第1，2趾間に発赤を伴う約1 cm径の腫脹があり，中央の1 mm径の瘻孔より希血性の浸出が見られた。1，2趾の運動機能には支障がなかった。通院にて消毒を続けたが症状はかわらず，1月27日，小ピンセットにて排膿洞内を探ったところ，内部より全長9.5 mm，3.5 mm径の淡黄色の虫体が採取された。瘻孔は，その後3日でふさがり，発赤，腫脹も消失した。

1 帝京大学医学部寄生虫学教室（東京都板橋区加賀2-11-1）
2 国立病院医療センター整形外科（東京都新宿区戸山1-21-1）

虫体所見

後端が摘出時にちぎれた虫体の大部分と，後端の表皮が一部付着した内蔵の断片2個が得られ，これらについて形態学的に検討した(図1)。虫体は前部が肥大した紡錘形で，ホルマリン浸漬のためか淡黄色を呈していた。後方気門を含む後端は摘出時に失われていたが，ホルマリン固定により収縮した状態での計測で体長9.5 mm，体幅3.5 mmであった。口鉤は明瞭に認められ(図2)，体表面には鉤状の棘が第7節までみられ尾端に向かっていた。形態的な特徴を写真記録した後，虫体を10%水酸化カリウム液で処理し，体表の棘(図3)，および咽頭骨格（図4）の形態を検討した。

前半が太く後半が括れた外部形態，体表面の棘の形態およびその配列，花房状の前方気門が見られないことなど(James, 1947)から，ヒフバエ科のヒトヒフバエ *Dermatobia hominis* の2齢幼虫と同定した。

考　察

ヒトヒフバエ *Dermatobia hominis* は，中南米のメキシコからチリ，アルゼンチンに及ぶ地域に限局して分布している。本虫は人のみでなく，ウシ，ウマ，ブタ，ヒツジなどの家畜や，イヌ，ネコなどのペット，さらに野生動物，鳥類にも寄生する。成虫は森林性で，雌は蚊，ダニなどの吸血性昆虫や虻を捕えて卵を産みつけ，運搬者が吸血あるいは汗を舐める時に，卵から孵った蛆が人に侵入し皮下に寄生する（Busck, 1912; Rook, 1986; Manson-Bahr and Bell, 1987)。したがって，流行地での感染機会は偶発的であり，ユカタン半島の遺跡巡り(大滝ら，1978)，コスタリカでのバードウォッチング（Everett *et al.*, 1977)，ブラジルでのラン採集（Iannini *et al.*, 1975）の際感染したと考えられる症例などが報告されている。また，その人体内寄生期間が2－3カ月と長いことから，流行地以外の世界各地で症例が認められ，すでに我が国でも5例が記録されている。本症例も，患者が1988年12月中旬ブラジル滞在中に感染した後，来日して発症し，受診したものと考えられる。

本症においては細菌による二次感染を合併する場合が多く，抗生剤の投与によって症状の一部は改善されるが，虫体の摘出によって初めて完治する。これまでに報告された症例では，抗生剤の投与を続けたが改善せず，1－2カ月後に自然排出された場合などが見られる。ヒトヒフバエなどの真性寄生種による皮膚蝿症では，頂点の穴を適当なもので塞ぎ幼虫の呼吸を抑制して這い出させることで，虫体の一部を残すことなく完全に取除くことが出来る。このため，流行地では，蜜蠟やチューインガム(James, 1947)，豚脂(Iannini *et al.*, 1975; Everett *et al.*, 1977）を厚く塗布する方法などが行われており，Everettらは，heavy creamを用いて良好な結果を得た事を報告している。本症例では，外科的に虫体を摘出したが，中南米への旅行歴などから本症が疑われる場合には，ワセリン基剤の軟膏を厚く塗布することにより，幼虫の自発的排出を計る方法を試みることが，患者への抗生剤長期投与を避けるためにも推奨される。

ま と め

ヒフバエ科のヒトヒフバエ *Dermatobia hominis* による蝿症の1例を報告した。

患者は10歳よりブラジルに在住の22歳の女性で，1988年12月中旬，右足第1，2趾間に小出血を認め，その後同部に希血性浸出液を含む瘻孔ができ，しだいに腫脹も出現してきた。1989年1月1日来日し，日本の知人宅に滞在中1月23日に国立病院医療センターで受診した。通院にて消毒を続けたが症状はかわらず，1月27日，瘻孔内より全長9.5 mm，3.5 mm径の淡黄色の虫体が採取された。瘻孔は，その後3日でふさがり，発赤，腫脹も消失した。摘出された幼虫は，ヒトヒフバエ *Dermatobia hominis* の2齢幼虫であった。

中南米への旅行歴などから本症が疑われる場合には，患者への抗生剤長期投与を避けるためにも，ワセリン基剤の軟膏を厚く塗布することにより，幼虫の自発的排出を計る方法を試みることが推奨される。

