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皮膚リーシュマニア症の治療
—Metronidazoleと温熱療法による治療例—

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はじめに

日本人の海外滞在地域の拡大と長期化に伴い，本来我が国には存在しない疾病の治療を，求められることがある。皮膚リーシュマニア症もそれらの疾患の1つである。同症の治療は，皮疹が少数の場合には，一般には局所療法単独または薬剤の全身投与を併用，皮疹が多数の時や局所療法が適さない部位などの場合には，主に薬剤の全身投与が用いられている。治療薬としては，古くからアンチモン剤が使用されているが，日本では入手しにくく，その副作用も考慮しなければならず，また皮膚リーシュマニア症に対する効力を，疑問視する報告（Convit and Kerdel-Vegas, 1965; Rahim and Tatar, 1966; 土田ら，1989）もあり，アンチモン剤に代わり得る薬剤が望まれている。今回，サウジアラビアで感染し，アンチモン剤の筋注と局所凍結療法を受けたにもかかわらず，皮疹からリーシュマニア原虫が培養された患者を治療する機会を得たので，アンチモン剤を使用しない治療法を試みた。まず，本症例より分離培養したPromastigote型原虫（以下KN株と略記す）を，*in vitro*で原虫の増殖性と運動性を示標に，5価のアンチモン剤(Pentostam®)と，現在日本で使用されている代表的な抗原虫薬であるMetronidazole (Flagyl®)との殺KN株効果を比較（矢後ら，1990）し，Metronidazoleが，アンチモン剤に変わり得る効力を持つことを確認した。実際に，Metronidazoleを皮膚リーシュマニア症患者に投与し，完治した症例報告（Peter, 1973; James and Stephen, 1975）があるので，皮疹より原虫を分離培養しながら，Metronidazoleによる治療を行った。Metronidazoleは，原則として1日750 mgを分3で10日間投薬後，10日間の休薬期間をもって1クールとし，3クール行った。さらにKN株が入浴湯温である42°Cで，2時間以上継続保温すると，死滅する実験結果（矢後ら，1989；矢後，1990）を応用して，患部を42°Cより43°Cに，合計30時間保温し，治癒した症例を経験したので報告する。

症　例

患者：51歳，日本人男性。会社員。

初診：1988年3月15日。

主訴：局所凍結療法を2回施行し，アンチモン剤を合計5,700 mg筋注したが，完治しない皮膚リーシュマニア症と考えられる皮疹の治療。

既往歴：特記事項なし。

現病歴：1987年6月より12月中旬まで，サウジアラビア，リヤドの西北約300 kmのブレイダ市郊外の砂漠地帯に滞在した。滞在中は，刺咬時に強い痛みを伴う，多数の虫刺を受けた。刺咬された直後に，腹部をピンク色にした白い小さな虫に気づき，捕らえて圧平すると，血液と思える液体が認められた。刺咬後，掻痒感は2または3週間位は持続したが，1カ月位後には消失した。1987年11月初旬，右大腿伸側中央部に掻痒感の強い，他の虫刺部位に比べて，出血しやすく治癒しにく

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い発赤腫脹に気づいたが，単なる虫刺と思い放置しておいた。時々，噴火口のように盛り上がった皮疹の中央の窪んだ部位から浸出液が流出し，下着を汚した。12月16日，帰国途中のバーレインのホテルで入浴中，同皮疹が少しも治癒していず，発赤腫脹部の中央が湿潤なのも気になり，ホテルで医師の診察を依頼したが，医師は見つからなかった。12月19日帰国し，某皮膚科を受診したが，蚊刺ではないかといわれた。しかし，サウジアラビアの人々が，サンドフライの刺咬によって起こる病気を，大変恐れていたことを思い出し，12月下旬，ブレイダ市の皮膚科医へ，皮疹の所見，すなわち周囲がピンク色の直径約2cmの皮疹で，中央の直径1cm位の部分は硬結を伴う濃い赤色で，その中心に1mm位のいつも湿潤な凹部が観察される状態や，皮疹の経過や写真なども送り，翌1988年1月初旬，皮膚リーシュマニア症との返信を得た。1月13日某病院皮膚科を受診し，皮膚リーシュマニア症とのサウジアラビアの医師の診断を伝えると，患部は外科的に切除するとのことなので，治療は希望しなかった。1月18日，某病院でリーシュマニア症についての説明を受け，内蔵型の混合感染の可能性も考え，1月23日，治療のためサウジアラビアへ再渡航した。1月24日，ブレイダ市の国立病院で，視診と触診のみで皮膚リーシュマニア症の初期と，再び診断された。発赤腫脹部は直径約1cm（写真1）と小さかったので，同24日直ちに，局所凍結療法が施行された。凍結療法は，直径1.9 cmの金属円板を，皮疹に強く押し付け，圧縮された炭酸ガスの気化熱を利用して，円板を−35°C〜−36°Cに1分位冷却後，一度円板を離して患部を観察し，再び1分位冷却した。冷却時，出血や疼痛はなかった。2月6日，長期間サウジアラビアで治療するのが困難な患者の事情も考慮され，5価のアンチモン剤（Pentostam®, Wellcome社，England，1 m*l*中に100 mgのPentavalent antimonyを含む）を，3 m*l*臀部に筋注した。翌2月7日より，6 m*l*ずつ毎日左右の臀部に交互に2月15日まで筋注し，合計10回，全投薬量は5,700 mgであった。アンチモン剤は，直接皮疹内には注入しなかった。同剤の筋注は大変不快で，投与後30分位すると，注射側の足がしびれ始め，1時間位続いた。また，注射を開始して2日目と3日目には，投与後1時間位から，軽度な頭痛，発熱を伴わない悪寒，数度の咳などの感冒様症状が出現し，また注射期間中は，両上肢と頭部に掻痒感を生じ，手には赤色の発疹が出た。医師に症状を訴えたが，頻発する症状とのことで，予定通り10回筋注した。食欲は良好であった。1月24日に施行した凍結療法部位に接して，2月16日，再び小さな発赤腫脹（5×2 mm位）が認められ，視診と触診のみで再発と診断され，前回と同様にして第2回目の凍結療法が行われた。凍結療法部は，冷却した金属円板を皮疹に強く圧迫したための白色の陥凹（写真2A）が形成され，ついで水疱（写真2B）になり，潰瘍，壊死を経て，白斑（写真3）になった。凍結療法後も，なお数カ月の経過観察が必要と言われたが，帰国を急ぐ患者の希望もあり，2月20日帰国した。帰国時，再々発に備え，Pentostamを100 m*l*持ち帰った。サウジアラビアの医師からは，完治を期待しても良いと言われたが，帰国後経過観察するも，皮疹が治癒していくようには思えないとのことで，相談を受けた。

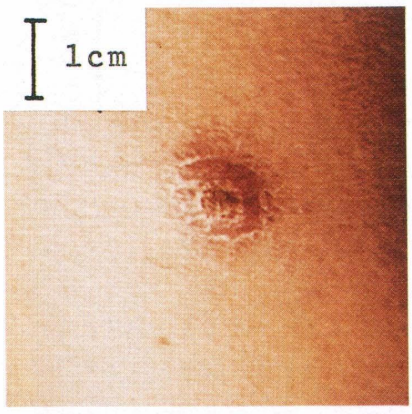
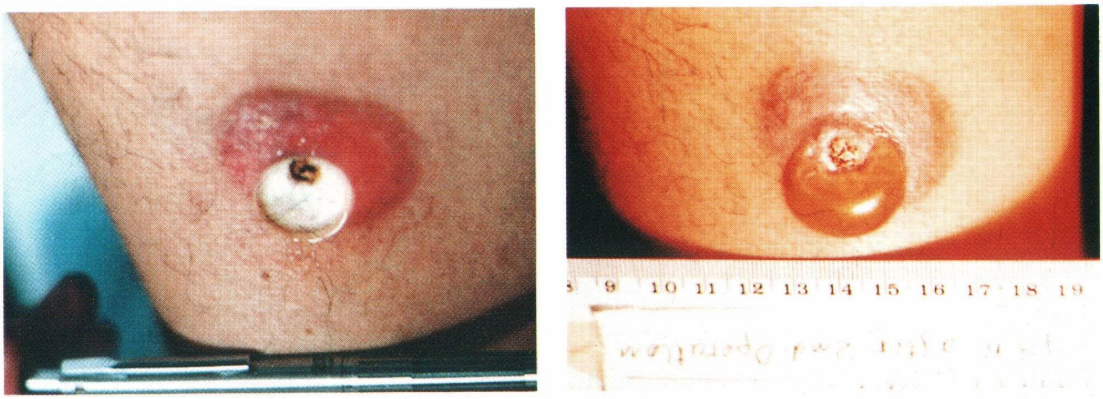


Photo. 1 Clinical appearance of the lesion on January 23, 1988, before initial cryotherapy in Saudi Arabia. Photographed by the patient.

初診時現症：1988年3月15日，右大腿伸側の膝関節より体幹寄り約15 cmの部位に，直径約3 cmの不正円形の脱色白斑（図1，March 31, '88および写真3参照）が観察された。白斑の周囲には，



(A) (B)

Photo. 2 Clinical appearance of the lesion after the second cryotherapy on February 16, '88, in Saudi Arabia.
(A) White concavity of the skin just after the cryotherapy. Necrosis in it and inflammation around it were traces of initial cryotherapy on January 24, '88.
(B) Bulla formation at 13 hrs after the cryotherapy.
Photographed by the patient.

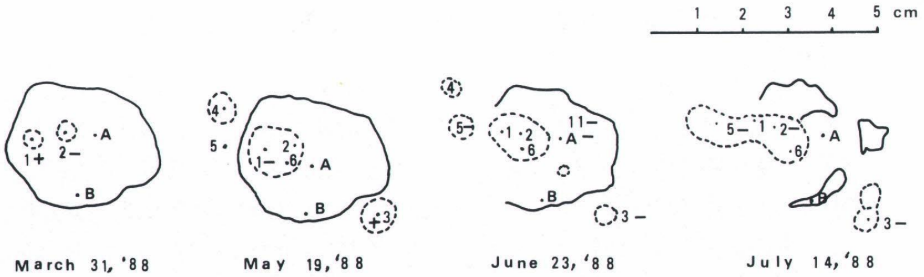


Figure 1 Changes in the clinical lesion traced on paper from the lesion. The portion surrounded by a dotted line indicates an area of redness and swelling with induration, and the portion surrounded with an uninterrupted line indicates leukoderma as a result of depigmentation. The mark "+" shows a point from which parasites were cultured and "−" shows that they could not be cultured. Number of eruptions refers to sites mentioned in the text. Refer also to the explanations of Photo. 3.

幅約0.5-1 cmの暗褐色の色素沈着が認められた。白斑部内には，直径約5 mmの暗赤褐色の硬結を伴う隆起が2カ所（図1，March 31, '88と写真3の皮疹番号1と2；以下図1および写真3と写真6の皮疹部位を示す番号と凍結療法痕AおよびBは共通で，皮番と略記す）観察された。掻痒感，易疲労感，疼痛，発熱，紫斑，貧血，リンパ節腫脹，肝脾腫または黄疸などはなく，一般状態は良好であった。

原虫の培養と診断：サウジアラビアでは，視診と触診のみで，皮膚リーシュマニア症と診断されたが，確定診断をするために，1988年3月31日，

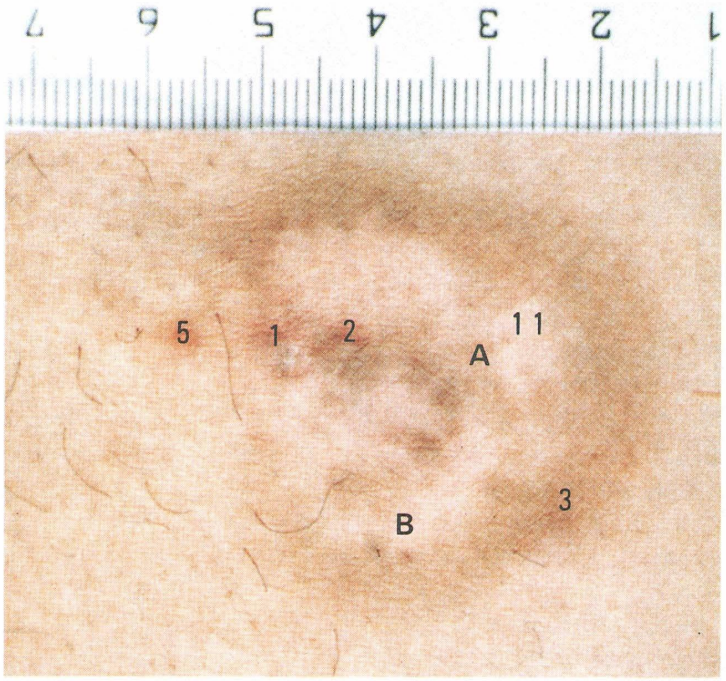


Photo. 3 Clinical appearance of the lesion on June 18, '88, just after the end of treatment with oral metronidazole for the second ten-day period. Parasites were cultured from a portion indicated by the number 1 on March 31, '88 and from the portion indicated by the number 3 on May 19, '88. Cryotherapy was applied at spot A on January 24, '88 and B on February 16, '88.

皮番1と2より原虫採取を行った。採取方法は，生理的食塩水（以下生食と略記す）約0.2 m*l*を入れた1 m*l*の注射器に静注針（22G×1¼）をつけ，発赤腫脹部の表皮に平行に浅く注射針を挿入し，静かに回転しながら針先で周囲の組織を破壊した後，生食の注入吸引を数回繰り返し，原虫を含む細胞を採取した。針を挿入した腫脹部は柔らかく，採取操作は容易であった。しかし，患者は約半月後の4月中旬まで，採取部位の鈍重感や違和感を訴えた。採取した材料は，斜面に固まらせた6.5 m*l*のNNN培地（Leventhal and Cheadle, 1989）に，生食を5 m*l*加えた中試験管に接種し，25°Cで培養した。培養1週目の4月7日，皮番1の採取材料より，多数のリーシュマニアPromastigote型原虫（KN株，写真4）が培養されたので，本症例を皮膚リーシュマニア症と確定診断した。

検査成績：1988年3月31日，5月19日，7月14日および10月6日と4回行った血液一般生化学検査値では，A/G比の軽度な低下が認められた以外は，異常は認められなかった。A/G比は3月31日1.3，5月19日と7月14日に1.4（正常範囲1.5—2.2）であったが，10月6日には1.5と正常値になった。電解質，脂質などは異常なく，肝機能異常もなかった。また貧血は認められず，白血球数，白血球分画，赤血球像なども正常で，検尿にも異常はなかった。1988年5月19日と同年10月6日に行った腹部エコー検査でも，肝腫や碑腫などは認められず，検査成績からは，内蔵型の感染は示唆されなかった。

治療と経過

1）Metronidazoleの経口投与：1988年3月31日，皮番1より原虫が採取されたが，その後経過観察するも，硬結を伴う発赤腫脹部の融合と増

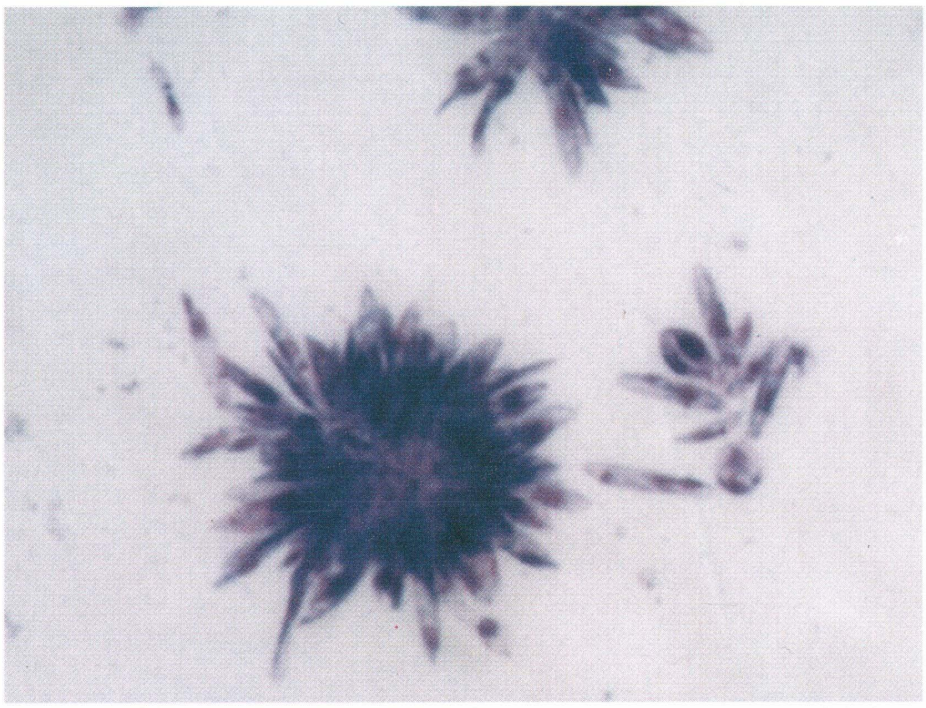


Photo. 4 Leishmania promastigotes separated from the lesion and cultured in NNN medium (Giemsa's stain; original magnification ×1,000).

大（図1のMay 19, '88.皮番1，2および6）と新生（同，皮番3，4および5）が認められたので，サウジアラビアで投与されたアンチモン剤の効果は，臨床所見からは認められなかったので，副作用も考慮し，以後のアンチモン剤の使用は見合わせた。そこで，KN株に殺原虫効果を示したMetronidazole（Flagyl®）による治療を5月19日より開始した。同日の投薬直前に，皮疹より原虫採取を行った。採取部位は，3月31日に原虫が培養された皮番1と，新たに出現した皮番3（図1，May 19, '88）である。培養5日目の5月24日，皮番3から多数のPromastigote型原虫が培養されたが，皮番1からは，6月4日まで16日間観察したが，原虫は検出されなかった。Metronidazoleの投薬は，原則として1日750 mgを分3で，10日間内服後，10日間の休薬期間をもって1クールとしたが，実際に服薬した1日当たりの薬量と分服回数の記録は，表1に示した。服薬開始後5日目の5月23日頃より，患者の観察によれば，手で皮疹を触れると硬結部が服薬前に比べ柔らかく浅くなり，また良く動くようになったという。5月30日，12日間かけて1クール目の服薬が終了し，Metronidazoleは合計7,500 mg服用した。服薬期間が2日間延長したので，休薬日数は8日間と短くなったが，予定どうり6月8日より2クール目の投薬を開始した。6月13日頃より，1日1—3回の下痢が始まり，口中に金属様の酸味感を覚えるようになった。特に，砂糖入りコーヒーを飲んだ直後に，酸っぱい金属様の味を強く感じた。しかし，他に副作用と思える症状は，認められなかった。6月18日に，11日間かけて2クール目が終了し，Metronidazoleの服薬量は1クール目と合わせ，15,000 mgになった。2クール目の休薬期間に入り5日目の6月23日に，皮疹内の4カ所（図1のJune 23, '88および写真3参照），すなわち，5月19日に原虫が培養された皮番3，硬結部が増大した皮番5，1988年1月24日施行された第1回凍結療法痕のA，および脱色白斑部の皮番11より第3回目の原虫分離を行い，7月14日まで3週間培養したが，原虫は培養されなかった。口内

Table 1 Schedule of oral administration of Metronidazole

Course	1			2			3		
Day	Date	Dose(mg)	Times†	Date	Dose	Times	Date	Dose	Times
1	May, 19(+)*	500	2	June, 08	500	2	June, 28	750	3
2	20	500	2	09	500	2	29	750	3
3	21	750	3	10	750	3	30	500	2
4	22	1,000	4	11	750	3	July, 01	500	2
5	23	500	2	12	750	3	02	500	2
6	24	750	3	13	750	3	03	750	3
7	25	500	2	14	750	3	04	750	3
8	26	750	3	15	500	2	05	750	3
9	27	500	2	16	750	3	06	500	2
10	28	500	2	17	1,000	4	07	500	2
11	29	750	3	18	500	2	08	500	2
12	30	500	2	19	0		09	500	2
13	31	0		20	0		10	250	1
14	June, 01	0		21	0		11	0	
15	02	0		22	0		12	0	
16	03	0		23(−)*	0		13	0	
17	04	0		24	0		14(−)	0	
18	05	0		25	0		15	0	
19	06	0		26	0		16	0	
20	07	0		27	0		17	0	
Subtotal		7,500			7,500			7,500	
Total		7,500			15,000			22,500	

* The mark "(+)" shows that the parasites were cultured from the lesion and "(−)" shows that they could not cultured.

