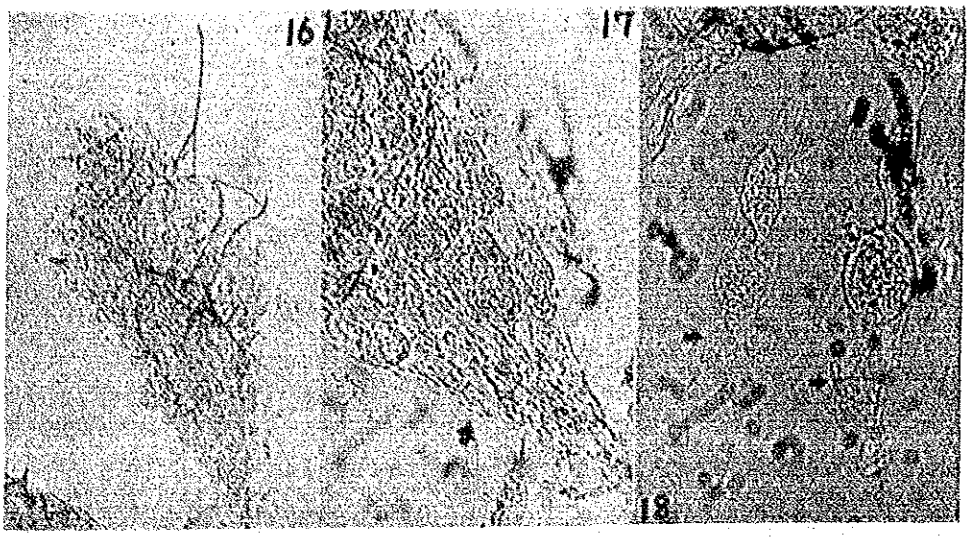
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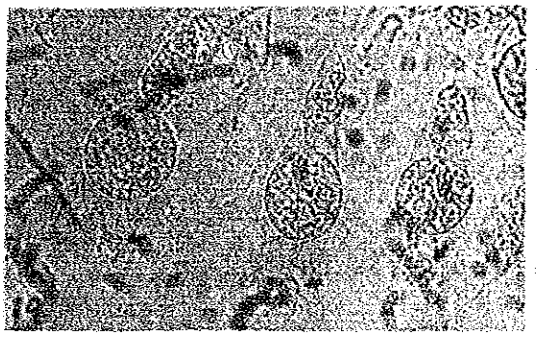
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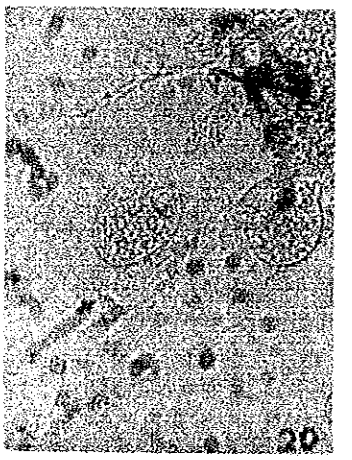
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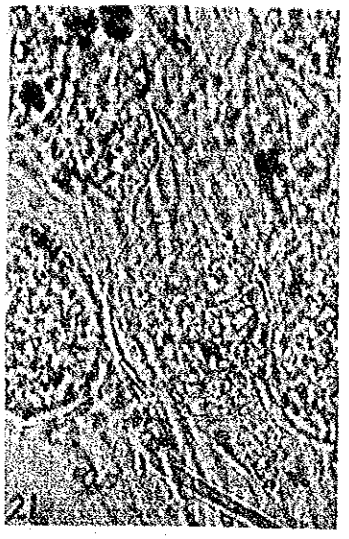
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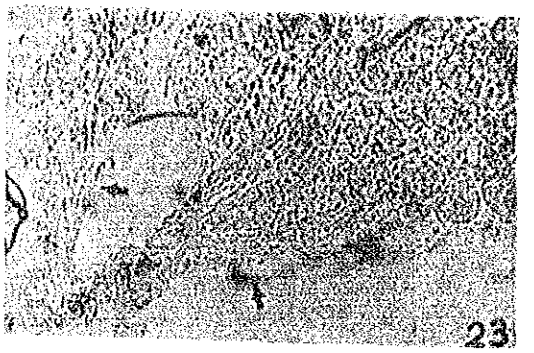
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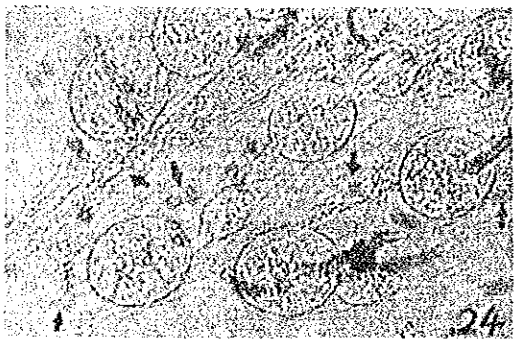
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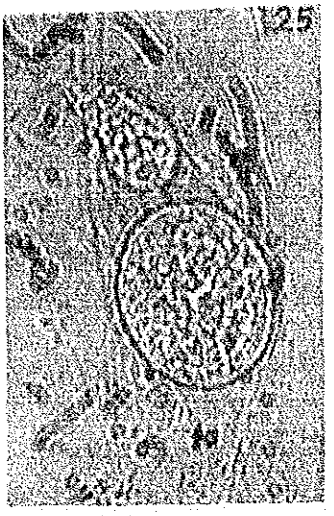
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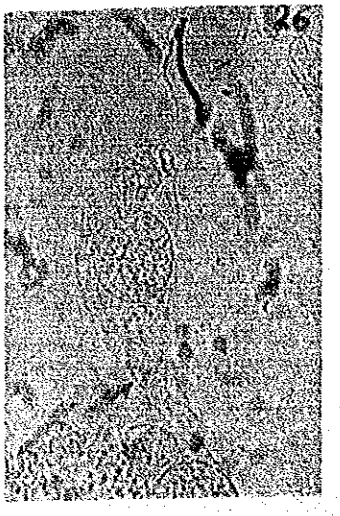
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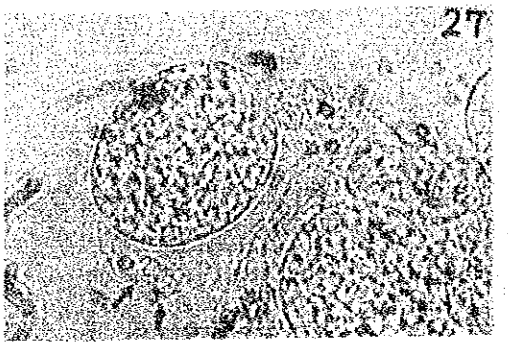

27

写 真 説 明

Inmediatamente después de emerger.

1: Ovariolas

2: Primer folículo es inmaduro en la etapa No. II.

Dos días después de emerger.

3: Ovarios íntegros

4: Oviducto lateral transparente, sin residuos celulares.

5: Primer folículo etapa Ib. pedicelo corto y difícil de ver.

6: Pedicelo no visible.

7 & 8: Algunas veces el epitelio folicular se rasga y los granulos foliculares se mueven dentro del pedicelo, causando dificultad en la identificación.

Inmediatamente después de la oviposición.

9: Integramente los ovarios se tornan amarillentos.

10: Oviducto lateral con considerable residuos celulares, consistentes del remanente de la célula nutricia y el epitelio folicular.

11, 12y 13: Mayoría de las ovariolas tiene saco parecido a túnica, conteniéndo muchos residuos celulares.

Aproximadamente 15 horas después de oviposición.

14: Túnica contraida

15: Algunas túnicas están contraidas completamente.

Un día después de oviposición

16: Ovario íntegro

17: Oviducto lateral contiene muchos residuos celulares

18, 19 y 20: Cada ovariola tiene una dilatación conteniendo reliquia folicular (unípara)

21: Dilataciones frecuentemente difícil de verlas, regularmente hay solamente residuos celulares.

Dos días después de oviposición.

22: Ovarios íntegros

23: Oviducto lateral aún no es transparente

24: Residuos celulares se encuentran dentro del cáliz.

25: Algunas ovariolas tienen dilataciones pequeñas, por eso es esencial poner mayor atención, para determinar la edad de la mosca.

26: Hembras paras, 2 días después de la oviposición se distinguen examinando los residuos celulares del folículo.

27: Ovarios que tienen dos dilataciones (diparas).

防除対象種 Simulium ochraceumの生態

1980年9月末グアテマラ国S.N.E.M.に着任した際の最大の課題は，プロジェクトエリアの南西にあるグアチピリン渓谷のオンコセルカ症ベクターの駆除の準備を進めることであった。

この目的を遂行するため，幼虫殺虫剤の剤型比較実験，薬剤投入間隔の再検討，ベクターの主発生水域の確認等の調査研究を行った。得られた知見を以下にまとめる。

1. 殺虫剤の剤型比較実験

プロジェクトエリアの北西に位置するバレタール渓谷においては，田原（1979）が設計し，松尾（1980）が改良したテメホス0.3%固型剤がベクターの駆除に使用され，目覚ましい成果を上げている。しかし，この剤型はTw.20のような非常に高価な表面活性剤が使用されること，製剤に手数がかかり，大量生産できないこと，等の点で問題がある。ベクター駆除地域を拡大するには大量の薬剤を必要とするので，現地で比較的入手容易な50%テメホス水和剤を採用したいという考え方が以前からあった。この問題解決のため，前任者の上村らが，雨期にグアチピリン渓谷の主要支流を使って広範囲にテメホス固型剤と同水和剤の効力比較実験を行った。その結果，水和剤はブユ幼虫駆除において有効であり，固型剤に替えて利用できるという結論を得ている。

われわれの行った実験は乾期における上村らの実験の追試に目的をおいた。試験方法は次の通りである。

1） グアチピリン渓谷の16支流を対象とし，3週間の間隔を置いて0.3%テメホス固型剤と5%同水和剤を10分間流水量あたり2 ppmの濃度で交互に散布する。2） 薬剤散布直前と1日後に，ブユ幼虫の10分間採集を100～150 m下流まで行い，その効果を判定する。

1980年10月から1981年1月までに2回の試験が行われた。得られた成績を第10表に示す。

水和剤のブユ幼虫に対する効力は上村らの（1980）成績とほとんど差がなく，有効であり，固型剤に替って使用できることを示唆している。これらの成績にもとづいて1981年3月3日から発足したグアチピリン渓谷のベクター駆除では，従来の固型剤と交替して，50%テメホス水和剤が全面的に使用されている。

2. グァチピリン渓谷におけるベクターS.ochraceumの幼虫の分布

剤型比較実験はテメホス水和剤の効力だけではなく，対象種S.ochraceumの幼虫の生態と分類について重要な示唆をわれわれに与えた。

第3図は1978年8月から1980年8月までの2年間のプロジェクトエリア内に設けられた9定点においての人囮を用いた3時間S.ochraceum雌成虫の採集成績である。この採集は2週間間隔で行われたが，作図の便宜上，月別にまとめられた。上記期間中に駆除事業の行われることのなかったグアチピリン渓谷のPeña Blancaと Guachipilin 23の成績は，この渓谷でのS.ochraceum雌成虫の季節的消長を代表して示すものと考えられる。この成績によれば，本効力実験の開始時期の10月下旬はS.ochraceumの雌成虫の数がピークにさしかかるシーズンに相当する。したがって各支流の条件は，本種幼虫の発育に好都合となり，幼虫の分布範囲も最大限に拡大しているものと予想された。

第1－6表は10月21日から11月7日までの効力実験の最初の1サイクルにおける，対象16支流に設定された各薬剤投入定点での薬剤投入前の幼虫10分間採集の成績である。

予想に反して，S.ochraceumの幼虫採集数は少く他の種に比べて優勢に発生していると確認できる定点は17−0−2（第3表）を除いてなかった。この期間中全定点の幼虫採集総数は5,193個体，そのうちmetallicumが67.5%を占め，第1位，S.ochraceumは僅か6.6%で第3位にとどまっている。従ってS.ochraceumを駆除対象種と考える立場では，この効力実験が薬剤散布対象河川の選択を誤まっていたことを示唆するものと考えられる。

第7表はS.ochraceum幼虫の採集数を散布定点における水量別に検討した成績である。

S.ochraceum幼虫の発生数は水量 6ℓ/sec 以下の定点で，とくに 1ℓ/sec 以下で多くなっている。1ℓ/sec 以下の水量であった定点での採集数の合計は総採集数の 70%以上を占めている。しかし奇妙なことに水量 9ℓ/sec のところで再び採集されている。この幼虫を詳細に調べた結果，体の色調，その他 2，3 の特徴で，0.3ℓ/sec以下の定点で発生する幼虫と明瞭に区別できることが判明した。そこで 0.3ℓ/sec以下の幼虫をA群，9ℓ/sec 以上の水域で出現するものをB群と呼び，両者の分布を検討した。A群は 1ℓ/sec 以下の水量の水域に出現し，0.3ℓ/sec以下ではA群のみとなる。一方B群は 0.3ℓ/sec以上の水域に出現し，1ℓ/sec 以上の水域ではすべてB群であった。したがってA，B両群は 0.3ℓ～1ℓ/sec の水域を推移帯としてすみ分けていることになる。

これまで発表された幼虫検索表では〔大西ら（1977）を含めて〕S.ochraceum と同定されるこれら両群は生態の異なる別種の疑いが持たれる。

グァチビリン渓谷におけるベクター駆除事業では，発生数の多いA群を駆除の対象とし，1ℓ/sec 以下の水域に薬剤散布を集中することになった。

3. S.ochraceum幼虫（A群）の主発生源の特徴

10 月 29 日，Gua 18—10 の支流に薬剤散布洩れとなって，S.ochraceumの幼虫が発生していることが発見された。そこでこの支流と同じく散布洩れの Gua 18—0の源流を使って，薬剤散布間隔を再検討する調査を行った。薬剤投入前と投入後 4 日以内の間隔で 10 分間幼虫採集を繰り返し行う方法が採用された。調査は 18－10 の支流が涸れるまで行われた。

その結果，水和剤の効力持続距離は 20～77m と予想外に短かく，一方幼虫，蛹の再出現までの日数はそれぞれ 16～22 日，28～30 日と長くなっている。この調査は反覆追試する必要がある。

各調査回の合計幼虫採集数とこれに対する S.ochraceum，S.metallicum，S.horacioi，S.payneiの占める割合（%）を第 1 図に示した。

Gua 18-10—1A と Gua 18—10—2の投入点での薬剤投入前の幼虫採集数は，それぞれ 193，150個体，うちS.ochraceumは 184，125個体となっている。これ

は先の効力比較実験における10月21日～11月7日までの期間の各定点でのS.ochraceumの採集数よりけた違いに多い。しかも他の種より圧倒的に多く採集されている。この傾向は薬剤の影響の全く及ばなかったGua 18−10−1Bで，流れの涸れる1週間前まで連続して観察された。ある種の発生数が多く，しかも他の種より優勢である期間が長いことが，その種の主発生源と認定できる条件とすれば，Gua 18−10の支流は，グアテピリン渓谷におけるS.ochraceum（A群）の主発生源の一部をなしているといえる。しかし同じ源流型の水域でも，18−0の支流のようにS.horacioiの主発生水域と推定されるような水域も存在する。

そこで，18−0と18−10の支流の相違点を分析し，表8に示す2点の特徴を見出した。S.ochraceum（A群）の主発生源の特徴は比較的背の高い滝にはさまれて河床勾配の緩やかな，いわゆるたなと呼ぶ部分が存在すること，水量が0.3ℓ/sec以下であることの2点に要約できる。

そこでプロジェクトエリアの内外で，これらの特徴を持つ水域を探し，そこでの幼虫の発生様相を調査した。その成績を表9に示した。観察例数はまだ少いが上記の特徴の要約が的外れでないことを示している。

4. S.ochraceum（A群）の産卵習性

1月中旬，グァテマラ市で開かれたオンコセルカ症に関する合同会議で，Maria Monroyが飽血したS.ochraceumの雌成虫を実験室で飼育して，産卵させるのに成功したことを報告した。その観察によれば雌成虫は卵塊をつくらずに，1個ずつばらばらに卵を産み落すとのことであった。しかしこの成績から，直ちに野外でもS.ochraceumは卵塊をつくらずに産卵するとみなすことはできない。何故なら野外で卵塊をつくって産卵するS.oitanumでも実験室のような人工環境ではしばしば1個ずつばらばらに卵を産みつけることが久納によって観察されているからである。

前項のS. ochraceum（A群）の主発生水域の特徴を具えたMonica Ivoneの支流3－1－5のブユ幼虫の採集調査を行った際，5個体の卵が採集された。この卵はシャーレの中で幼虫の附着した基物を洗った際，器底の泥の中に混じって沈

んでいる状態で発見された。このような卵の状態から，これらすべては雌成虫が1個ずつばらばらに，しかも基物に固着させずに産み落された沈性卵であると考えられる。そこでこの卵の同定を試みた。

カナダの Imhof（1979）はS.decorum他4種の卵の検索表を発表し，卵の形よりもサイズの方が変異の幅が少く同定に利用できると主張している。

そこで，これら5個の卵のサイズを測り，伊藤，Hansen から恵与された実験室で産下されたS.ochraceum 50 個の測定値との比較を行った。その成績を第2図に示した。実験室で産下された卵のサイズの変異は長径 0.20㎜から 0.25㎜の間に，また短径は0.15㎜から 0.20㎜の間にそれぞれ分布している。野外から採集された5個の卵のサイズは，実験室で産下された卵のサイズの変異幅の中に包含され，区別することはできなかった。したがってこれら野外の卵をS.ochraceumと同定しても誤同定である危険は小さいものと思われる。

このようにS.ochraceumの雌成虫が野外で沈性卵を1個宛，流れに産下する習性を持つのであれば，河床勾配のゆるやかなたなを具えた流れを選択する確率が高いことは，良く理解できる。なぜなら河床勾配の急なたなを具えた流れでは産下された卵が河床に落ちつくまでに，卵や幼虫の発育に不適な下流に流されてしまう危険が多くなるからである。

5. 今後の問題点

グアチビリン渓谷のベクター駆除事業では発生数が少いという理由でS.ochraceum（B群）を一時的に駆除対象から除外した。しかし発生数の多少だけで，B群のベクターとしての役割を評価しようとするのは，適当とはいえない。B群の吸血嗜好性や，O.volvulus Mf との親和性などについてもA群と比較し，ベクターとしての役割を判定しなければならないだろう。このため，成虫での同定が急がれる。またB群とA群の生態の比較についても更に検討する必要があると思われる。

（執筆者　上　本　駿　一）

Table 1.

The result of larval collection before application in the tributaries of Gunchipilín River Basin (21 st. Oct. - 7th Nov., 1980)

Tributary No.	Application Site No.	Water Amount ℓ/sec.	S. ochr. complex Nos. (%)	S. rot. complex Nos (%)	S. call. complex Nos. (%)	S. hons. Nos. (%)	Non-Onch. Nos. (%)	TOTAL (100 %)
22-0	1	1.33	11 (8.7)	87 (69.0)	26 (20.6)	0	2 (1.6)	126
"	2	1.00	9 (20.0)	32 (71.1)	4 (8.9)	0	0	45
"	3	0.67	0	1 (50.0)	1 (50.0)	0	0	2
"	4	0.15	0	10 (100.0)	0	0	0	10
"	5	0.15	1 (7.7)	12 (92.3)	0	0	0	13
"	6	0.50	1 (33.3)	0	2 (66.7)	0	0	3
"	7	0.10	0	0	11 (100.0)	0	0	11
22-1	1	0.03	0	0	4 (100.0)	0	0	4
23-0	1	0.23	2 (50.0)	2 (50.0)	0	0	0	4
"	2	0.13	9 (30.0)	6 (20.0)	15 (50.0)	0	0	30
"	3	0.07	17 (31.5)	30 (55.6)	5 (9.3)	0	2 (3.7)	54
"	4	0.03	0	9 (34.6)	2 (7.7)	1 (3.9)	14 (53.8)	26
"	5	0.17	0	15 (93.8)	0	1 (6.2)	0	16
23-1	1	0.03	0	0	0	1 (100.0)	0	1
28-0	1	1.00	14 (18.4)	56 (73.7)	6 (7.9)	0	0	76
"	2	1.00	12 (9.5)	108 (85.7)	4 (3.2)	0	2 (1.6)	126
"	3	0.67	1 (1.0)	96 (99.0)	0	0	0	97
"	4	0.67	0	4 (100.0)	0	0	0	4

Table 2.

The result of larval collection before application in the tributaries of Guachipilín River Basin (21 st Oct. - 7th Nov., 1980)

Tributary No.	Application Site No.	Water Amount ℓ/sec.	S.ochr. complex Nos. (%)	S. net. complex Nos (%)	S. call. complex Nos. (%)	S. horac. Nos. (%)	Nonanthropophylic species Nos. (%)	TOTAL (100 %)
21-0	1	2.00	3 (3.0)	89 (89.0)	8 (8.0)	0	0	100
"	2	0.50	3 (2.1)	104 (72.2)	37 (25.7)	0	0	144
"	3	0.40	8 (44.4)	10 (55.6)	0 (0.0)	0	0	18
21-1	1	1.50	0	13 (92.9)	1 (7.1)	0	0	14
"	2	0.33	0	23 (88.5)	3 (11.5)	0	0	26
20-0	1	5.00	0	215 (91.5)	6 (2.6)	0	14 (6.0)	235
"	2	3.33	0	326 (93.9)	14 (4.0)	0	7 (2.0)	347
"	3	3.33	3 (3.1)	86 (89.5)	0	0	7 (7.3)	96
"	5	0.83	0	49 (76.6)	13 (20.3)	2 (3.1)	0	64
"	6	0.83	11 (40.7)	8 (29.6)	1 (3.7)	7 (25.9)	0	27
"	7	0.83	3 (9.7)	11 (35.5)	0	17 (54.8)	0	31
20-1	1	0.16	7 (6.4)	58 (52.7)	0	45 (40.9)	0	110
20-2	1	0.16	0	1 (25.0)	3 (75.0)	0	0	4
"	2	0.13	1 (3.0)	17 (51.5)	0	15 (45.5)	0	33
"	3	0.20	17 (31.5)	30 (55.6)	2 (3.7)	5 (9.3)	0	54
"	4	0.16	18 (17.5)	16 (15.5)	1 (0.9)	68 (66.0)	0	103
20-4	1	0.83	9 (12.7)	12 (16.9)	0	50 (70.4)	0	71

Table 3.

The result of larval collection before application in the tributaries of Guachipilín River Basin (21 st Oct. - 7 th Nov., 1980)

Tributary No.	Application Site No.	Water Amount ℓ/sec.	S. ochr. complex Nos. (%)	S. pet. complex Nos (%)	S. call. complex Nos. (%)	S. horac. Nos. (%)	Nonanthropophylic species Nos. (%)	TOTAL (100 %)
37-0	1	2.66	4 (4.3)	38 (40.4)	52 (55.3)	0	0	94
"	2	3.33	7 (10.0)	20 (28.6)	40 (57.1)	0	3 (4.3)	70
"	4	1.00	20 (22.5)	51 (57.3)	17 (19.1)	0	1 (1.1)	89
37-1	1	1.00	2 (100.0)	0	0	0	0	2
17-0	1	16.00	7 (25.0)	4 (14.3)	17 (60.7)	0	0	28
"	2	16.00	33 (66.0)	8 (16.0)	9 (18.0)	0	0	50
26-0	1	1.50	0	31 (40.3)	43 (55.8)	0	3 (3.9)	77
"	2	0.33	0	20 (66.6)	10 (33.3)	0	0 (0.0)	30
"	3	0.13	1 (1.8)	47 (82.5)	4 (7.0)	0	5 (8.8)	57
"	4	0.33	0	163 (88.1)	1 (0.5)	0	21 (11.4)	185
"	5	0.33	9 (5.4)	80 (47.6)	23 (13.7)	0	56 (33.3)	168
"	6	0.33	1 (1.3)	76 (98.7)	0	0	0	77
32-0	1	0.16	0	4 (100.0)	0	0	0	4
30-0	1	1.50	5 (6.8)	38 (52.0)	25 (34.2)	5 (6.8)	0	73
14-0	1	7.00	0	196 (97.0)	6 (3.0)	0	0	202
"	2	3.50	5 (4.1)	105 (86.0)	9 (7.3)	0	3 (2.5)	122
14-1	1	0.33	0	1 (100.0)	0	0	0	1
14-2	1	0.33	0	3 (20.0)	0	12 (80.0)		15
14-3	1	0.16	0	6 (100.0)	0	0	0	6
			94	891	256	17	92	1,350

Table 4.

The result of larval collection before application in the tributaries of Guachipilín River Basin (21 st Oct. - 7th Nov., 1980)

Tributary No.	Application Site No.	Water Amount ℓ/sec.	S.ochr. complex Nos. (%)	S. set. complex Nos (%)	S. call. complex Nos. (%)	S. horse Nos. (%)	Nonanthropophylic species Nos. (%)	TOTAL (100 %)
11-0	1	3.00	1 (2.8)	35 (97.2)	0	0	0	36
"	2	2.00		7 (100.0)	0	0	0	7
"	3	2.00	5 (23.8)	15 (71.4)	1 (4.8)	0	0	21
"	4	1.33	12 (34.3)	12 (34.3)	11 (31.4)	0	0	35
10-0	1	5.00	0	72 (77.4)	21 (22.6)	0	0	93
"	2	5.00	0	10 (66.7)	4 (26.7)	0	1 (6.7)	15
"	3	4.00	0	71 (100.0)	0	0	0	71
10-3	1	1.00	0	11 (91.7)	1 (8.3)	0	0	12
9-0	1	5.00	0	14 (87.5)	2 (12.5)	0	0	16
9-1	1	3.00	0	40 (95.2)	2 (4.8)	0	0	42
"	2	1.00	0	22 (100.0)	0	0	0	22
9-2	1	1.33	0	1 (50.0)	1 (50.0)	0	0	2
9-2	2	0.67	0	69 (74.2)	24 (25.8)	0	0	93
"	4	0.33	11 (50.0)	3 (13.6)	8 (36.4)	0	0	22
"	5	0.40	0	1 (16.7)	5 (83.3)	0	0	6
19-2	1	0.03	0	27 (96.4)	0	1 (3.6)	0	28
19-3-0	1	0.07	0	26 (83.9)	0	3 (9.7)	2 (6.4)	31
19-3-1	1	0.76	0	2 (66.7)	1 (33.3)	0	0	3

Table 5.

The result of larval collection before application in the tributaries of Guachipilín River Basin (21 st Oct. - 7th Nov., 1930)

Tributary no.	Application (Site no.)	Water Amount ℓ/sec.	S. ochr. complex Nos. (%)	S. net. complex Nos (%)	S. call. complex Nos. (%)	S. horac. Nos. (%)	Nonanthropophylic species Nos. (%)	TOTAL (100 %)
19-0	1	5.33	0	20 (55.6)	11 (30.6)	0	5 (13.8)	36
"	2	4.33	0	8 (14.8)	34 (63.0)	0	12 (22.2)	54
"	3	3.00	1 (3.2)	9 (29.0)	16 (51.6)	0	5 (16.1)	31
"	4	0.83	1 (6.3)	15 (93.8)	0	0	0	16
"	5	1.00	1 (1.0)	45 (44.6)	46 (45.5)	0	9 (8.9)	101
"	6	0.33	0	1 (100.0)	0	0	0	1
"	7	0.67	0	0	2 (100.0)	0	0	2
"	8	0.50	11 (50.0)	8 (36.4)	3 (13.6)	0	0	22
18-0	1	10.00	0	122 (79.7)	27 (17.6)	0	4 (2.6)	153
"	2	8.33	0	62 (66.0)	30 (31.9)	0	2 (2.1)	94
"	3	6.67	0	37 (38.1)	41 (42.3)	0	19 (19.6)	97
"	4	6.67	0	39 (35.8)	55 (50.5)	0	15 (13.8)	109
"	5	5.00	0	11 (20.4)	31 (57.4)	0	12 (22.2)	54
"	6	5.00	0	60 (89.6)	3 (4.5)	0	4 (6.0)	67
"	6A	1.00	9 (13.6)	39 (59.1)	16 (24.2)	0	2 (3.0)	66
"	7	1.00	7 (6.4)	102 (93.6)	0	0	0	109
"	8	0.33	26 (50.9)	20 (39.2)	2 (3.9)	0	3 (5.9)	51
18-1	1	0.17	0	0	0	7 (100.0)	0	7

Table 6.

The result of larval collection before application in the tributaries of Guachipilín River Basin (21 st Oct. - 7th Nov., 1980)

Tributary No.	Application time	Water count l/sec.	S.ochr. complex Nos. (%)	S. pet. complex Nos (%)	S. cell. complex Nos. (%)	S. [illegible] Nos. (%)	[illegible] protozoic species Nos. (%)	TOTAL (100 %)
18-2	1	0.83	0	29 (74.4)	10 (25.6)	0	0	39
18-3	1	0.10	0	0	0	6 (100.0)	0	6
18-6	1	0.10	1 (33.3)	0	2 (66.7)	0	0	3
18-6B	1	0.06	0	0	17 (100.0)	0	0	17
18-6C	1	0.03	0	2 (18.2)	9 (81.8)	0	0	11
18-10	1	1.00	3 (21.4)	11 (78.6)	0	0	0	14
18-12	1	0.33	1 (33.3)	1 (33.3)	1 (33.3)	0	0	3
TOTAL (%)			344 (6.6)	3,506 (67.5)	862 (16.6)	246 (4.7)	235 (4.5)	5,193 (100.0)

TABLE 7.