稿を終るにあたり，虫体の同定に御教示をいただいた東京医科大学の篠永哲助教授に深謝する。

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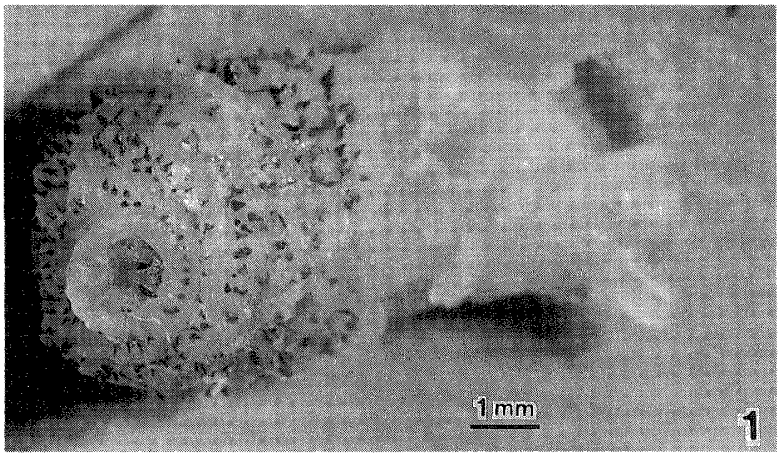

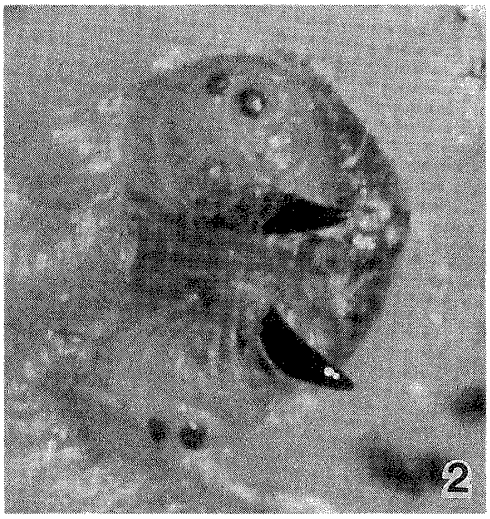

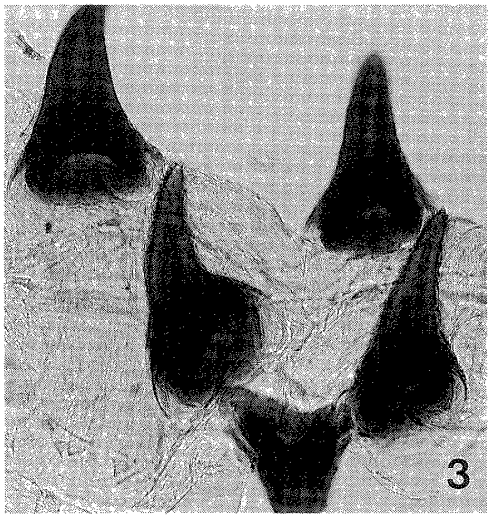

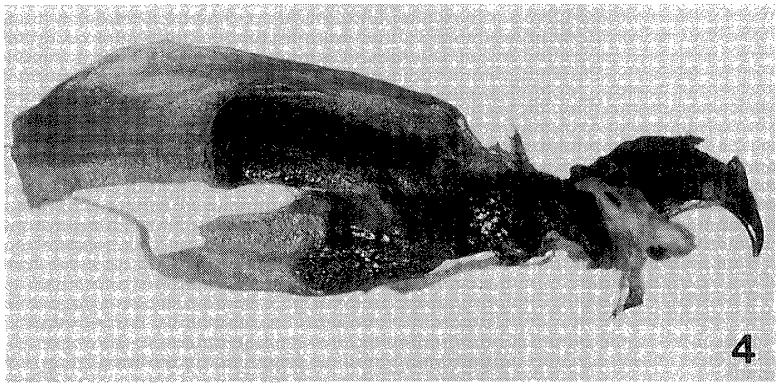

Figure 1 Second stage larva of *Dermatobia hominis* removed from the patient.
Figure 2 Anterior part of *Dermatobia hominis* second stage larva.
Figure 3 Spines on the surface of fifth segment of *Dermatobia hominis* second stage larva.
Figure 4 Cephalopharyngeal skeleton of *Dermatobia hominis* second stage larva.

Case report

A CASE OF MYIASIS CAUSED BY *DERMATOBIA HOMINIS*

RYUICHIRO MAEDA[1], EMI MAKITA[1], MITSURU SEGAWA[2],
TOSHIRO SHIBUYA[1] AND MIKIO OGINO[2]

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One case of human myiasis caused by *Dermatobia hominis* was reported.

The patient was a 22-year-old Japanese woman who had been in Brazil for 12 years. She lives in the suburbs of São Paulo City at present.

About the middle of December 1988, slight bleeding was noticed between the first and the second toe of right foot. She came to Japan for sightseeing on January 1 and visited National Medical Center on January 23, 1989. When she consulted the doctor, the swelling of 10 mm in diameter was noticed which was accompanied by the oozing of blood. The affected part was not cured after 3 days of sterilization. On January 27, a maggot was taken out from the lesion surgically. After the maggot was removed from the patient, the symptom disappeared rapidly. The maggot was 9.5 mm long and the color was yellowish white. The strong spines were distributed on the surface from the third to the seventh segment. The posterior spiracles were lost during the process of extirpation of the worm. The anterior spiracles were small and not flowerlike in appearance. It was identified as scond instar larva of *Dermatobia hominis* based upon above findings.

Occlusion of the punctum on the skin with vaseline causes the maggot to migrate out of the skin to breathe.

1 Department of Parasitology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173, Japan

2 Department of Orthopedic Surgery, National Medical Center, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162, Japan

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