† The dose a day was divided into the times and administered.
Refer to the explanations of Figure 1 and Photograph 3.

の金属様の酸味感は，2クール目の休薬期間中続いた。6月19日より27日まで9日間休薬後，6月28日より3クール目の投薬を開始した。服薬4日目の7月1日頃より，再び1日2－3回の下痢が生じたため，Metronidazoleは1日500 mgで分2にして7日間服用したので，3クール目は13日間かかった。口内の金属様の酸味感は，再び生じてきた。7月10日，3クール目の服薬が終了し，Metronidazoleの全服薬量は，22,500 mgになった。3クール目の休薬4日目の7月14日（図1，July 14,'88および写真3参照）には，皮疹の大腿外側の白斑部と色素沈着部の境界が不鮮明になり，白斑部が縮小し，発毛が認められた。同日，皮疹の4カ所，すなわち3月31日に原虫が培養されなかった皮番2，5月19日に原虫が採取され6月23日には採取されなくなった皮番3，6月23日には原虫が培養されなく，7月14日痂皮形成が認められた皮番5と皮番5の痂皮より，第4回目の原虫の分離培養を行ったが，8月4日までの3週間の培養では，原虫は検出されなかった。そして，7月14日の原虫採取操作時には，皮疹に注射針を挿入または生食を注入する時に，強い抵抗感があり，原虫が採取された3月31日や5月19日の注入操作時の柔らかさとは，明らかに異なってきた。

すなわち，原虫はMetronidazole投薬開始日である1988年5月19日には，皮番3より採取されたが，2クール投薬後と3クール投薬後には，試みた皮番1，2，3，5および凍結療法痕A，白斑

部11からは採取されず，Metronidazoleの投薬は有効であったと考えられた。

2）局所温熱療法：Metronidazoleの投薬により，皮疹より原虫が検出されなくなったが，皮疹のすべての部位から原虫採取を行ったわけではなく，また経過が緩慢なので臨床症状からは，すべての原虫が死滅したかどうかを判定することは困難であった。そこで，生存しているかもしれない原虫を殺すために，引き続き局所温熱療法を行った。42°Cの恒温水槽水面より，KN株を培養している試験管のNNN培地の最上部を0.7cm低く沈めて，2時間継続保温すると，原虫は死滅する実験結果（矢後，白坂，1989；矢後，1990）を治療に応用し，皮疹を保温した。保温方法は，表2に示したように，赤外線または温湯を使用して，合計30時間行った。まず，7月14日の第4回目の原虫採取直後に，赤外線ランプで皮膚表面の温度を43°Cより44°Cに，1時間継続保温した。第2回目の7月16日からは，写真5に示したように，皮疹の上にハンカチ大の布を数枚重ねた上に，55°Cより60°C位の温湯を入れたビニール袋を載せ，皮膚と布の間に置いた温度計が，42°Cより43°Cを指すように布の枚数で調節した。温湯の温度が低下するに従って，布を1枚ずつ取り除き，皮膚と布との間の温度は，常に42°Cより43°Cに保った。保温時間は，継続して1時間より5時間までで，10月2日まで6日間にわたり12回保温した。最後の温熱療法後4日目の10月6日に，皮番3と皮番5（写真6参照）に発赤腫脹が生じたので，同所より5回目の原虫分離を行ったが，原虫は培養されなかった。さらに，1988年11月26日，89年3月2日（写真6）そして1989年7月5日にも，同様な発赤腫脹が皮番3に生じたので，それぞれ第6回目，第7回目および第8回目の原虫採取を行ったが，いずれも原虫は培養されなかった。しかし，1988年11月26日の皮番3には，1×2mm大の膿瘍が認められ，89年3月2日の発赤腫脹部は，原虫採取操作時の出血量が多く，鬱血があったものと考えられ，1989年7月5日には痂皮形成が認められた。これらのことより，繰り返し生じた発赤腫脹は，残存原虫に起因するものではなく，細菌の感染などによる2次的反応と考えられた。なお，経過観察しても，1989年9月8日には発赤腫脹や硬結は触知されなく，90年10月現在，再発は認められず完治と判定した。

Table 2 Schedule of thermotherapy

No	Date	Method	Temperature(°C)	Duration(hrs)	Total hrs
1	Jul. 14,'88	infrared lamp	43-44	1	1
2	16,	hot water*	42-43	1	2
3	16,	hot water	42-43	1	3
4	16,	hot water	42-43	1	4
5	17,	hot water	42-43	3	7
6	17,	hot water	42-43	3	10
7	17,	hot water	42-43	1	11
8	24,	hot water	42-43	4	15
9	24,	hot water	42-43(over 43°C on occasion)	4	19
10	Sep. 25,	hot water	42-43	5	24
11	Oct. 2,	hot water	42-43	3	27
12	2,	hot water	42-43	3	30

* Lesion was covered by several pieces of cloth and warmed with a vinyl bag that contained hot water from 55°C to 60°C which was put on the cloth. As the water grew cool, the cloth was taken away piece by piece and the temperature between the cloth and the lesion was kept constant at 42°C or 43°C.

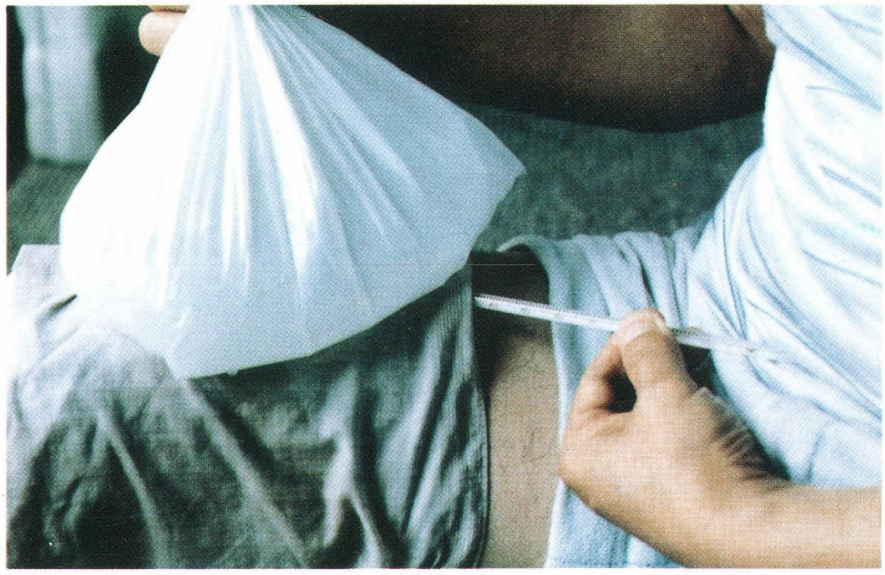


Photo 5. Patient treated by thermotherapy, using hot water, at 42°C or 43°C. Schedule and method of thermotherapy was given in Table 2.

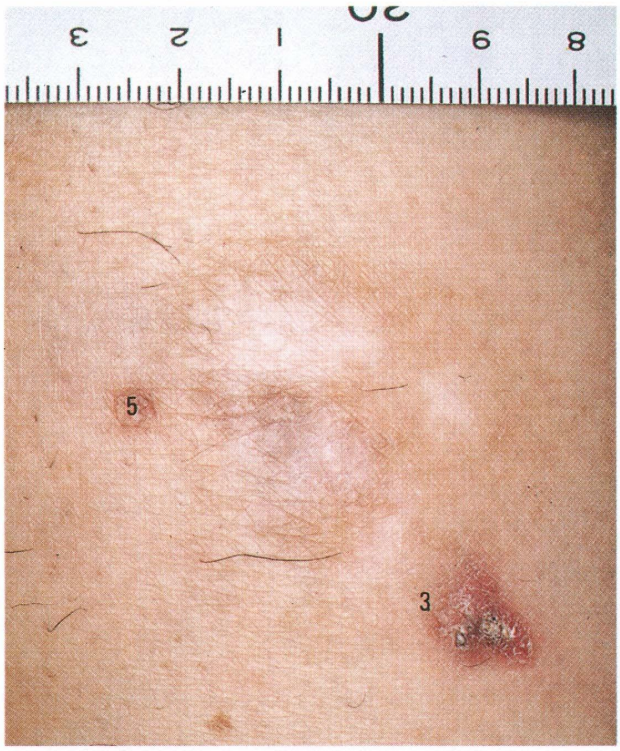


Photo. 6 Swelling with redness and desquamation on March 2, '89, at a time when no parasite could be cultured.

考　察

皮膚リーシュマニア症治療の第一選択剤は，アンチモン剤とされている（輸入熱帯病の薬物治療法に関する研究班, 1982； Paul *et al.*, 1984； 佐々ら，1986)。本症例は5価のアンチモン剤であるPentostam®を，1988年2月6日より，10日間かけて合計5,700 mg筋注したにもかかわらず，投与後約1カ月半の3月31日と約3カ月後の5月19日に，皮疹より原虫が培養され，同法によるアンチモン剤の効果は認められなかった。また，土田ら（1989）も3価のアンチモン剤であるFuadin®を，それぞれ1.5 m*l*, 2.5 m*l*, 3.5 m*l*と3日毎に，計4回筋注した症例で，注射後の悪心が強い上に硬結はむしろ増大し，同剤の効果は認められなかったと報告している。本患者も，多数の皮膚リーシュマニア症の治療経験を持つサウジアラビアの皮膚科医から，経験的にPentostamの投薬では，著しい効果は認められないので，アンチモン剤による治療は推奨しないと言われている。しかし，本患者の場合には，帰国を急いで，当時は特効薬と信じていたPentostamの投薬を強く望んだために投与されたものである。これらのことから，皮膚リーシュマニア症に対するアンチモン剤の使用については，その副作用を考慮しても，なお第一選択剤とすべきかどうか，その効果を再検討する余地があるものと思われた。

そこで，本患者の治療には，これ以上Pentostamを投与しても効果は少ないものと判断し，アンチモン剤以外の薬剤を検討した。Metronidazole（Flagylを使用）とアンチモン剤（Pentostamを使用）の薬効の差を比較した矢後ら（1990）の実験，すなわち，小試験管に斜面にしたNNN培地1.3 m*l*に生食1 m*l*を加え，5 mg, 7 mgおよび9 mgのMetronidazoleとアンチモン剤をそれぞれ入れ，KN株を培養すると，原虫の自発的な運動性は観察した14日後までには，両剤に認められなくなった。同様に，1 mg, 0.5 mgおよび0.1 mgの濃度では，両剤中で原虫は生存し，Metronidazoleとアンチモン剤の薬量による差異は，認められなかった。実際にMetronidazoleを使用して治療を行った症例では，パナマで感染した24歳の白人男性に1日750 mgを分3で10日間投薬後，10日間の休薬期間を1クールとし，2クールで治療に成功した報告（Peter, 1973）や，アフガニスタンで感染した24歳の白人男性に，同方法で3クール投薬し完治した報告（James and Stephen, 1975）などがある。本症例でも，Metronidazole投薬開始日である1988年5月19日には，皮疹から原虫が培養されたが，2クール投薬後の1988年6月23日には原虫は培養されず，その後1989年7月5日まで5回にわたり，原虫の採取培養を行ったが，原虫は培養されなかった。しかし，皮膚リーシュマニア症には，自然治癒があり得るので，Metronidazoleによる治療期間と，自然治癒が偶然重なったという可能性は否定できない。この点を明らかにするには，より多くのMetronidazoleによる治癒例が必要であるが，矢後，白坂（1990）のKN株を用いた*in vitro*の実験結果と合わせ考え，現時点では，Metronidazoleにより原虫は死滅したものと推定している。

Metronidazoleは日本では日常的に，膣トリコモナス症，ランブル鞭毛虫症やアメーバ性赤痢の治療に用いられている薬剤なので，入手が容易な上，経口投与が可能なので，患者はアンチモン剤の投与時のように，注射のために毎日通院したり，またその副作用を考慮して入院する必要はない。Metronidazoleの副作用は，常用量では軽微で，報告されている主なものには，末梢神経障害がある。同障害を生じた症例のMetronidazoleの全服薬量は，30 gより314 gで，必ずしも服薬量には関係していず，その障害も軽度で，服薬を中止すれば回復した（Bradley *et al.*, 1977）という。本症例の総服薬量は，1日750 mgで10日間服薬を3クール行ったので，22.5 gである（表1）が，Metronidazoleの蓄積と副作用を考慮して，原則として10日間投薬後，10日間の休薬期間を置いた。しかし実際には，表1に示したように，1クール目には2日間服薬が延びたので休薬日数は8日間，2クール目は1日延びたので9日間，3クール目の休薬日数は同様に7日間であった。本症例で観察された副作用と考えられる症状は，1日1－3回の下痢と金属様の酸味感である。酸味感は，Metronidazoleが唾液中に分泌されるためと説

明されている（Ralph, 1983）。これらのことから，Metronidazoleは皮膚リーシュマニア症の治療には，アンチモン剤に比して，副作用が軽微で，殺KN株効果もあるので，試みる価値がある薬剤と考えられた。

局所療法には，外科的切除，放斜線照射，凍結療法および局所への薬物注入などがある。外科的切除は，インドなどでは古くから施行され，小さな腫瘤では，醜い瘢痕も残さずむしろ自然治癒よりきれいに3週間以内に治癒する（Peter and Killick-Kendrick, 1987）という。本症例も，日本で外科的切除を勧められたが，既に皮膚リーシュマニア症には自然治癒もあり得，また全身に感染が広がる重篤な疾病ではないという知識を持っていたので，切除は希望しなかった。外科的切除は，顔面などの不適な部位もあり，また広範囲の摘出や部位によっては，機能障害を生ずる場合がある。有効で副作用が少ない内科的治療法が確立されれば，一般には外科的切除より優先されるべきであろう。放射線照射は，自然治癒が平均9カ月かかるのに，86例中84例で7または8週間で治療できたという報告（Peter and Killick-Kendrick, 1987）があるが，放射線の人体への影響も考慮する必要がある。凍結療法は，患者がサウジアラビアの病院で通院中観察していると，痕跡が残っても差しつかえない部位には，積極的に行っていたという。本症例は，サウジアラビアで2回凍結療法を受けているが，1988年2月16日に2回目が終了した1カ月半後の3月31日と，3カ月後の5月19日に，原虫が皮疹より培養された。矢後，白坂(1989, 1990)は，NNN培地で培養中のKN株を，7 ℃に10日間静置して観察したが，原虫は活発な運動性を保持していたので，低温には抵抗性を持つと考えている。また，50%にグリセリンを加えた培養液中のKN株を，−80℃で約1カ月間凍結保存した後融解してみると，運動性を取り戻し，また継代培養も可能であった。したがって，本症例に施行された−35℃1分間位を2回施行した温度と時間では，原虫は死滅しない可能性が考えられる。凍結療法が本症の治療に有効であるならば，低温のために原虫が死滅するのではなく，原虫が寄生している組織が，低温により破壊されたり壊死に陥り，その結果，原虫の生存条件が2次的に悪化し，弱化または死滅するのではないかと推察している。一方，温熱療法は，粘膜皮膚リーシュマニア症の治療にも用いられている（Rodbard, 1979）。Berman and Neva (1981)によれば，皮膚リーシュマニア症を生ずる*Leishmania* speciesは，37℃またはそれ以上の温度では，長期間生存できない。また，Ashi *et al.* (1980)は，サウジアラビアでの皮膚リーシュマニア症の発症数は，毎年10月または11月頃より増加しはじめ，1月または2月に最多数を記録し，4月頃より減少し，7月と8月に最少になるという。このような変動は，皮膚が直接触れる外気温の変化とは，無関係ではないと考えられる。そこで，温度負荷による殺KN株効果を，運動性と増殖数を示標に実験（矢後，白坂，1989；矢後，1990）し，42℃で2時間継続保温すれば，原虫が死滅するのを確認し，治療に応用した。患者が不動に近い姿勢を長時間保たねばならない負担と，低温火傷および保温時間を中断した場合の殺KN株効果の減弱などの実験結果（矢後，1990）から，実際には皮膚温は43℃を目標に，1日に合計3時間保温し，少なくとも1時間は継続し，1時間経過後に中断する場合も15分以内とし，3日間以上連続して保温するのが有効と考えられた。本症例では，第1回目は赤外線ランプを利用したが(表2)，赤外線ランプによる保温では来院しなければならず，多忙な患者には負担が大きかった。そこで，治療時期が7月より10月と，外気温の高い時期であったので，写真5のようにビニール袋に温湯を入れ皮疹を暖めたが，冬期には使い捨て懐炉の使用が便利と考えている。試みに，市販の使い捨て懐炉の中から，最高温度66℃，平均温度50℃（都条例による測定値），持続時間18時間（日本工業規格による測定値）の懐炉を用いて，成人男子の大腿伸側を保温すると，東京の11月下旬で，懐炉と皮膚の間に4枚のガーゼを挟んだ時，皮膚とガーゼの間の温度は約40℃であった。ガーゼの枚数や衣服を調節すれば，42℃の保温は可能であろう。懐炉による保温は，低温火傷に注意しさえすれば副作用もなく，仕事をしながらでもできる推薦できる治療法と考えている。本症例では，Metroni-

dazoleの内服治療を3クール行い,原虫が皮疹より検出されなくなってから，補助療法として温熱療法を行ったので，温熱療法が殺原虫効果を示したとする直接的な証明にはならない。しかし，原虫の採取培養を行いながら，温熱療法をMetronidazoleの投薬より先に行う症例数を増やせば,この点はより明らかにできるであろう。

サウジアラビアで，*L. tropica*を媒介するサンドフライの種は，主に*Phlebotomus*と*Sergentomyia*である（Ashi *et al.*, 1980)。日本では，*Phlebotomus*のような体長約5 mm（Viqar, 1983）という，患者の言によれば，60wの電球を近づけてやっと見えるぐらいの，埃のような小さな飛行する虫に刺される経験が乏しかったため，最初患者は，掻痒感を原因不明の体調不良によるものと思っている。そこで，健康に注意してみたが，なお同じ症状が続くので，次にはイエダニの発生を考え，衣類などは日光にあて，身のまわりの清潔に心掛けた。しかし，掻痒感は減少せず，なお注意して観察し，刺咬部位が露出部に多く，また空気中に浮かんでいる，白い小さな埃にしては動きの不自然な浮遊物に気がついた。また刺咬された直後に，白い腹部をピンク色に染め，フラフラしている小さなハエのような虫を捕らえて，圧平して血液を認め，初めて吸血する虫による刺咬と判明した。多いときには，1度に3匹も捕まえたことがあったという。そして，患者はサウジアラビアの皮膚科医から，皮膚リーシュマニア症の参考文献を入手し，自分の体験と合わせ，同地に引き続き滞在する同僚のために，以下のことを進言している。*Phlebotomus*の飛行距離は，約150 m(Ashi *et al.*, 1980)以内であるので,宿舎の周囲は150m以上空き地にし,窓には網目の細かい防虫網をはり，暑くても裸で屋外では眠らず，外出時には，長袖シャツ，長ズボンや靴下などを着用し，なるべく露出部を少なくする。敷地内では，イヌ，ネコ，ニワトリ，ウサギなどの動物は飼わず，ネズミの巣などは片づけ，また外部からもこれらの動物が侵入しないように囲いをし，*Phlebotomus*の繁殖のサイクルやヒトとの接触を妨げる。日中，*Phlebotomus*が潜んでいそうな暗い場所や，洗面所，風呂場，そして出勤時には自室にも殺虫剤を散布し，下水の蓋の隙間などはふさぐ。サウジアラビアに滞在中は，皮膚をよく観察し，発赤腫脹や硬結に注意し，帰国時には治療薬を持って帰ることなどである。患者は当時，Pentostamが特効薬で，日本では入手できず，治療経験のある医師はいないと考えていたが，その他のことは大変参考になる提言である。実際，*Phlebotomus*の飛行距離は短く，しかも休み休み飛行するので，殺虫剤は窓の周囲や扉などにも噴霧するのが有効である（Michael *et al.*, 1989)。しかし，どんなに用心しても，流行地で感染したサンドフライによる刺咬を避けるのは難しい。患者は，サウジアラビアの病院で，顔面に罹患した多くの人々，とりわけ鼻部が変色し，鼻下に4 cm四方位の，一部が黒化した潰瘍形成を生じた2歳位の女児を見た時には，その児が成人した時のことを考え，心が痛んだという。リーシュマニア原虫の感染のサイクルを断つ努力とともに，有効で副作用の少ない予防接種の開発と普及が望まれる。