Rate of S. ochr, larval occurrence in streams with different water volume

Water amount ℓ/sec	<0.30	0.30～0.59	0.60～1.00	1.10～3.00	3.10～6.00	6.10～9.00	9.00<	TOTAL
Nos. of application site	25	16	23	13	13	4	3	97
Nos. of S.ochraceum larvae % of occurring	74 (21.5)	71* (20.6)	102* (29.7)	42** (12.2)	15** (4.4)	0	40** (11.6)	344 (100.0)
Nos. of S.ochraceum larvae/site	2.96	4.43	4.43	3.23	1.15	0	13.3	3.55
Nos. of total collections	643	772	1111	658	1276	502	231	5193
Component ratio of S. ochraceum larvae (%)	11.5	9.2	9.2	6.4	1.2	0	17.3	6.6

* The collection mixtured with B-group.

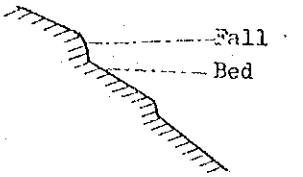
** The collection consists of only B-group

The population of S. ochraceum in Guachipilín River is consisting of both, A- and B-groups which can be differentiated from each one in larval stage excepting for the stream with less than 0.3ℓ/sec and more than/1ℓ/sec. water discharges.

Table 8 Characterization of main breeding streams of Simulium horacioi and S. ochraceum

A-type; main breeding streams of S. horacioi

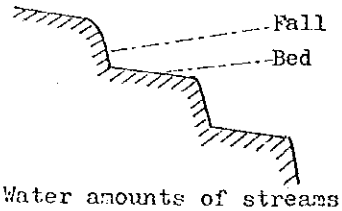
1. With small falls and precipitous stream-beds.



2. Water amounts of streams ordinarily more than 0.3 /sec.

B-type; main breeding streams of S. ochraceum

1. With rather large falls and rather flat stream-beds.



2. Water amounts of streams ordinarily less than 0.3 /sec.

Table 9 Component ratio of blackfly larvae in different streams belonging to each type.

Type	Streams	Date of coll.	Nos. and component ratio (%)				Total (100%)
			S. hora.	S. ochr.	S. meta.	other species	
A	Guachipilin	'80					
	20-1-1	X-21	45(40.9)	7(6.4)	58(52.7)	0	110
	20-2-4	X-21	68(66.7)	18(17.6)	16(15.7)	0	102
	20-4-1	X-21	37(63.8)	9(15.5)	12(20.2)	0	58
	Buena Vista						
	4-3-1	XII-16	64(82.1)	14(17.9)	0	0	78
B	Monica Ivo.						
	3-1-3	XII-3	0	41(68.3)	2(3.3)	17(28.3)	60
	3-1-4	XII-3	0	158(65.8)	0	82(34.2)	240
	3-1-5	XII-3	0	57(41.6)	2(1.5)	78(56.9)	137
	Buena Vista						
	1-0-2	XII-17	0	43(100.0)	0	0	43

Table 10 Effectiveness tests of 5% Abate water dispersible powder and 0.3 % Abate solid form with the dosage of 2 ppm per 10 minute water discharge against blackfly larvae.

Stream No.	Water dispersible powder		Solid form		Stream No.	Water dispersible powder		Solid form	
	Pre*	Pos**	Pre*	Pos**		Pre*	Pos**	Pre*	Pos**
22-)	7	0	3	0	20- 0	8	0	7	1
23-)	5	0	4	0	20- 1	1	0	1	0
18-)	9	2	6	1	20- 2	3	0	4	1
18- '	1	0	1	0	20- 4	1	0	1	1
18-	1	0	1	1	37- 0	5	1	4	1
19- (	8	2	5	1	10- 0	5	0	3	0
19-	1	0	1	0	10- 3	1	0	1	0
17- (	2	0	2	0	9- 0	2	0	2	0
26- (	6	2	7	3	9- 1	4	1	2	0
30- (	1	0	4	0	9- 2	4	0	4	0
14- (	2	1	2	1	11- 0	4	0	4	0
14- 2	1	0	1	0	Total	82	9	70	12

* No. of positive application sites before application.

** No. of positive application sites one day after application.

Fig. 1 Reappearance of blackfly larvae after larvicide application

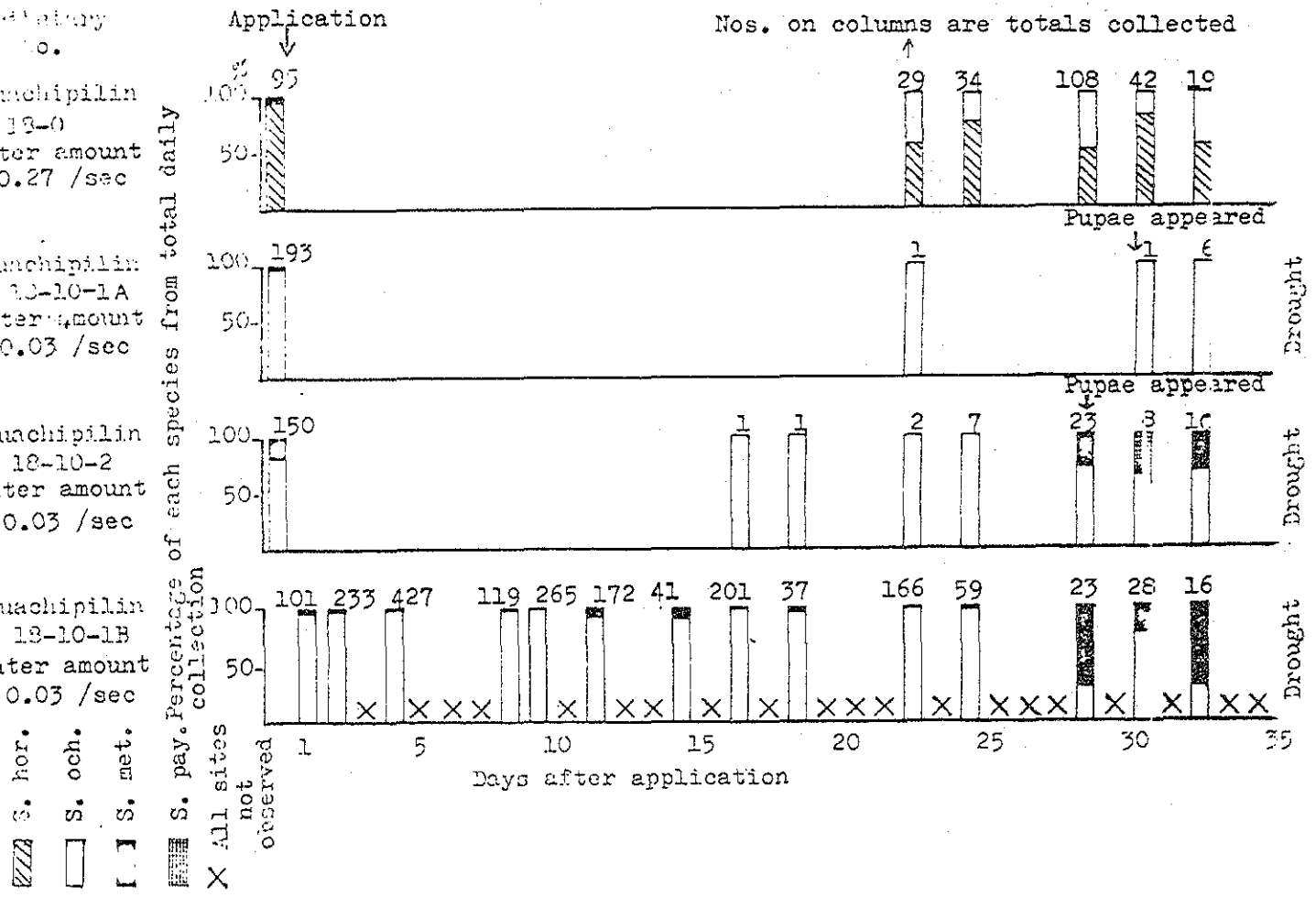


Fig. 2 Size comparison of S. ochraceum eggs deposited in laboratory with those collected in field.

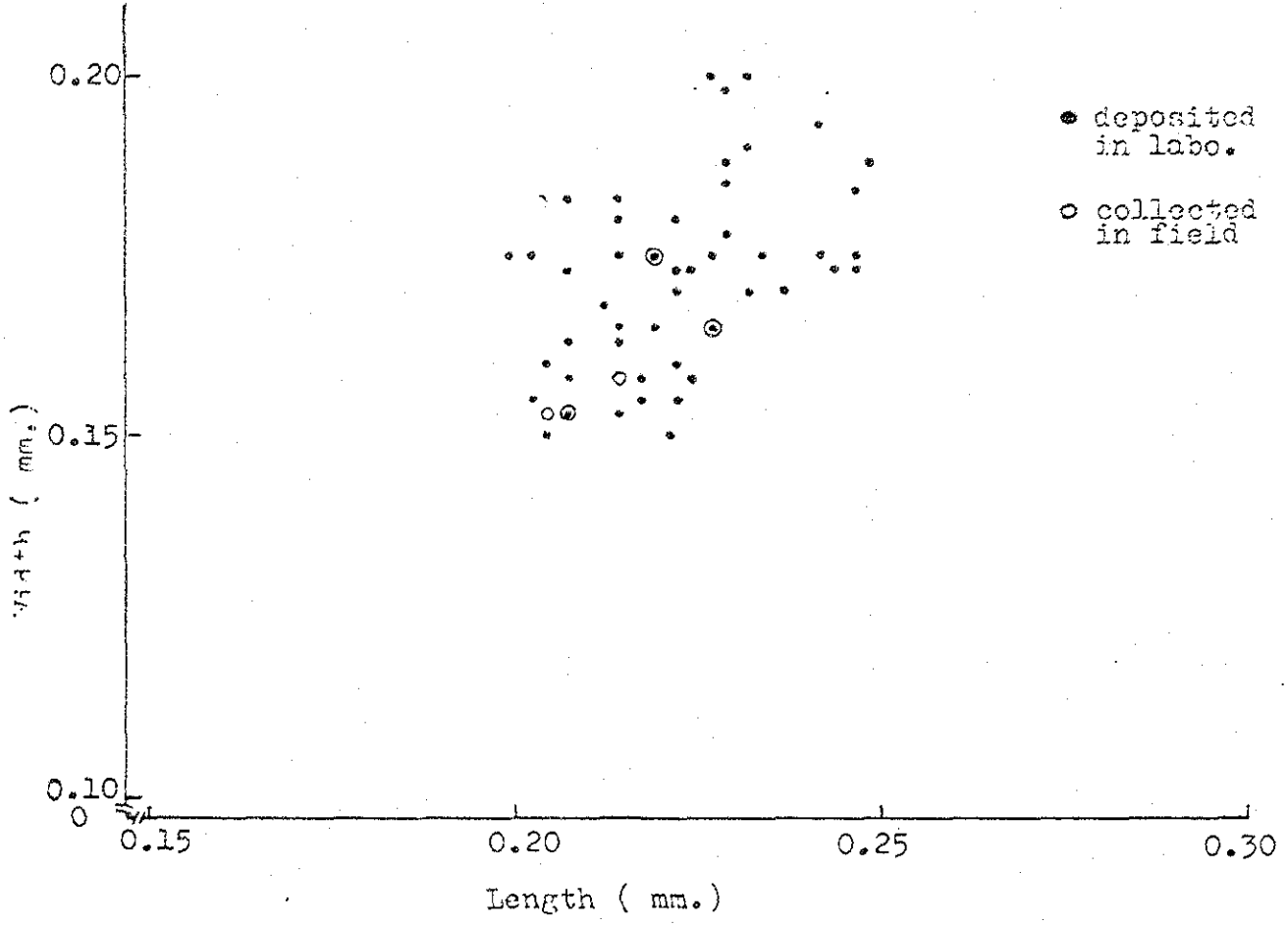
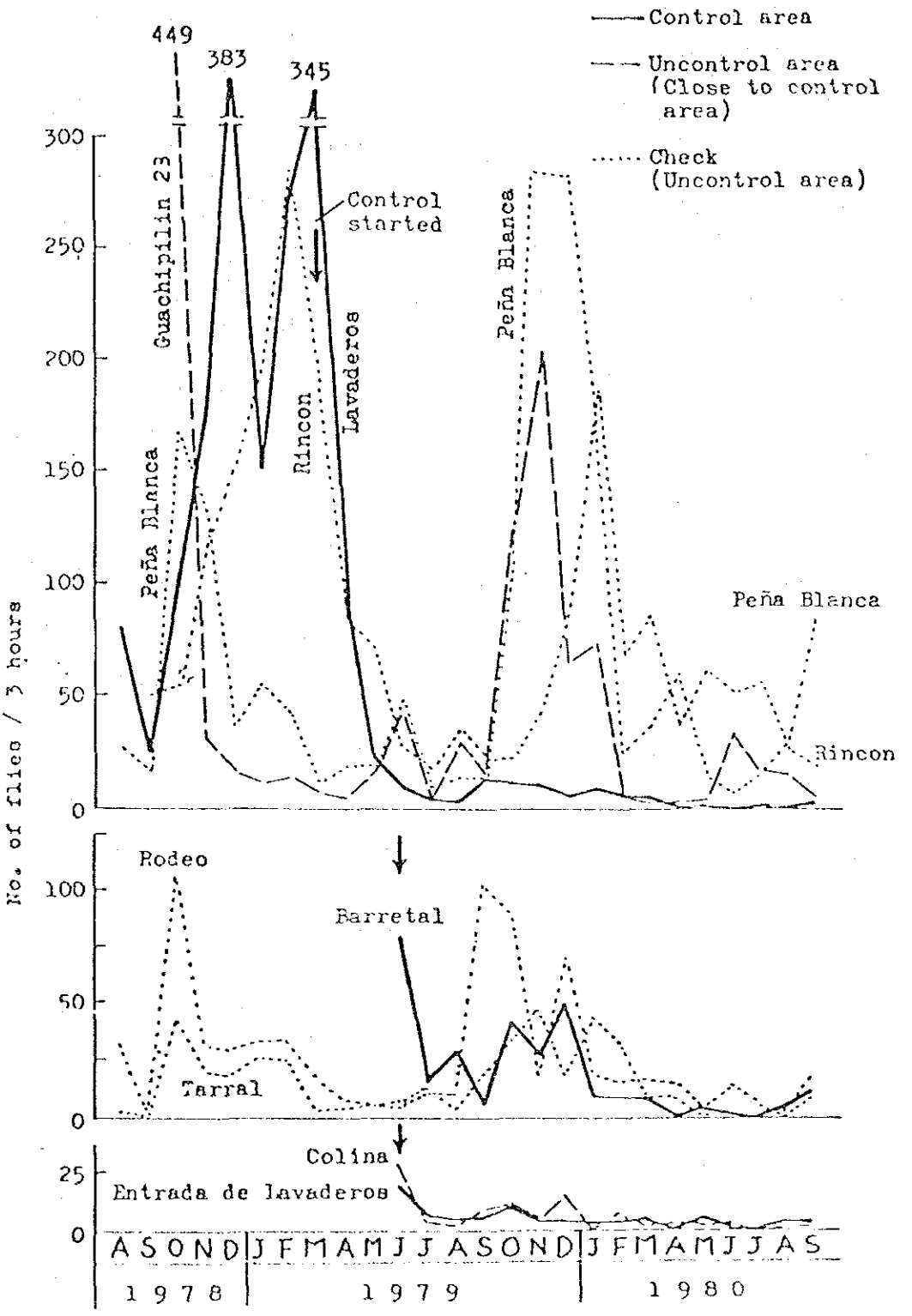


Fig. 3 Biting density of S. ochraceum (monthly)



ミニトラップによるブユ成虫の採集成績

ドライアイスを誘引源とするトラップでブユ成虫を捕集できることはすでに日本において筆者らによって報告されている。例えばTabla 1に示すように，日没前と日出前の各々2時間トラップを運転することで，数百個体のブユが採集でき，個体群調査の有力な手段となりうる。日本で使用したトラップは，蚊用ライトトラップのライトを消して代りにドライアイス2 kgを誘引源として用いたもので，交流100V電源を必要とする。これを単1乾電池2本で運転できるように改良したものが今回の試験に供したものである。このトラップの開発に当っては三共株式会社品川工場工務課に御協力いただいた。試験は4回おこない，第3回をフィンカ・プエナビスタでおこなった他は，他の3回をフィンカ・モニカイボネ（現称プロテ）でおこなった。

第1回試験は予備的に人囮との採集効率を比較するためにおこなった。トラップ5台運転したが，現地の電池は製品のばらつきが大きく試験中に止まってしまったものもあった。トラップによる採集数は人囮に劣るが，種構成においては両者で大差なかった（Tabla 2）。

第2回試験は2時間で採集できるブユの個体数を知るためにおこなった。同時間内に人囮から抱血ブユ約300個体を採集した。結果はTabla 3に示したように，5台のトラップで133個体採集した。すなわち，1人の人囮に相当する採集数を得るには約11台のトラップを設置すればよいという結果であった。

第3回試験は人と馬を使った吸血嗜好性試験と同時に実施した。Simulium horacioiが人に強く引かれることは伊藤専門家によりすでに報告されている。5台のトラップを運転して，S.ochraceumを中心に，22個体しか採集されなかった（Tabla 4）。これは電池の消耗による吸引力の低下が一つの原因であろう。

第4回試験は，トラップ設置場所による採集数の差をみる為におこなった（Tabla 5）。結果は図中④のカルダモモと木の陰では採集数が少なく，⑤の人家近くでは多く採集された。

以上まとめると：

(a) トラップの欠点

誘引力が人囮より劣る。しかし，これは多くのトラップを設置することにより補える。

バッテリーの性能により運転時間が制限される。単車用バッテリーを用いたトラップを開発試験すべきである。

トラップ設置場所によってはぬすまれるおそれがある。

(b) トラップの長所

人囮のような個体差がない。

ドライアイス1～2 kgの方が人囮より安価である。

人囮を使うことで刺咬による苦痛を与えるという人道的問題をトラップにより解決できる。

(c) さらに改良することにより，人囮と併用もしくは代替できるようになるであろう。

（執筆者　中　村　　譲）

Tabla 1.

Número de los simúlidos capturados por las trampas con hielo seco.

	Altura sobre el suelo	bidentatum	Japonicum	aokii	rufibasis	n.d.	Total
En la sombra	0.5	690	0	46	21	1	758
	1.0	229	2	35	7	0	273
	1.5	670	3	36	15	2	726
	3.0	240	1	10	13	1	265
	TOTAL	1,829	6	127	56	4	2,022
En el Sol	0.5	633	0	150	22	0	805
	1.0	546	0	161	8	0	715
	1.5	1,426	2	423	39	0	1,890
	3.0	335	0	17	5	0	357
	TOTAL	2,940	2	751	74	0	3,767

Fecha y hora:

19 de enero de 1,981 — 2 horas antes de la puesta del Sol

20 de enero de 1,981 — 2 horas después de la salida del Sol

Lugar:

Shuzenji, Shizuoka, Japón.

Tabla 2.

Comparación de capturas de los simúlidos
entre las trampas y el cebo humano

(1) Trampas.

FECHA	HORA	No. de trampas	SIMULIUM ochraceum	SIMULIUM metallicum	SIMULIUM sp-2	otro	TOTAL
22 de enero	13:30 - 14:50	4	43	2	0	0	45
" " "	15:00 - 16:00	4	42	0	0	1	43
23 de enero	08:10 - 10:10	3	60	6	2	0	68
		TOTAL	145	8	2	1	156

(2) Cebo humano

FECHA	HORA	Colector.	SIMULIUM ochraceum	SIMULIUM metallicum	SIMULIUM sp-2	otros	TOTAL
22 de enero	13:30 - 14:50	A	206	4	0	2	212
" " "		B	273	6	0	7	286
" " "	15:00 - 16:00	A	117	8	0	3	128
" " "		B	233	7	2	2	244
23 de enero	08:20 - 10:10	A	535	62	9	2	608
" " "		B	719	104	11	5	839
		TOTAL	2,083	191	22	21	2,317

Lugar:

Fca. Mónica Ivoné, Chicacao, Suchitepéquez.

Tabla 3.

Numeros de simúlidos capturados por las trampas con hielo seco

Trampa No.	ochraceum	metallicum	downsi	total simulium	otro insecto
1	26	5	-	31	5
2	52	1	-	53	2
3	25	1	-	26	0
4	5	0	-	5	2
5	17	0	1	18	4
Total	125	7	1	133	13
Promedio	25	1.4	0.2	26.6	2.6
%	93.98	5.26	0.75	100%	

Lugar:

Fca. Mónica Ivoné, Chicacao, Suchitepéquez.

Tabla 4.

Comparación del número de simúlidos atraidos
con los cebos y las trampas

Atrayente	horacios	metallicum	ochraceum	sp-2	otros	TOTAL
Hombre	4.5	193	232	22.5	0	452
Yegua	0	1274	37	23.8	10	1559
Trampa	0	2	17	0	3	22
Total	4.5	1469	286	463	13	2033

Fecha:

26 - 27 de febrero de 1,981.

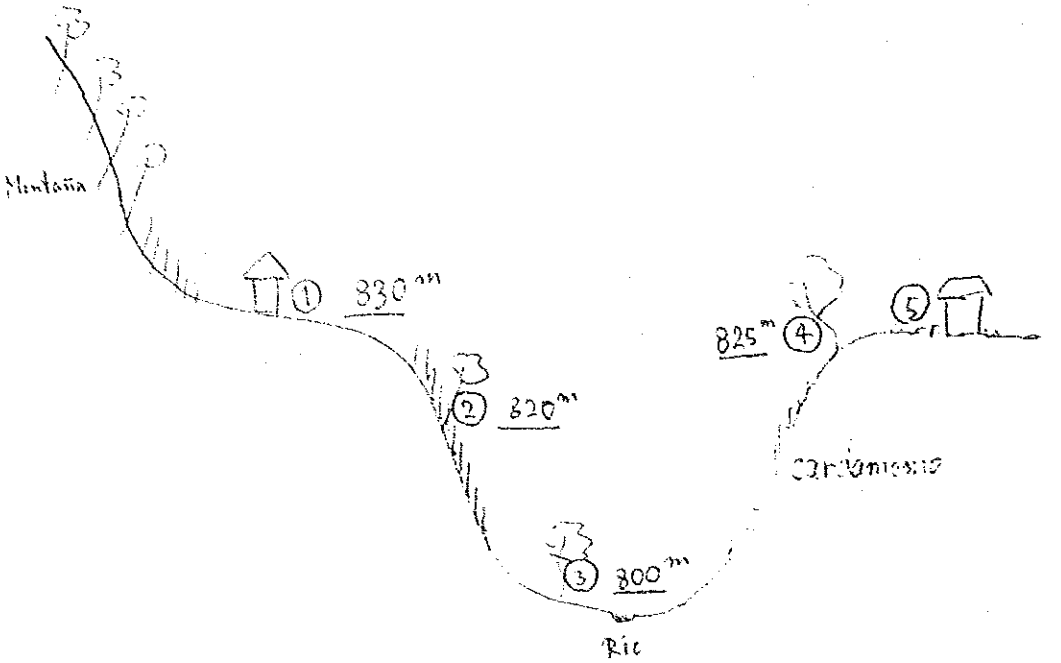
Lugar:

Finca Buena Vista, Acatenango, Chimaltenango.

Tabla 5.

Captura de los simúlidos
por las trampas localizadas en distintos lugares

Trampa	ochraceum	metallicum	otros	Total
1	8	1	0	9
2	4	3	2	9
3	2	5	1	8
4	0	2	0	2
5	13	2	0	15
Total	27	13	3	43



ブユ発生水域におけるユスリカ類の調査成績

オンコセルカ病を媒介するブユの幼虫は流行地の周辺の小渓流に生息しているが，このような水域にはブユの他多くの生物が共存して，それらが互に影響し合っている。従ってブユの生態を理解するためには，それらとの関係も明らかにする必要がある。また，ブユ駆除のために殺虫剤をその発生源に投入するさいに，これら随伴生物にどういう影響が出るかも調査する必要がある。

この目的の研究は，すでに長谷川淳一専門家が1979年2月より5月までGuatemala 滞在のさい開始してその成績の一部は第3次報告書（医二JR80-24）96-105頁に印刷されている。その第4表，第5表，第6表にいろいろな方法でブユ幼虫の発生源に見出される無脊椎動物の種類と個体数がおおむね科 family の単位で調べられている。この中に Chironomidae （ユスリカ科）としてあげられているものについて，さらに詳細な調査をおこなった。

調査方法　ブユ発生源の渓流に生息する動物群の採集には主として次の方法によった。

(1) 飽和食塩水浮游法：発生源の渓流に沈んでいる落葉などでブユ幼虫の付着していそうなものを集めてポリエチレン袋に入れ研究室に運ぶ。このさいなるべく低温に保存し虫の死滅融解を防ぐことを心掛ける。落葉など約200gをバットを用い約1ℓの0.1%石鹸水でよく洗う。この水を1ℓ入りビーカー（又は500ml入りビーカー2個）に移し，約5分放置して浮游物と上澄をすてる（この上澄でもとの落葉をくりかえし洗うと虫のロスが少ない）。沈渣にふくまれる虫，泥等を500ml入りの三角コルベンに移し，飽和食塩水を約200ml加えてよくかきまわした上，さらに飽和食塩水をビーカーの頂上近くまで加える。虫は食塩水の水面に浮上し，ごみや泥は大部分底に沈む。水面の粗大なごみはピンセットで除去した後，水面の浮上物を直径9cmの円形沪紙を設置したブフナー沪斗に移して沪過する。この沪紙を実体顕微鏡でしらべると，ブユ幼虫，ユスリカ幼虫，その他の水生昆虫，ダニ等が多数に検出される。この沪紙はフォルマリンを少量加えた

グリセリン数 ml でぬらしてシャーレに保存することができる。

この方法はすでに食品中の虫や，室内塵中のダニ等を回収するためにも用いられているが，ブユ幼虫等を大量に集めることができ，ブユ幼虫の駆除効果の判定にも一つのルティーンな，客観的な調査法としてすぐれていることが今回の実験で示された。

(2) ブユ発生源周辺での捕虫網による成虫捕集

ブユ発生源周辺の草叢などに捕虫網を無作意に振りまわすと，それに休息している多数の小昆虫を集めることができる。この中にはブユ成虫はほとんど見られないが，ユスリカ成虫はよくとれる。表1は各地のブユ幼虫発生源の周辺で約10分間の捕虫網採集にさいして集められたユスリカ各種の個体数（♂－♀）を示したもので，少なくも 11 種のユスリカが採集されたが，そのうち Cricotopus sp. A が最も普遍的に，かつ多数に見出された。

(3) ブユ発生源に生息する幼虫を飼育して成虫を羽化させる方法

(1)で述べたブユおよびユスリカ類の幼虫が付着している落葉等を研究室にもちかえり，これを直径 30cm，深さ 12cm のプラスチック水槽（市販の洗面器を利用）に入れ，水を加え，エアーポンプを用いて気泡を送り，上をナイロン布とゴムひもを用いて覆っておくと，落葉に付着していた幼虫ないし卵が発育して成虫となる。これを毎日1回吸虫管で捕集し，必要によりガムクロラール液封入標本として検査した。この方法で成虫として捕集された各種類の♂，♀の個体数は表2に示す通りである。

この方法によりブユ発生源9カ所のサンプルから3月 23 日までに 15 種，898匹のユスリカ成虫を羽化させることができた。ここでも Cricotopus sp. A と既に名づけた種類が最も多く，かつ普遍的に見出された。これらの種類については，サナギ，幼虫のぬけ殻を標本とし，これより羽化した雄成虫との関連により，それらの形態をも記録することができた。

ここに記録したユスリカの種類は現段階ではその属名までを検索することにとどめ，正確な種名については帰国後に検索をおこなうことになるが，中米の渓流に発生するユスリカについては，従来，ほとんど調査がおこなわれていないため，

多くの新種が記録されることと予想される。

なお，今回はユスリカ類の種と生息個体数を明らかにすることを主目的としたため，Temephos 撒布のユスリカ幼虫に対する影響等の調査は今後の機会にゆずることとなった。ただし，Rincon #20,#21 で2月9日に捕虫網で成虫がまったくとれなかったことは，2月3日にこの地域の小渓流に Temephos を 10 分間の水量に対し2 ppmの割で撒布したことの影響であろうと思われる。これら水域に見出されたブユ幼虫の個体数と種類の調査は中村専門家が担当した。