皮膚リーシュマニア症患者を治療する場合，内蔵型の合併の有無には，常に注意する必要がある。分離される原虫の形態からは，皮膚型と内蔵型の鑑別は困難なので，臨床症状や検査成績に加えて，渡航地や渡航経路が参考になる。内蔵型は，中近東ではイランなどサウジアラビア周辺には，一般的に分布しているが，サウジアラビアには少なく（Ashi *et al.*, 1980)，アラビア半島南西部のイエメンに近い地域にのみ発症例が報告されている（Peter and Killick-Kendrick, 1987)。患者が滞在していたサウジアラビア，カシーム地区の皮膚科医に，1988年1月に送った質問の手紙に対し，本症例が感染したと推定されるブレイダ市近郊には，内蔵型の発症例はないという返信が得られた。同市近郊には皮膚型が多く，1975年より1979年までの5年間に，サウジアラビア各地の病院より報告された4,352例の皮膚リーシュマニア症例の1,853例(42.6%)は，カシーム地区から報告されている（Ashi *et al.*, 1980)。内蔵型の症状は，持続する発熱，悪寒，紫斑，肝脾腫，貧血，好中球減少，肝機能異常，IgGの多クローン型増加によるA/G比の低下などであるが，本患者にはA/G比の軽度低下が観察された他には,相当する

検査成績や症状などはなく，また発生年齢も多くは9歳以下の幼児や乳児であることなどを考慮し，内蔵型の合併はなかったものと判断した。

皮疹から原虫が培養されなくなった1988年6月23日以後も，同10月6日，1989年3月2日および7月14日と皮番3に発赤腫脹が繰り返し生じた。しかし，原虫の採取培養を行っても，原虫は検出されなかった。発赤腫脹部には，膿汁，鬱血や痂皮などが確認されているので，細菌の感染などによる二次的な反応と推定しているが，患者は特に皮膚に傷をつけるようなことはしていないという。Peter and Killick-Kendrick (1987) によると，皮疹は傷を受けやすく，リンパ管炎，蜂窩織炎，丹毒などを起こし易く，また薬剤の注入により，アレルギーを伴った湿疹性反応を起こすことがあるという。本症例で，繰り返し生じた発赤腫脹も，感染のほかに何らかのアレルギー性反応を合併していた可能性もある。

皮膚リーシュマニア症は，日本では頻発する疾患ではないので，皮膚科医にも診断や治療の経験が少ないと思われる。本患者が，帰国直後に訪ねた皮膚科医には，蚊刺ではないかといわれ，また会社の同僚からも，小さな皮疹を恐れる患者は，理解されなかった。幸いサウジアラビアには，人々が大変恐れている皮膚病があることを知っていたので，患者は現地の医師に直接相談し，皮膚リーシュマニア症と診断された。治療経験の豊かなサウジアラビアの皮膚科医により，皮膚型が内蔵型に移行することはなく，また滞在していた地域には内蔵型の発症例はなく，皮膚型は生命の危険がない上，特別な治療をしなくとも自然治癒があり得，1988年1月24日の初診時には，小さな発赤腫脹が1カ所だったので，凍結療法が適応され得るなどの説明を受け，また皮疹の部位も成人男子の場合，あまり人目にさらす場所でない大腿であったことなどもあり，一応の落ち着きを得た。しかし日本では，特に熱帯病や特殊な皮膚病に関心を寄せていない医師には，皮膚リーシュマニア症の有効で副作用の少ない治療法や，治療薬などについての情報が得にくい。海外渡航が日常化され，日本にはない疾病が，即時に国内にもたらされる昨今，熱帯病についての知識や治療法，特殊な医薬品の入手方法などについても，日頃から準備しておく必要があると痛感させられた症例であった。

結　語

サウジアラビアに滞在し，皮膚リーシュマニア症に感染した51歳の日本人男子に，原則として，Metronidazoleを1日750 mg分3で10日間内服後，10日間の休薬を1クールとし3クール行い，その後，皮疹を42°Cより43°Cに合計30時間保温し，完治した症例を報告した。

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TREATMENT OF CUTANEOUS LEISHMANIASIS
—REPORT OF A CASE TREATED WITH METRONIDAZOLE AND LOCAL HEAT—

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A 51-year-old Japanese male who had spent six months in Saudi Arabia was diagnosed by biopsy in his right thigh as having cutaneous leishmaniasis and was successfully treated with metronidazole and local heat. The dose of metronidazole used was 250 mg three times a day, and the drug was given for three ten-day periods with intervening rest periods of ten days. Leishmania promastigotes could be cultured from the lesion on the first day that metronidazole was administered but could not be cultured after two ten-day periods of treatment with the drug. After medication was given, local heat therapy was applied at 42°C, using hot water in a vinyl bag, for a total of 29 hrs, and heating under an infrared lamp at 43°C for 1 hr on one occasion only. Details of heat treatment are given in Table 1.

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日熱医会誌.，第19巻　第1号　1991年，15-23頁

高知県の大複殖門条虫症
—12症例の追加—

鈴木　了司・今村　京子

平成2年10月19日受付/平成2年11月22日受理

大複殖門条虫症は日本にのみ見られ，いまだにその中間宿主が不明な寄生虫症であるが，高知県では本症例が多く，既に21例に達している（鈴木ら，1988)。これら症例は，県内医療機関から同定を依頼されて見出されたものであるが，県内の医療機関には，未同定，あるいは誤って同定されたままの本条虫の存在が推定される。

そこで，アンケート調査を行ってその存在を確かめるとともに，最近の本条虫症について若干の疫学的考察を行った。

方　法

県医師会の協力を得て，1989年5－7月に，寄生虫症に関連があると思われる462医療機関に対し，過去に条虫駆虫とその虫体の有無についての回答を求めた。虫体が存在する場合には，それらを借り受け，または恵与頂き，その虫体の形態を調べた。

また，鈴木ら（1988）の報告以降，1989年末までに寄生虫学教室に同定の依頼があった大複殖門条虫の症例をこれらに加えた。

成　績

1．アンケート調査による症例

462医療機関のうち，289機関から回答(62.6%)を得たが，条虫駆虫の経験があると答えたのは15機関であった。このうち，9機関は虫体が既に存在せず，6機関に11例からの12虫体（内2虫体は同一患者からの排出）が存在し，7例が大複殖門条虫，3例が無鉤条虫，1例が米子裂頭条虫と同定された。以下，それらの症例について簡単に記載する。

1）県中央部の山間地にある嶺北中央病院に保管してあった虫体で，患者の住所が大豊町以外は不明。長さ92.5cm，幅7mmで頭節はない。

2）県東部の宮田医院に保管してあった虫体で，45歳，男性，北川村在住。1973年4月18日に肛門より虫体の1/5をぶら下げた状態で受診。割箸で巻取ったもので，長さ43.5cm，幅8mm，頭節を欠く。虫体排出前後の症状は，特に異常ない。ウルメイワシ，マイワシ，アジ，カツオなどの刺身や酢のものを毎日のように食べていた。この患者は，上記2種のイワシを区別していた。サバはあまり食べない。

3）県東部の県立安芸病院の保管虫体で，安芸市在住の51歳の女性。1987年2月28日に腹痛。夜半に便意があり，排便とともに長さ576cm，幅16mmの頭節のない成熟虫体を排出した。魚の嗜好については不明。

4）症例3と同一病院に保管されていた虫体で，患者に関しては不明。長さは46.3cm，幅5mmの頭節のない未成熟虫であった。

5）県のほぼ中部に位置する西本医院に保管してあった虫体で，患者は年齢不明の男性，中土佐町在住。1966年11月初旬，長さ615.5cm，幅15mmの頭節のない成熟虫体を，排便とともに排出。軽度の貧血以外は特に異常はない。続いて同年12月30日に長さ772.0cm，幅14mmの頭節のない虫体

高知医科大学寄生虫学教室

を再び排出した。職業は漁師で，あらゆる海産魚を食べている。

6）県西部の県立西南病院に保管中の虫体で，33歳の男性，住所不明。1981年5月12日に条虫を排出したため，Bithionolで駆虫し，同18日に272.1cm，幅15mmの頭節を有する成熟虫を排出した。魚貝類の嗜好は不明。

7）症例6の病院に同じく保管中の虫体で，1982年5月末から1日4－5回の水様性の下痢。6月4日夜に下痢便とともに約1mの虫体を排出。続いて5日に長さ不明の虫体を，6日に270cm，幅8mmの頭節のある成熟虫を排出した。標本として保管されていたのは，6日の分である。患者は56歳の男性で佐賀町在住。魚貝類の嗜好状況は明らかではない。

2．同定依頼による症例

鈴木ら（1988）による本症の報告以来，直接に医療機関から同定を依頼された大複殖門条虫症例は5例あった。

1）68歳，男性，須崎市在住。菅野医院より同定依頼。1988年12月11日に下痢とともに虫体を排出したが，約1mを便槽内に切り捨て，その残りをもって受診。虫体の長さは89.5cm，幅16mmで頭節はない。駆虫はしていないが，その後の再三の検便で陰性。酢のものを含むアジ，サバ，カツオ，イワシなどの海産魚の生食を好み，イワシの稚魚であるドロメも頻繁に食べている。横川吸虫陽性。

2）53歳，男性，土佐市在住。伊藤医院より同定依頼。1週間前から下痢と腹痛があり，1989年12月21日に2回と，21日に1回の計3回にわたり虫体排出。長さの合計は499cm，幅15mmで頭節を欠く。後検便は陰性。海産魚を好み，カツオ，マグロ，ドロメをよく食べるという。

3）56歳，男性，高知市在住。高知市民病院より同定依頼。1989年6月15日朝，排便直後に未熟虫体を排出。長さ62cm，幅10mmで頭節はない。患者は健康で，異常は認められない。海産魚の刺身が好きという。

4）45歳，男性，高知市在住。高知医大第三内科より同定依頼。1989年6月28日に排便後，トイレットペーパーに付着している虫体を持参して受診。長さ47.1cm，幅10mmの未熟虫で頭節はない。3日前より下痢気味で，受診当日の夜にも虫体の排出があったが，その虫体はない。患者はほとんど毎日，カツオ，マグロ，ブリなどの刺身を，また，

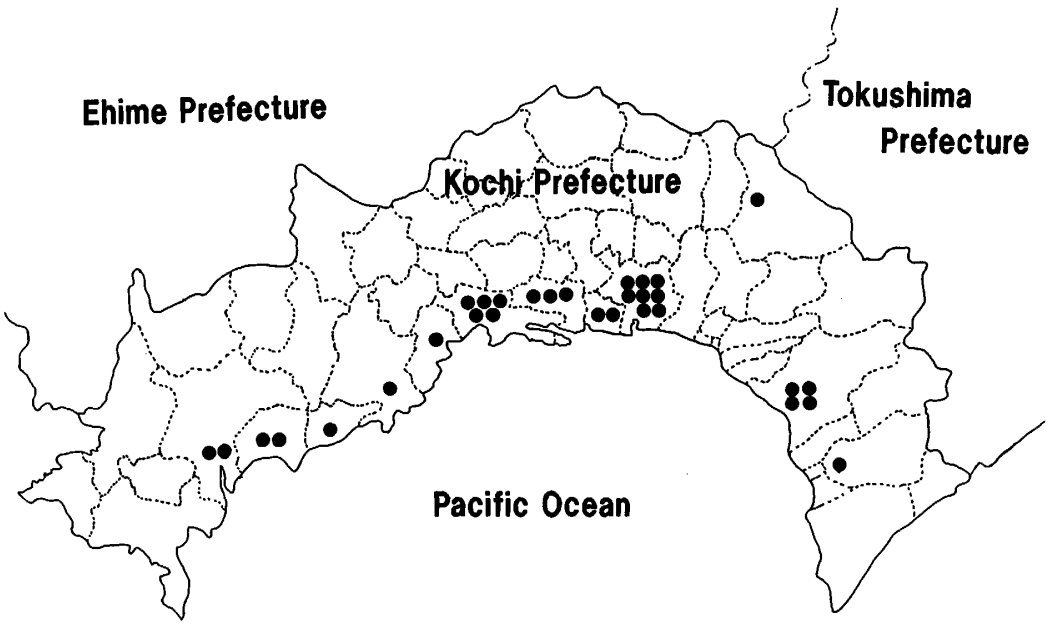

Figure 1　Geographical distribution of diplogonoporiasis within Kochi Prefecture.

約2週間前に3日ほど連続してドロメを食べている。

5）46歳，男性，春野町在住。1989年7月19日に，高知市民病院で糞便検査で虫卵が検出され，同月21—22日にParomomycin投与。翌23日に長さ284cm，幅13mmの成熟虫を排出。頭節を認めた。

これら12例の症例を含め，現在までに県内の本症患者の居住地を地図上に表したものが図1である。ただし，患者の住所不明の1症例は除いた。

考　察

1．アンケート成績

条虫駆除の経験があったと答えたのは，289機関中15機関であり，そのうち，虫体が存在したのは6機関で，その11例中7例が大複殖門条虫と同定され，予測したように新たに本症例を確認することができたが，大複殖門条虫と同定されていたものは全くなく，広節裂頭条虫と同定されていたもの3例，有鉤条虫と同定されていたもの1例，単に条虫とされていたもの3例であった。このような同定結果や，9機関で虫体が既に廃棄されており，検査し得なかったことは，条虫症に対する関心が必ずしも充分でないことを示している。

2．高知県下の本条虫症

県下の本症例は今回のアンケート調査による7例，同時期に同定依頼された5例を加えると33例に達した。岩田，安岡（1970）による第1例以来，1979年までは4例に過ぎなかったが，1980年代には27例（2例は虫体排出日不明で除外）となり，急激な増加が認められた。更に虫体標本が廃棄された9医療機関では，無鉤条虫としたもの3例，広節裂頭条虫としたもの1例，不明6例で，この中にも大複殖門条虫が存在していた可能性がある。

患者は従来指摘されているように，中間宿主が疑われる海産魚を手に入れやすい海岸に面した市町村に多いが(図1)，海岸から離れた山間地に1例の存在を認めた。この患者は大豊町在住者という以外，記録がないため，どこで中間宿主となる海産魚を食べたかは明らかではない。しかし，この病院では年間，30例近くのアニサキス症患者を認めていることから(神崎ら，1987)，海産魚類の流通機構の整備，嗜好魚類の変化，内陸部住民の海岸地方への旅行，その他により，今後は海岸部ばかりではなく，内陸部にも発生が認められる可能性が強い。

季節的には不明の3例を除くと，1980年以降，1—2月に8例，3—4月に3例，5—6月に7例，7—8月に2例，9—10月4例，11—12月に3例となり，1—2月と5—6月に多く，夏期に少ない傾向にある。特に冬期にも患者が見られることは，他県（前嶋ら，1990）と異なる。

本虫寄生による症状は，下痢，腹痛などの消化器症状があげられているが，今回の場合も，患者は数日前から下痢が続き，その後に自然排出しており，そのほかには症状としては特に異常は認められない。

3．全国的な症例の推移

鈴木ら（1985，1988）は全国の症例を整理したが，洩れていた症例（高田ら，1982；Terada *et al.*, 1985；板垣ら，1988）や，関東地方を中心に各地からの症例（安羅岡ら，1989；赤尾，1989；金子ら，1990；前嶋ら，1990；高尾ら，1990；影井ら，1990）が報告され，また，寺田ら（私信）の未発表分（4例）があり，1989年末までに本症の合計は162例となった（表1）。87例までは，鈴木ら（1985）の報告と同一のため表から省略した。これらの症例は論文としての発表順であることが望ましいが，学会のみの報告もあるため，すべて，最初に報告された時点の順に並べてある。本報告の12例は1989年に学会発表をしているため，教室での受け付け順で全国症例の130—141例に相当する。

これら症例を虫体の排出時期により分けると，最初に患者が見出された1894年以降1979年までの85年間に85例（排出日不明は10例あるが，著者の症例発告時が1979年以前の6例を加えてある）であったが，1980年以降の10年間には73例となり，全国的に症例数が増加している。これを本州東部太平洋域(福島—静岡)，本州西部太平洋域(瀬

Table 1 Summary of reported cases of diplogonoporiasis in Japan until 1989. All cases are re-numbered according to the date of report.

Case No.	Report		Patient					Worm found		
	Author	Year	Locality	Date found		Age	Sex	Length (cm)	Width (mm)	Scolex
88	Takada *et al.*	1981	Osaka	Dec	1980	63	M	174.0	13.0	−
89	Saiga *et al.*	1983	Kochi	Apr	1983	46	M	164.4	4.4	−
90	Sano	1984	Shizuoka	Aug	1983	49	M	380.0	18.0	−
91	Oshima and Amano	1984	Kanagawa	Apr	1984	58	M	145.0	4.0	−
92	Oshima and Amano	1984	Shizuoka	Jun	1984	65	M	410.0	14.0	−
93	Suzuki *et al.*	1984	Kochi	Jul	1982	74	M	341.0?	8.0	−
94	Suzuki *et al.*	1984	Kochi	May	1983	41	M	14.0	4.0	−
95	Suzuki *et al.*	1984	Kochi	Jun	1983	55	M	11.0	7.0	−
96	Suzuki *et al.*	1984	Kochi	Sep	1983	57	M	511.0	10.0	−
97	Suzuki *et al.*	1984	Kochi	Nov	1983	39	M	130.0	4.0	−
98	Suzuki *et al.*	1984	Kochi	Jan	1984	63	M	97.0	16.0	−
99	Suzuki *et al.*	1984	Kochi	Feb	1984	53	M	889.3	15.0	−
100	Suzuki *et al.*	1984	Kochi	Oct	1984	46	M	268.0	6.0	−
101	Kushima and Imai	1984	Miyazaki	Jun	1984	36	M	209.0	8.1	−
102	Ueta *et al.*	1985	Kochi	Jan	1985	39	M	67.0	4.5	−
103	Miyahara and Maejima	1985	Fukuoka	Feb	1984	42	M	80.0	6.5	−
104	Suzuki *et al.*	1985	Shizuoka	?	?	50	M	30.0	9.0	−
105	Kanazawa *et al.*	1985	Chiba	Dec	1984	50	M	343.0	7.3	−
106	Kagei	1985	Kanagawa	?	?	?	?	500.0	17.0	−
107	Terada *et al.*	1985	Shizuoka	May	1981	31	M	360.0	9.0	+
108	Mochizuki *et al.*	1986	Shizuoka	Aug	1983	4	F	200.0	10.0	−
109	Mochizuki *et al.*	1986	Shizuoka	Apr	1985	60	M	62.0	10.0	−
110	Mochizuki *et al.*	1986	Shizuoka	May	1985	81	M	280.0	10.0	−
111	Mochizuki *et al.*	1986	Shizuoka	May	1985	50	M	50.0	5.0	−
112	Mochizuki *et al.*	1986	Shizuoka	May	1985	43	F	180.0	7.7	−
113	Mochizuki *et al.*	1986	Shizuoka	May	1985	47	M	60.0	5.0	−
114	Mochizuki *et al.*	1986	Shizuoka	Jun	1985	42	F	45.0	3.0	−
115	Mochizuki *et al.*	1986	Shizuoka	Jun	1985	43	M	63.0	3.0	−
116	Inutsuka *et al.*	1986	Nagasaki	Feb	1986	60	M	692.0	9.0	+
117	Okamura *et al.*	1986	Kochi	Feb	1985	54	M	505.0	2.3	−
118	Okamura *et al.*	1986	Kochi	Nov	1985	32	M	118.0	4.3	−
119	Yamane *et al.*	1987	Shimane	Feb	1984	60	M	300.0	5.8	−
120	Nishiyama *et al.*	1987	Osaka	Sep	1986	55	M	273.0	13.0	+
121	Yamamoto *et al.*	1987	Hyogo	May	1987	64	F	200.0	20.0	+
122	Tanaka *et al.*	1987	Kanagawa	May	1987	58	M	1,067.0	12.0	+
123	Okamura *et al.*	1988	Kochi	Oct	1986	54	F	342.5	10.0	−
124	Okamura *et al.*	1988	Kochi	Mar	1987	48	M	306.4	9.0	−
125	Okamura *et al.*	1988	Kochi	Sep	1987	39	M	393.4	10.0	−
126	Okamura *et al.*	1988	Kochi	Jan	1988	43	M	118.0	13.0	−
127	Okamura *et al.*	1988	Kochi	Mar	1988	52	M	45.0	7.0	−
128	Itagaki *et al.*	1988	Yamaguchi	Jun	1988	26	M	248.0	6.1	+
129	Yasuraoka *et al.*	1989	Ibaraki	Aug	1988	57	M	490.0	15.0	−

Case No.	Report: Author	Year	Patient: Locality	Date	found	Age	Sex	Worm found: Length (cm)	Width (mm)	Scolex
130	Suzuki *et al.*	1989	Kochi	Dec	1988	68	M	89.5?	16.0	−
131	Suzuki *et al.*	1989	Kochi	Jun	1989	53	M	499.0	15.0	−
132	Suzuki *et al.*	1989	Kochi	?	?	?	?	92.5	7.0	−
133	Suzuki *et al.*	1989	Kochi	Apr	1973	45	M	43.5	8.0	−
134	Suzuki *et al.*	1989	Kochi	Jun	1989	56	M	62.0	10.0	−
135	Suzuki *et al.*	1989	Kochi	Jun	1989	45	M	47.1	10.0	−
136	Suzuki *et al.*	1989	Kochi	Feb	1987	51	F	576.0	16.0	−
137	Suzuki *et al.*	1989	Kochi	?	?	?	?	46.3	5.0	−
138	Suzuki *et al.*	1989	Kochi	Nov	1966	?	M	615.5	15.0	−
				Dec	1966	?	M	772.0	14.0	−
139	Suzuki *et al.*	1989	Kochi	Jul	1989	46	M	284.0	13.0	+
140	Suzuki *et al.*	1989	Kochi	May	1981	33	M	272.1?	15.0	+
141	Suzuki *et al.*	1989	Kochi	Jun	1982	56	M	270.0?	0.8	+
142	Kaneko *et al.*	1989	Fukushima	Aug	1989	49	F	100.0	8.0	−
143	Akao	1989	Ibaraki	Aug	1988	47	M	600.0	20.0	+
144	Akao	1989	Ibaraki	Sep	1988	50	M	200.0	20.0	−
145	Akao	1989	Shizuoka	Oct	1988	52	M	?	15.0	−
146	Akao	1989	Tokyo	Oct	1988	42	M	?	20.0	−
147	Maejima *et al.*	1990	Tottori	Jun	1978	41	M	67.0	6.0	−
148	Maejima *et al.*	1990	Shimane	Mar	1980	51	M	545.0	10.0	−
149	Maejima *et al.*	1990	Tottori	Jul	1984	82	M	64.0	5.5	−
150	Maejima *et al.*	1990	Tottori	Apr	1986	57	M	360.0	13.0	−
151	Maejima *et al.*	1990	Tottori	Jun	1987	39	M	94.0	5.5	+
152	Maejima *et al.*	1990	Nagasaki	Mar	1985	54	M	192.0	9.0	−
153	Maejima *et al.*	1990	Kagoshima	Apr	1985	62	F	51.0	15.0	−
154	Maejima *et al.*	1990	Yamaguchi	Apr	1987	41	M	89.0	5.0	−
155	Maejima *et al.*	1990	Oita	Apr	1988	58	M	12.0	20.0	−
156	Takao *et al.*	1990	Saga	Mar	1989	81	M	100.0?	?	−
157	Kagei *et al.*	1990	Tokyo	Feb	1989	23	M	224.0	?	−
158	Kagei *et al.*	1990	Chiba	Dec	1989	42	M	20.0	8.0	−
159	Terada *et al.*	unpublished data	Shizuoka	Nov	1980	?	?	135.0	7.0	−
160	Terada *et al.*		Shizuoka	Jun	1982	53	M	57.0	8.0	−
161	Terada *et al.*		Shizuoka	Jun	1983	83	F	36.0	6.0	−
162	Terada *et al.*		Shizuoka	May	1985	50	M	?	?	−

戸内海を含む愛知—宮崎)，日本海域（鳥取—山口)，九州西岸域の4域に便宜的にわけて，1979年以前と1980年以降とを比較すると，後者は前者のそれぞれ，1.40，1.83，0.32，0.20倍となり，本州東部域と西部域の太平洋岸では明らかに増加が認められるが，後二者は減少している。特に1960—1970年代に，多くの患者を認めた山陰地方を含む日本海域は近年少ない。本州西部域での増加は，主として高知県での著しい増加（6.8倍）による。本州東部域では，長い間，神奈川県以西に限られていたが，1984年以降，東京都2例，千葉県2例，茨城県3例，福島県1例が追加され，本症の分布地が北へと拡大していることは注目に値する。この地域に属する静岡県は，1979年までは20例で

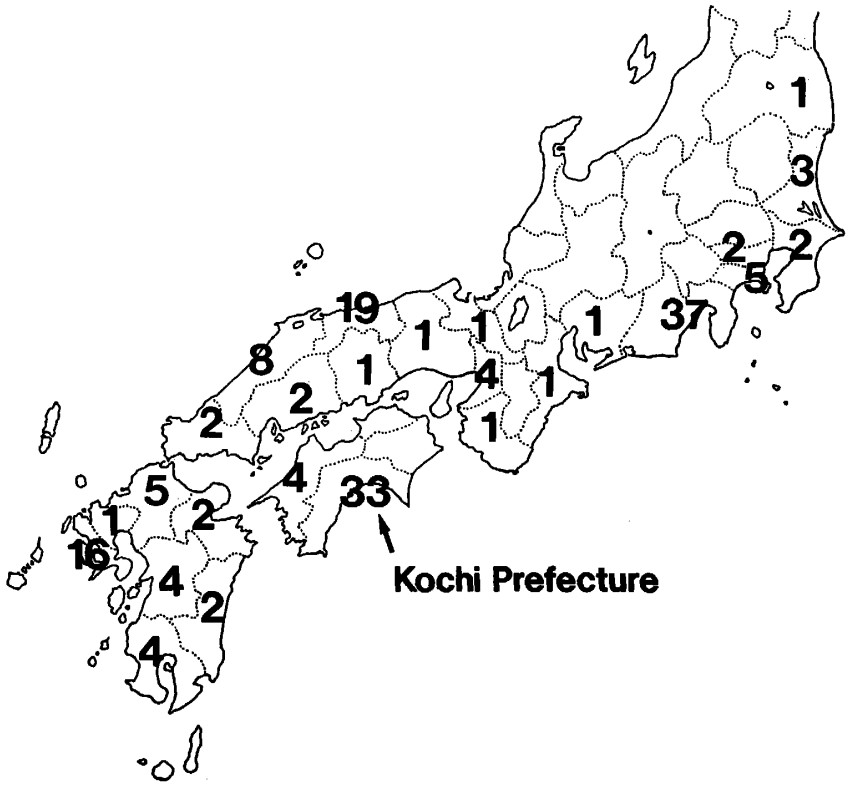

Figure 2 Prefectural incidence of diplogonoporiasis. Numbers of reported cases are indicated on the map.

あったが，1980年以降は17例で比較的平均して患者が見出され，実数としては日本で現在最も多い（図2）。

人口10万人当たりの発生数については，鈴木らが既に報告したが，1980年以降1989年までの10年間の本症例数を，患者数の多い県で改めて計算し直すと(1985年の国勢調査の人口に基づく)，長崎県0.12，島根県0.25，静岡県0.48，鳥取県0.49，高知県3.10で，高知県は静岡県の6.5倍となり，高知県が圧倒的に多い。

このように高知県に本症が多いことは，中間宿主となる海産魚の回遊状況と手に入れやすさ，それらの魚の生食の多寡や，慣習などが関与していると考えられる。

4．高知県の本症の中間宿主についての検討

本症がIijima and Kurimoto (1984) によって報告されて以来，中間宿主，つまり感染源は不明であるが，本来の終宿主と考えられるイワシクジラは北海道，三陸沖でカタクチイワシ，マイワシ，サンマ，サバ，オキアミ類を，コイワシクジラは日本海でカラヌス，オキアミ，イワシ，イカナゴを主要な餌としている（根本，1962：加茂，1969より引用)。一方，イワシ，アジ，サバ，カツオ，ハマチなどが患者の食生活から指摘され，なかでも，イワシ類が当初から中間宿主として疑われ，平井ら（1976)，安羅岡ら（1989）および前嶋ら（1990）はカタクチイワシの稚魚ではないかとしている。

高知県の症例からは，今回も魚を特定し得なかったが，南西海区水産研究所によると，高知周辺で比較的冬期にも漁獲があり，生で食べることが多く，本州北部には少ないが，もしくは見られない海産魚として，ゴマサバ，ウルメイワシ，カタクチイワシ，キビナゴ，ブリ（ハマチ）などが考えられる。このうち，キビナゴは高知県西部で

主として漁獲され，生で食べられる機会は多く，愛媛県の4症例中，3例が四国西部の患者ではあるが，図1に示されるように，高知県西部では本症の患者は少ないことから疑問視される。

一方，高知県では，新鮮なイワシの稚魚をヌタ，もしくは二杯酢で食べるドロメ料理による感染の可能性については，著者（鈴木ら，1985, 1988)が既に指摘している。この料理は季節により，イワシの種類の混合比が異なる。最近，漁獲高が多く，ドロメの大半を占めているマイワシは，冬から翌年5月位までが最盛期であるが，北方でも漁獲され，北海道でも獲れることから，本症の北限を考えると否定的である。ウルメイワシの稚魚は，高知では秋から翌年初夏にかけて採取され，カタクチイワシは年間採取されるが，6月から秋にかけて混合の割合が増加する。また，イワシは稚魚をドロメとして食べる以外にも，ウルメイワシ（秋から冬）の成魚は高知県で生で食べる習慣があるが，マイワシ（夏から冬）とカタクチイワシの成魚（冬から春）は生で食べない。ゴマサバ（秋から冬）やブリが，幼魚時代に自然感染して感染源となる推定，また，平井ら（1976）は自然感染したカタクチイワシを餌にすることによって，養殖ブリが感染するという推定も否定できないが，餌のイワシは冷凍されている場合が多く，また，サバやブリが遠隔地への輸送が可能なことから，患者が海岸部以外にも発生しなければならない。一方，イワシの稚魚は生での輸送が困難なこともあり，高知県での感染源としてはカタクチイワシやウルメイワシの稚魚，なかでも後者が疑われる。

そこで，1988—1989年に高知県沿岸で採取されたカタクチイワシの稚魚19,868匹，マイワシの稚魚17,550匹，ウルメイワシの稚魚6,952匹を圧平して解剖顕微鏡下で筋肉，内臓などを検査したが，plerocercoidを検出し得なかった。しかし，1回に食べるドロメ料理の量は魚の大きさにもよるが，約200—300匹であり，この検査数では約220—150回分にしか相当しないので，これによって寄生の有無を議論するには検査数が不足であり，本条虫の高知県の感染源の決定については，今後の研究に待ちたい。

5．米子裂頭条虫症ほか

今回のアンケート調査により，高知県西部の病院から1例の米子裂頭条虫が得られた。本条虫はYamane *et al.*（1981）および加茂ら（1982）により，広節裂頭条虫とは異なる種として記載され，四国からは平井ら（1988）によって愛媛県，および高知県から既に見出されているが，今回の症例で，高知県から第2例が得られたことになり，大複殖門条虫症例と共に，高知県が海産魚を中間宿主とする寄生虫に感染する機会が多いことを示している。また，無鉤条虫が3例認めたことは，高知県に本条虫症が存在することを示す。

要　約

高知県では既に21例の本症が報告されているが（鈴木，1988），今回12例が追加され，合計33例に達した。また，現在までの我が国の本症の報告を調べたところ，未発表を含めて162例であり，高知県は本症が極めて多く，特に近年になって症例が増加していることを明らかにした。

謝　辞

アンケート調査に当たり，高知県医師会および各医療機関から多大のご協力を得た。また，水産庁南西海区水産研究所古藤　力技官，花岡藤雄技官には魚類についての御教示を，高知県水産試験場山重政則海洋資源科長に，イワシの稚魚の御供与を得た。ここに深謝する。

さらに，鳥取大寄生虫学教室前嶋條士博士に，米子裂頭条虫の同定をして頂いたことを付記して謝意を表する。

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ADDITIONAL 12 CASES OF DIPLOGONOPORIASIS IN KOCHI PREFECTURE

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Twenty-two cases of *Diplogonoporus grandis* infection have already been reported from Kochi Prefecture, and in this report, additional 12 cases were described.

In contrast to other regions, Kochi Prefecture aparently exhibits a marked increase in the incidence of cases in recent years.

Since the first description of this tapeworm was reported by Iijima and Kurimoto (1984) in Nagasaki Prefecture, the 162 cases of human infection with the worm were recorded in Japan in the literatures until 1989.

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ELECTRON MICROSCOPICAL OBSERVATIONS ON GAMETOGENESIS AND FERTILIZATION IN CULTURED *PLASMODIUM FALCIPARUM*

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Abstract: Electron microscopical studies were performed on gametogenesis and fertilization of the gametes developed in the prolonged culture of *P. falciparum* gametocytes which appeared during the culture of the parasites in the gametocytogenesis-induction medium. When the erythrocyte plasma membrane separated from the gametocyte during gametogenesis, a small part of the gametocyte cytoplasm which was enveloped with the multilayered sheaths of the erythrocyte plasma membrane, was released from the gametocyte into culture medium. We could observe the process of penetration of the macrogamete cytoplasm by the microgamete and then the fusion of the membrane surrounding microgamete with the nuclear envelope of macrogamete. From the findings of two microgamete nuclei were present in the cytoplasm of a macrogamete at fertilization, it is assumed that the multiple nuclear-fusion may occur.

INTRODUCTION

The process of gametogenesis and fertilization of *Plasmodium* have been examined with electron microscope (Sinden *et al.*, 1976, 1978; Aikawa *et al.*, 1984). Sinden *et al.* (1976) described the fusion of the plasma membrane of male and female gametes at fertilization in *P. yoelii*. Aikawa *et al.* (1984) observed in *P. gallinaceum* that the male nucleus appeared to travel through a channel of the endoplasmic reticulum to a region of the female nucleus. However, these workers did not observe the process of penetration of the macrogamete cytoplasm by the microgamete and the subsequent nuclear fusion of these gametes. Since Ono *et al.* (1986) introduced the gametocytogenesis induction method for *P. falciparum* strains which seldom produce gametocytes in the routine culture, it has become easy to study gametocytogenesis *in vitro*. Ono and Nakabayashi (1989) found that the gametocytes which appeared in the gametocytogenesis-induction medium developed subsequently to the ookinetes in the prolonged culture medium. In the present experiment, ultrastructural observations were carried out on mature gametocytes and the gametes which developed in the

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prolonged culture of *P. falciparum* gametocytes. Studies were focussed upon (1) the release of a small part of the gametocytes cytoplasm into culture medium during gametogenesis, (2) the process of penetration of the macrogamete cytoplasm by the microgamete at fertilization, (3) the fusion of the membrane surrounding the microgamete nucleus with the outer membrane of the macrogamete nucleus.

Materials and Methods

Species of *Plasmodium*: Falciparum-Vietnam-Oaknoll (FVO) strain of *Plasmodium falciparum* was provided by courtesy of Dr. W.A. Siddiqui, Department of Tropical Medicine, University of Hawaii in 1983. This strain has been used in the previous reports (Ono *et al.* 1986; Ono and Nakabayashi, 1989, 1990). Gametocytes are seldom produced in this culture strain under normal culture conditions. The strain was cultured by the method of Siddiqui (1979) in 100-m*l* Erlenmeyer flasks, but Falcon tissue culture dishes were used for the prolonged culture of gametocytes which appeared after the gametocytogenesis-induction.

Induction of gametocytogenesis: Gametocytogenesis-induction medium (RPMI-FSC reactive medium) was used for induction of gametocytogenesis. This medium was prepared by the method described in our previous report (Ono and Nakabayashi, 1989). To put it briefly, this medium consists of a mixture of 4 m*l* regular RPMI 1640 medium, 4 m*l* RPMI-FSC medium, and 1 m*l* horse serum. RPMI-FSC medium was prepared by dissolving powdered RPMI 1640 medium in the mixture of the culture supernatant of the anti-*P. falciparum* antibody producing hybridoma cells and the hybridoma cell lysate. The hybridoma cells used in the present study were the same cell line as used in the previous report (Ono and Nakabayashi, 1989).

Prolonged culture of gametocytes: The method for the prolonged culture of gametocytes was described previously (Ono and Nakabayashi, 1989). On day 5 of culture in the regular RPMI 1640 medium containing 10% horse serum, the medium was replaced by a mixture of 9 m*l* RPMI-FSC reactive medium containing 10% horse serum, 0.5 m*l* concanavalin A (200 μg/m*l*), and 0.2 m*l* caffeine (100 mM/m*l*) to induce gametocytogenesis. After culturing for 24 hr, the medium was replaced by Waymouth's MB 753/1 medium with para-aminobenzoic acid (final concentration, 1 μg/m*l*) containing 10% horse serum; thereafter the medium was renewed every 24 hr. The parasites, which had been maintained in 100-m*l* Erlenmeyer flasks until the day 3 of culture in Waymouth's medium, were transferred into 60-mm Falcon tissue culture dishes for candle jar culture and cultured for 10 days. The temperature was constantly maintained at 37°C through the cultivation. The exflagellating medium (Carter and Beach, 1977) was not used in the present experiment.

Transmission electron microscopic observations: On day 13 of culture in Waymouth's medium, the parasites were collected by centrifugation at 3,000 rpm for 10 min and the sediment was fixed at 4°C for 1 hr in 0.01 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde, washed for 1 hr with 0.01 M phosphate buffer (pH 7.4) containing 0.25 M sucrose and postfixed at 4°C for 1 hr with 1.5% osmium tetroxide in isotonic buffer. After washing by centrifugation, the pellet was stained with 1% uranyl acetate solution for 1 hr. Then, the samples were dehydrated in absolute ethanol, treated with propylene oxide and embedded in epoxy resin. The sections were double stained with uranyl acetate and lead citrate.