（執筆者　佐々　学）

Table 1 . Numbers of chironomids collected by sweeping resting places near breeding sites of blackfly vectors of onchocerciasis. (left, males; right, females)

Locality	Lava-deros	Lava-deros	Barre-tal	Medio monte	Rincon #20	#21	#20	#31	#20 #21	#30	#31	#31	#30
Date of collection	1/22	1/22	1/22	1/28	2/02	2/03	2/03	2/03	2/09	2/09	2/09	2/25	2/25
Cricotopus sp. A	61-32	32-16		7- 2	16- 1	15- 2	17- 3	7- 5		9- 4	8- 3	20- 8	7- 4
Cricotopus sp. B					5- 1								
Metriocnemus sp.		1- 1	1- 1										
Parametriocnemus sp. D						1- 0	0- 1						
Limnophyes sp. E					2- 1	1- 0	1- 0					2- 1	
Popypedilum sp. H											1- 0		
Polypedilum sp. J		1- 0			5-10								
Polypedilum sp. K	1- 0				0- 1								
Tripodura sp.							1- 0	1- 0			1- 0		
Chironomus sp. R					1- 2	2- 2							
Chironomus sp. S					4- 0		1- 4	0- 3				1- 0	

Table 2[illegible]. Numbers of adult chironomids reared from riverbed samples (mainly fallen leaves) collected at breeding sites of blackfly vectors of onchocerciasis in Guatemala, January-March 1981 (left, number of adult males - right, number of adult females)

Locality	Lava-deros	Barre-tal	Medio-monte	Rincon #20	Rincon #31	Rincon #30	Rincon #21	Medio-monte	Medio-monte	
Date of collection	1/22	1/22	1/28	2/02	2/03	2/25	2/25	2/25	2/25	TOTAL
Cricotopus sp. A	6-10	18-20	12- 6	29-33	22-30	13-10	55-59	6-11	2- 3	163-193
Cricotopus sp. B.	1- 4	18-13		8- 7			2- 0			29- 24
Metriocnemus sp. C				1- 0	3- 2	2- 0	4- 5	1- 1	3- 1	14- 9
Parametriocnemus sp. D		1- 0	0- 1		5- 3		2- 4	0- 1	0- 1	8-10
Limnophyes sp. E				1- 0	1- 0	1- 0				3- 0
Corynoneura sp. F					3- 7	1- 0	2- 3	2- 5		8-15
Thienemaniella sp. G						1- 0	1- 3			2- 3
Polypedilum sp. H			12- 8		0- 1		2- 0	7- 6	1- 0	22-15
Polypedilum sp. I			5- 3				1- 0	55-51		61-54
Polypedilum sp. J			1- 0	5- 3			4- 2	71-44	27-46	108-95
Polypedilum sp. K	1- 0			2- 0			1- 1		3- 1	7- 2
Polypedilum sp. L							1- 3	6- 2	3- 0	12- 5
Tanytarsus sp. M					2- 0	1- 0				3- 0
Tanypodinae sp. P					3- 2		7- 2	4- 2	3- 2	17- 8
Tanypodinae sp. Q								1- 5	1- 1	2- 6

- March 23 459 - 439

ユスリカ類の発生源から得たブユ幼虫の種構成

(a) フィンカ・メディオモンテにおけるブユの種構成

パイロット地区南西部を流れるパハル川とメディオモンテ山脈で隔てられた西側にこのフィンカは位置する。グアテマラ市からエスクイントラを経てサンホセ港へ至る国際道 CA 9 が西側を通っている。傾斜 30°～35°の岩壁より水が湧き出し，その水を管で引いて牛の飲み水としている。本年1月 28 日に当地で定性的に調査した結果はTabla 1に示したように，S.ochraceumとS.metallicum の2種のみであった。

(b) フィンカ・リンコンにおけるブユ個体群の変動

このフィンカはアマティトラン湖の東に位置し，パイロット地区外北側の定期定点調査地となっている。ここで，本年2月3日に一部支流に殺虫剤テメフォス（アベイト）を投入し，その後のブユとユスリカの個体群の変動を観察した。ユスリカに関しては佐々専門家が報告しているので，ここではブユについて記す。調査はほぼ10mの区間を 10 分間にわたって採集する方法によった。2人で調査した場合には平均値を出して1人の値に換算した。殺虫剤は5％テメフォス水和剤を用い，水量1ℓ／秒当り 4g を使用した。調査地点をFigura 1 に，殺虫剤投入状況をTabla 2に示した。すなわち，処理区は，＃21下流，＃20，＃30下流の3カ所で，対照区は，＃21上流，＃30上流，＃31の3カ所であった。

これらの支流のうち＃21は水量約0.8ℓ／秒でS.ochraceumの主発生源であった。その他の種ではS.metallicumとS.horacioiとが多かった（Tabla 3，4）。支流＃20は水量約6ℓ／秒でS.metallicumを優占種として他の多くの種が出現した（Tabla 5）。支流＃31と＃30とはともに水量10ℓ／秒以上の流れで，対照区の＃31にはS.metallicumが多く，処理区の＃30にはさらにS.ochraceum が多数出現した（Tabla 6, 7, 8）。

いずれの支流でも殺虫剤処理後個体数は減少したが，上流部を処理しなかったために，流下による個体群の回復があった。とくに，殺虫剤投入点直下ではほと

んどの個体が生存しており，同一薬量ならば短時間高濃度の暴露では有効でないことを証明しているといえよう。調査したすべての支流からオンコセルカ症の媒介者であるS.ochraceumが出現したことは，当地を定期定点調査地に選定したことの妥当性を裏づけている。とくに年間を通じて水量変化が小さい点，ならびに成虫の季節消長曲線が類似している点で，現在薬剤処理中であるラバデロス川流域の対照区としての意義は大きい。

（執筆者　中　村　　譲）

Tabla 1.

Numero de larvas y pupas de los Simúlidos en la Finca Medio Monte

Especie	Larva	Pupa	Total
S. ochraceum	25	0	25
S. metallicum	27	3	30

Fecha : 28 de Enero de 1,981.

Observación: Agua se sale de la peña.

Tabla 2

Aplicación del insecticida a los afluentes de la Finca Rincón

No. de Afluente	Descarga de agua (ℓ/seg.)	Cantidad de insecticida*	Temperatura de agua	Hora de Aplicación
# 21	0.8	4g.	14.5	10:10
# 20	5.7	24g.	14.0	10:20
# 30	13.3	56g.	16.8	11:10

* Cada bolsa contiene 4g. de Abate polvo humectable al 5%.

Fecha: 3 de Febrero de 1,981.

Tabla 3.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el afluente # 21 arriba en Fca. Rincón

Especie de Simulium	Fecha de Observación									
	feb. 2	feb. 3	feb. 4	feb. 9	feb. 19	feb. 25	mar. 16	aplicación	mar. 16	mar. 25
S. ochraceum			73/3	28	39/3	81/1	28			16
S. metallicum			3	0	22	0	0			0
S. horacioi			24/1	11	17	22	6			8
S. sp-2			1	1	0	1	0			0
S. (Hemicnetha) sp.			0	0	0	0	0			0
TOTAL	-	-	105/4	40	78/3	104/1	34		-	34

NOTA: Números indican larvas/pupas.

Tabla 4.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el afluente # 21 abajo en Finca Rincón.

Especie de Simulium	Fecha de Observación										
	feb.2	1a. aplicación	feb.3	feb.4	feb.9	feb.19	feb.25	mar.16	2a. aplicación	mar.16	mar.25
S. ochraceum	66		10/2	0	11/2	5	31	28			7/1
S. metallicum	29/1		10/1	3/3	3/1	0	1	4			0
S. horacioi	9		6	3	7/2	3	24	15			2/1
S. sp-2	0		1/1	0	6	2	2	6			0
S. (Hemicnetha) sp.	0		1	0	1	10	0	0			0
TOTAL	104/1		28/4	6/3	28/5	20	58	53		-	9/2

NOTA: Números indican larvas/pupas.

Tabla 5.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el afluente # 20 en Finca Rincón

Especie de Simulium	Fecha de Observación									
	feb.2	aplicación	feb.3	feb.4	feb.9	feb.19	feb.25	mar.16	mar.16	mar.25
S. ochraceum	10		2	2	4	15	4	28		16
S. metallicum	42		20	7	10	15	5	27		13
S. horacioi	0		0	0	0	0	0	1		0
S. sp-2	14		3	11	3	25/1	5	4		6
S. (Hemicnetha) sp.	4		1	5	2	15	2	6		7
S. (S.) sp.	1		0	0	0	0	0	1		0
TOTAL	71		26	25	19	70/1	16	67	-	42

NOTA: Números indican larvas/pupas.

Tabla 6.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el afluente # 31 en Fca. Rincón

Especie de Simulium	Fecha de Observación									
	feb.2		feb.3	feb.4	feb.9	feb.19	feb.25	mar.16	mar.16	mar.25
S. ochraceum			0	0	0	3	1	6/1		2
S. metallicum			43/1	9	26	138	17	26		9
S. horacioi			0	0	0	0	0	0		0
S. sp-2			0	8	0	0	0	0		0
S. (Hemicnetha) sp.			0	1	0	0	0	0		0
TOTAL	-		43/1	18	26	141	18	32/1	-	11

NOTA: Números indican larvas/pupas.

Tabla 7.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el afluente # 30 arriba en Fca. Rincón

Especie de Simulium	Fecha de Observación										
	feb.2		feb.3	feb.4	feb.9	feb.19	feb.25	mar.16		mar.16	mar.25
S. ochraceum				71/6	36/2	95/2	51/1	22/1			23/2
S. metallicum				93/2	36/2	72	31/1	23			23
S. horacioi				0	0	0	0	0			0
S. sp-2				33/4	9	8	12	10			16
S. (Hemicnetha) sp.				9	1	0	0	2			5
S. downsi				0/3	5	8	2	1			8
TOTAL.	-		-	206/15	87/4	183/2	96/2	58/1		-	75/2

NOTA: Números indican larvas/pupas.

Tabla 8.

Cambio de números de larvas y pupas de simúlidos capturados durante 10 min./hombre en el alfuente # 30 abajo en Fca. Rincón.

Especie de Simulium	Fecha de Observación										
	feb.2	1a. aplicación	feb.3	feb.4	feb.9	feb.19	feb.25	mar.16	2a. aplicación	mar.16	mar.25
S. ochraceum	48/1		5	18/2	13/1	41	49	18		12/2	7
S. metallicum	22/1		12	37/1	24/1	19	33	13		9/1	13
S. horacioi	0		2	0	0	0	0	0		0	0
S. sp-2	8/1		2	5/3	1	5	9	2		5	3
S. (Hemicnetha) sp.	1		0	0	0	0	0	2		3	0
S. downsi	13/1		0	6	3	1	3	3/1		4/4	0
TOTAL	302/4		21	66/6	41/2	66	94	38/1		33/7	23

NOTA: Números indican larvas/pupas.

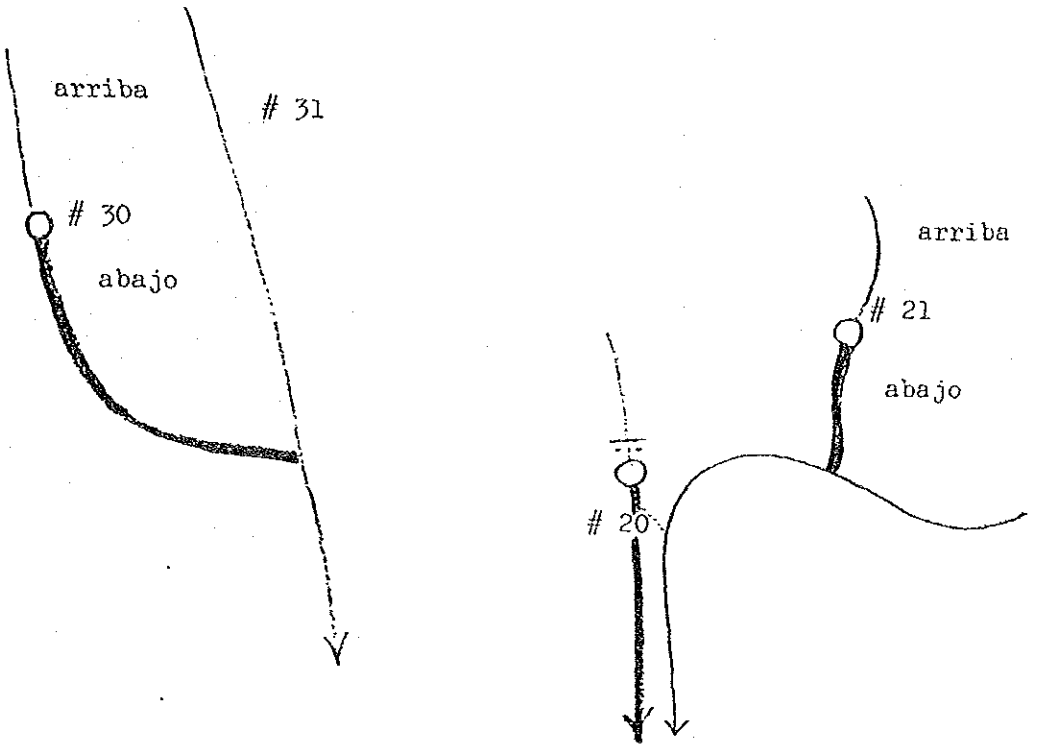


Figura 1.

Los afluentes de la Finca Rincón

NOTA: Los circulos indican sitios de la aplicación.
Líneas gruesas indican corriente tratada y
Líneas delgadas indican corriente no tratada.

Ⅲ　寄生虫学・疫学部門

REVIEW OF PARASITOLOGICAL, EPIDEMIOLOGICAL AND IMMUNOLOGICAL INVESTIGATIONS IN ONCHOCERCIASIS CONTROL PROJECT IN GUATEMALA, 1976-1980

INTRODUCTION

A cooperative project was initiated between Japan International Cooperation Agency (JICA) and the Government of Guatemala in an attempt to control onchocerciasis. The first five-year program of this project was started in May, 1975 and terminated in September, 1980. The objective of this publication are to review advances in the initial 5 years and orient future investigations. The results were based on the First Report on Onchocerciasis Control Project in Guatemala published by JICA in 1978, in addition to quarterly reports submitted to JICA by Japanese experts of palasitology. Emphasis was placed on basic biological and immunological studies of this parasitic diseases. Ophthalmological, dermatological and epidemiological aspects are not treated in depth in this review although there is much available data.

Japanese experts who participated in the present project of parasitology and epidemiology are as follows:

Hiroshi Takahashi (1976-1980); Project leader

Yoshiki Aoki (1976-1977); Parasitology

Teruaki Ikeda (1976-1977); Immunology

Yoshihisa Hashiguchi (1977-1979); Parasitology

Masato Kawabata (1977-1979); Parasitology

Shigefusa Sato (1976); Immunology

Isao Tada (1976); Parasitology

Akihisa Hasebe (1977); Epidemiology

Shigeo Nonaka (1977, 1978, and 1979); Dermatology

Hiroto Yamada (1977, 1978, and 1979); Ophthalmology

Hitoshi Kasuga (1977); Epidemiology

Tsuguyoshi Suzuki (1977); Epidemiology

Takesumi Yoshimura (1978-1979); Epidemiology

Masatoshi Takaoka (1978-1979); Immunology

Makoto Sakamoto (1979-1980); Parasitology

Yohichi Ito (1979-1980); Immunology

Kaoru Kondo (1980); Parasitology

Guatemalan participants in the present project of Parasitology and epidemiology are as follows:

Juan José Castillo O.

Hector A. Godoy Bonilla

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Julio César Ramos V.

José Ernesto Estrada F.

René Del Cid

Enrique Chester

Aracely Luján Trangay

Hilda Olivia Reynozo

Guadalupe Sandoval

María del Carmen de Rodrigues

Jaequline W. de Cruz

Contents

IV. Indirect haemaggultination test (IHA)

1) Materials and methods

2) Comparison of blood samples from ear lobes and veins

3) Standard for IHA positiveness

4) Variation of titer during storage of blood samples in filter paper

5) Cross reaction with other intestinal helminths

6) Titer of IHA and microfilaria density

7) IHA positive rate in relation to ages

8) Stability of IHA test

9) IHA positive rates and microfilaria positive rates

V. Double diffusion test

1) Materials and methods

2) Reliability of D D test

3) Specific precipitation bands

VI. Skin test

1) Specificity of purified antigen from dog filariae (Dilofilaria immitis)

a) Preparation of antigen

b) Methods of skin test

c) Antigenic activity

d) Protrin concentrations

e) Non-specific reaction

f) Reliability of antigen FST-3

g) Positive rates with skin test using antigen FST-3

2) Antigen from onchocerca microfilaria

a) Materials and methods

b) Protein content for antigen

c) Skin reaction with time

d) Skin reaction in relation to intestinal parasitic helminths

e) Distribution of wheal sizes

3) Antigen from O. volvulus adults

a) Materials and methods

b) Protein contents for skin test

c) False negative rate

d) False positive rate

e) Stability

f) Fractionation of antigen from O. volvulus adults

VII. Inoculation of microfilariae into mouse and chemical treatment

1) Materials and methods

2) Migration of microfilaria into different organs of mice

3) Survival of microfilariae in mice

4) Sites for microfilaria inoculation

5) Chemical treatment of mice

I. Onchocercoma

1) Distribution of onchocercal nodules in patients bodies.

In Nigeria, Onuigbo (1979) studied 78 cases of onchocercoma and found only 14 (16.7%) in the head and the other 64 in the trunk. In Guatemala, however, formation of the onchocercal nodules occurs generally in the head, rather than the trunk, rarely in the legs and arms. Out of the 530 nodules diagnosed by palpation, 56.5% were located in the head, particularly on the occipital and parietal bones, the remaining in the trunk, especially near the iliac (TABLE I-1). Thus, there was an observable difference between the location of nodules in patients' bodies from Guatemala and Nigeria.

2) Size of nodule

In Guatemala, the diameter of 338 nodules removed from the head and trunk was measured, sampling patients who had not had a nodulectomy for more than one year. The nodules examined ranged from 4 to 20 mm, although 82.5% had a diameter of 5 to 12 mm, with the majority measuring 6-8 mm (TABLE I-2). The average diameter of the nodules removed from the head was 7 mm, whereas 8 mm from the trunk. Moreover, 13% of the nodules removed from the trunk measured greater than 15 mm (FIGURE I-1). Essentially, nodules from the torso tend to grow larger than those from the head.

TABLE I-1

Distribution of Human onchocercal nodules (530 nodules were examined by palpation).

Location		Frequency (%)
Head	Occipital	25.2
	Parietal	22.1
	Temporal	7.2
	Others	2.1
Sub-Total		56.5
Trunk	Iliac	16.4
	Scapular	6.2
	Costal	5.4
	Coxal	4.0
	Dorsal	2.4
	Cervical	2.3
	Trochanter	2.2
	Extremity	1.0
	Others	3.6
Sub-Total		43.5

(Aoki, Onchocerciasis Control Project in Guatemala, First Report, 78-2(186), JICA, 1978).

TABLE I-2

Size of Nodule

Diameter (mm)	Frequency (%)
- 4	7 (2.1)
4 - 5	28 (8.3)
5 - 6	54 (16.0)
6 - 8	109 (32.2)
8 - 10	75 (22.2)
10 - 12	41 (12.1)
12 - 15	20 (5.9)
15 -	4 (1.2)
Total	338

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 78-2(186), JICA, 1978).

3) Microfilariae detection in relation to nodule size.

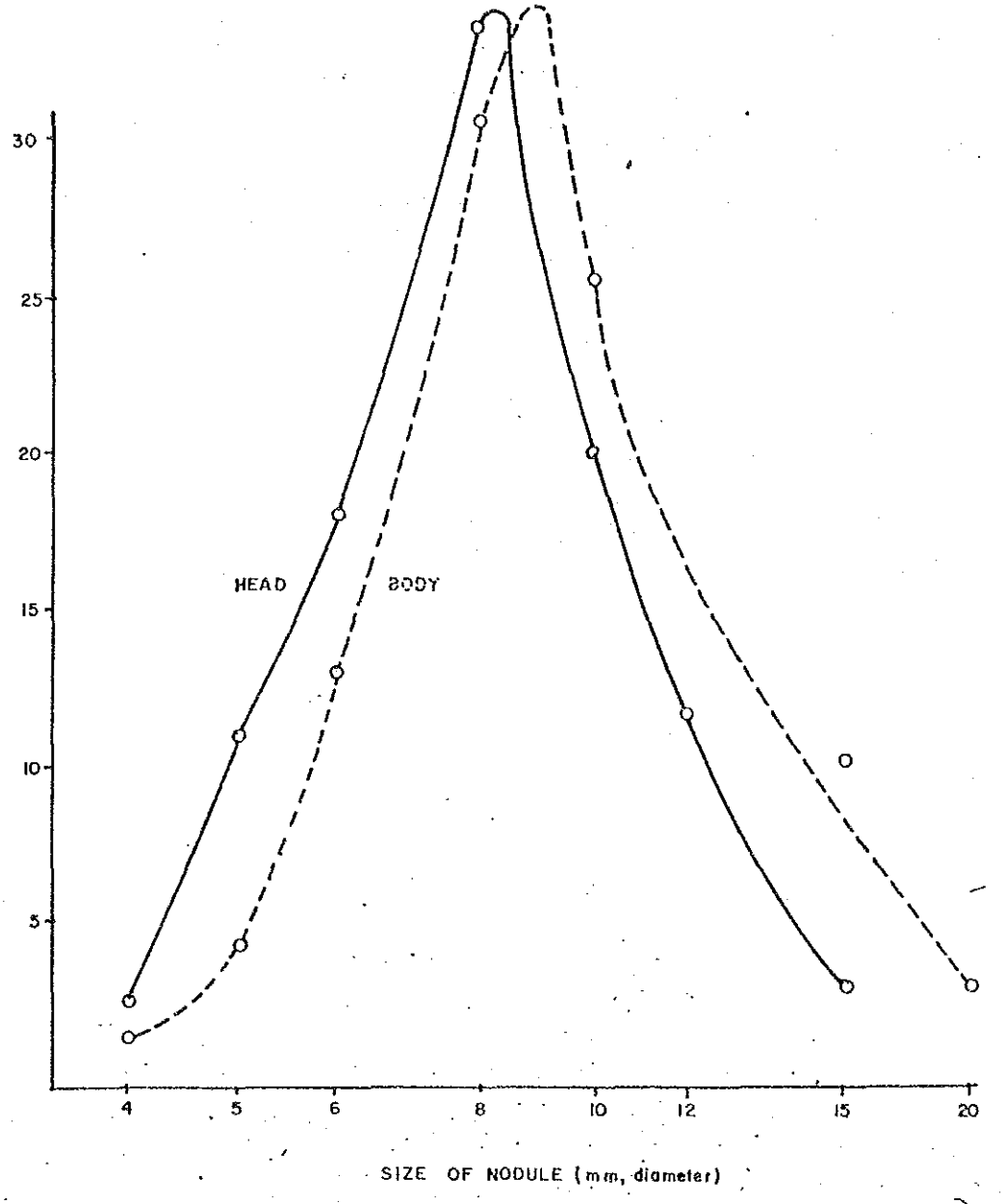
Various sized nodules were examined for presence of microfilariae (mf) and no 4 mm nodules exhibited mf. Percentage of nodules containing mf increases proportionately, with size.
Eighty percent of the 12 mm nodules contained mf, while all 20 mm nodules were positive for mf. (FIGURE I-2). These reports suggest that large nodules are the primary source of mf.

4) Non-onchocercal nodules among diagnostic onchocercomae.

373 nodules were diagnosed as onchocercal, using Brigader's palpation, then they were excised and microscopically examined for helminth adults (TABLE I-3). In the study, approximately 10% of the diagnosed onchocerca nodules were later determined as non-onchocercal, due to the absence of the O. volvulus adults.

5) The relationship between the microfilariae and the sex of the adult O. volvulus in the nodule.
The sex of the adult O. volvulus from 62 colagenase-digested nodules was determined. One-half of the nodules contained females, while the other half sheltered both sexes (TABLE I-4). The presence or absence of mf was determined by skin biopsy of persons harboring adult helminths within nodules. Among the mf infected patients, 55.6% of the 45 nodules harboured both male and female heminths, while in the non-infected mf cient, 35.3% of the 17 nodules had both sexes.

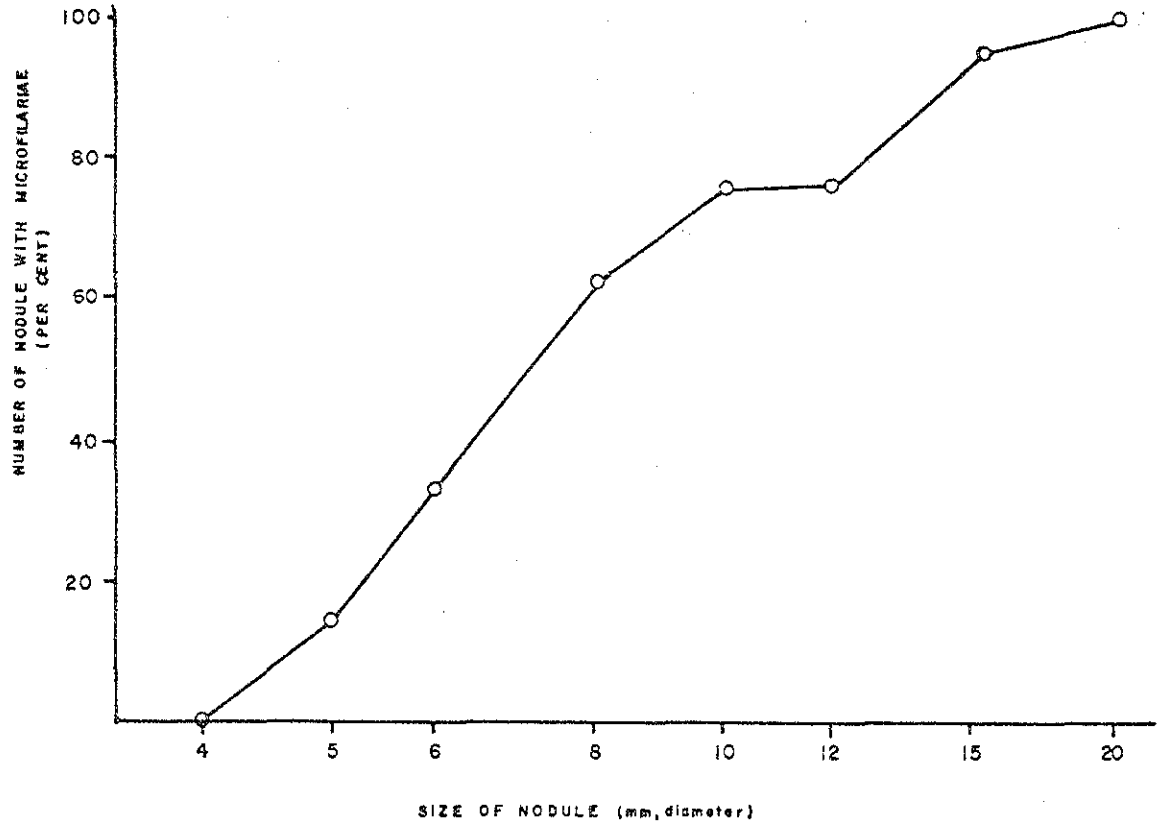
FIG. 1-1 **COMPARISON OF SIZE OF NODULE FOUND IN HEAD AND BODY**



(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 73-2 (186), JICA, 1978)

FREQUENCY OF NODULE WITH ACTIVE MICROFILARIAE

Fig. I-2



Aoki; Onchocerciasis Control Project in Guatemala, First Report, 78-2 (186), JICA, Aoki et al (1978)

TABLE I-3

Certainty in Diagnosis of Onchocercoma by palpation

Location	No.of Nodule Examined	No. of Onchocercoma	Location	No.of Nodule Examined	No. of Onchocercoma
Patrocinio	104	95	Chilar	363	344
Rios	13	11	Positos et al	10	10
Cedro	16	11	Caracol et al	26	22
Amate, Chilcas	22	19	Pepinal et al	3	2
Guachipilin et al	52	49	Soleda et al	5	4
Hamburgo et al	27	21	Arabia et al	24	23
Camaron, Chaguites	7	5	Ramon et al	19	17
Berlin, Caña Viena	49	42	S.J. Guachipilin	64	62
Pacaya Grande et al	20	18	Alejandria et al	49	46
Total	310	271(87.4)	Total	563	530(94.1)

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 78-2 (186), JICA, 1978).

TABLE I-4

Sex of Onchocerca containing in nodules

Results of Skin biopsy	sex of Onchocerca containing in nodule		TOTAL
	both sex	single sex (female)	
Mf-positive	25	20	45
Mf-negative	6	11	17
TOTAL	31	31	62

(Sakamoto, Ito & Yoshimura, Quarterly Report. 1979).

This suggests that the mf emerge more frequently from nodules containing both sexes of O. volvulus.

6) Male and female helminths in nodules.