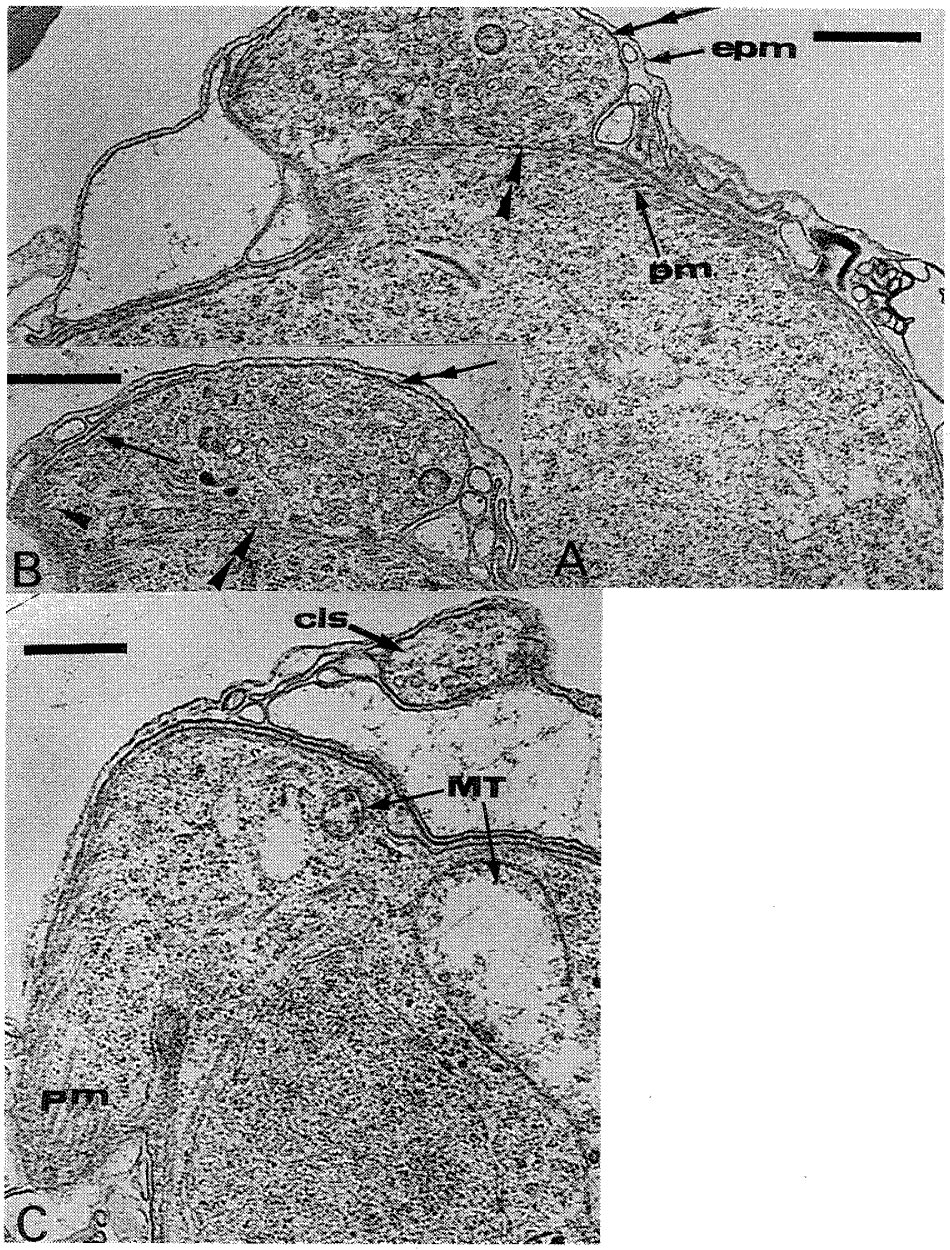

Figure 1A shows same gametocyte during gametogenesis as Fig. 1B but a different section. The extended inner membrane (double arrow heads) of the gametocyte plasma membrane divides the cytoplasm into two parts of a protruding part and a major part. The cytoplasm of protruding part contains ribosomes, a large number of small vesicles. In Fig. 1B, remnant of the inner membrane (arrow) and a few pellicular microtubules (arrow head) are visible clearly beneath the outer membrane (double arrow) in the cytoplasm of a protruding part. epm=erythrocyte plasma membrane; pm=pellicular microtubules. In gametocyte during gametogenesis shown in Fig. 1C, the cytoplasm-like structure (cls) which is enclosed by the disrupted erythrocyte plasma membrane, contains the similar contents as seen in the protruding part of gametocyte shown in Figs. 1A and B. A small mitochondrion and many pellicular microtubules (pm) are present in the cytoplasm of the protruding part. MT=mitochondrion. Scale bar equals approx. 0.5 μm in Figs. 1A, B, C.

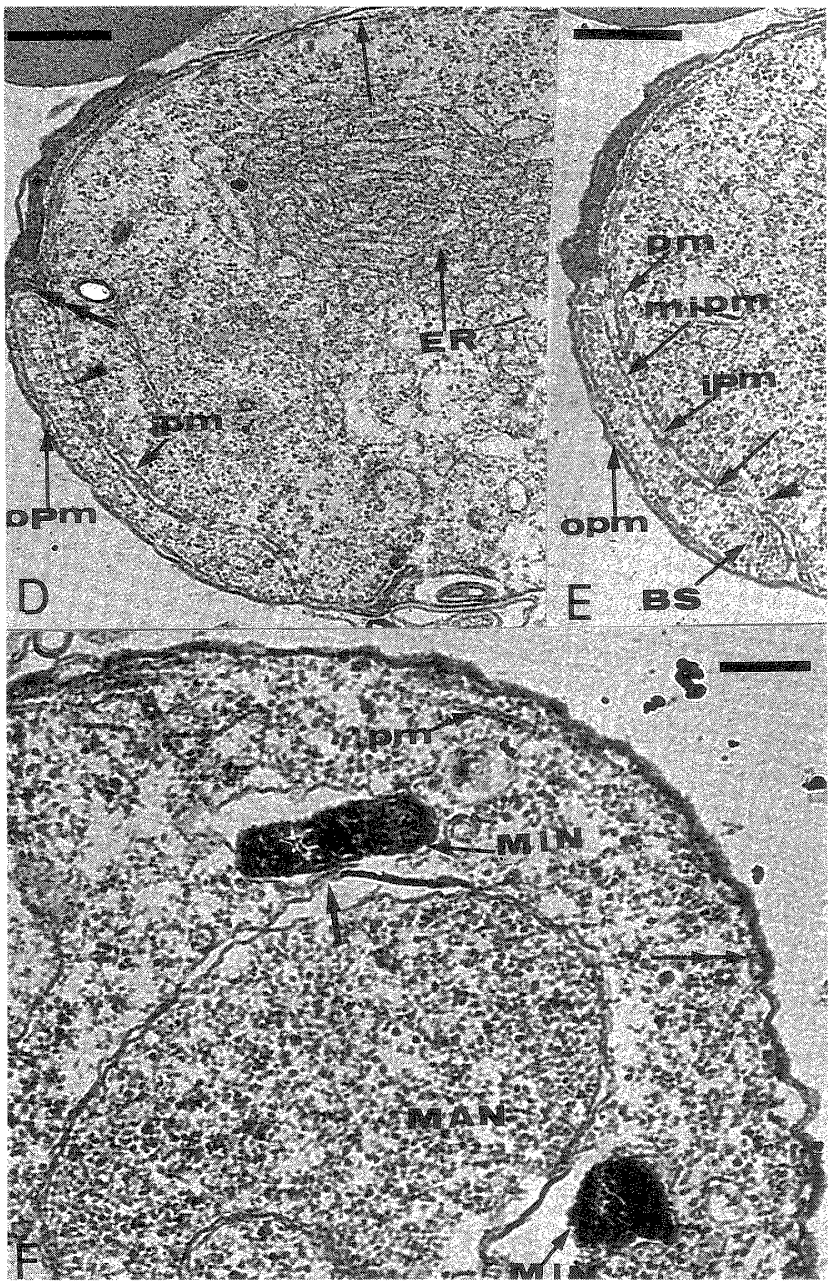

Figure 1D is the same macrogamete as Fig. 1E but a different section. A microgamete is seen between the outer (opm) and the inner (ipm) plasma membrane of a macrogamete. Pellicular microtubules (pm), partially disrupted inner membrane (arrow in Fig. 1D), extensive endoplasmic reticulum (ER) and macular desmosomes (arrow head in Fig. 1D) are observed in a macrogamete. The microgamete has a joint structure (double arrow in Fig. 1D). Fusion of a microgamete plasma membrane (mipm) and the inner membrane of macrogamete plasma membrane (ipm) can be seen (arrow in Fig. 1E). In the vicinity of this part, two membranes are indistinct in part (arrow head in Fig. 1E). BS=bulbous swelling. In Fig. 1F, two condensed microgamete nuclei (MIN) are observed near the nucleus of the macrogamete (MAN). The membrane enclosing a cigar-shaped nucleus of the microgamete is fused (arrow in Fig. 1F) with the outer one of two dissociated membrane of the macrogamete nucleus. The disrupted inner membrane of the macrogamete can be seen only in some places (double arrow in Fig. 1F). pm=pellicular microtubules. Scale bar equals approx. 0.5 μm in Figs. 1D and E; 0.25 μm in Fig. 1F.

RESULT

Figures 1A and B are the same gametocyte during gametogenesis but in the different sections. In Fig. 1A, a gametocyte is surrounded with the disrupted plasma membrane of an erythrocyte visible as multilayered sheaths. The extended inner membrane of the gametocyte plasma membrane divides the cytoplasm into two parts of a protruding part and a major part. Pellicular microtubules are observed below the inner membrane surrounding the cytoplasm of the major part. In Fig. 1C, a gametocyte during gametogenesis has a protruding part, as in Figs. 1A and B. The cytoplasm-like structure which is enclosed by the disrupted erythrocyte plasma membrane, contains many small vesicles. The contents of the structure are similar to it of the cytoplasm of the protruding part shown in Figs. 1A and B. A large vacuolated mitochondrion which contains a few tubular crista, is present in the cytoplasm of a major part. Figures 1D and E are the same macrogamete but in the different sections. A microgamete is seen between the outer and the inner plasma membrane of the macrogamete. The microtubules remain beneath the pellicular membrane. But, the macrogamete is not more enclosed in the erythrocyte plasma membrane and the inner membrane of the macrogamete is disrupted partially. A joint structure is observed in a microgamete. Macular desmosomes are present between the membrane of a microgamete and the inner membrane of macrogamete plasma membrane. Fusion of these two membranes can be seen in the neighborhood of the bulbous swelling of microgamete. In the vicinity of a fused part, two membranes are indistinct in a part. Figure 1F shows the fusion of the membrane enclosing the microgamete nucleus with the nuclear membrane of the macrogamete. In Fig. 1F, two condensed microgamete nuclei are observed in the cytoplasm of macrogamete. The macrogamete is surrounded by two layered plasma membranes, the outer membrane and the disrupted inner membrane. The latter can be seen only in some places. But, a small number of the pellicular microtubules still remain beneath the plasma membrane. The erythrocyte plasma membrane is invisible in the neighborhood of the macrogamete.

DISCUSSION

When the erythrocyte plasma membrane separated from the gametocyte during gametogenesis, a small part of the gametocyte cytoplasm which was enveloped with the multilayered sheaths of the erythrocyte plasma membrane, was released from the gametocyte into culture medium. This phenomenon is unknown until now. We concerned about whether the mature gametocytes developed in the midgut of the mosquitoes which engorged a *P. falciparum* patient release a part of the cytoplasm as those developed in the *in vitro* culture or not. Penetration of the macrogamete by the microgamete is so rapid that the ultrastructure of the microgamete just after penetration has not been observed until now. In the present study, however, we could observed a microgamete between the outer and the inner plasma membrane of the macrogamete. The microgamete is about to penetrate to the cytoplasm of the macrogamete by fusion of the microgamete plasma membrane and inner membrane of macrogamete plasma membrane. Furthermore, we observed the fusion of the membrane surrounding the microgamete nucleus with the outer membrane of the macrogamete nucleus. A joint structure (double arrow in Fig. 1D) in a microgamete has not been observed until now. This structure is regarded as a convenient structure to detach the nuclear part of

microgamete which is essential for nuclear fusion.

The multiple fertilization has not been observed in *Plasmodium*. Nijhout and Carter (1978) found light microscopically in *Plasmodium gallinaceum* that after the fusion of a microgamete and a macrogamete, entry of other microgametes into the same macrogamete was apparently prevented. But, Aikawa *et al.* (1984) could not find any evidence for this prevention by electron microscopic observation of a fertilized macrogamete of *P. gallinaceum*. Electron microscopy in the present study revealed two microgamete nuclei surrounded by the membrane in the cytoplasm of a fertilized macrogamete. It is interested in the whether multiple fusion occurs or not.

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培養熱帯熱マラリア原虫における生殖体形成および受精に対する電子顕微鏡的観察

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熱帯熱マラリア原虫赤内型の *in vitro* 培養に際し，これに抗熱帯熱マラリア原虫抗体産生ハイブリドーマ細胞の上清液を加えて誘発した生殖母体を長期培養すると，生殖体形成，受精，融合体形成，虫様体形成と発育が進む。この論文では生殖体形成中の生殖母体，雄性生殖母体による雌性生殖体侵入，更に雌雄生殖体による受精を，電子顕微鏡によって観察した。その結果，次の所見が得られた。

1．生殖母体の2枚の細胞質膜の中，内膜が伸展して生殖母体の細胞質の一部が分割される。そして，この部分は生殖母体を包んでいた赤血球膜が生殖母体から剝れる時，赤血球膜に包まれて虫体から培地中に放出された。

2．雄性生殖体は雌性生殖体侵入に際して，まず雌性生殖体細胞質外膜下に入り，その後雄性生殖体膜と雌性生殖体細胞質内膜の融合によって，細胞質に入るように思われた。両膜の間に macular desmosome が見られた。雄性生殖体は，雌性生殖体に入ると核部分がはずれるが，それを容易にすると思われる構造が見られた。

3．雄性生殖体の核を包む膜と，雌性生殖体の核膜の融合，すなわち受精を形態的に初めて観察することが出来た。雌性生殖体細胞質に核が2つ認められ，多受精の可能性が示唆された。

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DEFECT OF POST-TRIGGERING NATURAL KILLER CELL MEDIATED CYTOLYTIC ACTIVITY BY SYNTHETIC HIV ANALOGUE PEPTIDES

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Abstract: Three kinds of peptides termed ABJ917, ABJ918 and ABJ919 were synthesized and tested their capability of inhibition of Natural Killer (NK) cell mediated cytotoxicity. ABJ917, which is conserved among various retroviral transmembrane envelope proteins inhibited NK activities of the peripheral blood lymphocytes (PBLs) of both asymptomatic carriers and normal controls. ABJ918 or ABJ919 which correspond to this conserved region also inhibited NK activities. To know the mechanism of inhibition of NK cells by these synthetic peptides, conjugate formation assay and triggering assays were then performed. ABJ917 which inhibited overall NK cytotoxicity did inhibit neither NK and target tumor cell binding nor NK cell triggering by target K562 tumor cells. These results show that the inhibition of NK cytotoxic activity by synthetic HIV peptide is caused in the stage of post-triggering. Peripheral blood $CD4^+$ cell rate of the asymptomatic carriers of HIV was almost 0% when we obtained enough NK activities and these carriers were still quite healthy. Taken together, the immunosuppression of AIDS patients is thought to be caused at least partly from some defects of post-triggering lytic activity of NK cells by HIV transmembrane peptides.

INTRODUCTION

The hallmark of AIDS is thought to be a selective depletion of CD4 positive helper/inducer lymphocytes (Fauci, 1988). But, as far as from our knowledge and experiences, asymptomatic carriers whose CD4 positive lymphocytes are almost 0% in the PBLs are still healthy with no symptoms untill the onset of opportunistic infections. After disease manifestation, NK cytolytic activities are known to be inhibited as other immunological functions (Rook *et al.*, 1983; Wong-Staal and Gallo, 1985).

Therefore the NK cells are thought to play some important roles for initiating disease manifestation of AIDS. On the other hand, the retroviral transmembrane envelope protein p15E and its synthetic analogue peptides are reported to inhibit NK cytotoxicity (Cianciolo *et al.*, 1985; Harris *et al.*, 1987). And the mechanism of this NK suppression was the

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inhibition of post-effector target binding. Here, we tried to analyze further-*i.e.* if synthetic HIV peptide inhibit NK cell triggering or not.

Materials and Methods

Effector cells: For the NK cell source, PBLs from HIV asymptomatic carriers or normal volunteers were prepared after Ficoll Hypaque centrifugation and depletion of plastic dish adherent cells for 30 min at 37°C from heparinized blood.

Target cells: A human myelogenous leukemia cell line K562 was maintained in 5% Fetal Bovine Serum containing RPMI1640 medium and was used as a target tumor cell.

Cytolytic assay: Cytotoxicity assays were performed in a standard 4-hr ^{51}Cr-release assay as previously described (Pross and Baines, 1981). The following equation was used to calculate cytotoxicity: % specific lysis=[(cpm test−cpm medium)/(cpm max−cpm medium)]×100. The cpm max was determined by counting an aliquot of resuspended target cells. The cpm medium was determined in wells counting targets only with no effectors added. Lytic units (LU) were calculated from cytotoxic titration curves; 1 LU was defined as the number of effector cells required to cause 20% lysis of 5×10^3 targets.

Binding assay: Effector PBLs were mixed with target K562 cells at a 1:1 ratio as described previously (Roder and Kiessling, 1987). The cell suspension was centrifuged at $200\times g$ for 5 min at room temperature, followed by incubation on ice for 30 min. The pellet was gently resuspended and 200 lymphocytes were counted in triplicate samples.

Triggering assay: To detect the triggering events, phospholipid methylation assay (Hirata *et al.*, 1979) was employed. PBLs (2.5×10^4 cells) with or without target K562 cells (5×10^3 cells) were incubated in a total volume of 200 μl with 20 μCi of L-[methyl-^{3}H] methionine (87 ci/nmol, 1 Ci=3.7×10^{-4} MBq, Amersham, Amersham, UK) in 1.5 ml Eppen dorf tubes. After 1 hr of incubation at 37°C, the cells were washed once with cold phosphate-buffered saline (PBS) containing 5 mM L-methionine (Sigma Chemical Co., St. Louis, MO) and twice with cold PBS to stop the reaction. The lipids in the cell pellet were extracted overnight with 750 μl of cold chloroform/methanol (v/v=2/1). After centrifugation at $27{,}000\times g$ for 15 min, the chloroform/methanol extract was transferred to borosilicate tubes (Corning Lab. Sci. Comp., Corning, New York) and evaporated to dryness in a Rotavapor RE 120 (Buchi, Toronto, Canada). Dried samples were dissolved in 100 μl of chloroform/methanol (v/v=2/1) and aliquots (50 μl) were chromatographed by thin-layer chromatography (TLC) on 20×20 cm silica gel G plates (Analtec Inc., Newark, Denver), using a solvent system of chloroform/propionic acid/n-propyl alcohol/distilled water, 2/2/3/1 (v/v). The front migrated approximately 15 cm. After drying and staining with iodine (Sigma), phospholipid spots were scraped and the radioactivity was measured in a liquid scintilation counter (Beckman LS 7500, Beckman, Irvine. CA).

Synthetic peptides: Three kinds of peptides composed by 17 amino acids were prepared. ABJ917 is homologous to retroviral transmembrane glycoprotein p15E (correspond to gp41 for HIV). ABJ918 and ABJ919 peptides are homologous to ABJ917 but precise structures are minimally changed.