Since it was previously difficult to separate the entangled helminths from the nodules, few measurements of the adult length had been taken. However, in recent years, with the use of colagenese (0.1 mg/ml diluted in 199 medium), enzymatic digestion of the nodules occurs, allowing separation of intact adults and their measurements. The length of the adult males ranged from 1.0 to 5.2 cm. (mean 2.5 ± 0.7 cm), while females measured from 16.8 to 69.7 cm. (mean 33.8 ± 10.7 cm). The majority of the females harbored mf and eggs in their uteri although two small females, with lengths of 8.9 and 12.4 cm, were considered to be uncopulated, early fifth-stage adults, without mf or eggs. These small females were responsible for nodule formation clearly detectable by palpation. Within the males observed, all were mature possessing two spicules.

7) Rate of new nodule formation.

New nodules were observed 6 to 8 months in 90 out of the 883 patients (10.8%) reexamined after removing all detected nodules. Considerable variation in rate of nodule formation was observed among the different localities. In San Nicolás and Camarón, where mf infection was low, no new onchocercoma were formed. In Guachipilín, where mf positive rate was 80.3%, new nodules were found in 26 out of 48 patients (54.2%).

In general, the rate of new nodule formation increased, as mf positive rate increased (TABLE I-5).

An additional survey was conducted to determine the nodulation rate among nodulectomized patients with or without mf. The rate of nodule formation was 37.0% (for 50 out of 135 patients) for mf and nodule positive patients within 6 to 8 months after the initial nodulectomy. For mf positive and extirpated nodule, the rate was 28.4% and 4.5% for mf and nodule negative patients. The data demonstrates that nodulation rate was higher among patients positive with mf and nodules, than among those negative, in addition, the rate remains high even after nodulectomy. (TABLE I-6).

A third examination was conducted 6 months after the second and approximately one year after the initial examination to determine the nodulation rate among patients who had been negative in the previous examinations.

New nodule formation was common among patients younger than 15 years old (TABLE I-7).

In summary, the rate of nodule formation was determined by periodic examinations and surgical extirpations, which allowed appraisal of dissemination in a given area and is useful for determining an effect of control measures.

8) Nodule occurrence in relation to sex and age of patients.

Frequency of onchocercal nodules was determined according to age and sex of patients. In all age groups, males were more frequently infected than females. In either sex, infection rate augmented with age. The

TABLE I-5.

Frequency of Nodule appearance, formed within 6-8 months from endemic areas

Locality	mf pos. (1976)	nodule pos. (1976)	newly-formed nodule. No. exam.	No. patients showed newly formed nodule	%
San Nicolas	11.3	15.1	19	0	
Camaron	7.3	9.7	34	0	
Cedro	8.3	9.8	254	4	1.6
Chaguites	18.2	11.6	68	3	4.4
Pacaya Grande et al	20.4	14.4	108	8	7.4
Tiguimay et al	28.4	33.3	40	3	7.5
Amate	35.6	32.9	64	8	12.5
Los Rios	49.0	46.7	83	14	16.9
Berlín	69.0	36.2	25	4	16.0
Caña Vieja	62.3	46.3	39	8	20.5
San José de la Cruz et al.	68.6	31.1	35	9	25.7
Chilcas	76.0	58.6	16	5	31.3
Guachipilín	80.3	67.6	48	26	54.2
Total			883	90	10.8

(Tada, Aoki, Hashiguchi, Ibeda and Kawabata, Quarterly Report No. 4, 1977).

TABLE I-6.

Correlation between newly-formed nodules and persons with or without mf and nodules in the examination of 1976.

Results of skin biopsy in 1976	Nodule (1976 → 1977) (+)→(+)	(-)→(+)	(+)→(-)	(-)→(-)	Total
mf (+)	50	15	85	79	229
mf (-)	6	20	42	511	579
Total	56	35	127	590	808

		% Posibility
mf- positive	50+15/229	28.4%
mf- negative	6+20/579	4.5%
Nodule and mf positive	50/50+85	37.0%

(Tada, Aoki, Hashiguchi, Ikeda and Kewabata, Quarterly Report No. 4, 1977).

TABLE I-7

Newly-formed nodules among persons with received two previous examinations

Group*	No. persons examined	No. positive palpations	Previous examination: Positive-mf with nodule	Positive-mf without nodule	negative-mf	age for patient with neither nodule nor mf.
1	89	10	6	2	2	16, 54
2	77	6	4	1	1	12
3	155	20	13	2	5	2, 2, 3, 10, 33
4	120	12	3	1	8	4, 4, 7, 8, 10, 13, 15, 43.

Group* 1; Aldea Los Ríos

2; Finca Hamburgo, Finca Los Chorritos, Finca Puerta de Oro, Finca San José de la Cruz.

3; Casa Caña Vieja, Finca Berlín, Finca Guachipilín, Finca San Gregorio, Finca San Rafael Coyolito.

4; Aldea Los Chagüites, Finca El camarón.

(Hashiguchi, Kawabata, and Tada; Quarterly Report No. 5, 1977).

infection rate increased rapidly in male age groups 3-4 and 10-14, while in female age groups 5-9.

Very few onchocermae were observed in ages 0 to 2 years, although it is interesting to note that nodules formed after 2 years.

9) Diagnosis by palpation and skin-biopsy.

The objective of this study was to compare two methods for diagnosis of onchocerciasis infection; 1) palpation of nodules and 2) Skin-biopsy for presence of mf. The former proved slightly less effective than the latter. Use of only one method failed to detect from 20 to 30% of the cases (TABLE I-8).

In areas with low infection rate, the two methods were comparably efficient. Palpation and skin-biopsy which could not be detected by each other method detected 27.1 and 30.2% of the cases, respectively. On the other hand, in areas with high infection, palpation was less efficient than skin-biopsy. Palpation and skin-biopsy diagnosed 15.0 and 33.9% of the cases, respectively, which the other method failed to detect. Among patients younger than 15 years old, palpation tended to be more efficient (TABLE I-9). In conclusion, the two approaches should be used complimentarily for more precise onchocerciasis diagnosis.

TABLE I-8.

PARASITOLOGICAL FINDINGS IN 587 CASES OF ONCHOCERCIASIS

Findings	No. of cases	Percent of total infected cases.
Nodule-positive	412	70.3
Mf-positive	485	82.8
Mf-positive with nodule	311	53.1
Mf-positive without nodule	174	29.7
Nodule-positive without Mf	101	17.2
Total	586	

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, M-78-2 (183), JICA 1978).

TABLE I-9.

Frequency of infected persons exhibiting only Onchocercoma

LOW ENDEMIC AREA

Age group (years)	No. of persons infected	Nodule-positive with Mf	Nodule-positive without Mf	Nodule negative with Mf	Frequency of patients with nodule only (%)
0- 4	3	0	3	0	100.0
5- 9	34	10	19	5	55.9
10-14	49	18	22	9	44.9
15-19	37	12	10	15	27.0
20-	301	141	61	99	20.3
Total	424	181	115	128	27.1

HIGH ENDEMIC AREA

Age group (Years)	No. of persons infected	Nodule-positive with Mf	Nodule-positive without Mf	Nodule-negative with Mf	Frequency of patients with nodule only (%)
0- 4	12	4	6	2	50.0
5- 9	47	21	15	11	31.9
10-14	41	20	8	13	19.5
15-19	26	13	4	9	15.4
20-	181	99	13	69	7.2
Total	307	157	46	104	15.0

(Tada, Sato, Aoki, Ikeda, Quartely report, No. 4 1977).

II. MICROFILARIAE

1) Microfilariae density in the skin.

Microfilariae (mf) density was determined by snipping skin from different parts of the patient's body to establish a skin-biopsy methodology for reliable onchocerciasis diagnosis.

Picq and Jordal (1974) reported different mf densities in scapular, pelvic and fibular skin with a uniform mf population near the pelvis upon examining 50 onchocercal patients in Upper Volta, Africa.

In Guatemala, at Finca Monte de Oro, skin samples were taken by a Holth-type sclero-punch from 14 different body locations of 14 patients (FIG. II-1).

After the samples were immersed in saline solution (0.85%) for one hour, emerged mf were microscopically counted.

Mf density was high in scapular and pelvic skin, particularly high (52 mf/snip) from the right pelvis. All 12 mf-infected patients were diagnosed positive by examiming skin from left pelvis. Mf density was low in skin from the head, arms and legs, and snips from these areas failed to detect 5 out of 12 mf positive patients. It is interesting to note a low mf density in the head, where nodules are most frequently formed (TABLE II-1).

Thus, skin-biopsies from scapula and pelvis give most reliable diagnosis.

Similar results were obtained in Palín, Guatemala, by skin-snipping 6 different body locations from 22 patients previously diagnosed positive by eye examination. Skin from left pelvis had high mf

TABLE II-1

Mf density per skin snip taken by holth-type sclero-punch from various parts of the body (shown in Fig.II-1), Finca Monte de Oro,Guatemala.

sites of snip in Fig.II-1 / No. Nodule	A	B	C	D	E	F	G	H	I	J	K	L	M	Total
1	7	0	88	66	6	5	22	38	66	17	24	1	0	340
2	0	1	8	15	8	0	0	0	0	32	47	2	0	113
3	0	0	8	8	2	66	39	0	0	9	47	12	0	191
4	1	0	0	0	0	0	0	5	0	3	1	2	0	12
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	7	20	44	79	180	12	42	45	24	2	108	0	0	565
7	0	0	0	7	2	0	0	0	0	66	1	0	0	76
8	0	0	9	23	14	0	0	0	4	12	1	1	0	64
9	0	0	0	0	0	0	0	0	0	7	0	0	1	8
10	-	2	-	-	88	0	3	0	3	247	329	12	1	685
11	0	-	26	4	1	0	0	0	1	348	122	10	9	521
12	1	1	0	86	140	3	0	4	13	40	130	1	2	421
13	2	0	7	11	18	0	12	21	4	42	59	55	0	231
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	18	24	190	299	459	86	118	113	115	825	869	96	13	3225
Average	1.39	1.85	14.61	23.00	32.79	6.14	8.43	8.07	8.21	52.93	62.07	6.86	0.93	
%	0.56	0.74	5.89	9.28	14.24	2.67	3.66	3.50	3.56	25.58	26.94	2.98	0.40	

(Tada, Aoki, Hashiguchi, Kawabata, Quarterly Report, No.5, 1977).

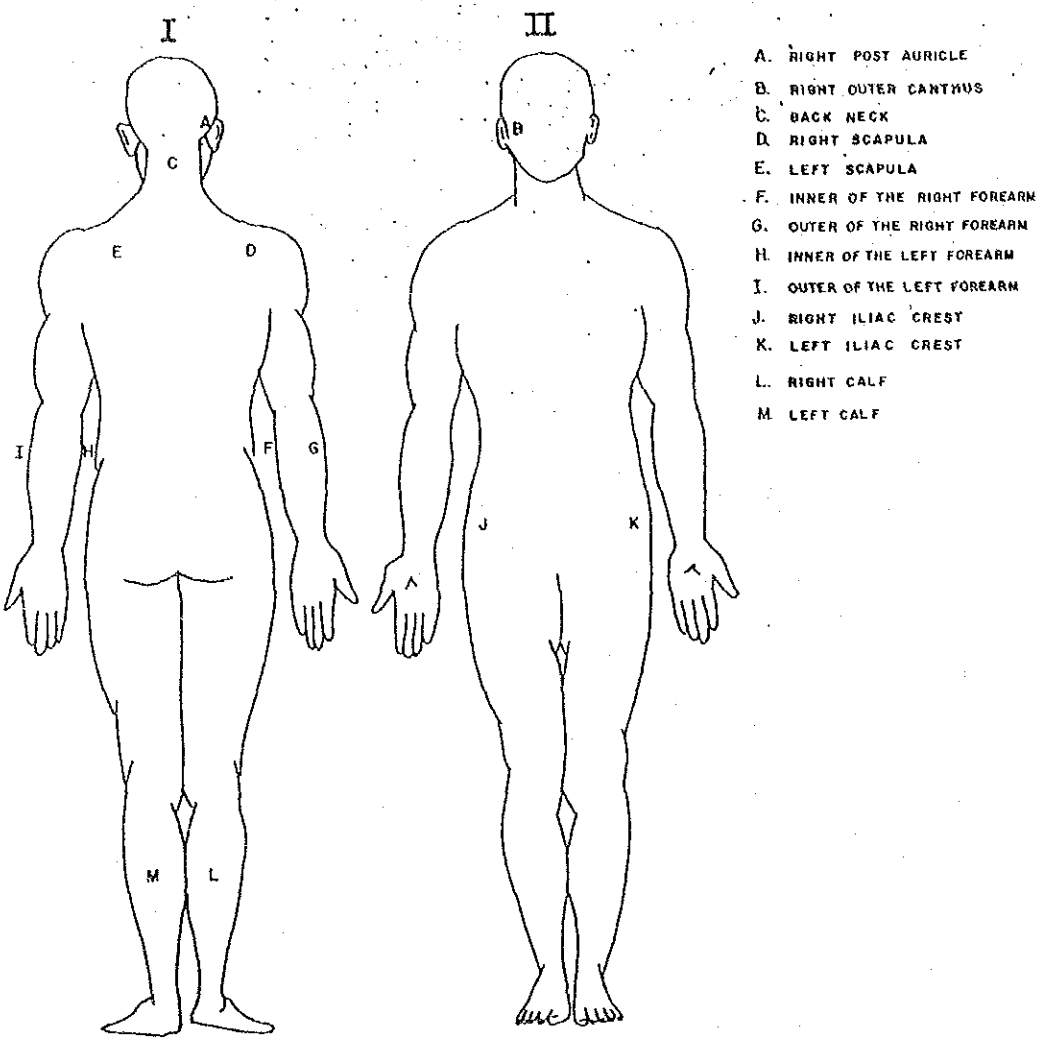


FIG. II-1 LOCATION OF SKIN SNIPS TAKEN BY: HOLTH-TIPE SCLERO-PUNCH.

I: DORSAL VIEW II: VENTRAL VIEW

(Tada, Aoki, Hashiguchi & Kawabata; Quaterly Report, No. 5, 1977)

density and most efficient for detecting onchocerciasis (TABLE II-2). Skin from the left scapula and pelvis were compared for mf density and mf detection using 96 women from a highly infected area. No significant difference was found between the two specimens (TABLE II-3). Therefore, it is considered appropriate to skin-snip both left and right scapulas, rather than inconvenience the women with pelvic skin sampling.

Accuracy of skin-biopsy in determining mf positive patients was compared using snips from one versus two areas. Skin samples were removed from men's left scapula and left pelvis and from women's right and left scapulas (TABLE II-4). Examination of either one sample detected 85-95% of the cases, which were diagnosed by the two complimentary biopsies. For more informative results, two biopsies were taken from patients to diagnosis onchocerciasis.

2) Microfilariae density surrounding onchocercoma.

Microfilariae (mf) density in skin tissue near the onchocercoma was studied to determine if mf produced by O. volvulus adults emerged from nodule and penetrated the surrounding skin. Mf were frequently found in skin near nodules in non-hairy head and trunk, however not in skin from hairy heads.

No mf were observed in half of the skin-snips taken from hairy heads, while mf density was 20 in legs, 50 in trunk and 6 in hairy heads (TABLE II-5).

TABLE II-3.

Comparison between skin from scapular and iliac crest and rate of Mf-positive and Mf density for 96 women from highly endemic areas in Guatemala..

No. of persons examined	No. of positive by skin biopsy	Average No. of Mf per 10 mm^2 skin snip which show positive by skin biopsy		Percent of mf-positive from skin of	
		Scapular	Pelvis	Scapular	Pelvis
96	74	21.4	13.7	66.7	71.9

(Hashiguchi & Kawabata, Quartaly Report No. 8, 1978).

TABLE II-2.

Microfilaria density per snip taken by holth-type scleropunch from the various parts of the body (as shown in Fig. II-1) Palín Guatemala.

Sites of snip / No. Patients	A	B	C	E	K	K	TOTAL
1	144	66	108	130	44	1	493
2	5	30	1	1	1	2	40
3	410	85	370	60	50	0	975
4	9	13	52	47	194	16	331
5	9	0	39	24	2	3	77
6	96	41	66	167	573	3	946
7	0	0	5	3	10	0	18
8	60	166	154	277	91	39	777
9	18	0	0	2	21	0	41
10	57	95	9	5	22	0	188
11	8	0	5	2	8	0	23
12	0	3	12	42	77	5	139
13	93	86	687	319	729	118	2032
14	588	10	262	245	197	4	1306
15	15	0	0	110	162	1	288
16	127	165	869	22	158	21	1362
17	17	73	177	40	139	1	447
18	2	5	22	33	316	3	381
19	14	124	29	21	8	0	196
20	271	531	37	112	101	24	1076
21	0	5	3	3	6	0	17
22	11	0	6	10	173	0	200
Total	1,954	1,493	2,913	1,675	3,082	241	11,363
Average	88.82	68.09	132.41	76.14	140.09	10.95	
%	17.20	13.13	25.13	14.74	27.13	2.12	

(Tada, Aoki, Hashiguchi, and Kawabata; Quarterly Report, No.5, 1977).

TABLE II-4.

Mf positive rate of double skin snip taken from Onchocerciasis patients

Sex	Examination of microfilaria in skin samples taking from			No. of cases	%
	Left-scapular	Right-scapular	Left-pelvis		
Male	+		+	186	(80.8)
	+		-	10	(4.3)
	-		+	34	(14.8)
Total				230	
Female	+	+		102	(80.3)
	+	-		11	(8.7)
	-	+		14	(11.0)
Total				127	

Skin samples were removed from left-scapular left-pelvis from man and left-scapular and right scapular for woman.

(Tada, Aoki, Hashiguchi & Kawabata, Quarterly Reported No. 5, 1977).

TABLE II-5.

Result of skin biopsy at the site of onchocercoma

Location of onchocercoma	No. of cases	Positive Rate
Head		
Frontal, Parietal, Temporal, Occipital.	101	58 (57.4)
Head		
Auriculal, Zygomatic	12	11 (91.7)
Upper part of the body		
Cervical, Dorsal, Scapular, Acromial, Clavicular, Arm, Costal, Abdominal	34	34 (100)
Lower part of the body		
Sacral, Coxal, Iliac, Trochanterian	55	48 (87.3)

(Aoki & Ikeda, Quarterly Report, No. 3, 1977).

Thus, since mf density is low in hairy heads where nodules often form, this indicates that mf density is not necessarily high around nodules. It is open to question how low mf density in head skin is related to the translocation of mf to the eyes.

3) Microfilariae density in scapular skin and around nodules.

Mf density was observed in scapular skin-snips and in skin surrounding nodules from various regions in the body. Mf density was higher in skin surrounding trunk nodules, than in scapular skin-biopsies (10 out of 18 cases), in although mf density was lower around nodules from hairy heads, than from compared with scapular skin-biopsies (22 out of 35 cases). Mf were found in scapular skin but not around head nodules in 14.3% of patients, while no mf were detected in scapular skin nor in tissue surrounding hairy head nodules in 22.9% of the cases. In 15.9% of nodules formed in legs, mf were observed near nodule, but not in scapular samples. Approximately 10% of onchocercoma patients show a mf negative reaction to skin autopsy from scapula or pelvis, and can be accounted for by previously described non-onchocercal nodules.

4) Comparison of three skin-biopsy methods.

The traditional method for sampling mf in the skin was to raise the

skin with a lancet (or needle) and cut with a scapel, a sample with a 3 - 5 mm diameter. In recent years, however, two types of sclerocorneal punch (Holth and Walser) have been employed for skin sampling. The objective of this study was to compare the practicality and efficiency of the three methods.

At Finca Valle de Oro in Guatemala, skin-snips were collected, 10 mm apart, from three locations on the left scapula of 109 patients, using the three methods 1) lancet and scapel 2) Holth-type, and 3) Walser-type sclerocorneal punch. After skin samples were soaked in physiological saline solution for one-hour, emerged mf were counted. Skin-snips were measured for thickness after fixed in 10% formaldehyde. The use of the lancet and scapel produced the thickest skin-snip, while no significant difference in thikness was observed between the two sclerocorneal punches (TABLE II-6). Lancet and scapel was most efficient for detecting mf, followed by Holth-type, then Walser-type sclerocorneal punches.

Although lancet and scapel was most efficient, it was least practical. There was great variability in skin-snip thikness and often bleeding resulted, scarring patient and causing pain. Considering attributes and drawbacks, the Holth-type sclerocorneal punch was chosen for epidemiological studies in the field.

5) Appearance of mf in the Urine.

Anderson (1973), Buck (1973) and Piq & Roux (1973) reported the

TABLE II-6.

Results of skin snipping in the residents of Finca Valle de Oro using three instruments.

Instruments	Microfilarial density* in positives		Positive rate for MF (%)	False negative rate (%)
	Arithmetic mean	Geometric mean		
Scalpel	5.79 ± 12.67**	2.37	66/108 (61.1)	8/76 (10.5)
Holth punch	2.86 ± 4.60	1.55	60/108 (55.6)	11/76 (14.5)
Walser punch	2.51 ± 3.94	1.39	56/108 (51.9)	18/76 (23.7)

* Microfilarial density per 1 mm^2 skin snip.

** Standard deviation.

(Hashiguchi, Kawabata, and Tada; Quarterly Report No. 5, 1977).

excretion of mf in urine of African onchocercal patients. Pazen et. al (1975) examined 65 patients, older than 10 years, from a coffee plantation in Yepocapa Guatemala and detected mf in the urine in 17 - 30%.

In the present project, mf presence in the urine was microscopically examined, particularly in relation to skin-biopsy, in 46 and 41 Guatemalan inhabitants from Finca Berlín and Finca San Rafael Sumatán, respectively.

Urine samples were allowed to settle several hours, the supernatant decanted, residue centrifuged and the precipitate microscopically examined for mf.

One to four mf were detected in the urine of 8 (17.4%) and 7 (17.1%) in inhabitants from Finca Berlín, and Finca San Rafael Sumatán, respectively (TABLE II-7). Mf were found in urine 23.5%, and 20.6% of mf positive patients from Fincas Berlín, and San Rafael Sumatán respectively. Three patients diagnosed mf-negative by skin-biopsy, exhibited mf in the urine. A complimentary urine examination may improve between mf density in skin-biopsy and appearance of mf in urine.

6) Periodicity of microfilaria density.

Thomas et al (1973) studied a daily fluctuation of mf density in skin snips from onchocercal patients in Cameroon, Africa. They reported that mf density doubled from early morning toward noon. On the other

TABLE II-7.

Results of examinations on microfilarae of O. volvulus in urine and in skin snip

Locality	No. of persons examined	No. of persons positive with mf in skin snips	No. of persons positive with mf in urine.
Berlín	46	31 (67.4)	8 (17.4)
San Rafael Sumatán	41	31 (75.6)	7 (17.1)

(Hashiguchi, and Kawabata; Quarterly Report No.6, 1977).

hand, Picq and Jardel (1974) concluded that there was no daily periodicity of mf density in patients in Upper Volta, Africa. Duke and Moor (1974) inoculated chimpanzees with Guatemalan and African strains of Onchocerca volvulus and studied daily fluctuations of mf density in skin-snips. Mf of the Guatemalan strain peaked from 5:00 to 12:00 in the morning. They mentioned that during this time of day, Simulium ochraceum and S. metallicum, two major vectors of Guatemalan onchocercosis, most actively suck the blood from humans.

Anderson et al (1975) reported that mf density peaked around 10 a.m. coinciding with the highest blood-sucking activity of S. ochraceum in Guatemala. On the other hand, Tada and Figueroa (1974) found no daily periodicity in mf density of 21 onchocercal patients, and no correlation in periodicity between mf density and blood-sucking activity of S. ochraceum in Guatemala.

In summary, there are conflicting reports concerning daily periodicity of mf density in skin-snips.

In the present project a daily periodicity was studied in skin-snips from 14 onchocerciasis patients from Finca San Rafael Sumatán and Finca Nimayá, Guatemala. Skin samples were snipped at different hours and soaked in physiological saline solution for one (Finca San Rafael Sumatán) or two hours (Finca Nimayá) at room temperatures (18-27 °C). Emerged mf per 10 mm^2 were microscopically counted (TABLE II-8, and II-9).

There was much variation from each patient and no clear daily periodicity was observed. In mean counts per sampling hour, however, mf density

TABLE II-8.

Daily fluctuation of microfilariae density in skin snips taken from patients at Finca San Rafael Sumatán, Guatemala.

Hour	No. of microfilaria/10mm^2				Mean
	Patient-No.1	No. 2	No. 3	No.4.	
18:00	63	398	66	20	136.8
21:00	51	297	142	31	130.3
24:00	114	99	181	20	103.5
4:00	70	183	121	56	107.5
6:00	45	114	237	18	103.5
9:00	78	179	68	14	84.8
12:00	144	217	69	6	101.5
15:00	31	317	64	43	113.5

(Hashiguchi & Kawabata, Quarterly Report, No. 8, 1978).

TABLE II-9

Daily fluctuation of microfilariae density in skin snips taken from patients at Finca Nimayá, Guatemala.

Hour	No. of microfilaria/10mm^2										Mean
	Patient-No.1	2	3	4	5	6	7	8	9	10	
17:00	56	36	0	116	147	14	729	179	36	150	146.3
19:00	64	49	9	146	153	2	83	88	22	137	75.3
21:00	27	39	27	44	0	9	266	110	25	186	73.3
23:00	40	82	11	29	75	14	2	129	100	41	52.3
1:00	22	12	25	121	73	25	66	150	103	71	66.8
3:00	20	33	0	145	91	15	14	75	18	153	56.4
5:00	15	28	2	20	7	0	93	77	44	8	29.4
7:00	10	5	38	84	7	2	161	11	0	75	39.3
9:00	33	6	4	72	52	6	5	14	27	88	31.3
11:00	21	1	13	54	12	0	5	85	2	0	19.3
13:00	61	4	79	20	4	3	49	32	30	66	34.8
15:00	6	3	26	27	41	4	78	61	2	105	35.3

(Hashiguchi & Kawabata, Quarterly Report, No. 8, 1978)

was lowest during 9:00 - 13:00, and highest during 17:00 - 21:00. It was difficult to drew a definite conclusions due to high variation in mf-density among skin-snips. This may account for the previously-reported conflicting results on daily periodicity of mf-density.

7) Penetration of microfilaria into anterior chamber of eye.

Presence of mf in anterior chamber was studied in relation to mf density per skin-snip, using 587 residents from onchocerciasis-infested area in Guatemala. More mf were found in anterior chamber, proportional to incresing in patient's skin. When mf-density surpassed 500 per skin-snip, mf were observed in anterior chamber of nearly half of the patients (TABLE II-10).

8) Microfilaria infection in relation to sex and age of inhabitants.

More men were infected with mf, than women in all age groups. Infection rate increased with age, both with men and women, rapidly rising in 3 to 4 and 15 to 19 male age brackets and in 5 to 9 and 10 to 14 female age groups. Thus, men were infected with mf at younger ages, compared with women. Mf were detected in skin-snips from patients of 0-2 age group, indicating that mf were produced within two years after the patient was infected with O. volvulus (TABLE II-11).

TABLE II-10.

Relation between Microfilarial Rate in Anterior Chamber and Microfilaria Density in the Skin.

MFD*	No. of Examined	No. of positive in Anterior Chamber	Percent positive in Anterior Chamber
0 -	102	5	4.9
1 - 10	166	5	3.0
11 - 50	151	23	15.2
51 - 100	63	13	15.2
101 - 200	52	13	20.6
201 - 300	16	4	25.0
301 - 500	21	6	28.6
501 -	16	7	43.8
Total	587	76	12.9

* No. of Microfilariae per Single Snip.

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 1978).

TABLE II-11.

Sex and Age Specific Prevalence of Infection with Onchocerca volvulus using Skin Biopsy Method.

	Male			Female		
Age	No. Exam.	No. Posi.	Percent Posi.	No. Exam.	No. Posi.	Percent. Posi.
0- 2	35	1	2.9	35	0	0
3- 4	76	10	13.2	65	3	4.6
5- 9	202	30	14.9	176	20	11.4
10-14	146	41	28.1	188	35	18.6
15-19	103	40	38.8	89	19	21.3
20-29	151	63	41.7	166	51	30.7
30-39	143	91	63.6	110	38	34.5
40-49	109	71	65.1	97	34	35.1
50-59	99	50	50.5	59	17	28.8
60-	86	46	53.5	50	12	24.0
Total	1150	443	38.5	1035	229	22.1

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 1978).

III. Dissemination of onchocerciasis in Guatemala.

1. Disease survey in Guatemala.

The present project conducted a survey on the extension of onchocerciasis in different areas of Guatemala from 1976 through 1980, The disease was diagnosed by presence of nodules using palpation, and mf in skin-biopsy. The results are summarized in Table III-1, and III-2.

2. Rate of infection and altitude.

A disease survey was undertaken at different altitudes in San Vicente Pacaya, Guatemala. Infection rate was highest at altitudes from 1,200 to 1,400 m. above sea level; nodules and mf were detected from 32.2 - 67.6%, and 44.4 - 80.3% of the residents examined, respectively. The second highest infection rate was located around 700 m; nodules and mf were found in 17.8 - 58.6%, and 35.6 - 76.0% of the habitants surveyed, respectively. The infection rate was lowest below 450 m and above 1,700 m; nodules and mf were detected only in 9.7 - 15.1% and 7.6 - 18.2% of the residents examined, respectively (TABLE III-3).

TABLE III-1.

Positive rate of onchocercoma by the palpation, surveyed at the different localities in Guatemala from 1976 to 1980.

Locaties	No.Exam.	No.Posi.	Percent Posi.	Date Exam.
Agua Blanca	6	0	0	4-6/'77
Agua Dulce	140	18	12.9	5/'77
Alejandría	20	8	40.0	4-6/'77
Amate	64	8	12.5	4-6/'77
Arabia	53	12	22.6	4-6/'77
Berlín	47	17	36.2	5-9/'76
	25	4	16.0	4-6/'77
	54	8	14.8	8/'79
	64	1	1.6	7/'80
Buena Vista	99	77	78.6	7/'79
	80	31	38.8	8/'79
	120	8	6.7	1/'80
	79	13	16.5	5/'80
	92	14	15.2	7/'80
Caña Vieja	82	38	46.3	5-9/'76
	39	8	20.5	4-6/'77
	65	14	21.5	8/'79
	54	4	7.4	7/'80
Caña Vieja-Berlín	104	54	51.9	10-12/'77
Camarón	93	9	9.7	5-9/'76
	34	0	0	4-6/'77
Camaron-Chagüites	129	23	17.8	10-12/'77
Caracol	29	9	31.0	4-6/'77
Cedro	325	32	9.8	5-9/'76
	254	4	1.6	4-6/'77
Chaguites	95	16	11.6	10-12/'76
	68	3	4.4	4-6/'77
Chilar	22	20	90.0	4-6/'77
Chilcas	16	5	31.3	4-6/'77
Chilco	163	15	8.9	4/'80
Diamante	88	2	2.3	4-6/'77

El Amate	79	26	32.9	10-12/'76
El Milagro	8	2	25.0	5-9/'76
El Rabón	10	2	20.0	4-6/'77
El Silencio	142	10	7.0	10-12/'76
El Teguimay	46	16	34.8	5-9/'76
Guachipilín	71	48	67.6	5-9/'76
	48	26	54.2	4-6/'77
	52	33	63.5	10-12/'77
	25	8	32.0	8/'79
	18	6	33.3	7/'80
Guachipilín Area	210	38	18.1	8/'79
Hamburgo	129	23	17.8	10-12/'76
	74	25	33.8	10-12/'77
	63	8	12.7	2/'79
	59	7	11.9	9/'79
	79	0	0	7/'80
Hoja Blanca	347	42	12.1	10-12/'77
INDE	33	0	0	4-6/'77
La Cruz	236	21	8.9	10-12/'77
	179	9	5.0	3/'79
	241	12	5.0	7/'79
	204	3	1.5	6/'80
La Moka	292	0	0	4/'80
Las Chilcas	29	17	58.6	5-9/'76
Las Ilusiones	29	2	6.9	5-9/'76
Las Parasitas	41	7	17.1	5-9/'76
Las Victorias	75	16	21.3	3/'80
Londres	32	9	28.1	8/'79
	19	2	10.5	7/'80
Los Chorritos	6	2	33.3	10-12/'76
Los Rios	105	49	46.7	5-9/'76
	83	14	16.9	4-6/'77
	85	38	44.7	10-12/'77
	121	18	14.9	8/'79
	112	8	7.1	7/'80
Monte Quina	112	13	11.6	8/'80
Nimaya	73	3	4.1	3/'80
Pacaya Grande	125	19	15.2	5-9/'76

Pacaya Grande et al.	108	8	7.4	4-6/'77
Pacayal	359	41	11.4	11/'79
Pacayalito	145	11	7.6	2/'80
Palín(Chilar)	622	275	44.2	10-12/'77
Patrocinio	546	188	34.4	5-9/'76
	486	48	9.9	6/ '79
	511	32	6.3	6/'80
Puerta de Oro	15	2	13.3	10-12/'76
Rincón de Pacaya	35	6	17.1	4-6/'77
Rodeo	25	8	32.0	4-6/'77
San Francisco de Sales	319	35	11.0	10-12/'76
San Gregorio	12	4	33.3	10-12/'76
San Isidro	43	7	16.3	4-6/'77
San José Bejucal	114	0	0	4-6/'77
San José de la Cruz	24	10	41.7	10-12/'76
San José de la Cruz et al	35	9	25.7	4-6/'77
San José Guachipilín	77	39	50.6	4-6/'77
San Nicolás	73	11	15.1	5-9/'76
	19	0	0	4-6/'77
San Rafael	56	13	23.2	4-6/'77
San Rafael Coyolito	10	6	60.0	5-9/'76
	36	7	19.4	8/'79
San Rafael Sumatán, Rosario	228	38	16.7	10-12/'77
San Roman	34	10	29.4	4-6/'77
Santa Clara	17	2	11.8	4-6/'77
Santa Emilia	109	13	11.9	11/'79
Santa Eulalia	15	5	33.3	5-9/'76
Santa Fé	5	2	40.0	5-9/'76
Sibajá	88	27	30.7	5/'80
Soledad	22	1	4.5	4-6/'77
Tequimay et al	40	3	7.5	4-6/'77
Valle de Oro	64	9	14.1	9/'79
Valle de Oro Esperanza	271	27	10.0	10-12/'77

TABLE III-2

Positive rate of microfilaria by the skin-biopsy, surveyed in the different localities in Guatemala.

Locality	Population	No.Exam.	No.Posi.	Percent Posi.	Date exam.
Agua Blanca	7	5	1	20.0	4-6/'77
Alejandría	35	18	13	72.2	4-6/'77
Arabia	55	45	12	26.7	4-6/'77
Berlín	52	42	29	69.0	5-9/'76
Buena Vista	79	79	68	86.1	1/'80
		76	71	93.4	5/'80
		92	78	84.8	7/'80
Camaron Chaguites		129	22	17.1	10-12/'77
Caña Vieja	92	53	33	62.3	5-9/'76
Caña Vieja, Berlín		104	74	71.2	10-12/'77
Caracol	44	27	10	37.2	4-6/'77
Carrizal		82	0	0	1-3/'79
Chilar	25	20	18	90.0	4-6/'77
Chilar (Pelín)		590	283	48.0	4-6/'77
Cuilco		163	11	6.5	4/'80
Diamante	95	75	4	5.3	4-6/'77
El Amate	90	73	26	35.6	5-9/'76
El Camaron	107	76	6	7.6	5-9/'76
El Cedro	412	331	25	8.0	5-9/'76
El Coro		76	0	0	1-3/'79
El Milagro	14	8	1	12.5	5-9/'76
El Rebón	12	8	4	50.0	4-6/'77
El Silencio	174	139	3	2.2	5-9/'76
Guachipilín	84	66	53	80.0	5-9/'76
		52	37	71.2	10-12/'77
Guachipilín Area (Guachipilín Berlín, Caña Vieja Coyolito).	232	168	110	65.4	7-9/'78
		210	107	51.0	3/'79
		191	81	42.4	7/'80
Hamburgo	177	117	45	38.5	10-12/'76
		106	40	37.7	5-9/'76
		74	40	54.1	10-12/'77
		64	27	42.2	1-3/'79

		59	28	47.5	9/'79
		81	28	34.6	7/'80
Hoja Blanca		347	32	9.2	10-12/'77
INDE	36	33	8	24.2	4-6/'77
La Cruz		236	28	11.9	1-3/'78
		150	14	9.3	1-3/'79
		252	29	11.5	7-9/'79
		238	20	8.4	6-8/'80
La Foka		291	0	0	4/'80
La Victoria		75	52	69.3	3/'80
Las Chilcas	42	27	21	77.8	5-9/'76
Las Ilusiones	32	28	2	7.1	5-9/'76
Las Parasitas	59	40	9	22.5	5-9/'76
Los Chaguites	119	98	16	18.2	5-9/'76
Los Chorritos	8	6	5	83.3	5-9/'76
Los Ríos	146	96	47	49.0	5-9/'76
		85	34	40.0	10-12/'77
		118	45	38.1	3/'79
		112	34	30.4	7/'80
Medio Monte		89	18	20.2	10-12/'79
		107	30	28.0	1-3/'79
		93	21	22.6	8/'79
Monte Quino		208	77	37.0	10-12/'79
		112	61	54.5	8/'80
Mineyá		72	28	38.9	3/'80
Pacaya Grande	164	120	27	22.5	5-9/'76
Pacayal		359	202	56.3	11/'79
Pacayalito		144	68	47.2	2/'80
Palín (School-children).		322	17	5.3	7-9/'79
Palín (Inhabitants)		434	112	25.3	7-9/'79
Patrocinio	607	507	196	38.7	5-9/'76
Patrocinio Area (Patrocinio, Rodeo, Caracol).	641	357 49	98 20	27.5 40.8	7-9/'78 10-12/'79
		486	139	28.6	6/'79
		511	127	24.9	6/'80
Puerta de Oro	24	4	3	75.0	5-9/'76
Rincón de Pacaya	104	33	8	24.3	4-6/'77
Rodeo	39	23	10	43.5	4-6/'77
San Gregorio	12	12	4	33.3	5-9/'76

San Francisco El Amate		72	26	36.1	5-9/'76
San Francisco de Sales	390	279	27	9.7	5-9/'76
San Isidro	349	278	91	32.7	4-6/'77
San José de la Cruz	29	25	16	64.0	5-9/'76
San José Bejucal	181	107	0	0	4-6/'77
San José Guachipilín	93	67	41	61.2	4-6/'77
San Nicolás		71	8	11.3	5-9/'76
San Rafael Coyolito	11	10	5	50.0	5-9/'76
San Rafael	60	53	29	54.7	4-6/'77
San Rafael Sumatán		229	145	63.6	10-12/'77
Santa Clara	25	19	8	42.1	4-6/'77
San Román	40	29	10	34.5	4-6/'77
Santa Fé	18	5	3	60.0	5-9/'76
Santa Eulalia		15	4	26.7	5-9/'76
Sibajá		31	24	77.4	1-3/'79
		88	61	69.3	5/'80
Soledad	27	20	15	75.0	4-6/'77
S.V.P.		487	14	2.9	10-12/'78
Tiquimary	49	44	9	20.9	5-9/'76
Valle de Oro Esperanza		271	141	52.0	10-12/'77
Valle de Oro		64	24	37.5	9/'79

TABLE III-3.

Relation among Microfilarial Rate, Nodule Rate and the Altitude of Villages in S.V.P.

Location	Altitude (m) (above sea level)	Mf rate	Nodule rate
Ald. S. F. Bejucal		0	0
Fnc. Camaron	400	7.6	9.7
Ald. C. Cedro	1720	8.3	9.8
Ald. S.F. Sales		9.6	10.9
Fnc. S. Nicolás	400	11.3	15.1
Ald. Chaguites	450	18.2	11.6
INDE		25.0	0
Fnc. S.F. Amate	725	35.6	32.9
Fnc. Hamburgo	700	38.5	17.8
Ald. Patrocinio	1600	38.7	34.4
Cas. Caracol et al	1500	43.6	30.2
Fnc. Santa Fé et al	1200	44.4	44.4
Ald. Los Ríos	1350	49.0	46.7
Fnc. S.F. Guachipilín et al		61.8	44.7
Cas. Caña Vieja	1200	62.3	46.3
Fnc. Chorritos et al	650	68.6	31.1
Fnc. Berlín	1370	69.0	32.2
Fnc. Chilcas	750	76.0	58.6
Fnc. Guachipilín	1200	80.3	67.6
Cas. Chilar		90.0	90.9

(Aoki; Onchocerciasis Control Project in Guatemala, First Report, 1978).

IV. INDIRECT HAEMAGGLUTINATION TEST (IHA)

Indirect haemagglutination test (IHA) is considered one of the most sensitive and realiable methods, frequently use in immunological diagnosis for parasitic diseases. There have been, however, few reports using IHA for diagnosing onchocerciasis, hence, several basic points must be checked to study the feasibility of this method for this particular disease. The principal objective of the present study was to determine if IHA could be used to measure the effect of control measures for onchocerciasis.

1. Materials and methods.

Antigen: O. volvulus adults from onchocercomae were macerated by mortar and pestle, treated with acetone to remove fats, and extracted for 2 days in 0.015 M phosphate buffer, pH 6.8. The extract was centrifuged for 30 min at 27000g and the supernatant was used as the antigen.

Sensitized red cells: Unless otherwise mentioned, human erythrocytes from O blood type were used.

The erythrocytes were fixed with formaldehyde, treated at 37 °C for 15 minutes with tannic acid solution (1:40,000 dilution) and sensitized at 37 °C for 30 min with the same volume of antigen solution (2 mg dry weight/ml).

Blood samples: Filter paper for blood-sampling (Toyo Roshi Co., type I) was used to collect the blood from patient's ear lobes,

dried at room temperature, and stored at -20 °C. For assay, the blood was extracted from the filter paper in 0.015 M phosphate buffer, pH 7.2 to give a 1:20 dilution. Sera from veins of some patients was also assayed for comparison.

IHA TEST: The blood samples of 0.025 ml each were pipetted in microtiter plate (Tomy) and diluted in a series of 3-fold dilutions. To each well 0.025 ml of 2.5% sensitized red cells were added. Reactions were observed 24 hrs. later.

2. Comparison of blood samples from ear lobes and veins.

Blood sample collected from ear lobe by filter paper and serum from veins removed by needle and syringe were compared in titers of IHA test, from 63 residents of Finca La Torre, Guatemala. The two samples were closely correlated (r=0.96) (Fig. IV-1). Many patients refused blood sampling from vein using needle and syringe. Spearing ear lobes, on the other hand, was less painful and more easily accepted by patients. Moreover, blood-stained filter paper is more easily transported and stored than sera. Threfore, it was decided to use filter paper for collecting the blood for IHA in the present project.

3. Standard for IHA positiveness

IHA tests were performed on 208 habitants from Fincas Nicolás,

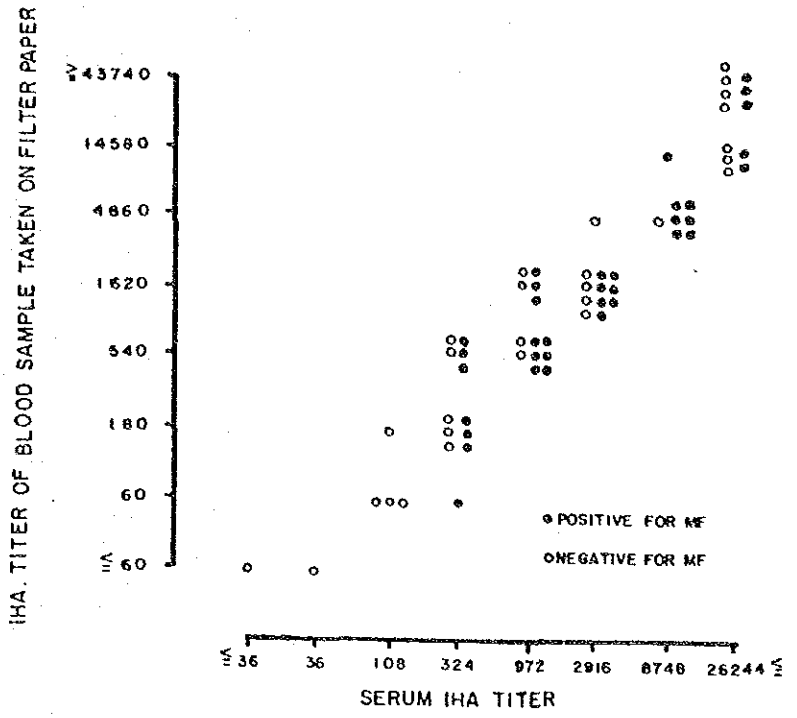


FIG. IV-1. Comparison of IHA titers obtained with serum collected by venipuncture and blood collected on filter paper (Finca La Torre, Guatemala).

(Ikeda, Tada and Aoki; Journal of Parasitology, 64(5), 1978)

Guachipilín, and El Amate, three Guatemalan plantations in the endemic onchocerciasis-zone. Skin-biopsy and palpation for nodules were also conducted simultaneously to compare with IHA (TABLE IV-1).

The residents examined were divided in four groups depending on mf infection and nodules; group I= both mf and nodule positive, II=mf positive and nodule negative, III=mf negative and nodule positive, and IV=both mf and nodule negative. Setting a titer 1:180 as standard for IHA positiveness, a 85.0 % of 107 patients of onchocerciasis from groups I, II, and, III were IHA positive. The remaining 15.0% can be accounted for by presence of approximately 10% of non-onchocercal nodules, which was previously stated. By the same standard, very high proportion (94.4%) of mf positive patients (true patients of onchocerciasis) from groups I and, II were IHA positive. Only a 6.2% of healthy inhabitants from group IV were IHA positive. Therefore, a titer 1:180 or higher was an acceptable standard for judging IHA positiveness with using our method.

4. Variation of titer during storage of blood samples in filter paper.

A variation of titers was studied by storing blood samples in filter paper. The filter paper absorbed blood samples from patients of onchocerciasis, or serum was collected from rabbit, which was immunized with O. volvulus antigen.

The rabbit serum was used for comparison. The filter papers were stored at different temperatures in air-tight jars which had silica

TABLE IV-1

Result of IHA test on dried blood taken on filter paper , Guatemala.

Group	Diagnosis: Microfilaria	Diagnosis: Nodule	No. Exam.	Reciprocal IHA titer: 60	60	180	540	1620	4860	14580	Positive Rate
I	Positive	Positive	59	0	1	2	5	14	15	22	98.3
II	Positive	Negative	28	2	1	1	5	3	7	9	89.3
III	Negative	Positive	20	5	7	4	0	2	0	2	40.0
IV	Negative	Negative	101	32	40	11	8	3	5	2	28.7
V*	Negative	Negative	50	39	7	3	1	0	0	0	8.0
Control sera**			15	0	0	0	0	0	0	0	0.0
Total			273	93	56	21	19	22	27	35	

* Control population from nonendemic area.

** Nonparastic deisease from Roosevelt Hospital , Guatemala City.

(Ikeda, Tada & Aoki; Journal of Parasitology, 64(5), 1978)

gel inside, to determine the effect of humidity on titers.
Sensitized sheep red cells were used in this test.
There was no change in titers of the rabbit serum up to 90 days when the filter papers were stored at -20°, 4°, and 26 °C with or without silica gel, and, at 30°, and 35°C with silica gel. The titers dropped sharply when the filter papers were kept without silica gel at 30°C for 60 days, and at 35 °C for 30 days. Thus, low temperatures and low humidity helped maintain titers high (TABLE IV-2).
The blood samples in filter paper from patients of onchocerciasis lost titers more rapidly, than the rabbit serum.
However, low temperatures and humidity were again favorable for maintaining high titers. There was no change in titers of the blood samples up to 60 days when the filter papers were stored at 4 °C with silica gel. The titers dropped when the filter papers were stored at 4°C without silica gel for 30 days, or at 26 °C and 30 °C with or without silica gel for 30 days, and at 35 °C without silica gel for only 7 days (TABLE IV-3).

5. Cross reaction with other intestinal helminths.

The objective of this study was to see if IHA test for O. volvulus interacted with other intestinal helminths.
Ninety-seven residents from onchocerciasis-endemic and other areas were checked for intestinal helminths by fecal examination.
There was no significant difference in fecal examinations between

TABLE IV-2 Variation of titer during storage of rabbit sera samples in filter paper at the different temperature and humidities.

Temperature	With or without Silicagel	Days of strage 1	2	3	7	30	50	90
-20°C	+	1920*	-	-	-	-	-	1920
	-	1920	-	-	-	-	-	1920
4°C	+	1920	-	-	-	-	1920	1920
	-	1920	1920	-	-	-	1920	1920
26°C	+	1920	1920	-	-	1920	1920	1920
	-	1920	1920	-	-	1920	1920	1920
30°C	+	1920	1920	-	-	1920	1920	1920
	-	1920	1920	-	-	1920	240	240
35°C	+	1920	1920	-	-	1920	1920	1920
	-	1920	1920	-	-	480	240	240

* Reciprocal IHA Titer.

(Takaoka et al.; Personal Comunication, 1979)

Table IV-3 Variation of titer during storage of onchocerciasis patients blood samples in filter paper at the different temperature.

Temperature	with or without Silicagel	Days of Strage 1	2	3	7	14	30	60
-20°C	+	480*	–	–	–	–	–	–
	–	480	–	–	–	–	–	–
4°C	+	240	240	480	–	480	480	480
	–	–	240	480	480	–	480	240
26°C	+	240	240	240	480	480	480	240
	–	240	480	480	240	480	480	240
30°C	+	240	480	480	480	480	480	240
	–	240	240	480	480	240	480	120
35°C	+	480	480	480	480	480	480	240
	–	120	120	120	120	60	60	60

* Reciprocal IHA Titer.

(Takaoka et al.; Personal Comunication, 1979)

the two areas, or between IHA-positive and -negative habitants of endemic area in infection rate with intestinal helminths (TABLE IV-4).

This suggests that intestinal infection with other helminths has no effect on IHA reaction for O. volvulus.

6. Titer of IHA and mf density.

IHA titers were studied in relation to microfilarial density (MFD). MFD was categorized into 4 groups; 1-3, 4-20, 21-100, and over 101 mf per skin snip. In each group, frequency of IHA titer was studied. The results are shown in FIG. IV-2. Frequency of high titers increased with increasing MFD. More than 60% of the patients with MFD over 101 per skin snip had a titer equal or higher than 1: 14,580, when MFD was low (1-3 per skin snip), some patients showed very low titer (1:60) or negative reaction of IHA.

7. IHA positive rate in relation to ages.

Habitants with various ages from high, medium, and, low endemic areas were examined by IHA tests, in addition to skin-biopsy and palpation for nodules for comparison.

IHA positive rates increased with age in all three areas (FIG. IV-3). In the high endemic area, the IHA positive rate jumped sharply

TABLE IV-4 IHA test prevalence of intestinal parasites among inhabitants of coffee plantations in endemc and nonendemic areas.

Parasites*	Coffee plantation in endemic area (finca El Amate)			Coffee plantation in nonendemic area (Finca Colomba)
	IHA positive	IHA negative	Total	
Ascaris lumbricoides	22 (75.9%)	17 (68.0%)	39 (72.2%)	29 (67.4%)
Trichuris trichura	20 (69.0%)	16 (64.0%)	36 (66.7%)	27 (62.8%)
Hookworm	6 (20.7%)	9 (36.0%)	15 (27.8%)	23 (53.5%)
No. examined	29	25	54	43

* No significant statistical differences were seen(P 0.05), except for hookworm infection between inhabitants of El Amate and Colomba.

(Ikeda, Tada & Aoki; Journal of Parasitology, 64(5), 1978)

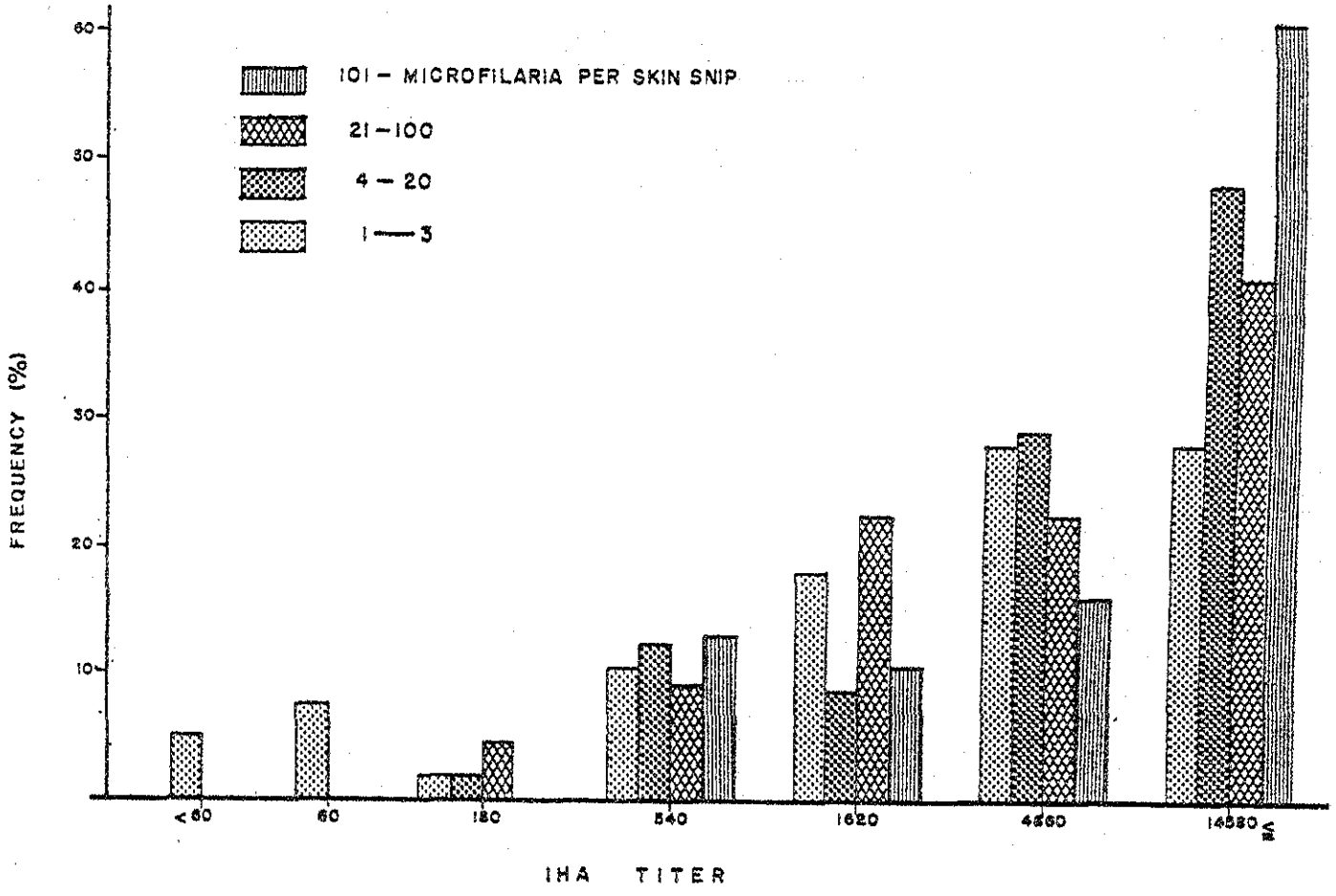


FIG. IV-2 FREQUENCY DISTRIBUTION OF IHA TITER FOR FOUR GROUPS OF MICROFILARIA DENSITY

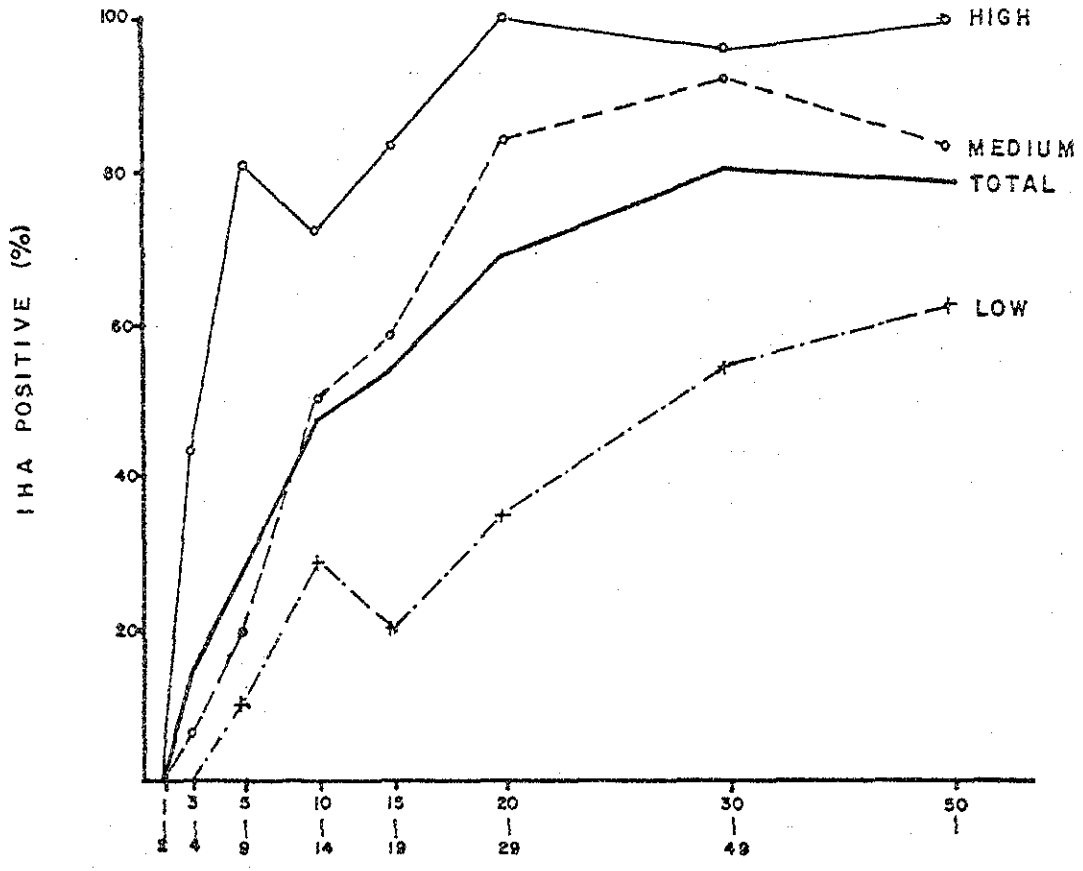


FIG. IV-3

IHA POSITIVE RATES BY AGE GROUP OF THE INHABITANTS IN HIGH, MEDIUM AND LOW ENDEMIC AREAS

in age group 3-4 reaching 80 and 100% in age groups 5-9, and, 20-29 respectively, then leveled off. In the medium endemic area the rates increased gradually with age, reaching approximately 80% in 20-29 age group, then leveled off. In the low endemic area the rates continued, increasing slowly and reached 60% in the over 50 age group.