Table 1 Amino acid sequence of the synthetic peptides

HIV (gp41)	(ABJ917)	Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu
CKS17	(ABJ918)	Leu-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Leu-Lys-Glu-Gly-Gly-Leu
	(ABJ919)	Leu-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu

Table 2 Inhibition of NK activities of a normal volunteer during incubation

	without	+ABJ917*	+ABJ918*	+ABJ919*
NK activities	1,280†	853	1,067	1,000
(% inhibition)		(33.4)	(16.7)	(21.9)

*Concentration of the synthetic peptides were 5 μM
† Lytic Unit

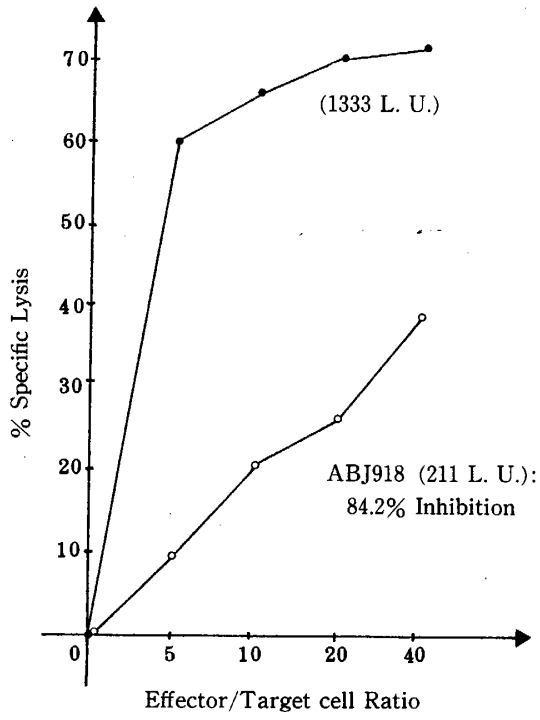

Figure 1 NK activities of the peripheral blood lymphocytes of an AIDS asymptomatic carrier before and after adding a synthetic peptide ABJ918.
Closed circle shows NK activity (1333 Lytic Unit) against ^{51}Cr labelled target K562 cells before adding ABJ918. Open circle shows NK activity (211 Lytic Unit) after adding the 918 peptide. 84.2% of NK activity was inhibited by 918 peptide.

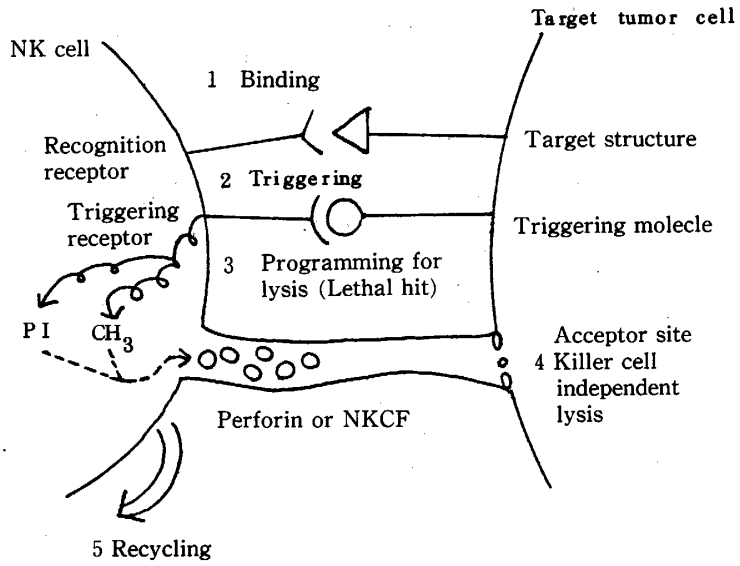

Figure 2 Schematic representation of NK cell mediated cytolytic process.
First, NK cell recognizes and binds with target tumor cells. Then, some saccharides structure (We call it triggering structure) triggers NK cell metabolic processes (Kiyohara *et al.*, 1985). Some lytic molecules are thought to be released from NK cells followed by formation of tubular structure (poly perforin) between NK and target cell and two different kinds of pores are produced on the target cell membrane. Killer cell independent lysis is the stage of target cell death accompaning DNA fragmentation (Apoptotic death). After target cell death, NK cell detach from the target and recycles to another targets.

Table 3 Percent conjugate formation of NK cells and target K562 cells

	% conjugate formation
without peptide	42.1±3.0
with 917 peptide	42.8±3.1

Concentration of the synthetic peptide 917 was 5 μM.

Table 4 The effect of a synthetic peptide 917 on the phospholipid methylation (triggering) of NK cells with K562 target cells

Cell mixture	^{3}H-methyl incorporation into phospholipid fractions (cpm)
NK alone	0
NK+K562	6,871
NK+K562+917	7,020

Results and Discussion

Inhibition of NK activities of HIV asymptomatic carriers: As mentioned before, Harris *et al.* (1987) showed that NK activity of the normal volunteer was inhibited by the synthetic peptides. But, if the peptide block NK activity of the AIDS asymptomatic carrier or not has not been reported yet. So, we decided to see if our synthetic peptides inhibit NK activity of the carriers. We added 918 peptide (Table 1) to the NK assay system at the concentration

of 5 μg (Fig. 1). This 17 mer peptide inhibited NK activity of an AIDS asymptomatic carrier as much as 84%. This is surprisingly high value.

Inhibition of NK activities of a normal volunteer: Next, we tested if these peptides could inhibit NK activities of normal person instead of asymptomatic carrier. As shown in Table 2, ABJ917 as well as ABJ918 and ABJ919 inhibited normal NK activities. The difference of the magnitude of inhibition between normal persons and asymptomatic carriers may suggest some tendency or difference of sensitivity is present.

The effect of 917 peptide to NK effector target conjugate formation: NK cell mediated cytotoxicity is known to proceed via several discrete stages including: 1. effector-target cell recognition and binding, 2. triggering and activation of the NK effector cells, 3. release and binding of the lytic factor to acceptor sites on the tumor cell surface, 4. target cell death, and 5. effector cell recycling to another target cell (Fig. 2). To know which stage of these killing process is involved in peptide mediated inhibition phenomenon, we performed Roder's conjugate formation (binding) assay. As shown in Table 3, conjugate formation between NK cells and target K562 cells was not inhibited at all. Therefore, the synthetic peptide seemed to block after binding stages.

The effect of 917 peptide to NK effector cell triggering: We defined the triggering stage of NK cell cytotoxic reaction as the stage of some biochemical reactions including membrane phospholipid methylation coupled to phosphatidyl inositol turnover (Kiyohara *et al.*, 1985). So, we examined if the peptide can block phospholipid methylation (*i.e.* triggering) of NK cells. As shown in Table 4, 917 peptide did not influence NK cell triggering by K562 target tumor cells. Therefore, 917 peptide block NK cytotoxicity after triggering. Taken together, the stage of action of the synthetic peptide seemed to be after binding and after triggering. As the lytic molecules are not determined (there are some evidences that tumor necrosis factors play some roles in NK cytotoxicity and some people have reported the importance of the perforins or esterases in NK cytotoxic reaction) actual site of these synthetic peptide should be clarified in future.

Acknowledgement

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HIVgp41の構成ペプチドおよびその類似合成ペプチドによるヒト末梢血NK活性の抑制機構

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AIDS患者末梢血NK活性が抑制されている事が知られているが，このNK活性の抑制機構を解析した。無症候性キャリアのNK活性は抑制を受けておらず，その時のキャリアの末梢血のCD4⁺細胞は0％であった。つぎに，HIVの外殻糖蛋白質gp41の一部で，レトロウイルスに保存されている17個のアミノ酸から成るペプチドと，その類似構造をしたペプチドを3種合成した。これらの合成ペプチドは，正常コントロールのNK活性も，無症候性キャリアのNK活性も共に抑制したが，キャリアに対する抑制が著しかった。正常コントロールNK活性に対する合成ペプチドの作用段階は，NK細胞と標的癌細胞の結合にも，標的細胞によるNK細胞のTriggeringにも合成ペプチドが影響を与えないことから，Triggering以後の段階であると考えられた。一般には，AIDSの発症には，HIVがCD4⁺細胞に感染し，その機能が損われる事が重要だと考えられており，多くの研究が為されているが，以上の結果より，無症候性キャリアが発症するに至るプロセスには，それ以外のNK等の免疫機能の抑制が何らかの役割を果たしていると考えられた。

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AN ETIOLOGICAL STUDY OF BACTERIAL DIARRHEA AMONG INFANTS IN PARAGUAY IN 1990

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Abstract: An etiological study of bacterial diarrhea in infants under five years of age in Paraguay from May to July of 1990 was done. Enteropathogenic *E. coli* (EPEC) strains of known serogroups were isolated from 35.8% of the patients with diarrhea, *Salmonella* spp. were isolated from 15.1%, enterotoxigenic *E. coli* (ETEC) strains were isolated from 5.7%, and *Shigella* and *Campylobacter* spp. were isolated from 3.8% respectivery. No enteroinvasive *E. coli* (EIEC) or *Vibrio* spp. was isolated. Out of 53 diarrheal patients, 34 cases (64.1%) had bacterial diarrheal diseases. The infants under one year of age with diarrhea were predominant among inpatient and outpatient children under five years of age and the total number of male diarrheal cases was greater than that of female cases. Enteropathogenic organisms were isolated from nine out of 12 water samples taken from the Paraguay River. The major serotype (O18) of the EPEC isolated from the water samples of the Paraguay River corresponded to the major serotype isolated from patients with diarrhea.

INTRODUCTION

Bacterial diarrheal disease is still one of the main public health problems with high morbidity and mortality rates, especially in the tropics and subtropics (Merson, 1982).

It is reported that diarrheal diseases accounted for 8.2% of the infant motality rate in Paraguay in 1989, according to a report by the Ministry of Health, however the etiology of diarrhea as well as its prevalence is still unknown. Surveys and studies conducted so far on diarrhea in Paraguay have been confined mainly to traditional enteropathogens such as *Salmonella* and *Shigella*. The first four cases of diarrhea due to *Campylobacter jejuni* in Paraguay were reported by Achucarro *et al.* (1989) and this was the first report on

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campylobacter gastroenteritis in Paraguay. However, an etiological investigation including one concerning gastroenteritis due to *E. coli, Campylobacter* and *Vibrio* sp. has not been done.

In this report we tried to clarify the present status of diarrhea, especially the etiological agents of bacterial diarrheal diseases among children under five years of age in Paraguay. In order to treat and control diarrheal diseases, we also investigated the water of the Paraguay River which is closely associated with the life of the people. In addition, the drinking water was studied bacteriologically to clarify the degree of enteropathogen contamination resulting in diarrhea, because diarrheal diseases are recognized to be a fecal-oral infection, so that drinking water and food are considered major sources of infection.

Materials and Methods

Rectal swabs (Carrymate media, Ono Pharmaceutical Co. Ltd., Japan) were taken from children under five years of age having diarrhea who visited or were admitted to the Department of Pediatrics, at the School of Medicine of the Asunción University and LACIMET Hospital from May to July of 1990 and were processed bacteriologically in the Bacteriology Section of the Instituto de Investigaciones en Ciencias de la Salud. The rectal swabs were inoculated directly on TCBS, SS, EMB and Skirrow media and incubated at 37°C overnight.

The specimens were, at the same time, subcultured in alkaline peptone water (pH 8.5) and selenite broth. After being incubated at 37°C overnight, three loopfuls of bacterial culture were inoculated on TCBS and SS media. A suspicious colony from each plate was examined with the following media for identification, Kligler iron, SIM, Voges-Proskauer (VP), lysine, ornithine and Simmons citrate, and was simultaneously checked for the level of citochrome oxidase. Ten colonies of *E. coli* were inoculated on BTB media to check purity and three colonies each were checked for serotyping with diagnostic antisera for enteropathogenic *E. coli* (EPEC) (Denka Seiken, Japan) when *E. coli* was predominant on the EMB media. The serotyping for *Shigella* and *Salmonella* was done with diagnostic antisera (Denka Seiken, Japan).

Skirrow agar medium for *Campylobacter* containing 5% horse blood, trimethoprim (5 mg/m*l*), vancomycin (10 mg/m*l*) and polymyxin B (2.5 μ/m*l*) was incubated at 37°C for 3 days or 42°C for 2 days in a microaerobic atmosphere containing 5-10% oxygen generated by a CampyPak (BBL Microbiology System, Cockeysville. Maryland). The minimum criteria used for presumptive identification of *C. jejuni/coli* were: Gram-negative, spiral -shaped, motile, oxidase positive and growth at 42°C but not at 25°C. The sodium chloride tolerance test was also employed for further confirmation and classification of *Vibrio* sp.

Thousand milliliter samples of water were collected in sterile bottles from 12 water points in the Paraguay River and from nine water points for drinking in two areas in Asunción (Fig. 4).

Each water sample was concentrated to 10 m*l* with a milipore filter and 3 m*l* each was subcultured in double strengthened alkaline peptone water (pH 8.5), peptone water (pH 7.2) and selenite broth at 37°C overnight. A portion of the concentrated water sample was also inoculated on EMB media and incubated at 37°C overnight. The filter paper used for concentration was placed onto SS media and incubated at 37°C overnight. The bacterial culture in alkaline peptone water was streaked on TCBS and that in the peptone water and

selenite broth was both streaked on SS media.

A suspicious colony from each plate was examined with identification media after being incubated at 37°C overnight. To test the sensitivity against several antibiotics, a single disk method (Bauer *et al.*, 1966) was employed and the results were reported to the pediatric ward.

The production of LT and ST was checked by GM_1-ELISA and suckling mouse assay, respectively, as previouly described (Honda *et al.*, 1984; Takeda *et al.*, 1979).

Results

The isolation rate of different organisms and the percentage of all the detected agents are shown in Fig. 1. From 53 diarrheal patients, 34 cases were recognized to be bacterial diarrheal diseases. The most dominant bacterial pathogen was enteropathogenic *E. coli* (EPEC) with 19 isolates (35.8%) which was followed by *Salmonella* with eight isolates (15.1%). No *S. typhi* or *S. paratyphi-A* was isolated. Serotyping of the *Salmonella* could not be done thoroughly because there were not enough serotyping kits for diagnosis when they were isolated. Out of eight isolates identified biochemically as *Salmonella,* four isolates were serotyped, two strains were serogroup O4, and one strain each was serogroup O13 and O35. However, the remaining four isolates were not adequately serotyped because of contamination during stock. Three strains of enterotoxigenic *E. coli* (ETEC) were isolated. Heat labile enterotoxin (LT) was not detected by GM_1-ELISA from all the strains serotyped as EPEC or ETEC. Two EPEC strains (FA ratio: 0.141, 0.85) and one strain serotyped as ETEC (FA ratio: 0.096) were revealed to be positive in the suckling mouse assay. Two strains each of *Shigella flexneri* 2a and *Campylobacter jejuni/coli* were isolated. Three mixed infection cases were found, the included; EPEC O1 and 18; EPEC O86a and O157; *Salmonella* serogroup

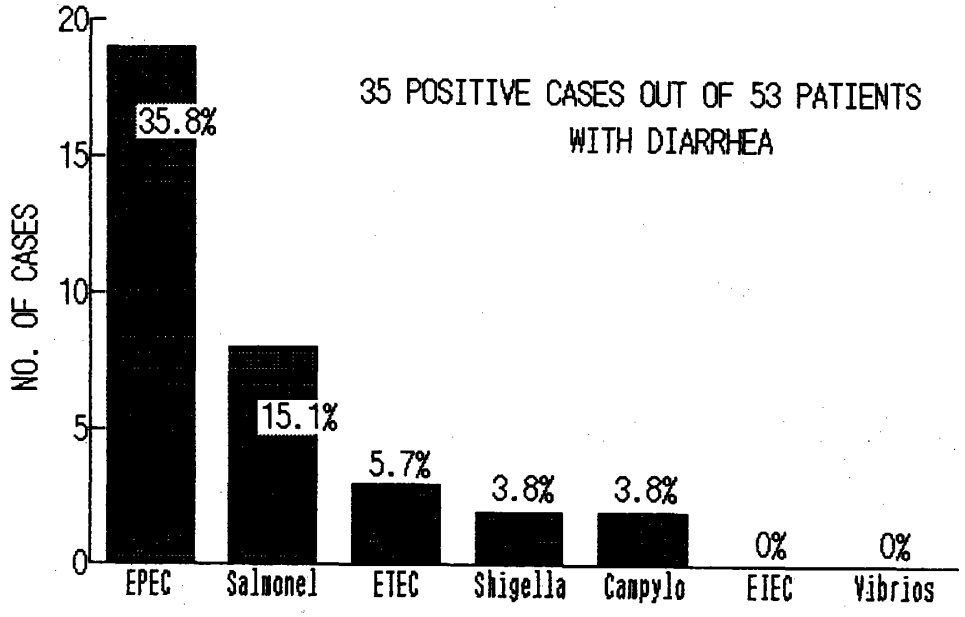

Figure 1 Pathogenic bacterial agents isolated from diarrheal patients under five years of age obtained between May and July of 1990.

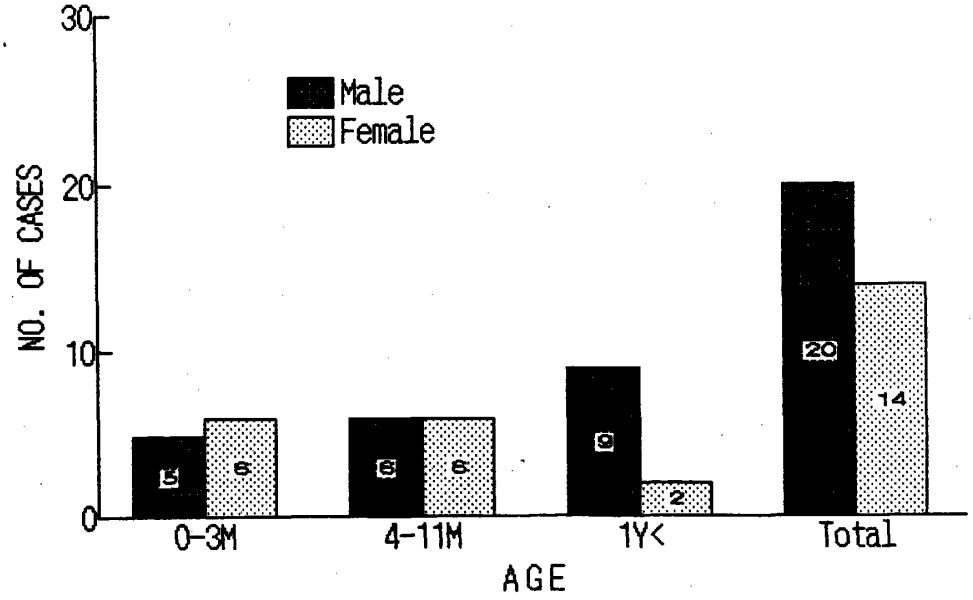

Figure 2 Age and sex distribution of 34 children with diarrhea.