Means of IHA positive rates of each age group from the three areas were compared with those of mf-and nodule positive, mf-positive and nodule positive rates.

The IHA positive rates were always higher than the mf-positive rates in all age groups. The IHA and mf positive rates increased gradually in the same fashion with increasing age, reaching 80 and, 60%, respectively, in 20-49 age group, then leveled off. The mf and nodule positive, and nodule positive rates were higher in young ages (less than 9 years), but lower in age groups over 10 years, than the IHA positive rates. The mf and nodule positive rates were always higher than nodule positive rates. However, these two rates increased slowly in a similar fashion with age and reached plateaus in 20-49 age group (FIG. IV-4).

8. Stability of IHA test.

This study was undertaken to determine if IHA test give stable titers with same patients throughout the year. Eight patients from Fca. Sibajá, Guatemala were examined monthly by IHA test. Formald[illegible]-fixed sheep red cells were used in this test.

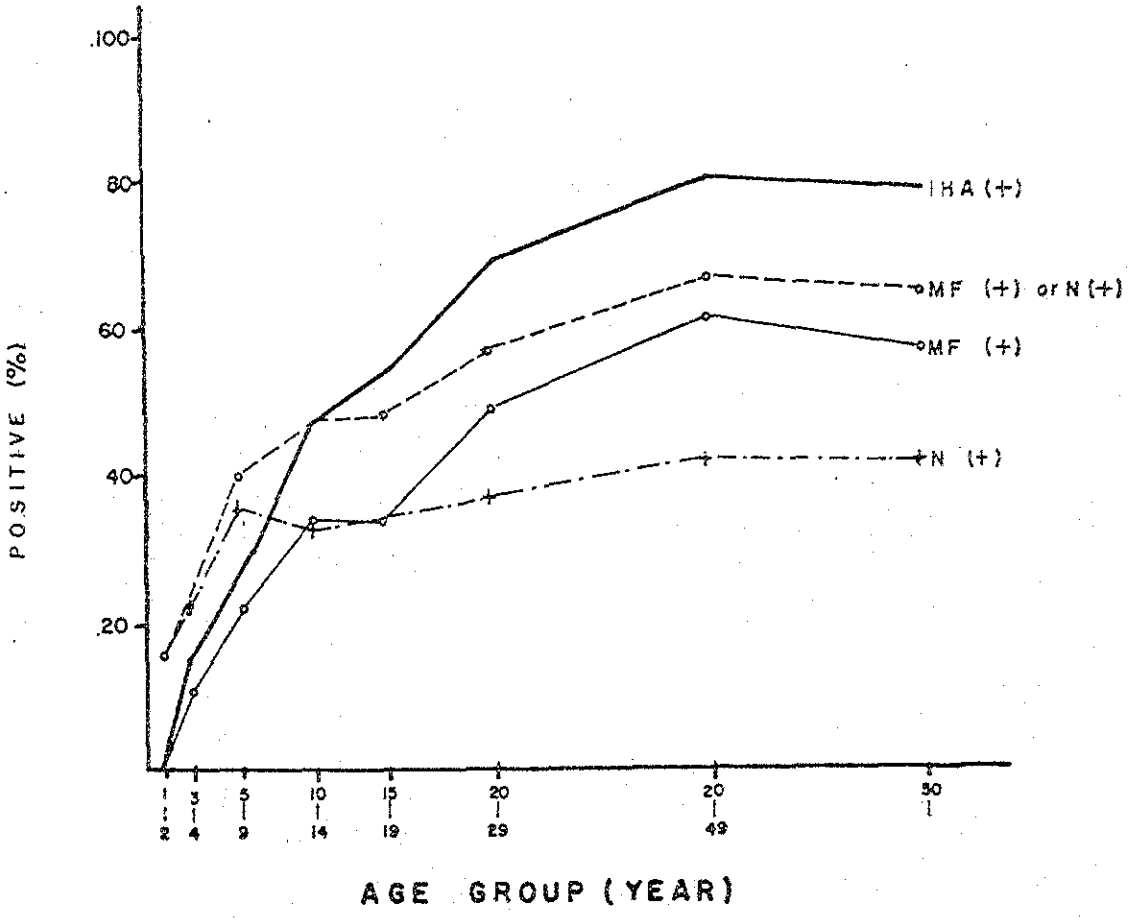


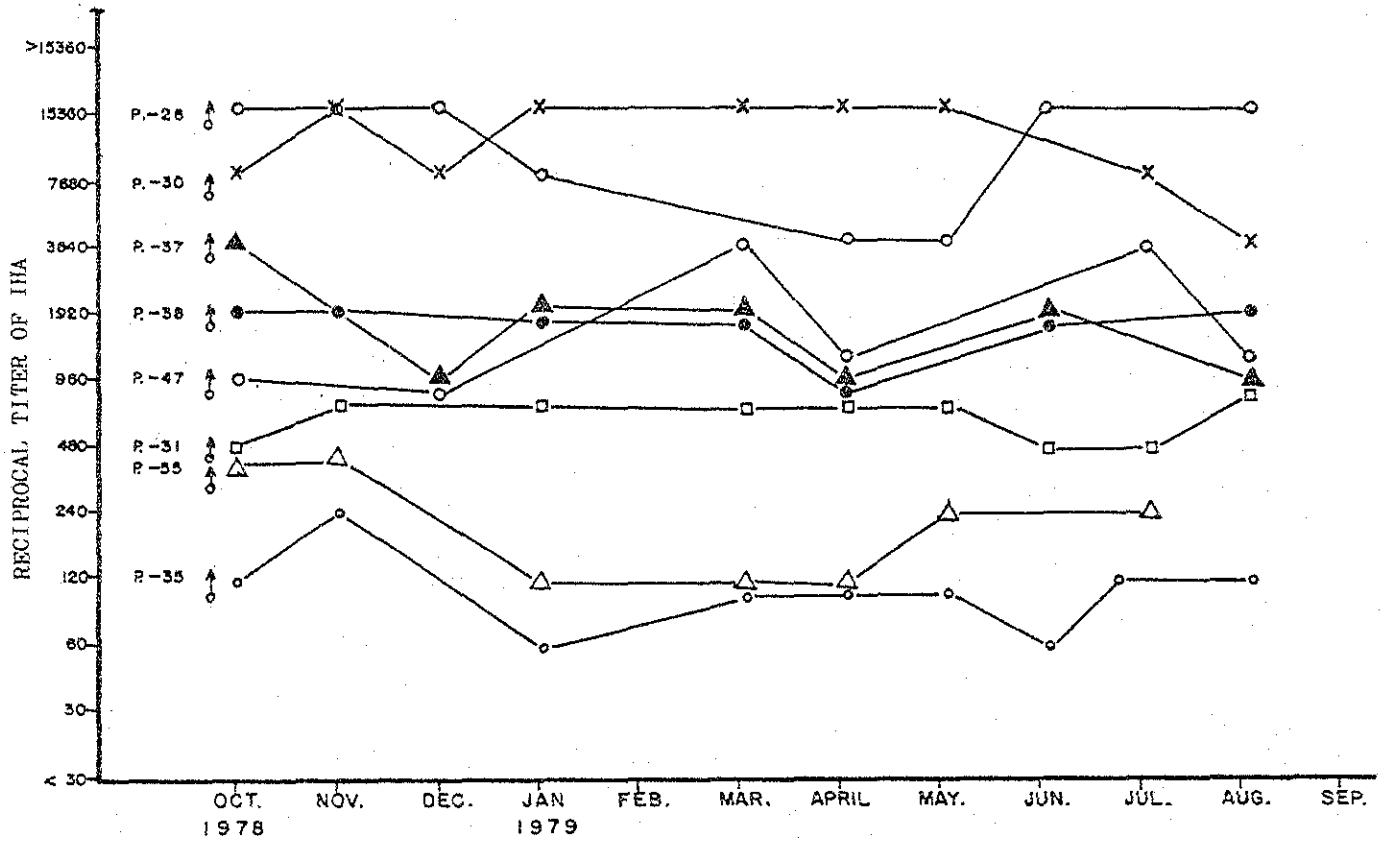
FIG. IV-4 COMPARISON OF AGE SPECIFIC PREVALENCE AMONG IHA, MICROFILARIA AND NODULE POSITIVE

Relatively constant titers were observed for each patient throughout the year (FIG. IV-5). Some patients with initial high titers maintained high titers, while others with low titers, continued low. Therefore, IHA test tends to be a stable method.

9. IHA positive rates and microfilaria positive rates.

IHA test was compared with skin-biopsy to determine which method was most efficient in detecting onchocerciasis and if results obtained by these two methods agreed with each other.
More than 560 inhabitants from 5 endemic areas were examined by IHA tests and skin-biopsy. One hundred forty-seven residents from two onchocerciasis-free areas were also included as comparison. Formaldehyde-fixed sheep red cells were used in this test. A titer 1:60 or higher was set as standard for IHA positiveness. IHA positive rates were closely correlated with mf positive rates confirming reliability of IHA test for diagnosing onchocerciasis. More-over, the IHA positive rates were always higher than the mf positive rates, indicating that IHA test is more sensitive than skin-biopsy. No disease was detected in the two control areas by these methods (TABLE IV-5).

FIG. IV-5 VARIATION OF IHA TITER OF 8 ONCHOCERCIASIS PATIENTS FROM NINE CONSECUTIVE EXAMINATIONS THROUGHOUT A YEAR (FCA. SIBAJA. GUATEMALA)



(Takaoka et al.; Personal Comunication, 1979)

TABLE VI-5 Results of 560 inhabitants from 5 endemic areas in Guatemala to the examination with skin biopsy for microfilaria and IHA test.

Location	IHA(+)mf(+)	IHA(+)mf(-)	IHA(-)mf(+)	IHA(-)mf(-)	Total
La Cruz	11 (8.0)	11 (8.0)	0 (0.0)	116 (84.1)	138
M. Monte	24 (22.2)	14 (13.0)	6 (5.6)	64 (59.3)	108
Hamburgo	27 (42.4)	15 (23.4)	0 (0.0)	22 (34.4)	64
Ganchinilín	112 (50.5)	30 (13.5)	12 (5.4)	68 (30.6)	222
Sibajá	25 (78.1)	6 (18.8)	0 (0.0)	1 (3.1)	32
Total	199 (35.3)	76 (13.5)	18 (3.2)	271 (48.1)	564

V. DOUBLE DIFFUSION TEST

Gel-double diffusion test (DD test) is a relatively simple method and is often used for diagnosing parasitic diseases. The object of this study was to determine if DD test could be used for estimating the effect of control measures for onchocerciasis.

1. Materials and methods.

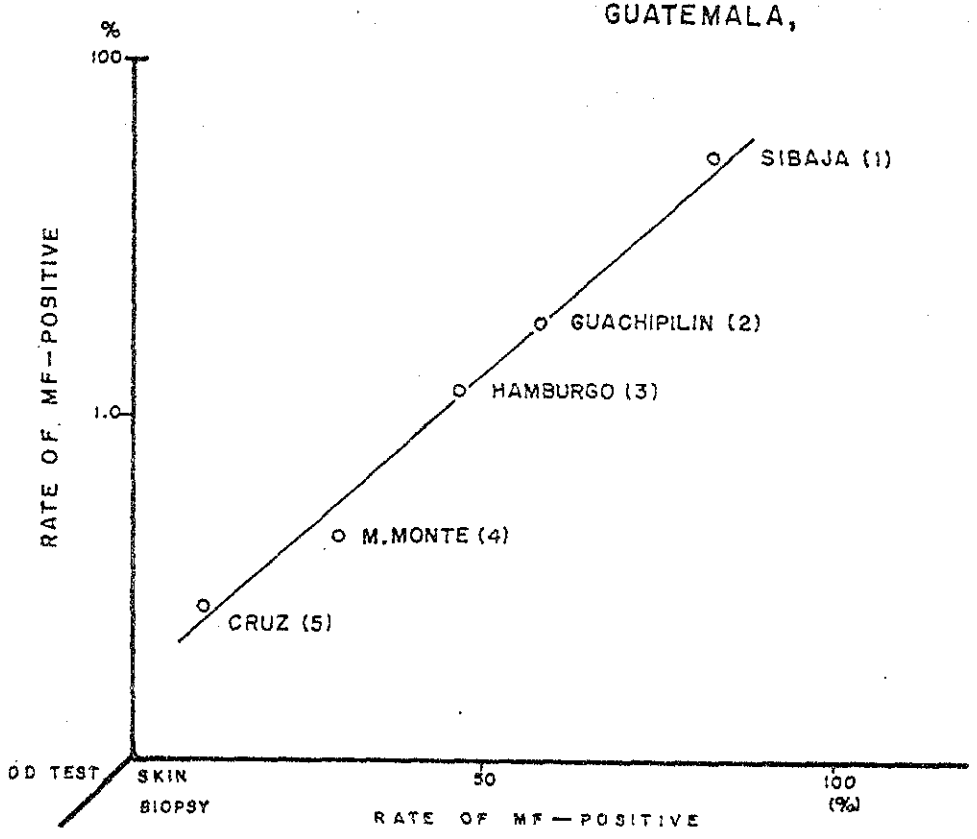
Antigen: O. volvulus adults were removed from onchocercomas by colagenase and macerated by glass homogenizers. The homogenate was treated with ether to remove fats and extracted in 0.15 M Veronal Buffer Saline, pH 7.8. After the extract was centrifuged at 10,000 rpm for 30 minutes, the supernant was dialyzed and concentrated by lyophilization. Antigen was standarized at protein content 3,000 - 5,000 µg/ml.

Agar: Agar (Difco's Agar Noble) was dissolved in Veronal Buffer at the rate of 1.0%. Four ml of agar solution were poured to cover a slide glass.

Serum for assay: The blood was collected from ear lobes in microtubes(Thermo VC-C110 P, volume 150 µl) and the serum was prepared by centrifugation.

D D Test: Ouchterlony method was used. After the agar solidified,

FIG. V-1. CORRELATION BETWEEN MF-POSITIVE RATE OF SKIN BIOPSY AND O D TEST FOR DETECTING THE SPECIFIC ANTIBODIES OF O. VOLVULUS AT FIVE ENDEMIC AREAS IN GUATEMALA,



(Takaoka et al.; Personal Comunication, 1979)

7 wells with 3 mm diameter were punched in agar by agar cutter, 7 mm apart from one on other. Antigen was pipetted in central well, and sera were placed in six surrounding wells.

2. Reliability of D D Test.

D D test and skin-biopsy were compared to determine reliability of D D Test.

Two hundred and sixty-nine habitants from five onchocerciasis-endemic areas in Guatemala were examined by D D test and skin-biopsy. One hundred and fifty-eight residents in El Paro and, Carrizal, onchocercosis free-areas, were also checked as controls.

D D test and mf positive rates for five endemic areas were dotted in FIG. V-1. D D test positive rates were very closely correlated with mf positive rates (r=0.99), indicating reliability of this test for diagnosing onchocerciasis.

In summary, of the five endemic areas, 83 (30.9%) and 58 (21.6%) residents were D D test-and mf-positive, respectively, suggesting that D D test is more sensitive than skin-biopsy for mf detection. Fifty patients (18.5%) were negative for both D D test and mf, 228 peoples(84.8%) in total showed the same reaction to D D test and skin-biopsy, indicating high reliability of D D test. Of 58 mf positive patients only 8 patients were not recognized by D D test (TABLE V-1). Among 158 people from the onchocercosis-fre area, only 3 individuals (1.9%) showed a weak positive reaction to D D test.

TABLE V-1 Reactions of 269 inhabitants from onchocerciasis endemic area to skin biopsy and D. D. test.

Skin biopsy / D. D. test	Positive	Negative	Subtotal
Positive	50 (18.5%)	333 (12.3%)	83 (30.9%)
Negative	8 (3.0%)	178 (66.2%)	186 (69.1%)
Subtotal	58 (21.6%)	211 (78.4%)	269

(Takaoka et al.; Personal Communication, 1979)

3. Specific precipitation bands.

Studies were undertaken to determine what specific and common precipitation bands were formed by interacting antigens and antibodies of three different parasites; Onchocerca volvulus, dog filaria (Dirofilaria immitis), and roundworm (Ascaris lumbricoides). Antigens were prepared from adults of these parasites. Antibodies were from rabbit sera immunized with one of the three parasites and from onchocercosis patients. Specific and common precipitation bands were determined by immuno electrophoresis and Ouchterlony method (D D test).

A band specific for O. volvulus was observed in immuno electrophoresis between O. volvulus antigen and sensitized rabbit serum, as well as between O. volvulus antigen and serum from onchocercal patients. This specific band is numbered No. 2 (FIG. V-2).

Several bands common for different antigen-antibody combinations were also observed in immuno-electrophoresis and Ouchterlony methods (FIGS. V-2 & V-3). Common bands that could interfere with diagnosing onchocercosis arise from interaction between O. volvulus antigen and roundworm antibody. To clarify this point, D D test was conducted on habitants in El Faro, and Carrizal, where onchocercosis is unknown, although intestinal parasitic helminths, such as roundworm, whipworm, and hookworm are quite common. Only 2% of the residents examined were positive to D D test, indicating that weak precipitation band between O. volvulus and roundworm antibody interfere slightly with diagnosing onchocercosis with D D test.

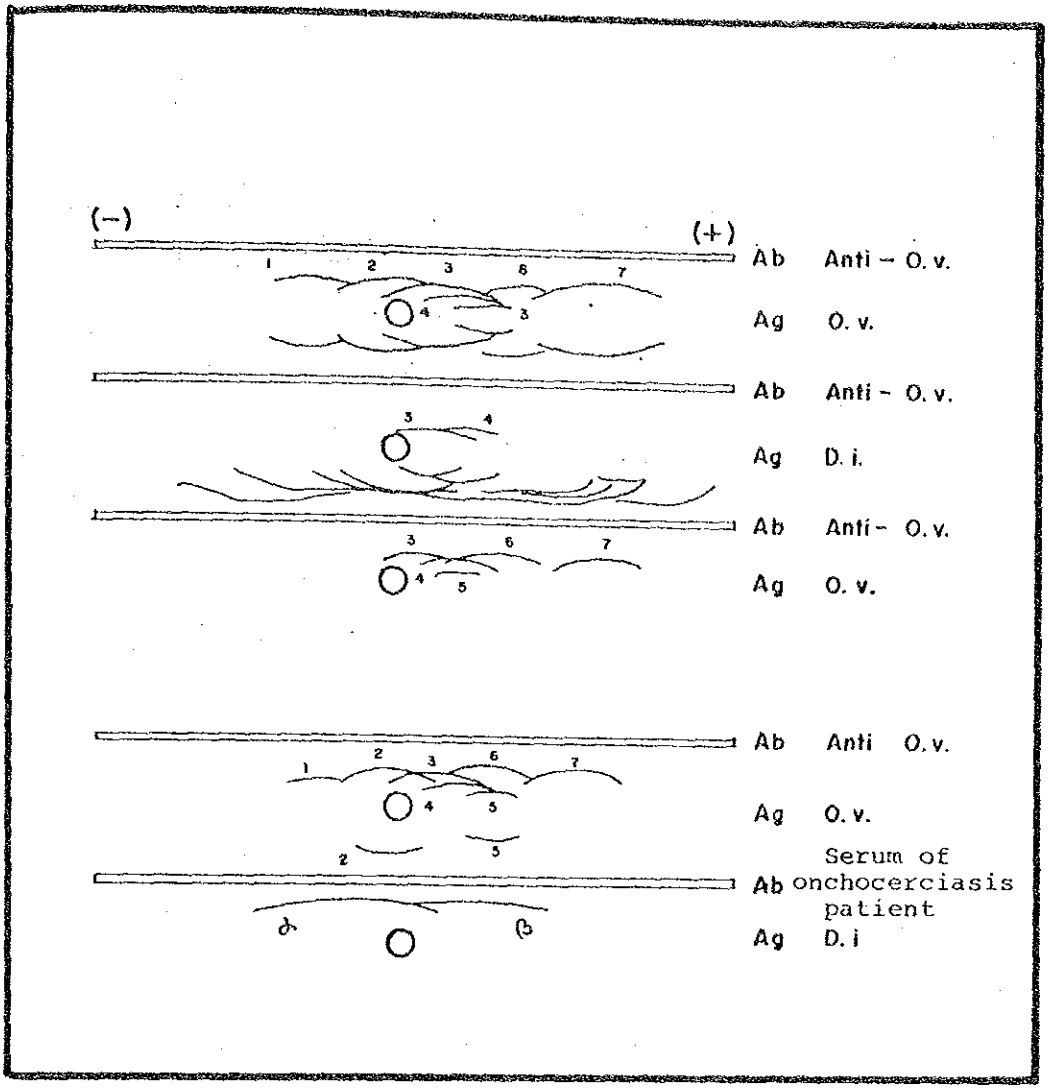


FIG. V-2. Immuno-electrophoretic pattern formed by interaction between antigens and antibody of Onchocerca volvulus or Dirofilaria immitis

(Takaoka et al.; Personal Communication, 1979)

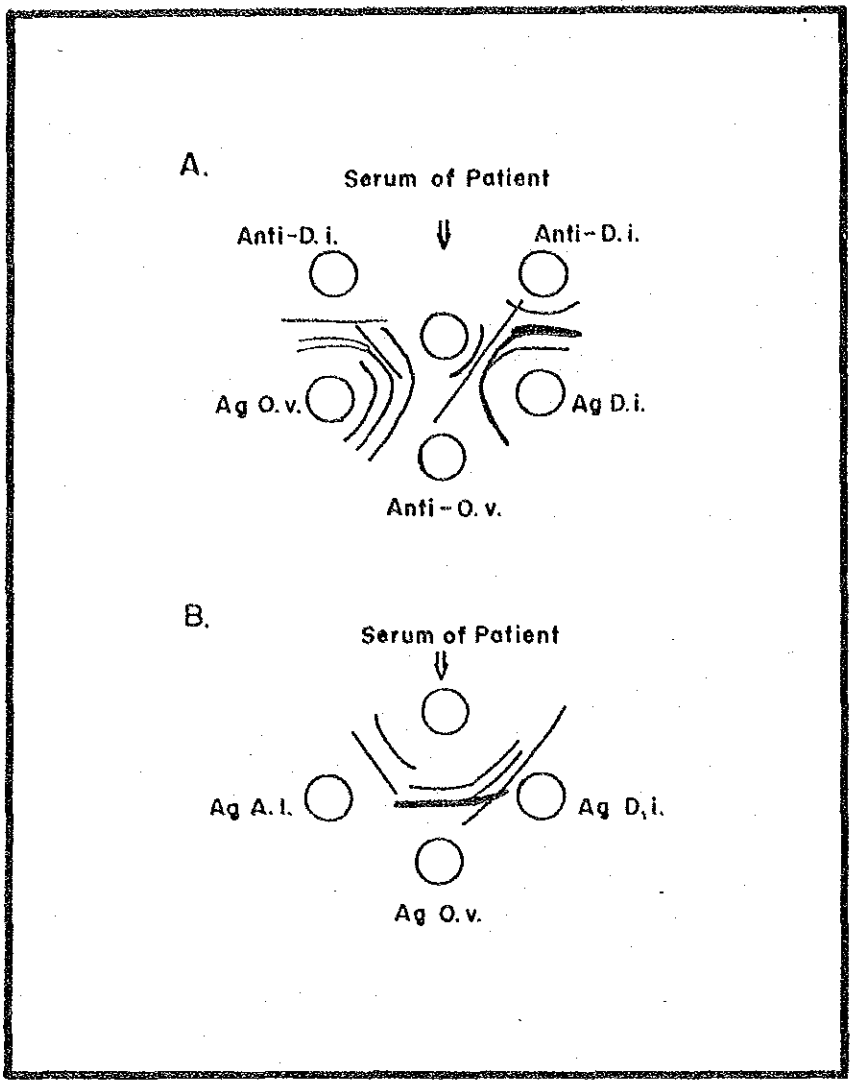


FIG. V-3. Precipitation bands produced by antigens and antibodies of three different parasites from Ouchterlony test

Anti-D.i.; Immunized rabbit serum of D. immitis
Anti-O.v.; Immunized rabbit serum of O. volvulus
Ag A. l. ; Antigen of A. lumbricoides
Ag D. i. ; Antigen of D. immitis
Ag O. v. ; Antigen of O. volvulus

(Takaoka et al.; Personal Communication, 1979)

VI. SKIN TEST

Since many patients can be assayed during a short period of time, skin test is often used for diagnosing parasitic diseases. This method consists of relatively easy procedures, which include preparing antigen from parasites and injecting it into patient's skin. In some species of filariae, it is often difficult to collect true parasites for antigen, and therefore filariae from other mammals must be used as antigens to determine skin reaction based on cross-reactions. In onchocercosis, however, O. volvulus adults are located in nodules near skin, thus can be easily obtained and used for preparing antigen.

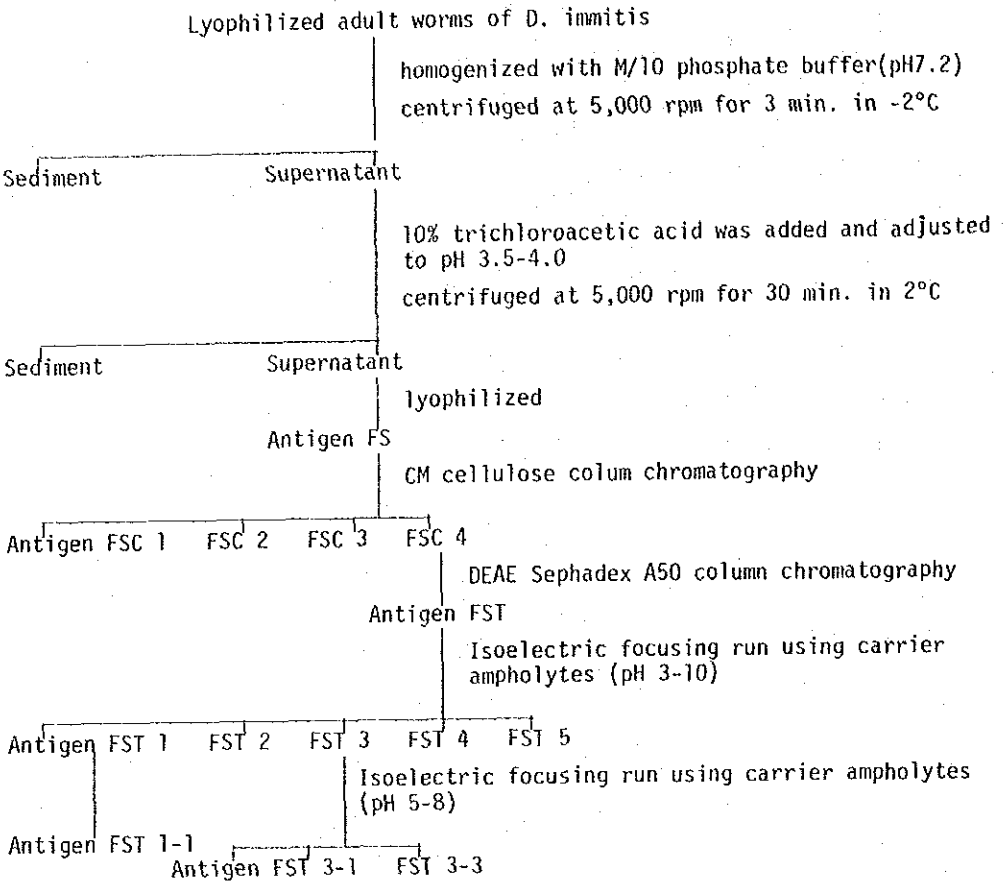
In the present project, three species of antigen were prepared from O. volvulus and dog filaria for comparison.

1. Specificity of purified antigen from dog filaria.

Preparation of antigens: Thirteen fractions of antigen were prepared from dog filaria, Dirofilaria immitis adults by column chromatography and isoelectric focusing run (TABLE VI-1).