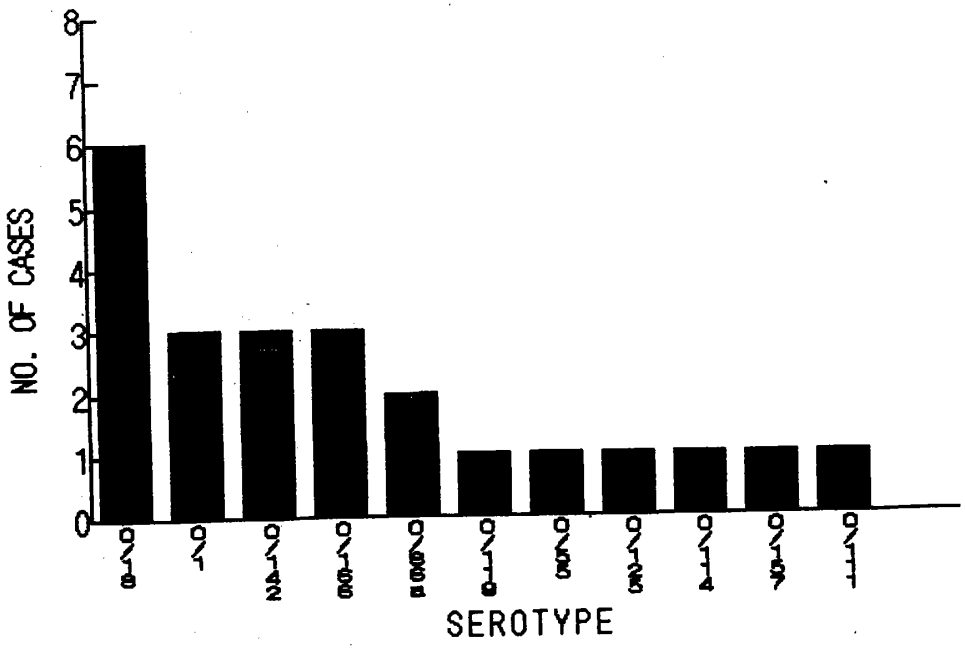

Figure 3 Distribution of serotyping of enteropathogenic *E. coli* isolated from children with diarrhea.

O4 and EPEC O86a, and the last case was thought to be a *Salmonella* infection (Fig. 1). No enteroinvasive *E. coli* or *Vibrio* sp. was isolated. A total of 41.5% of the diarrhea cases was, thus, considered to be due to *E. coli*. The infants under one year of age with diarrhea were predominant among inpatient and outpatient children under five years of age and the total number of male diarrheal cases was greater than that of female cases (Fig. 2).

Eleven kinds of EPEC serotypes were isolated and O18 in the EPEC isolates were predominant (Fig. 3). Twelve water samples from the Paraguay River were taken at the

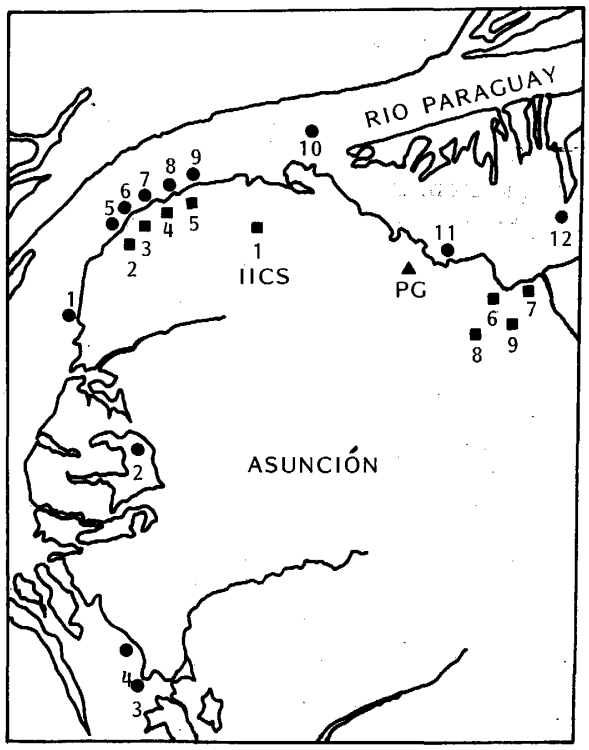

Figure 4 A map of Asunción, Paraguay. The numbers represent the points where the water samples (●) and drinking water (■) were obtained. PG: The Palace of the President of Paraguay, IICS: Instituto de Investigaciones en Ciencias de la Salud.

Table 1 The enteropathogens isolated from water of the Paraguai River

Point 2	EPEC (O18, O44)
Point 4	EPEC (O18, O26, O142)
Point 5	*Salmonella* (serogroup O13)
Point 6	*Salmonella* (serogroup O21), EPEC (O18, O146)
Point 7	*Salmonella* (ND)
Point 8	EIEC (O28a,c)
Point 9	*Salmonella* (serogroups O1, 3, 19), *Vibrio mimicus*
Point 10	*Salmonella* (ND)
Point 12	EPEC (O18, O128)

ND: The isolates were identified biochemically but serotyping of the isolates was not done.

points shown in Fig. 4. These points were selected as they were near to the areas where people in relatively low-socioeconomic conditions live. Enteropathogenic organisms were isolated from nine points out of the 12 points. Two or three EPECs were isolated from four points and the serotype O18 was isolated from all four points. *Salmonella* was isolated from five points.

Each strain of EIEC and *Vibrio mimicus* was isolated. Enteropathogenic organisms were isolated from all the points where people in low-socioeconomic conditions live. The major serotype of the EPEC isolated from the water samples of the Paraguay River corresponded to the major setotype isolated from the patients with diarrhea (Table 1).

In the analysis of the drinking water, enterobacteria such as *Salmonella, Enterobacter* and *Proteus* spp. were isolated from three points out of nine points tested. No enteropathogens were isolated from the tap water.

In the drug sensitivity test, six or seven kinds of discs containing ampicillin, cephaloridine, cefotaxime, ceftazidime, gentamicin, amikacin, chloramphenicol and trimethoprim were used. Four strains of EPEC showed resistant against three or four kinds of antibiotics including ampicillin and cephaloridine and the other pathogenic *E. coli* strains were relatively sensitive. One strain of *Salmonella* was resistant against five kinds of antibiotics including ampicillin and cephaloridine and the other strains were relatively sensitive to all the antibiotics except for ampicillin and cephaloridine. One strain of *Shigella* was resistant against aminoglycosides and chloramphenicol but the other was sensitive to all the antibiotics except for ampicillin. Two strains of *Campylobacter* were sensitive against aminoglycosides and tetracycline but resistant to all the other antibiotics.

Discussion

This etiological study of bacterial diarrhea was performed over a short period of time and the instruments and the materials for making diagnosis were not adequate enough that we could identify the organisms in detail and thoroughly analyze characteristics such as toxin productivity. However, additional investigations over an extended period of time will provide more meaningful results.

The following findings are from the results of this study. Firstly, more than 60% of the diarrheal patients were considered to have bacterial diarrhea, although this study was conducted during the dry and cold season. Since the rotavirus is also an important organism causing diarrhea among children during the dry and cold season as previously reported (Koopman *et al.*, 1984; Brandt *et al.*, 1982; Brandt *et al.*, 1983), it is necessary to investigate viral enteropathogens as well as bacterial enteropathogens. Secondly, the dominant bacterial pathogen that caused diarrhea among inpatients and outpatients was EPEC. It is reported that EPEC is the leading cause of diarrhea in black South African children and that diarrhea in white children is largely attributable to rotavirus (Robins-Browne, 1984). Of course, it is difficult to consider it to be the overall infection rate of EPEC, because the diarrheal patients who came to the Asunción University Hospital and LACIMET Hospital were severe cases and diarrheal patients in low-socioeconomic areas who do not visit a hospital unless their condition becomes severe. Thirdly, the isolation rate of *Campylobacter jejuni/coli* was 3.8% in this study, which is almost the same as that in other countries (Chyou *et al.*, 1988; Tang *et al.*, 1984).

Shimotori *et al.* (1986) reported that the overall isolation rate of *Campylobacter jejuni* from children with diarrhea in Kenya was 12.6% but in the cold and dry season it was 6.3%. It is considered that the infection rate of *C. jejuni/coli* in Paraguay is within the ranges reported earlier for developing countries. Forthly, the EPEC serotype of the enteropathogenic organisms isolated from nine out of 12 water points in the Paraguay River corresponded to that of the diarrheal patients. The outbreak of diarrheal diseases seems to be closely related to the usage of contaminated water from the Paraguay River. The water level of the Paraguay River was elevated because of rainfall during the spring, so a fairly large number of people who lived on the banks of the river had found refuge on a hill overlooking the river. The people had to build their houses temporarily in this area. Chlorinated tap water was, of course, supplied to the people of this area. The drinking water was kept in their houses because the water supply was not connected to individual houses, thus resulting in it becoming contaminated. The water from the Paraguay River is used for domestic use such as washing dishes and clothes and bathing. It is, therefore, believed that the establishment of a water supply to this area as well as health education is required. The last point of note is on the high mortality rate due to diarrheal diseases. Firstly, an effective and cheap drug for diarrheal patients should be chosen, with due consideration being given to the most appropriate drug for pediatric cases. Secondly, particular attention should be paid to the care of hospital patients, and to the improvement of laboratory diagnostic techniques. Thirdly, effective use of oral rehydration solution in rural areas should be promoted because of the high mortality rate. Finally, it is important to continue the etiological examination of diarrheal diseases.

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パラグアイ，アスンシオン市における幼児の細菌性下痢症

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1990年5月から7月までの3カ月間，パラグアイ・アスンシオン市の医療施設において，5歳以下の下痢症例患者を対象に，下痢便の細菌学的検査を行った。53症例から34名（64.1%）に下痢原因菌を検出したが，その結果は病原性大腸菌の19株（35.8%）を筆頭に，サルモネラ8株（15.1%），毒素原性大腸菌3株（5.7%），赤痢菌とカンピロバクターがそれぞれ2株（3.8%）が検出され，このうち混合感染が3例あった。

診断用抗血清による凝集試験で，病原性大腸菌では11種に型別され，O18型が最も頻度が高かった。血清学的抗原構造で毒素原性大腸菌に属するものからは，易熱性毒素(LT)は検出できなかったが，乳のみマウスによる耐熱性毒素（ST）の検査で，毒素原性大腸菌から1株が陽性を示し，病原性大腸菌も2株が陽性を示した。

河川水や飲料水の細菌学的検査において，病原性大腸菌，組織侵入性大腸菌，サルモネラ，ビブリオ属などの病原菌の他に，腸内細菌科の細菌も検出された。特にパラグアイ川からは，下痢便で最優先であったものと同型の，O18型病原性大腸菌が検出されたことは，この地域住民に水系感染の危険性があることが推察された。

またディスク法によって，抗生物質7種類に対する薬剤感受性試験を行ったが，同一菌種間においても，その感受性パターンは多様性を示した。

パラグアイにおける細菌性下痢症の調査は，従来，サルモネラや赤痢菌などの古典的下痢症については行われてきた。しかしカンピロバクターについては，1989年において，初めて4症例の報告がなされた状況であり，腸管病原性大腸菌やビブリオ属の調査については，まったくなされていない。

パラグアイでの下痢症の疫学的・病因的調査は不十分な状況であるので，この調査を機会に，今後も継続して行っていく必要性を強調したい。

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臨床的研究

熱帯熱マラリア治療における開始遅延の意義

海老沢 功[1]・田辺 清勝[2]・小原 博[3]

平成2年10月24日受付/平成2年12月1日受理

はじめに

現在日本で報告されるマラリア患者は年間約60人, 実際は約100人, 死亡例は1965—1987年の23年間の平均が3人である。その他に瀕死の重症例が毎年1—2人ある。これらの重症, および死亡例はすべて熱帯熱マラリアで治療開始が遅れたものである(海老沢ら, 1980; Ebisawa *et al.*, 1980; Ebisawa, 1989)。治療開始遅延日数と血液の諸検査所見を比較して, 熱帯熱マラリアの重症化の関係を述べ, 早期診断と治療の重要性を強調したい。

患者資料

1966—1989年の24年間に東大医科学研究所内科, 東邦大学その他の施設で我々が直接, あるいは間接に関与したマラリア患者432人である。血液内マラリア原虫の算出法その他は既に報告した(海老沢ら, 1990a, b)。

使用述語の定義

瀕死の重症：意識混濁, 昏睡, 全身痙攣などの意識障害, 腎不全, 高度の貧血, 黄疸, 血色素尿, DIC, 肺水腫などを単独あるいは合併して, 生命に危険な兆候を示したものを瀕死の重症とした。治療にはクロロキンの筋注, またはキニーネの点滴静注を行い, 腎不全合併例では腹膜, あるいは血液透析などの積極的治療を必要とした症例を指す。全症例中23人が瀕死の重症に分類された。死亡した13人はすべて瀕死の重症例であり, その中にはマラリアの治療を受けないで死亡した者もいる。

遅延日数(損失)日数：はじめて発熱した日を第1病日として起算し, その後適切な抗マラリア薬を用いて治療を開始するまでに経過した日数を, 遅延日数とした(病日—1日に相当する)。遅効性あるいは感染熱帯熱マラリア原虫がR3耐性を示して, 効果を示さなかった時は有効な治療薬を開始した日までの日数とした。

結 果

Ⅰ. 瀕死重症例の発生要因

瀕死重症例はすべて熱帯熱マラリアで, その他の原虫種感染によるものはなかった。

1) 感染地域別発生頻度

瀕死の重症例は, アフリカ大陸で感染したマラリア患者122人中17人(14%), その他の地域で感染したマラリア患者は310人中8人(3%)で, アフリカ大陸で感染したものに多く発生している。その理由は, 熱帯熱マラリアの頻度がアフリカ大陸では70%, その他の地域では30%と, アフリカ大陸に熱帯熱マラリアが多いためである。

熱帯熱マラリア原虫に感染した場合には, 瀕死重症マラリアの発生率は, アフリカで感染した86人からは20%, その他の地域で感染した90人からは9%で, 有意な差はなかった。即ち感染地域に関係なく, 熱帯熱マラリアに感染することが危険である。

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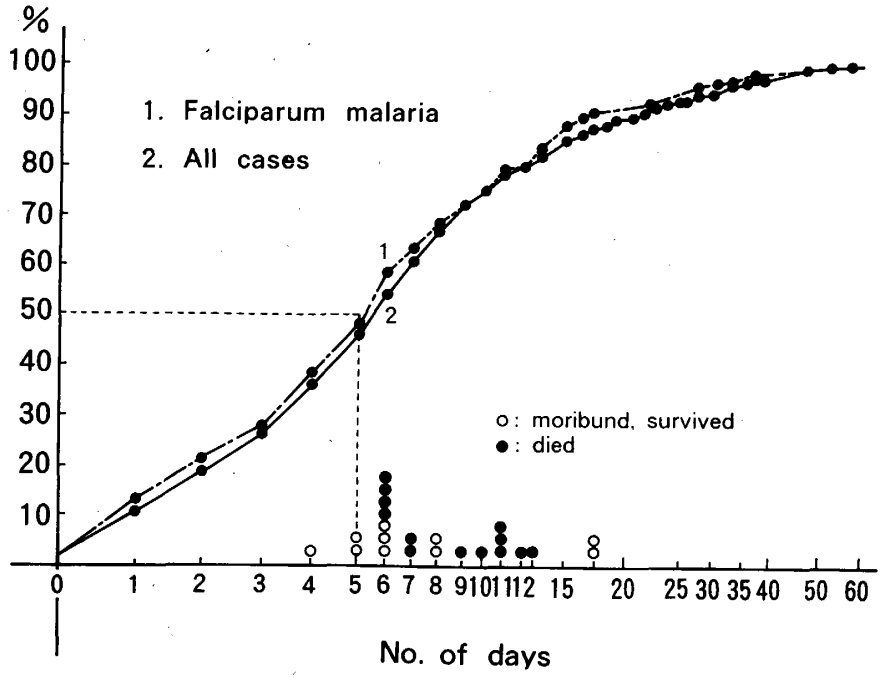

Figure 1 Cumulative percentage of time lag in the treatment of malaria patients and development of moribund conditions after a delay of 4 days or more.
All moribund and fatal cases are falciparum malaria patients.

2）遅延（損失）日数

発熱発作時に悪寒戦慄を伴う三日熱，卵形および四日熱マラリアは症状が定型的であり，素人でもマラリアを疑う。これに反して熱帯熱マラリアは，発病初期に悪寒だけを訴えることが多く，その他の症状が乏しい。

そこで，熱帯熱とその他のマラリアを含めた全症例について遅延日数を分析し，その累積百分率を図1に示した。これによると，遅延日数は感染原虫の種に関係がない。また発病後5日以内，即ち6病日以内に治療を開始された者は，全体の約50%に過ぎない。

他方，瀕死の重症に陥ったが生存したものを白丸で，死亡例は黒丸で，それぞれの遅延日数を図の下部に記入した。瀕死症例中には，遅延日数4日で治療を開始したが重症であった例もある。死亡例の中には，図に示した日に治療を受けることなく死亡したものがある。従って熱帯熱マラリア患者が重篤な合併症を起こさず，順調な経過をとって治癒するには，少なくとも遅延日数4日以内に治療を開始することが必要である。

3）遅延日数に相関する諸種検査成績

予後に最も関係する血液内最大原虫数，腎機能の指標としてのBUNとクレアチニンの最大値，および赤血球数と血色素の最低値について，遅延日数との関連を検討した。

1．血液内熱帯熱マラリア原虫数

a）熱帯熱マラリア原虫の自然増加

抗マラリア薬を予防内服しない患者：血液検査で熱帯熱マラリアと診断のついた患者の治療開始が遅れたため，マラリア原虫が急激に増殖し患者は意識不明，腎不全を合併し瀕死の重症になった。血液1 μl当たりの原虫数は，7日間に6,760から1,549,000に230倍増加した(海老沢ら,1978)(表1)。

効力不完全な抗マラリア薬を内服した場合：熱帯熱マラリア原虫にピリメタミン耐性があるナイジェリアに滞在中，発病予防にピリメタミンを内服していた男性が，次第に貧血となり脾腫を発見され，その原因追求のため入院した。その間血液内には，少量の熱帯熱マラリア原虫があったが発

Table 1 Increase of *P. falciparum* in untreated patients

Case 1.	35 y.m.	Case 2.	38 y.m.
Day of dis.	Parasite count (/μl)	Day of dis.	Parasite count (/μl)
2	6,760	−5	550
4	91,200	−3	2,290
5	56,200	−2	830
6	371,500	−1	1,780
8	380,200	1	400
9	1,548,800	2	5,000
		3	6,030
		4	10,000

Day 1 is the day on which the patient became febrile. The sign − indicates day before onset of fever. Case 2 had been admitted for examination of anemia and splenomegaly of unknown cause.

熱がなかった。そのうち発熱したので血液標本を遡って鏡検したところ，入院時より少量の原虫が血液内にいたことが分った。この例では，9日間に1 μl当たり550から10,000に18倍増加しただけである。ピリメタミンで不完全に抑制されていた，少量の原虫の長期感染によりある程度の免疫ができ，熱帯熱マラリア原虫の増殖が抑えられたためであろう。

b）多数例における観察—治療開始前の最大原虫数

検討した対象は，非マラリア流行地に生まれて成人したもので，主として日本人である。治療開始前10日以内に，何らかの抗マラリア薬を内服したものは除外した。

80人の成績を，遅延日数を横軸に，最大原虫数の対数を縦軸にとって図2に箱ひげ（box whisker)図で示した。縦長の矩形は各群50%の症例が入る範囲を，上下にのびる直線は最高と最低値の範囲を，箱を横切る線分は平均値を示す。

遅延日数と血液内原虫数の間には1—10日の間では，正の相関関係が見られた。

$y=4.031+0.124x$, $r=0.359$, $p<0.01 (n=80)$

ただし11日以後になると異常に高い値を示すものが少数あるが，一般に遅延日数が延びても原虫数は増加しない傾向が見られた。

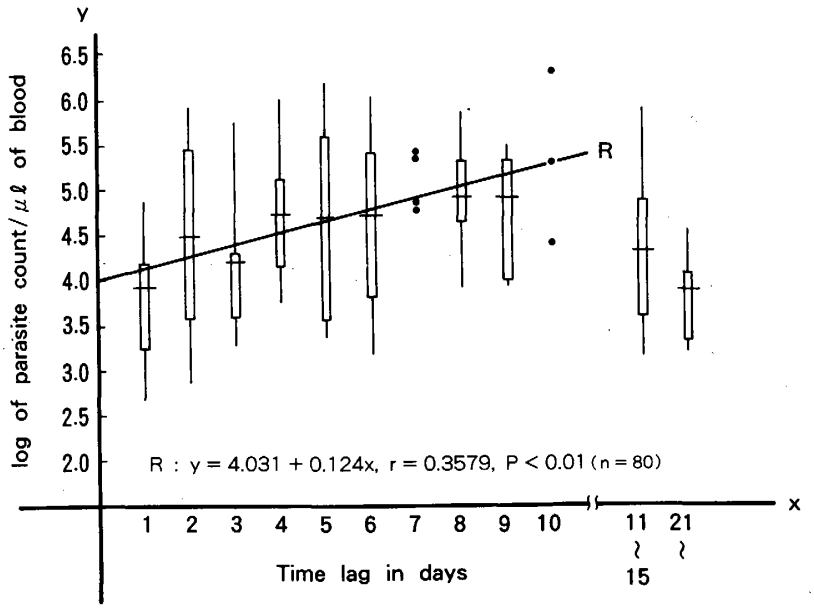

Figure 2 Time lag in the treatment *vs* maximum parasite count in falciparum malaria patients.
See text for explanation of box-whisker figures. The regression line R indicates, $y=4.031+0.124x$, $r=0.3579$, $p<0.01$.