Method of Skin Test: Ten microgramas of dry antigen powder were dissolved in 4 ml of saline solution (protein concentration; 2.5 μg/ml), then 0.02 ml were injected into patient's skin. Long

TABLE VI-1 Fractionation of FST skin test antigen from D. immitis



(Sato et al.; Personal Communication, 1976)

and narrow diameters of wheal, taken at right angles to each other, were measured in millimeters 15 minutes after the injection, then mean, equal to or over 7 mm was considered positive.

Antigenic activity: Seventy-nine mf-positive patients from Fincas Valle de Oro and, Armonías were assayed with 13 fractions of dog filaria antigen. Fractions FS, FSC-4, FST, and FST-3 showed high antigenic activities, resulting in 100, 95.2, 100 and, 88.9% (or 84.2%) positive rates, respectively (TABLE VI-2).

Protein concentrations: The effect of protein concentrations of antigen on skin reaction was studied by injecting 21 mf-positive patients with 2.5 and 5.0 µg/ml of protein fractions of FST and FST-3. The fraction FST was purified by CM cellulose and DEAE Sephadex column chromatography, later the fraction FST-3 was further purified by isoelectric focusing run. There was no significant difference in antigenic activity between two protein concentrations of the further purified fraction FST-3. When the less purified fraction FST was used, antigenic activity increased with higher protein concentration. (TABLE VI-3).

Non-specific reaction: Fifty non-onchocercosis SNEM employees, Guatemala City, were skin-injected with the fractions FST (protein 5 µg/ml) and FST-3 (protein 2.5 µg/ml) to determine non-specific reaction. Only six and two individuals reacted positively to FST and FST-3, respectively, indicating a higher specificity of

TABLE VI-2

Results of skin test with 13 antigens purified from Dirofilaria immitis for mf-positive inhabitants by skin biopsy in endemic areas of onchocerciasis, Guatemala.

Antigens	No. of exam.	No. of positive	percent of positive
FS	21	21	100.0
FSC-1	21	13	61.9
FSC-3	21	10	47.6
FSC-4	21	20	95.2
FST	21	21	100.0
FST-1	18	11	66.1
FST-2	18	12	66.7
FST-3	18	16	88.9
FST-4	18	14	77.8
FST-5	18	0	0.0
FST-1	19	13	68.4
FST-1-1	19	14	73.7
FST-3	19	16	84.2
FST-3-1	19	15	79.0
FST-3-3	19	15	79.0

(Sato et al.; Personal Communication, 1976)

TABLE VI-3

Results of skin tests with different protein concentration of antigens FST and FST-3 for mf-positive inhabitants by skin biopsy in endemic area of onchocerciasis, Guatemala.

Antigen	Protein concentration/ml	No. examined	No. positive	% positive
FST	2.5µg	21	14	66.7
FST	5.0µg	21	19	90.5
FST-3	2.5µg	21	20	95.2
FST-3	5.0µg	21	19	90.5
Saline control		21	0	0.0

(Sato et al.; Personal Communication, 1976)

the purified fraction FST-3 (TABLE VI-4).

Reliability of antigen FST-3: Skin reaction with antigen FST-3 was compared with skin biopsy for mf to determine reliability of skin test with this particular antigen for diagnosing onchocercosis. Nine-hundred and twenty-three residents in San Vicente Pacaya were examined by the two methods. Of 237 people who were mf positive by skin-biopsy, a high proportion, 70 patients (29.5%) were diagnosed as negative by skin test (TABLE VI-5), indicating that antigen FST-3 is less not so high specific for O. volvulus and therefore unreliable for skin test.

Positive rates with skin test using antigen FST-3: Residents in onchocercosis-endemic areas near San Vicente Pacaya were examined by skin biopsy for mf and skin reaction using antigen FST-3 and resulted as shown in TABLE VI-5.

2. Antigen from onchocerca microfilaria.

Since numerous mf area produced from O. volvulus adults and migrate through patient's skin, mf protein is considered the most important antigenic substance in forming antibodies. The protein was prepared from mf constituents of nodules and assayed for antigenic activity in skin reaction.

TABLE VI-4

Results of skin tests using antigens FST and FST-3 for 50 healthy Guatemalan persons (SNEM employees) as the control.

Antigens	No. examined	No. positive (%)
FST (5.0µg/ml)	50	6 (12.0)
FST-3(2.5µg/ml)	50	2 (4.0)

(Sato et al.; Personal Communication, 1976)

TABLE VI-5

Reaction of 923 inhabitants in San Vicente Pacaya to skin biopsy for microfilaria and skin test using antigen FST-3

Skin biopsy / Skin test	Positive (%)	Negative (%)	Total (%)
Positive	167 * (18.1)	194 (21.0)	361 (39.1)
Negative	70 (7.6)	492 (53.3)	562 (60.9)
Total	237 (25.7)	686 (74.3)	923

* No. of inhabitants

(Sato et al.; Personal Communication, 1976)

Materials and methods: Onchocercomae were removed from patients, sliced into several pieces, and soaked in saline solution at 4 °C for 24 - 72 hrs. After filtering the saline solution through gauze, it was centrifuged.

The precipitate was washed several times in saline solution centrifuged then macerated by tissue grinder, later extracted in saline solution for 72 hrs. The extractant was repeatedly frozen and thawed, then centrifuged for 30 min at 8,000 r.p.m. The supernatant was adjusted at protein content 10 or 20 µg/ml by Lowry's method and was used as antigen for skin reaction. Skin test was administered subcutaneously injecting 0.02 ml of the antigen in the inner forearm. Fifteen or twenty minutes after injection, the skin surrounding reaction site was traced onto paper with ball-point pen and measured with planimeter.

Protein content for antigen: Two different protein concentrations, 10 and 20 µg/ml were compared in skin reaction using 23 mf-positive patients. There was no significant difference between the two concentrations (FIG. VI-1).

Skin reaction with time: Changes with time in wheal sizes were studied by injecting 11 adult patients, 10 apparently healthy women, and 24 apparently healthy children from endemic area, and 24 healthy children from onchocercosis-free area, Guatemala City. The largest wheal reached maximum size 15 and 20 minutes after injection in children and adults, respectively, then gradually

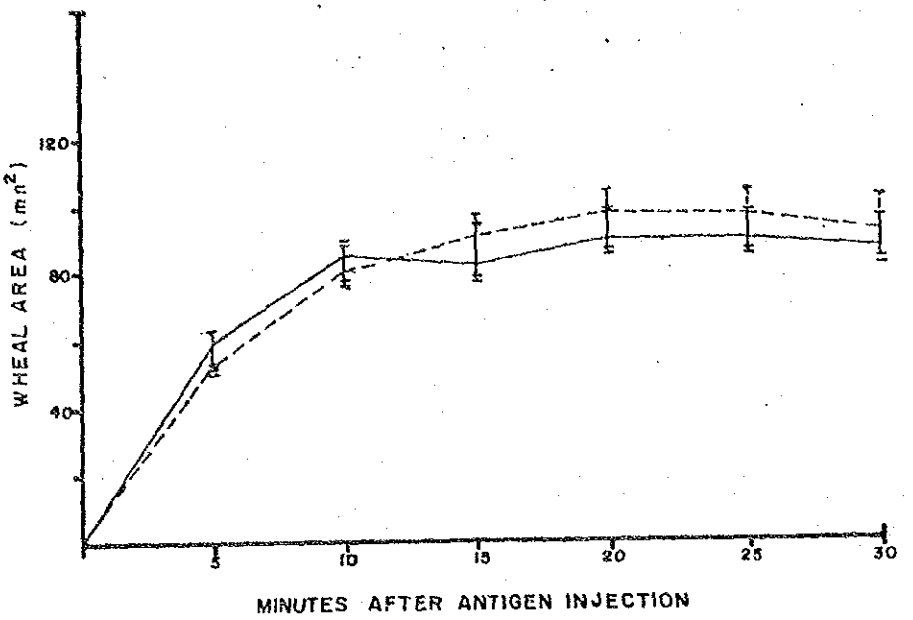


FIG. VI-1. Changes with time in the mean wheal size of skin test injected with two different protein concentrations of microfilaria antigen.

Dotted line: 10µg/ml of protein
Solid line : 20µg/ml of protein

(Yoshihisa Hashiguchi, Masato Kawabata, Guillermo Zea F., Manuel M. Recinos C. and Otto Flores C.; Transactions of the Royal Society of Tropical Medicine and Hygiene, 73(5), 1979)

diminished. (FIG. VI-2).

Skin reaction in relation to intestinal parasitic helminths: One-hundred and forty-seven habitants from endemic area and 75 from non-endemic area were checked for intestinal helminths such as roundworm, whipworm, hookworm, and tapeworm by fecal examinations, in addition to skin test. There was no correlation between intestinal parasitic helminths and skin test using mf antigen, indicating that antigen does not react with antibodies originated from other parasitic helminths.

Distribution of wheal sizes: Frequency of different wheal sizes was studied by skin-testing 484 residents of endemic area and 106 of non-endemic area. Of 484 examined from the endemic area, 136 (28.1%) were mf or nodule positive.

The habitants of non-endemic area had wheals frequently extending 20 mm^2. Those from endemic area had much larger wheals, whether they were mf or nodule positive or not. A wheal size over 56 mm^2 was considered positive, when mf antigen 20 μg/ml was used for skin test (FIG. VI-3).

Men had wheals significantly larger than women.

Skin test, skin biopsy, and palpation were conducted in five areas which had different degrees of onchocercosis infestation. Wheal sizes increased as mf or nodule positive rates increased (FIG. VI-4).

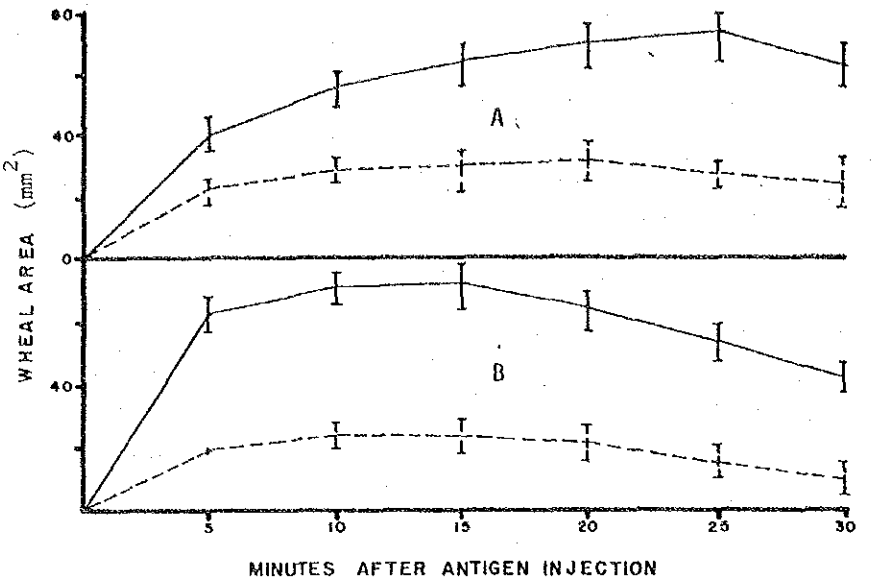


FIG. VI-2. Changes with time in the mean wheal size of skin test using microfilaria antigen.

A: Reactions of adults from endemic areas (solid line) and those from non-endemic areas (dotted line)

B: Reactions of schoolchildren from endemic areas(solid line) and those from nonendemic areas(dotted line)

(Hashiguchi et al.; Trans. Roy. Soc. Trop. Med. Hyg., 73(5), 1979)

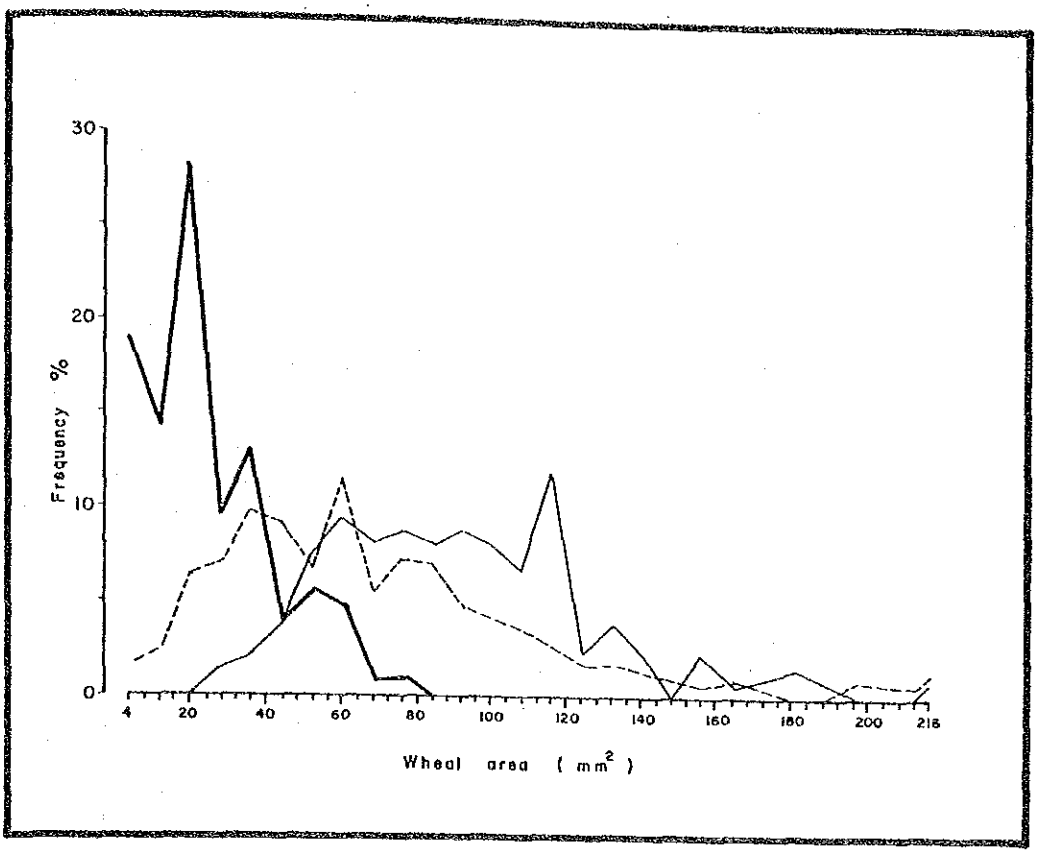


FIG. VI-3 Frequency distribution of wheal areas exicited by skin tests.

_____: Reaction of the control group in non-endemic areas

_____: Reactions of the persons with mf and/or nodules in an endemic area

-----: Reactions of the persons without mf and/or nodules in an endemic area

(Yoshihisa Hashiguchi, Masato Kawabata, Guillermo Zea F., Manuel M. Recinos C., and Otto Flores C.; Transaction of the Royal Society of Tropical Medicine and Hygiene, 73(5), 1979)

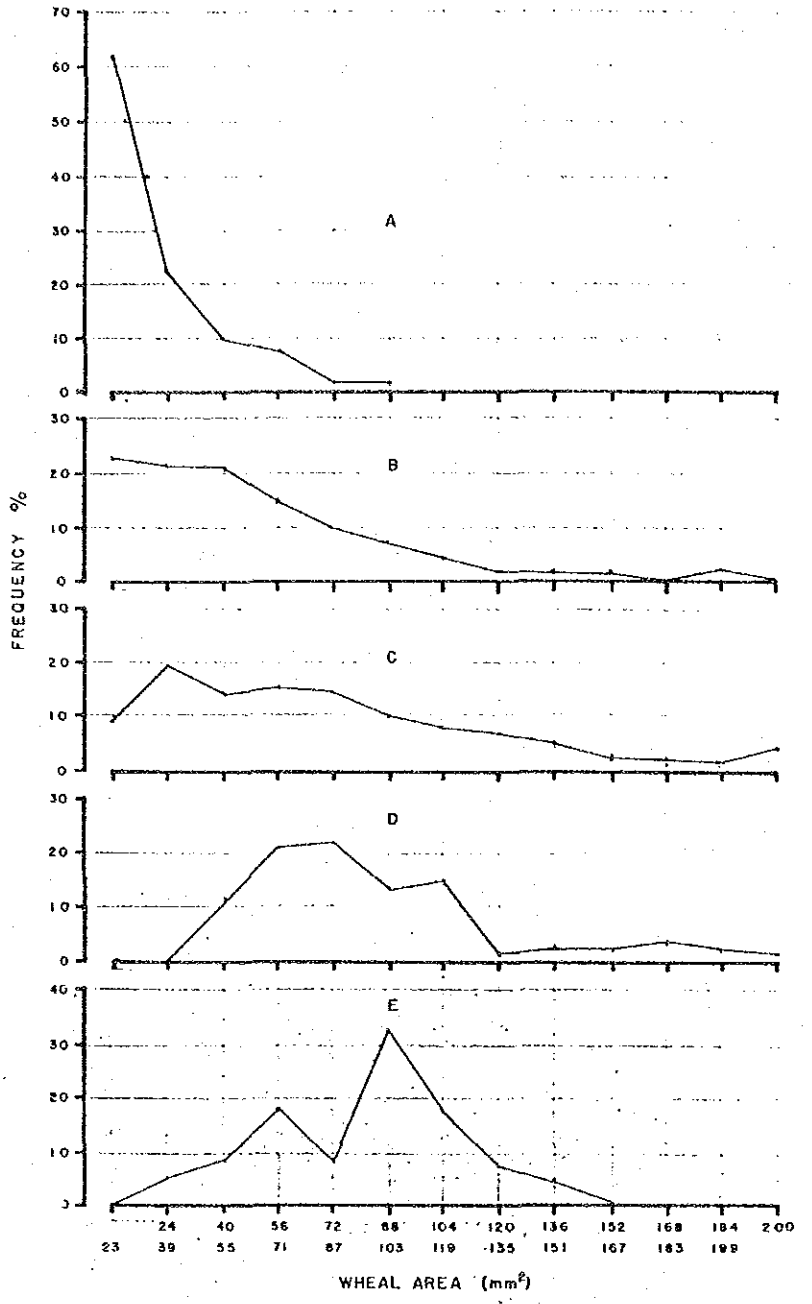


FIG. VI-4. Frequency distribution of wheal areas by skin tests arranged by the grade of infection. A: Non-endemic areas, B: Aldea Calderas, C: Aldea L Cruz, D: Fincas San Rafael Sumatan, Victoria and Sibaja, E: Pueblo Palin. (Hashiguchi et al.; Trans. Roy. Soc. Trop. Med. Hyg., 73(5), 1979)

3. Antigen from O. volvulus adults.

As stated before specificity of skin test antigen from dog filaria (D. immitis) was low for onchocercal patients.
Antigen from onchocerca mf was specific for O. volvulus, but it was difficult to obtain sufficient amount of protein originating from mf due to contamination. Proteins from nodule tissue contaminated mf suspension in preparation procedures and thus changed the proportion of protein originating from mf in the total protein content of antigen.
To eliminate these drawbacks, antigen from O. volvulus adults was studied for skin test.

Material and methods: Onchocercomae were removed from patient's subcutaneous tissues and digested in 0.01% collagenase (collagenase type I, Sigma Chem. Co., U.S.A.), pH 7.2 overnight. O. volvulus adults were separated from nodule tissues using stereo-microscope, washed several times in saline solution, weighed after lyophilization, macerated in 100-fold volume of ethylether by tissue homogenizer, and centrifuged for 20 minutes at 3,000 r.p.m. The precipitate was suspended in 100-fold volume of Véronal-Buffered saline, pH 7.8. The suspension was agitated at 4°C for 24 hrs by magnetic stirrer to extract soluble proteins, and centrifuged at 10,000 r.p.m for 30 minutes. The supernatant was dialyzed against physiological saline solution and bacteria removed through disposable millipore filter, pore size 0.4 μm (Acrodisc, Gelman,

U.S.A.). The protein content of the sterilzed solution was determined by Lowry-Folin's method an adjusted at 20 µg/ml with sterile saline solution. This protein solution was used as skin test standard antigen from O. volvulus adults.

Skin test was conducted by injecting antigen interdermally to inner side of forearm. Fifteen minutes after injection, wheal diameter 9 mm and greater was determined as positive.

Protein content for skin test: Antigen of different dilutions was assayed to determine optimum protein content for skin test. One-hundred and seventy-seven habitants in Fincas Sibajé and Buena Vista were injected interdermally to inner side of forearm with 1.0, 2.5, 5.0, or 10.0 µg/ml of proteins.

Standard antigen (20 ug/ml) was applied to the other forearms as checks.

Diluted antigens resulted in significantly smaller wheals than standard antigen. Antigens 1.0 and 2.5 µg/ml gave wheals smaller than 9 mm, antigens 5.0 and 10.0 µg/ml produced wheals larger than 9 mm, whereas standard antigen resulted in wheals of approximately 12 mm in mean diameter (TABLE VI-6).

One-hundred thirty-six patients were mf-positive by skin biopsy. Of these mf positive patients, 133 (97.8%) were positive to skin reaction using standard antigen. When antigen was diluted, the percentage of positive patients gradually decreased. 90.0% of the patients were positive to antigen 10 µg/ml, whereas only 38.3% were positive to antigen 1 µg/ml. A coincidence rate was

TABLE VI-6.

Wheal sizes of skin test using standard (protein 20 μg/ml) and diluted antigens from Onchocerca volvulus adults.

(Fincas Sibajá and Buena Vista, Guatemala, 1980.)

Protein content of diluted antigen (μg/ml)	Number of patients assayed	Wheal diameter (mm) ± S.D.*	
		Diluted antigen	Standard antigen
1.0	87	8.3 ± 2.4	12.3 ± 2.9
2.5	32	8.8 ± 1.7	11.9 ± 2.1
5.0	30	9.7 ± 2.5	12.2 ± 3.3
10.0	28	9.9 ± 2.1	12.6 ± 2.9
Total	177		

S.D.; Standard deviation.

(Yoichi ITO, Makoto SAKAMOTO; Quarterly Report, No. 15, 1980).

calculated by taking a percentage of patients who had same (positive or negative) reactions to standard and diluted antigens, over the total number which was assayed with a particular dilution. The coincidence rate decreased gradually, as antigen was diluted (TABLE VI-7).

Therefore, the protein content 20 µg/ml was considered appropriate for conducting skin test with antigen from O. volvulus adults.

False negative rate: The reliability of skin test was studied by injecting antigen (protein 20 µg/ml) from O. volvulus adults to mf-positive patients from San Vicente Pacaya. Two antigens purified from dog filaria (D. immitis), FST-3 and FSC-1 were also included for comparison.

Antigen from O. volvulus adults resulted in a much higher positive rate (98.8%), or a lower false-negative rate, than antigens from dog filaria (TABLE VI-8). This high rate is in accordance with the results obtained in Fincas Sibajá and Buena Vista (97.8%), which were reported in the previous section on protein content for skin test.

False positive rate: A false positive rate was determined by skin-testing 291 residents in onchocerciasis-free Finca La Mocá with antigen from O. volvulus adults (protein 20 µg/ml).

The residents were also examined for onchocerca by skin biopsy, D D test, and palpation.

In Finca La Mocá, black fly (Simulium ochraceum), vector of

TABLE VI-7.

Reactions of microfilaria-positive patients to skin test using standard (protein 20 μg/ml) and diluted antigens from Onchocerca volvulus adults.

(Fincas Sibajá and Buena Vista, Guatemala, 1980.)

Protein content of diluted antigen (μg/ml)	Number of patients assayed	Reaction: Diluted antigen	Standard antigen: Positive(+)	Standard antigen: Negative (-)	Number of patients with same reactions: Posi.+ Neg.	Coincidence rate
1.0	60	Positive(+)	23*(38.3%)	0	23+2=25	25/60=41.7%
		Negative(-)	35 (58.3%)	2(3.4%)		
2.5	29	Positive(+)	15 (51.7%)	0	15+1=16	16/29=55.1%
		Negative(-)	13 (44.8%)	1(3.5%)		
5.0	25	Positive(+)	19 (76.0%)	0	19+0=19	19/25=76.0%
		Negative(-)	6 (24.0%)	0		
10.0	22	Positive(+)	20 (90.9%)	0	20+0=20	20/22=90.9%
		Negative(-)	2 (9.1%)	0		
Total	136		133 (97.8%)	3(2.2%)		

*) Number of patients. Positive reaction = wheal over 9 mm in diameter.

(Ito & Sakamoto; Quarterly Report No. 15, 1980).

TABLE VI-8.

Reactions of microfilaria-positive onchocerciasis patients to skin test using three species of antigen prepared from onchocerca (*Onchocerca volvulus*) and dog filaria (*Dirofilaria immitis*) adults, (San Vicente Pacaya, Guatemala).

Antigen	Protein content (μg/ml)	Number of patients examined	Number of positive patients	Positive rate (%)
O. volvulus	20.0	246	243 a)	98.8
D. immitis Fraction FST-3	2.5	477	342 b)	71.7
D. immitis Fraction FSC-1	2.5	55	44 b)	80.0

a) Positive reaction = wheal diameter 9 mm or over 15 min after injection

b) Positive reaction = wheal diameter 7 mm or over 15 min after injection

(Ito, Sakamoto & Yoshimura; Quarterly Report, No. 13, 1979).

onchocerciasis, is often found, although few onchocerciasis patients are seen.

None of the 291 habitants examined were positive by biopsy, D D test, and palpation. However, approximately 40% of them produced positive skin tests, a false-positive rate with skin test was 40% (TABLE VI-9).

This false positive rate is much higher than that Ito, et al (1969) reported surveying in Japan with antigen from Japanese blood flukes (Schistosoma japonicum), lung flukes (Paragonimus westermani) and dog filaria (Dirofilaria immitis). The high rate can be explained by 1) cross reaction with intestinal helminths and 2) contamination of antigen with proteins originating from or similar to S. ochraceum. The residents examined are frequently bitten by black flies and probably have acquired reaginic antibodies to S. ochraceum. The contaminant mentioned above may react with the reafinic antibody, giving an apparently positive skin reaction. Further studies are needed to account for a high false-positive rate of skin test using antigen from O. volvulus adults.

Stability: Stability of skin test with antigen from O. volvulus adults was studied by injecting monthly for 3 months, 70 mf-positive patients from Finca Buena Vista. Antigen from dog filaria (D. immitis) fraction FST-3 was interdermally applied to other forearms of the same patients for comparison.

Antigen from O. volvulus resulted in much more consistent skin

TABLE VI-9.

Results of examinations to 291 residents in onchocerciasis-free Finca La Mocá with skin biopsy, D D test, palpation, and skin test using antigen fron Onchocerca volvulus adults.

Assay	Number of positive patients	Porcentage of positive patients
Skin biopsy	0	0
D D Test	0	0
Palpation	0	0
Skin test	116 a)	39.9

a) Positive reaction = wheal over 9 mm in diameter 15 minutes after injection.

(Ito, Sakamoto & Kondo; Quarterly Report, No. 16, 1980).

reaction than from D. immitis adults. 95% of the patients assayed were positive in 3 consecutive test using onchocerca antigen, whereas only a 44% of them were positive in 3 consecutive tests with dog filaria antigen (TABLE VI-10).

Fractionation of antigen from O. volvulus adults:

To lower a false-positive rate, antigen from O. volvulus adults was fractionated by DEAE-Sepharose CL-6 B ion-exchange chromatography and was assayed in skin test using the following procedures;

1) Onchocercomae were removed from patient's subcutaneous tissues and treated with collagenase. O. volvulus adults were washed several times with saline solution, lyophilized, and weighed.

2) The adults were suspended in a 100-fold volume of ethylether, macerated by tissue homogenizer, and centrifuged at 3,000 r.p.m. from 15 minutes.

3) The precipitate was extracted in a 100-fold volume of Veronal buffer saline solution, pH 7.8, overnight.

4) The extracted was centrifuged at 10,000 r.p.m. for 30 minutes. The supernatant was dialyzed against 0.015 M phosphate buffer solution.

5) A protein content of the dialyzate was determined by Lowry

TABLE VI-10.

Reactions of 70 microfilaria positive onchocerciasis patients to 3 consecutive skin tests with monthly intervals, using antigen from Onchocerca volvulus and Dirofilaria immitis adults.

(Finca Buena Vista, Guatemala, 1980)

Antigen	Protein content (ug/ml)	Number of patients assayed	Number of patients with positive reaction in 0	1	2	3 tests
O. volvulus	20.0	70	0	0	3 (4.3%)	67 (95.7%)
D. immitis Fraction FSP-3	2.5	70	8 (11.4%)	16 (22.9%)	15 (21.4%)	31 (44.3%)

(Ito, Sakamoto & Yoshimura; Quarterly Report, No. 14, 1980).

Follin's method. A column, 1.5 x 28 cm, was packed with ion-exchange resin, DEAE-Sepharase CL-6B, which was previously equilibrated in 0.015 M phosphate buffer, and charged with the dialyzate containing 10 mg of protein. The column was eluted stepwise with increasing concentrations of sodium chloride solution.

6) Each fraction was dialyzed against saline solution, adjusted at protein content 20 µg/ml with saline solution, and sterilized with Acrodisc filter, pore size 0.45 µm, prior to skin test. Five fractions were obtained by ion-exchange chromatography (FIG. VI-5).

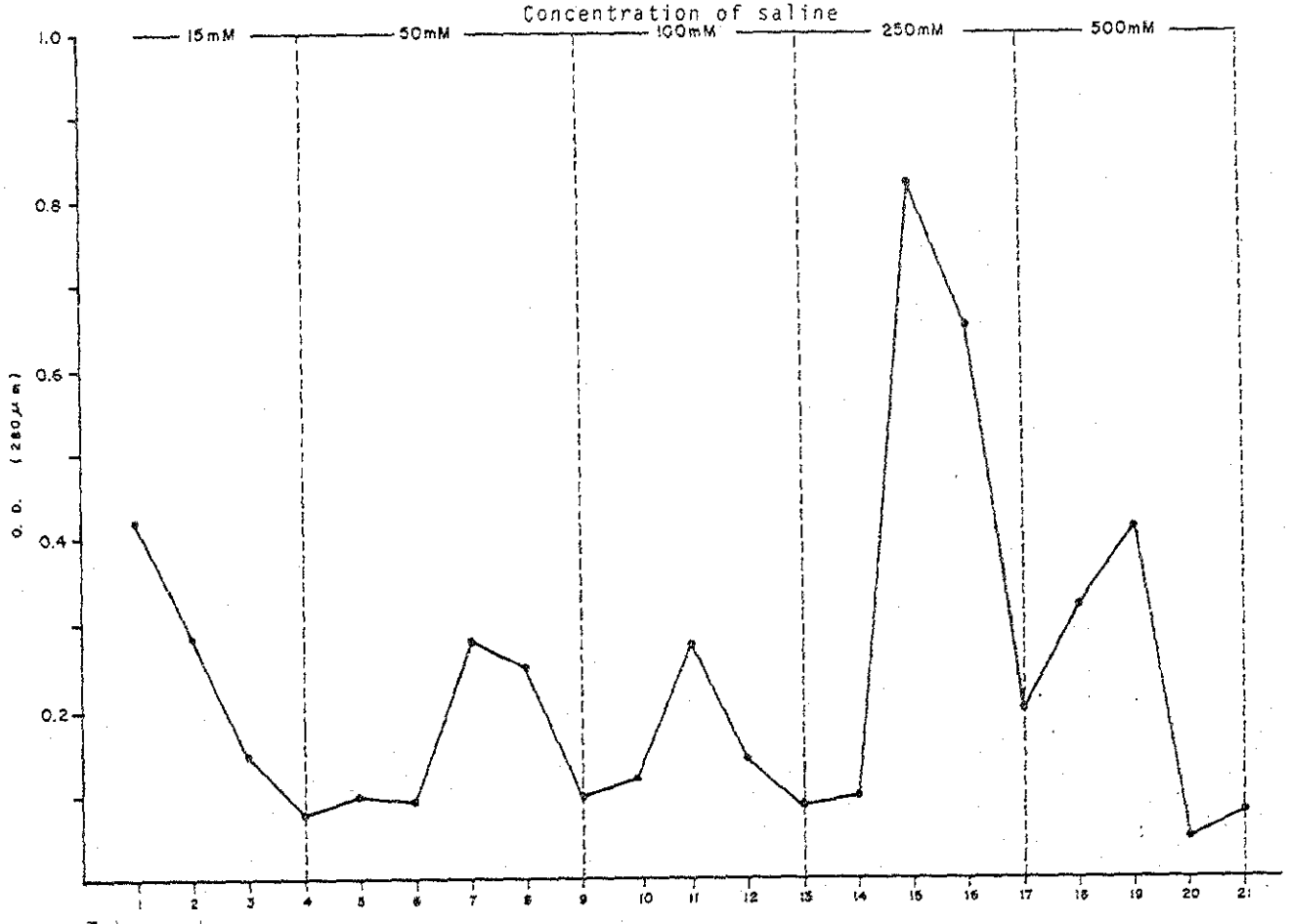
Habitants of Finca Pacayalito were inoculated interdermally in left forearms with 2.5, 10.0 or 20.0 µg/ml of protein of the fractions.

Standard antigen without fractionation (20 µg/ml) was injected into right forearms as controls.

Only the fraction F-2 at 2.5 and 10 µg/ml gave a significantly smaller wheal than standard antigen. There was no significant difference in wheal diameter between standard antigen and the fraction F-2 at 20 µg/ml or all other fractions at any three protein contents assayed (TABLE VI-11).

Standard antigen decreased activity when it was diluted to 5 µg/ml or more (See the section on protein content for skin test). The fractions F-1, F-3, F-4 and F-5 maintained activity when diluted up to 2.5 µg/ml, indicating that these purified fractions have greater activity than standard antigen. False-

FIG. VI-5 Elution pattern obtained from extraction of O. volvulus adult worms by ion-exchange chromatography



(Ito & Sakamoto; Nonpublished data, 1980)

TABLE VI-11.

Reactions of onchocerciasis patients with skin test using with standard antigen (20 µg/ml) and fractions of antigen from Onchocerca volvulus adults.

(Finca Pacayalito, Guatemala, 1980).

Fraction	Protein content (µg/ml)	Number of patients assayed	Wheal diameter (mm) ± S. D.	
			Fraction	Standard antigen
	2.5	10	10.2 ± 1.8	11.3 ± 2.0
F-1	10.0	5	13.2 ± 2.3	13.6 ± 1.7
	20.0	15	13.6 ± 3.2	13.3 ± 2.8
	2.5	13	10.1 ± 3.0*	12.9 ± 2.7
F-2	10.0	5	9.8 ± 1.9*	12.9 ± 2.7
	20.0	15	10.8 ± 2.4	12.2 ± 2.9
	2.5	5	10.4 ± 2.2	12.6 ± 1.0
F-3	10.0	5	11.4 ± 1.5	12.0 ± 2.1
	20.0	15	11.0 ± 3.1	12.2 ± 3.0
	2.5	5	11.4 ± 1.9	11.6 ± 1.9
F-4	10.0	5	11.0 ± 1.7	12.0 ± 1.3
	20.0	15	11.1 ± 1.3	12.2 ± 1.9
	2.5	5	14.4 ± 5.4	15.8 ± 3.9
F-5	10.0	5	11.8 ± 3.1	11.0 ± 2.6
	20.0	15	11.3 ± 2.0	12.7 ± 3.4

*) significantly different from standard antigen by F test.

(Ito & Sakamoto; Nonpublished data, 1980)

positive and false-negative rates must be studied with these fractions.

VII. Inoculation of microfilariae into mouse and chemical treatment

The greatest barrier in carrying-out investigations on O. volvulus is the lack of adequate experimental animals for infection. Since there is no specific medication for onchocerciasis, it is urgently needed to find an experimental animal in order to develop chemical treatment.

Duke (1962) used chimpanzees as experimental animals to study O. volvulus. A chimpanzee, however, is expensive and difficult to rear in a large numbers. In the present project, a mouse was studied as an experimental animal by inoculating it with mf and tracking their migration from the point of inoculation and their survival.

1. Materials and methods.

Onchocercomae were removed from patient's subcutaneous tissues, sliced, and soaked in saline solution for 30 min. A mf suspension was prepared by centrifuging at 1,500 r.p.m. for 5 min and washing the precipitate several times with saline solution. Mf density was determined by counting active mf in the suspension under microscope. Mice were incoulated subcutaneously with 7,500 - 63,000 mf in the inguinal region, in the scalp, or intraperitoneally.

Inoculated mice were dissected at different intervals, examing various organs for mf using the same prodedure as preparation of mf suspension for inoculation. Mf in the blood were counted by

Knott's concentration technique (Knott, 1939).

2. Migration of microfilaria into different organs of mice.

Fourteen mice were subcutaneously inoculated in the inguinal region with 30,000 mf and two were dissected each at different intervals up to 60 hrs. after inoculation. Mf were counted in different organs.

Mf migrated from the inguinal region to lungs, kidneys, and tail within 1 hr and to ears and spleen within 3 hrs after inoculation. An increasing number of mf were found in tail with time; more than 95% of the mf recovered were localized in tail in 24 hrs after inoculation (TABLE VII-1).

A similar experiment was conducted for a longer period of time, up to 12 weeks. A percentage-recovery peaked at one-week, then tapered off. Almost no mf were recovered after 4 weeks.

More mf were recovered from tail than any other organ, confirming the previous results. Few mf were recovered from ears, eyes, and the pelt, but no mf were found in the blood (TABLE VII-2).

3. Survival of microfilariae in mice.

In the previous experiment on migration of mf in different organs of mice, mf were recovered from tail and other organs up to 8 weeks after inoculation, indicating that mf can survive in mice

TABLE VII-1.

Visceral migration of O. volvulus microfilariae from the inguinal region to tail and other organs of mice with time after inoculation. Two mice per treatment were subcutaneously inoculated with 30,000 microfilariae in inguinal region.

Time after inoculation (hrs)	Number of microfilariae recovered per mouse (per cent of recovery)					
	Ears	Lungs	Spleen	Kidneys	Trail	Total
1	0 (0%)	5 (0%)	0 (0%)	2 (0%)	11 (0%)	18 (0%)
3	2 (0%)	2 (0%)	1 (0%)	0 (0%)	33 (0.1%)	35 (0.1%)
6	2 (0%)	4 (0%)	1 (0%)	5 (0%)	60 (0.2%)	72 (0.2%)
12	8 (0%)	18 (0.1%)	2 (0%)	6 (0.1%)	219 (0.7%)	253 (0.8%)
24	28 (0.1%)	11 (0%)	1 (0%)	4 (0%)	1163 (3.9%)	1207 (4.0%)
36	49 (0.2%)	7 (0%)	2 (0%)	1 (0%)	1386 (4.6%)	1445 (4.8%)
60	82 (0.3%)	27 (0:1%)	9 (0%)	7 (0%)	2400 (8.0%)	2525 (8.4%)

(Tada, Aoki, Hashiguchi, Ikeda & Kawabata; Quarterly Report No. 4, 1977).

TABLE VII-2.

Visceral migration of O. volvulus microfilariae from the inguinal region to tail and other organs of mice with time after subcutaneous inoculation.

Time after inoculation	Number of mice assayed	Number of microfilariae inoculated per mouse	Number of microfilariae recovered per mouse (per cent of recovery)						
			Eyes	Ears	Pelt	Tail	Blood	Other	Total
36 hrs	2	47,625	1 (0%)	40 (0%)	1,429 (3.0%)	2,080 (4.5%)	0 (0%)	3,122 (6.6%)	6,672 (14.1%)
1 week	2	23,433	1 (0%)	201 (0%)	407 (1.7%)	2,059 (8.8%)	0 (0%)	900 (3.8%)	3,568 (15.2%)
2 weeks	3	25,603	0 (0%)	102 (0.4%)	115 (0.4%)	775 (3.0%)	0 (0%)	141 (0.6%)	1,133 (4.4%)
4 weeks	3	45,125	0 (0%)	6 (0%)	8 (0%)	261 (0.6%)	0 (0%)	10 (0%)	285 (0.6%)
6 weeks	3	35,500	0 (0%)	0 (0%)	5 (0%)	35 (0.1%)	0 (0%)	4 (0%)	43 (0.1%)
8 weeks	4	20,420	0 (0%)	2 (0%)	5 (0%)	83 (0.4%)	0 (0%)	7 (0%)	96 (0.5%)
12 weeks	4	14,166	0 (0%)	0 (0%)	2 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (0%)

(Tada, Aoki, Hashiguchi, Ikeda & Kawabata; Quarterly Report No. 4, 1977)

for this period of time.

4. Sites for microfilaria inoculation.

Mice were inoculated subcutaneously with mf in three sites; groin, peritoneal cavity, or scalp, and were dissected 1 or 2 weeks after inoculation to count mf in different organs. When mf were incoulated in the inguinal region and peritoneal cavity, more mf were found in tail, than in ears. When mf were inoculated in the scalp region, more mf were found in ears, than in tail. Very few mf were found in other organs (TABLE VII-3).

5. Chemical treatment of mice.

Previous experiments showed that inoculated mf migrated principally to tail and ears, and survived in mice up to 3 weeks. Mice were inoculated with mf, then treated with diethylcarbamazine (DEC) to see if microfilaricidal activity could be determined using mice.

Mice were subcutaneously inoculated with mf in the inguinal region then administered DEC orally. After 2 days of DEC administration, they were dissected to count number of mf in ears and tail. The same number of mice were inoculated with mf and dissected without drug treatment as controls.

TABLE VII-3

Visceral migration of microfilariae from different sites of inoculation to ears, tail and other organs of mice.

Site for inoculation	Number of mice assayed	Number of microfilariae inoculated	Time after inoculation (weeks)	Number of microfilariae recovered (per cent of recovery)							
				Blood	Eyes	Ears	Lungs	Spleen	Kidneys	Tail	Total
Groin	1	46,000	1	0	0	54 (0.1%)	4 (0%)	0	0	1,030 (2.2%)	1,089 (2.4%)
	2	20,500	2	0	0	70 (0.2%)	2 (0%)	2 (0%)	1 (0%)	968 (3.3%)	1,045 (3.5%)
Peritoneal cavity	1	32,000	1	0	0	187 (0.6%)	11 (0%)	4 (0%)	11 (0%)	1,550 (4.8%)	1,764 (5.5%)
	2	44,000	2	0	0	27 (0%)	1 (0%)	0	0	412 (0.9%)	442 (1.0%)
Scalp	2	23,000	1	0	1 (0%)	216 (0.9%)	19 (0%)	3 (0%)	4 (0%)	113 (0.5%)	337 (1.6%)
			2	0	1 (0%)	336 (1.5%)	19 (0%)	2 (0%)	13 (0%)	186 (0.8%)	557 (2.4%)

(Tada, Aoki, Hashiguchi, Ikeda & Kawabata; Quarterly Report, No. 4, 1977)

There was a significant reduction in number of mf when mice were administed with 300 or 500 mg/kg of DEC.

DEC showed little microfilaricidal activity at 5 and 50 mg/kg.

In a second experiment, mice were subcutaneously inoculated with mf in the inguinal region, 7 days after inoculation they were administered DEC at different doses in 2 different manners 1) single administration, and 2) five consecutive administrations at one-fifth dose for 5 days.

There was no observable difference in number of mf between single and five consecutive administrations. There was a significant reduction in number of mf when mice were administered with 300 and 500 mg/kg. of DEC.

These two preliminary experiments suggest that chemicals can be screened for microfilaricidal activity using mice.

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References

1. Anderson R. I., Fazen L. E. & Buck A. A.; Onchocerciasis in Guatemara III. Daytime periodicity of microfilariae in skin, Am. J. Trop. Med. Hyg., 24, 62-65, 1975.

2. Buck, A. A.; Microfilaruria in onchoserciasis in Africans. Review and research recommendations, Ztschr. Tropenmed. Parasit., 24, 336-338, 1973.

3. Duke B. O. L.; Experimental transmission of Onchocerca voluvulus to chimpanzee, Tran. R. Soc. Trop. Med. Hyg., 51, 37-44, 1962.

4. Duke B. O. L. & Moore P. J.; The concentration of microfilariae of a Guatemalan strain of Onchocerca volvulus in skin snips taken from chimpanzees over 24 hours, Tropenmed. Parasit., 25, 153-159, 1974.

5. Fazen L. E., Anderson R. I., Figueroa M. M., Arthes F. G., & Buck A. A.; Onchocerciasis in Guatemala I. Epidemiological studies of microfilaruria, Am. J. Trop. Med. Hyg., 24, 52-57, 1975.

6. Hashiguchi Y., Kawabata M., Guillermo Zea F., Manuel M. Recinos C. & Otto Flores C.; The use of an Onchocerca volvulus microfilarial antigen skin test in an epidemiological survey of onchocerciasis in Guatemala, Tran. R. Soc. Trop. Med. Hyg., 73, 543-548, 1979.

7. Ikeda T., Tada I. & Aoki Y.; The indirect hemagglutination test for onchocerciasis performed with blood collected on filter paper, J. Parasit., 64, 786-789, 1978.

8. Ito Y., Hosaka H., Ishizaki T. & Kutsumi H.; Specificity of the intradermal test on the parasitic disease, with the reaction on inhabitants in the endemic or non-endemic areas of shcistosomiasis and paragonimiasis, Jap. J. Parasit., 21, 266-274, 1972.

8. Japan International Cooperative Agency; First Report on Onchocerciasis Control Project in Guatemala, 1978.

9. Japan International Cooperative Agency; Quaterly Reports No. 1-No. 15.

10. Picq J. J., Jardel J. P.; Une methode d'evaluation des densites microfilariennes d'Onchocerca volvulus, Leuckart, 1893, chez des onchocerquiens repotition des densites microfilariennes suivant les sites et niveaux de prelevement des biopsias cutanees. Variations des densites microfilariennes an cours des 24 heures, WHO/ONCHO/73, 103, 1973.

11. Picq J. J. & Roux J.; La microfilarurie, sa répartition géographique, ses rapports avec les densites microfilariennes cutanees, l'albuminurie et la chimitotherapie. Primiers resultats, Med. Trop., 33, 451-461, 1973.

12. Tada I. & Figueroa M. H.; The density of Onchocerca volvulus microfirariae in the skin at different times of the day in Guatemala, Jap. J. Parasit., 23, 220-225, 1974.

13. Thomas D. B., Anderson R. I., & MacRae A. A.; Daytime variation in the density of Onchocerca volvulus microfilariae in human skin, Bull. Wld. Hlth. Org., 49, 493-498, 1973.

Onchocerca 症の寄生虫・疫学的調査成績

（1980年4月－8月）

グアテマラ共和国におけるOnchocerca症の研究対策プロジェクトは，本症流行地の伝播ブユに対するコントロールを行ない，その成果を寄生虫・疫学的見地から判定すべく進められてきた。本プロジェクトは1980年9月をもって一応終結をみたが，その事業は更に同年10月に発足した第2次プロジェクトへと引き継がれた。この間に多くの派遣専門家によって多面的な調査研究がなされ，それぞれの分野で多くの成果が報告されてきた。

本報告は第1次プロジェクトの最終年度にあたる，1980年4月－8月に行なわれたパイロットエリア内の総合調査，およびその他の地域で行なわれた寄生虫・疫学的調査成績をまとめ解析したものである。

調査地区および調査方法

調査地区は表1，2に示したが，パイロットエリア内では5地区で，ESCUINTLA県San Vicente Pacaya郡のAld. El Patrocinio，Canton Santa Cruz(La Cruz)，Ald. Los Rios，Fca. Hamburgo，Fca. Berlín area（Fca. Guachipilín，Cas. Caña Vieja，Fca. Berlin，Fca. El Coyolito，その他5つの小フィンカを含む）である。その他の地区ではQUEZALTENANGO県のFca. La Moka，HUEHUETENANGO県のAld. Cuilco地域（Ald. Cuilcoの他22の小フィンカを含む），CHIMALTENANGO県のFca. Sibaja，Fca.Buena Vista（5月と7月の2回調査），SOLOLA県のFca. Monte Quina，SUCHITEPEQUEZ県のFca.Olas de Mocaの6地区で調査を行なった。

調査は従来から行なわれてきた方法と同様に皮内反応，皮膚生検によるMfの有無ならびにその密度，採血後実験室内でのゲル内沈降反応として二重拡散法（Ouchterlony法を用い，D.D testと略す）による検討，ブユの刺口，眼科的検診，腫瘤保有者を検出する触診，腫瘤摘出手術によって行なった。

調　査　成　績

1)　受診者について

今回調査を行なった11地区で，住民の生活人口を知ることができたのはパイロットエリア内5地区（1979年調べ）のものと，Olas de Moca 地区(1980年調べ）の6地区であった。パイロットエリア内5地区の年令的人口分布と受診者数については，ピラミッド型人口分布図として図1に示した。パイロットエリア内での受診者率は必ずしも高いものではなく，1,684名中1,129名（67.0％）が我々の検診を受けたが，受診者は相対的に女性が多く811名中626名(7.7.2％)であったのに対し，男性は873名中503名（57.6％）であった。男性は図1に示されているように，労働年令層とみられる30～39才およびその前後の年令層で受診率が特に低かった。このことは既に報告されているように，人口移動の理由の1つとして，本エリア外に労働者として出ていっているものと思われる。

2)　調査項目別陽性者率

各地区の調査項目別陽性者率は表1，2に示した。Mf 陽性者率とMf密度は従来から行なわれてきた方法に従って算出したが，Mf 密度はこの項ではふれない。

調査11の地区のうちLa Moka では全くMf 陽性者を見出せなかったが，その他の地区のうちBuena Vista（5月検診分）では75名中71名（94.7％）と最も高いMf 陽性者率を示した。これに対しCuilco 地域では167名中11名（6.6％），パイロットエリア内ではSanta Cruzの234名中19名（8.1％）と最も低いMf 陽性者率であった。

腫瘤保有者では，パイロットエリア内の地区でSanta Cruz，Hamburgo，その他の地区はLa Moka でみとめられなかったが，Olas de Moca の142名中19名（18.8％），Buena Vista（5月検診分）で75名中13名（18.3％）と最も多く，パイロットエリア内Patrocinio では506名中24名（4.7％），Berlin では188名中13名（6.9％），Los Rios で112名中8名（7.1％），その他の地区でCuilcoの167名中11名（6.6％）といずれも少なかった。

皮内反応陽性者率をみると，一般に高い検出率がみられた。Buena Vistaでは5月検診時には75名中72名（96.0％），7月検診時には91名中90名(989

％）と最も高く，パイロットエリア内では89.9～71.4％の陽性者率であった。一方，Cuilco，Monte Quina では56.3，42.0％とやや低く，Mfが全く陰性であったLa Mokaでは52.5％の陽性者率であった。

D.D test陽性者率では受診者のうち検査ができなかったものもあるが，Buena Vista（7月検診分）では75名中32名（42.7％）と最も高く，Sibaja，Monte Quinaでは28.1，27.7％であった。パイロットエリア内の各地区では12.8～3.9％とあまり高い陽性率ではなかった。

3) 検査項目の組合せによる陽性者率の比較

Mf，腫瘤，皮内反応，D.D testの検査ができたものは1,571名である。そのうちMf陽性者は493名（31.4％），腫瘤保有者は109名（6.9％），皮内反応陽性者は1,176名（74.9％），D.D test陽性者は150名（9.6％）であった。これらの人達について，検査項目の組合せからそれらの陽性者率をみたものが表3である。

これら検査法の関係をみると，Mf陽性者で皮内反応が陽性であったものは，493名中446名（89.9±8.4％）でよくMf陽性者をとらえているが，D.D testでは493名中109名（23.6±12.1％）と低かった。また，腫瘤陽性者で皮内反応が陽性であったものは109名中90名（88.5±15.8％）と高いが，D.D testでは109名中29名（28.4±14.5％）と低い結果であった。このような結果から，免疫学的検討を行なうにあたって，Onchocerca成虫抗原の抗原性，反応の感度および交叉反応性など，今後さらに検討されねばならない問題が多々あるように考えられた。

4) Onchocerca症浸淫地のMf密度指数による解析

Onchocerca症の浸淫状況をみるにあたって，今回Mf密度指数による検討を試みた。Mf密度指数とはMf密度係数をMf陽性者率に乗じたものとした。Mf密度係数は表4に示すように，Mf密度が1～9/mm³の場合は（+），Mf密度係数を1とした。以下，Mf密度が10～29/mm³の場合は（++），係数を2とする様に，Mf密度を6段階にわけ，それぞれの段階について1～6の係数を付し，Mf密度が陰性の場合はMf密度係数を0とした。表5は例としてBuena Vistaの男性

表4　Mf 密 度 係 数 表

Mf数／mm^2	係　数
1 ～　9(+)	1
10 ～　29(++)	2
30 ～　49(+++)	3
50 ～　99(4+)	4
100 ～ 499(5+)	5
500以上　(6+)	6

Mf 密度指数 ＝ Mf 陽性率×Mf 密度係数

受診者について，Mf 密度指数を算定したものを示したものである。即ち，各年令層にわけたMf 陽性者を更にMf 密度別6段階にわけ，それぞれMf 陽性者率とMf 密度指数を算出した。年令層別Mf 密度指数は年令別に集計し，地区別Mf 密度指数は年令層別Mf 密度指数を総計したものである。

各地区のMf 密度指数の年令的推移は，図2，3に示した。Mf 密度指数は全般的にみて年令が増加するに従って上昇し，Onchocerca 症濃厚浸淫地に行くに従って高値を示す傾向がみられた。また，男女間の差についてみると，年少者においてはあまり差がみられないが，年令の増加に従い差がひらいて行く地区が多かった。

パイロットエリア内のSanta Cruz ではMf 密度指数はあまり高くなく，40～49才の男性では200以下であり，女性では50～59才頃まで非常に低い値であった。その他の4地区では，最も高い値を示したものはPatrocinioで20～59才（男性），Berlín では30～69才（男性）で，共に300～400の間の値を示した。またLos Rios では60～69才代（男性）でMf 密度指数が高かったのは，Mf 密度指数が4＋で200.0，5＋で250.0の2人があったためと思われる。一方，Sibaja では15～49才代でMf 密度指数が400.0を越え最高450.0（20～29才男性）に達し，Buena Vista では15才（男性）以上で428.1 ～

500.0の値であった。またBuena Vistaのように女性でも15～49才で300.0～464.5の高いMf密度指数を示したところもあった。それらに比べCuilcoでは男性にのみMf陽性者がみられ，Mf密度指数においても60～69才代で125.0と低く，Onchocerca症の低い浸淫地と考えられた。

これらの成績から，Mf密度指数が若年層から高く，高年令層に至っても高値がみられる地域では，他の検査成績も全般的に高く，濃厚なOnchocerca症浸淫地ということができる。

5) 4検査成績とMf密度指数との図形的解析

今回行なわれた11地区の寄生虫・疫学的調査成績から，本症の浸淫状況を図形による解析把握を試み，図4，5にまとめた。この図形は4検査成績，受診者率あるいは受診者数，Mf密度指数の6項目のそれぞれの成績を，6角形を基準とした中心を通る6対角線上にプロットしたものである。図形の中心から12時方向には受診者率（パイロットエリア内各地とOlas de Mocaの6地区），あるいは受診者数（その他の5地区）をプロットし，2時方向にはMf陽性率，4時方向には地区のMf密度指数，6時方向には腫瘤陽性者率，8時方向には皮内反応陽性者率，10時方向にはD.D test陽性者率をそれぞれプロットした。

パイロットエリア内の5地区のうち，最も低い浸淫地と思われるパターンを示した地区はSanta Cruzであった。Santa Cruzでは皮内反応陽性者率（76.1％）で他地区のそれにほぼ近い値を示していた以外は，何れの検査成績も低い値であった。特に腫瘤保有者は検出されておらず，Mf密度指数も574.5と他4地区と比べてかなり低い値であった。一方，パイロットエリア内でも高い浸淫地と思われる地区はBerlín地域，Los Riosであった。Berlín地域ではMf陽性者率（42.0％），Mf密度指数（2,797.2）と最も高く，Los RiosではMf密度指数（2,699.7）と高い値を示した。

パイロットエリア以外の6地区についてみると，La Mokaでは皮内反応陽性者率（52.5％）のみの成績しかみられず，Cuilco地域ではMf密度指数（267.7）の値が小さく，共に前述のSanta Cruzの示したパターンと類似している。これに対し，Buena Vista，Sibaja，Olas de Moca，Monte Quinaでは

Mf 密度指数が 5,175.5， 4,313.1， 3,383.8， 3,113.1 と何れもパイロットエリア内各地の値よりも高く，その他の検査成績もMonte Quina以外はかなり高い値であった。

これらの成績から，Onchocerca 症の極低浸淫地か低い浸淫地と思われる La Moka，Santa Cruz，Cuilco では 12 時方向と 8 時方向に長い，いわゆる時計が 8 時を指す型である。逆に濃厚浸淫地と思われるBuena Vista，Sibaja, Olas de Moca，Monte Quina では 4 ～ 6 角型のパターンを示した。Patrocinio，Hamburgo，Los Rios，Berlin ではこれら低浸淫地型と濃厚浸淫地型の中間型として，三角型のパターンを示したものと考えられた。

結　　論

本報告は 1980 年 4 月から 8 月までに行なわれたパイロットエリア内 5 地区と，その他の 6 地区について，Onchocerca 症の寄生虫・疫学的調査成績を，Mf 密度指数，図形による浸淫パターンから解析したものである。

Mf 密度指数も 3,000 以上で，4 ～ 6 角型パターンを示す Sibaja，Buena Vista，Monte Quina，Olas de Moca は本症の濃厚浸淫地であった。一方，La Moka，Cuilco，Santa Cruz では Mf 密度指数も 1,000 以下と低く，図形パターンも低浸淫地型を示した。また，Patrocinio，Los Rios，Hamburgo，Berlin では，これら浸淫地区の中間型を示した。

これらの結果から，Onchocerca 症の浸淫程度を把握し，今後本症に関するコントロールが進められる場合，効果判定の指標となり得ると考えられる。

本報告は，伊藤洋一，坂本信，石田誠夫・各専門家と共に行った成績から寄生虫・疫学的解析を試みたものである。

（執筆者：近藤力王至）

文　　献

1) 青木克巳他：グァテマラ共和国・オンコセルカ症研究対策プロジェクト・第 1 次報告書　71~133，1978。