Table 2 Time lag in days *vs* the incidence of the patients with parasite count of over 100,000 per μl of blood

Time lag in days	Parasite count/μl		Total
	$\geqq 10^5$	$<10^5$	
1～2	3	15	18
3～4	6	15	21
5～6	7	16	23
7～8	4	5	9
9～11	6	8	14
Total	26	59	85

$x^2=3.490$, $p>0.05$, No significant difference.

c）高い原虫数の出現頻度

血液1μl当たりの原虫数が10万を越えるものの出現頻度を，2日間隔で区切って調べたところ，2病日ですでに10万を越える患者があり，以後その割合は増加の傾向はあるが，統計学的には有意差は見られなかった（表2）。

d）遅延日数4日以内と，5—11日の患者の最大原虫数

原虫数の対数を両群について検討し，遅延日数が多い群の原虫数が，有意に多いことが認められた（$p<0.05$）。平均値を実数で示すと，それぞれ23,400と69,200である。

2．赤血球と血色素

a）一般的傾向

赤血球と血色素の値を遅延日数ごとに箱ひげ図を作り，さらにそれらの値と遅延日数との間の相関の有無を検討した。図3に示すように，これら検査値と遅延日数との相関は遅延日数が1—3日と4—8日の間では，いずれも有意な相関関係（$p<0.01$）が証明された。すなわち赤血球については遅延日数1—3日（$r=-0.480$），4—8日（$r=-0.408$），血色素については1—3日（$r=-0.524$），4—8日（$r=-0.444$）の値が得られた。いずれも有意な相関係数である。

遅延日数が9日以上の者は，数が少なく検討できなかった。ただし今回の分析で，赤血球と血色素が最低の98万/μlと3.2g/dlの値を示した者は遅延日数が11日で，原虫数は1μl当たり728,500に達して死亡した（海老沢ら，1990a，b）。

b）遅延日数と高度貧血出現頻度

遅延日数が4日以内と5—11日の群について，赤血球数は1μl当たり299万以下，血色素は9.9 g/dl以下の貧血を示すものの割合を比較すると，いずれも5—11日のものに多くでる結果が得られた（表3）。また両群の平均値の間に赤血球では約50万，血色素では2.5g/dlの差が認められた

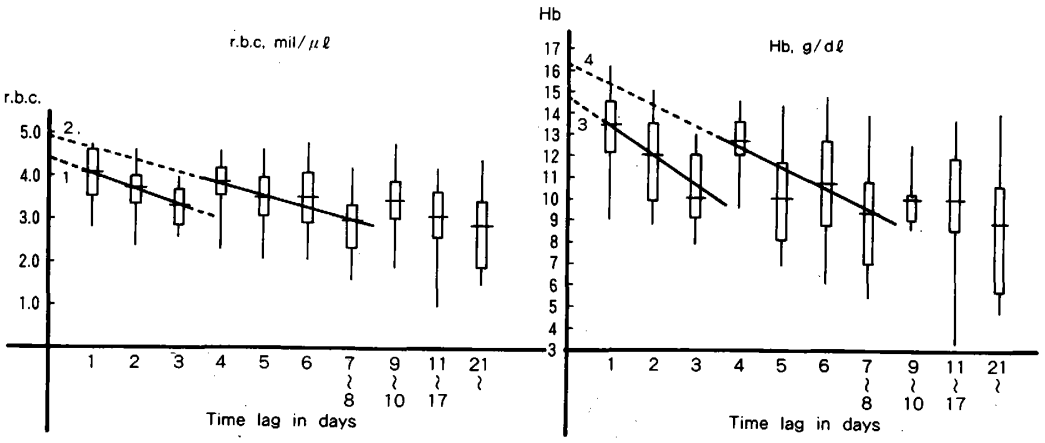

Figure 3 Time lag in the treatment *vs* minimum r.b.c. counts and Hb concentration in falciparum malaria patients.
Regression lines are 1 ($y=4.39-0.36x$, $r=-0.480$), 2 ($y=4.89-0.25x$, $r=-0.408$), 3 ($y=14.66-1.33x$, $r=-0.524$), and 4 ($y=15.93-0.89x$, $r=-0.444$). P values are all <0.01.

Table 3 Time lag in days *vs* degree of anemia

Time lag in days		≦4	5～11	Total
r.b.c. /μl	≦2.99 mil	8	16 (10)	24 (10)
	≧3.00 mil	45 (2)	35 (7)	80 (9)
Total		53 (2)	51 (17)	104 (19)
Hb g/dl	≦ 9.9	7	21 (10)	28 (10)
	≧10.0	42 (1)	28 (5)	70 (6)
Total		49 (1)	49 (15)	98 (16)

The number in parenthesis indicates moribund but survived or fatal cases.
$x^2=3.88$, $p<0.05$ for r.b.c. *vs* time lag
$x^2=9.800$, $p<0.01$ for Hb *vs* lag time
The incidence of moribund cases was not related to the degree of anemia, but was more frequent among the patients with longer time lags than with shorter time lags ($p<0.01$).

Table 4 Comparison of the lowest mean r. b. c. count and Hb concentration *vs* time lag in days

Time lag	≦4 days	5～11 days	Difference
r.b.c.* mil/μl	3.81 (0.69)	3.28 (0.82)	$p<0.01$
Max～Min	5.53～2.25	4.76～0.98	
Hb* g/dl	12.4 (2.1)	10.0 (2.5)	$p<0.01$
Max～Min	18.1～7.9	14.8～3.2	

*( ) Standard deviation in parenthesis.
There was a significant difference in the means but not in the variances ($p>0.05$).

(表4)。

3．尿素窒素とクレアチニン

a）両者の度数分布

両者の度数分布は，箱ひげ図によって図4に示した。平均値が上方にずれているものが多い。こ

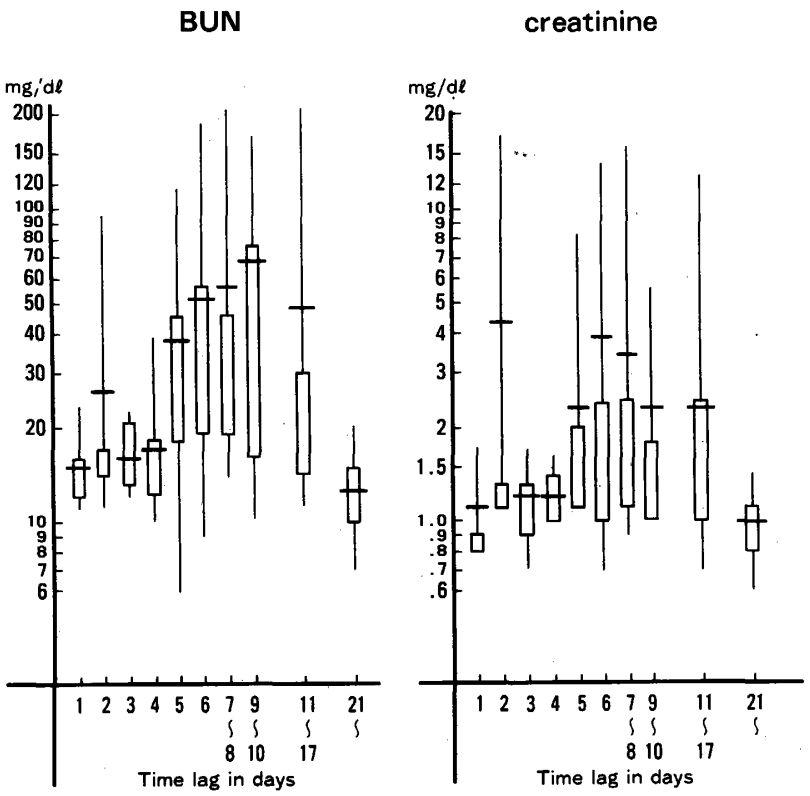

Figure 4 Time lag in the treatment *vs* maximum BUN and creatinine in falciparum malaria patients.
Note abrupt increase of abnormal values of BUN and creatinine beyond time lag of 5 days or more.

れは，極端に高い値を示すものがあるためである。例えば遅延日数2日で，他の例は大部分正常範囲内にあったのに，BUNが92mg/d*l*，クレアチニンが16.9 mg/d*l*に達していた症例があった。ここでは平均値はそれに引きずられて上方にずれている。

一般的傾向としては，BUNとクレアチニンいずれも遅延日数が5日以上になると，平均値と分散が大きくなっていることがわかる。この傾向は原虫数，赤血球数，血色素量いずれの場合よりも顕著な差として認められる。

b）遅延日数と異常BUN，クレアチニン値の出現頻度

統計学的分析には，2病日で例外的異常値を示した例を除いて検討した。この例を除いた者のBUNとクレアチニンの平均値±標準偏差は，それぞれ15.3±2.7と1.2±0.1 mg/d*l*であり，平均値に標準偏差の3倍の値を加えても，異常高値例の数字に達しない。すなわち，このような異常値を示す例は，統計学的に0.3%以下に過ぎないので除外して計算した。

遅延日数が4日以内と5日—11日の2群に分けて，BUNが21 mg/d*l*以上，およびクレアチニンが1.6 mg/d*l*以上になったものの割合を検討した。その割合は危険率1%以下で後者に多いことが確認された（表5）。

両群の差は，平均値に最も顕著に見られた。BUNの平均値は，両群でそれぞれ16.0と57.1 mg/d*l*，クレアチニンの平均値は1.2と3.4 mg/d*l*で，有意差が認められた（$p<0.01$，表6）。

Table 5 Incidence of abnormal kidney function (BUN and creatinine) *vs* time lag in days

BUN, mg/d*l*		≧21.0	≦20.0	Total
Time lag in days	≦4	6	30	36
	5～11	30	19	49
	Total	36	49	85
Creatinine, mg/d*l*		≧1.6	≦1.5	Total
Time lag in days	≦4	3	28	31
	5～11	20	25	45
	Total	23	53	76

The incidence of abnormally high BUN and creatinine was significantly higher in patients with longer time lags than with shorter time lags ($p<0.01$).

考　察

図1に示したように，熱帯熱マラリア患者が重症化するのは，遅延日数が5日以上になる時が多い。この5日を境として何が起こるかが，今回の分析の主要な目的であった。

本論文では原虫数，貧血および腎機能を示すBUNとクレアチニンと遅延日数との相関，および遅延日数4日以内と5—11日の群の各検査項目の平均値を検討した。

まず注目すべきことは，図2に示した回帰直線で遅延日数0，即ち第1病日の原虫数の平均値は，約10,000と推定されることである。これは初めて発熱した日を第1病日としたからで，実際にはその数日前から，原虫は血液内で増殖をしていたことを示すものである。いわゆるprepatentの終り

Table 6 Time lag in days *vs* BUN and creatinine (mg/d*l*)

	Time lag	No.	Mean	S.D.*
BUN	≦4	36	16.0	5.1
	5～11	49	57.1	60.5
	Total	85	39.7	45.8
	Time lag	No.	Mean	S.D.
Creatinine	≦4	31	1.2	0.3
	5～11	45	3.4	4.0
	Total	76	2.5	3.0

Both BUN and creatinine were significantly higher in patients with longer time lags than with shorter time lags ($p<0.01$). A patient with BUN of 92 mg/d*l* and creatinine of 16.9 mg/d*l* on the second day of illness was excluded from the analysis as the values were greater than mean+3 S.D. of the patients whose treatment was started on the same day of illness.

から抹梢血液のマラリア原虫は出現するが，一定の数に達しないと発熱しない。prepatent period は潜伏期よりも少なくとも2日以上短いという（Garrham, 1988）。この時期には臨床的に倦怠感，頭痛，食欲不振など非特異的症状を訴えるものが多い。本来はこれら症状の出現時を初発日とすべきであるが，発熱は最も明らかな客観的所見であるから，初めて発熱した日を第1病日とした。実際にはこれは4—5病日になっている可能性は理解しておくべきである（表1，症例2）。

諸検査項目は，いずれも遅延日数の増加に伴って悪化する相関関係が認められたが，最も顕著であったのはBUNとクレアチニンである。両者とも遅延日数が5—11日のものは4日以内のものに比べて，顕著な増加ぶりを示している。Marsden (1989) は熱帯熱マラリア原虫の標的臓器は脳と腎臓で，意識障害と尿量に注意するよう述べているが，的を得た表現である。

小児では，脳性マラリアと貧血が最も普通に見られる致死的合併症であるという（Molyneux, 1990）。我々の症例には小児は少なく，貧血が死因と思われた例は26歳の女性1人で，この患者の遅延日数は11日であった。

従って熱帯熱マラリア治療上最も注目すべき事項は，臨床的に遅延日数を確定し，意識障害の有無と尿量に注意する。最小限の項目として原虫数，BUNとクレアチニン，赤血球と血色素の検査は実施しておくべきである。

遅延日数が12日以上のものでは，検査所見に異常に高いものが少なかったのは，重症例はそれまでに死亡してしまっているが，ある程度の免疫ができて病勢が進展しなかったものであろう。

結　語

1）全体約420人のマラリア患者中，発病後5日以内に治療を開始された者は，熱帯熱でもその他のマラリアでも約50%である。熱帯熱マラリア患者で瀕死の重症になったものは，遅延日数が4日以上になったものである。

2）発病後11日までの間では原虫数，貧血の程度（赤血球と血色素の減少），腎機能の低下（BUNとクレアチニンの増加）は遅延日数に平行して悪化している。発病後4日以内と5—11日以内の群では，検討した項目すべてに有意な増加，または低下が見られた。特に5日を境にした，腎機能の急激な悪化が目立つ。

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Clinical study

THE SIGNIFICANCE OF TIME LAG IN THE TREATMENT OF FALCIPARUM MALARIA

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We have previously reported that there exists a time limit for effective treatment of falciparum malaria. In short, four days after the onset of the illness was found to be the time limit beyond which severe, life-threatening complications may develop.

In order to elucidate the level of laboratory variables beyond which the patient's prognosis is poor, we examined the correlation between time lag in days *vs* maximum parasite count, minimum red blood cell (r.b.c.) count, minimum hemoglobin (Hb), maximum blood urea nitrogen (BUN) and creatinine concentration of each patient during the period of admission. A linear correlation was found between time lag (x) and the maximum parasite count in log units (y), $y=4.031+0.124x$, $r=0.3579$. All variables among patients whose treatment was started after 5 to 11 days of time lag (6th to 12th day of illness) were significantly worse than the variables of patients whose treatment was started after only 1 to 4 days of lag time. However, the most conspicuous change was observed in the abrupt increase of BUN and creatinine in the members of the late-treatment group: the mean BUN and creatine levels in the early and late treatment groups were 16.0 *vs* 57.1 and 1.2 *vs* 3.4 mg/d*l* ($p<0.01$), respectively. The mean parasite counts of the early and late treatment groups were 23,400 *vs* 69,200/μl of blood ($p<0.05$), while the mean r. b. c. count and Hb concentration of the two groups were 3.81 *vs* 3.28 million per μl and 12.4 *vs* 10.0 g/d*l* ($p<0.01$), respectively.

In addition to the level consciousness of the patient, special attention should be paid to kidney function in the treatment of falciparum malaria patients whose treatment is delayed beyond the 5th day of illness.

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臨床的研究

マラリア患者の黄疸について

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はじめに

マラリアでは一定の間隔をおいて溶血が起こり，黄疸を起こすことが知られている。しかしマラリア患者の血清ビリルビンを，各感染原虫ごとに比較した研究は少ない。

重症熱帯熱マラリアは，しばしば激症肝炎，急性腎不全を伴った閉塞性黄疸などの死亡診断名がつけられているので，これらの疾患との鑑別を念頭において，マラリア患者の血清ビリルビンについて述べる。

症例と検索方法

症例は前報(海老沢ら，1990a，b)に述べた419人のマラリア患者のうち，血清ビリルビンを測定してあった127人である。患者は複数の病院で検査されているので，各検査室で行われている成績をそのまま用いた。また各検査項目につき，その患者が入院中に示した最高値を，その患者の代表値とした。患者の80%は男性で，20—40歳のものが80%を占める。

マラリア原虫数の半定量的検査法は，すでに述べた（海老沢ら，1990a，b)。検査データの統計的処理で原虫数，総ビリルビンとLDHは計測値が広い範囲にわたるため，対数変換を行って分析した。データの処理は，群間の検査値の分散と平均値の差，度数分布の差，および2つの変数の相関は相関係数を求めて，その有意差を検定した。計算には脇本たちのソフトウェア（脇本ら，1987)を用いて行った。

成　績

1）感染原虫別に見た血清総ビリルビン

各感染原虫別に調べた血清総ビリルビンの比較は，一部をすでに報告した（海老沢ら，1990c)。それによると，熱帯熱マラリアでは血清総ビリルビンの値が0.2～42.3 mg/dlと広い範囲にわたっている。血清総ビリルビンの平均値と標準偏差は，熱帯熱マラリア患者が6.2±8.6 mg/dl (n=59)，三日熱と卵形マラリア患者が1.2±0.9 mg/dl (n=57)で，平均値と分散ともに有意の差がある。

表1にその後の資料を加えて，血清総ビリルビンの値を1.0 mg/dl以下，1.1～5.0，5.1以上の3群にわけて各原虫ごとに示した。熱帯熱マラリア患者には，5.1 mg/dl以上の値を示すものが多い。最高値は熱帯熱マラリアで42.3 mg/dl，三日熱マラリアでは6.4 mg/dlであった。症例を熱帯熱と，それ以外のマラリアの2群に分けて検定すると有意の差が認められた（$p<0.01$)。

2）血清総ビリルビン値と予後

意識混濁，昏睡，痙攣などの意識障害，腎不全，血色素尿，DIC，肺水腫などを単独あるいは複数合併し，生命に危険な兆候を呈したものを，死亡例を含めて瀕死重症と定義した。瀕死重症例は，すべて熱帯熱マラリアである。そこで熱帯熱マラリ

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Table 1 Serum total bilirubin and the incidence of severe illness in malaria patients

Total bilirubin mg/d*l*	Infecting malaria parasite				Total
	P. falciparum	*P. vivax*	*P. ovale*	*P. mal.*	
≦1.0	19 (0)	28	2	1	50
1.1～5.0	33 (5)[a]	24	4	1	62
≧5.1	14 (14)[b]	1	0	0	15
Total	66 (19)	53	6	2	127

The number in parenthesis indicates moribund or fatal cases.
[a]: 3 of 5 patients died [b]: 7 of 14 patients died.

ア患者を血清総ビリルビン値により3群にわけ，瀕死重症例の起こる頻度を見ると，血清ビリルビンが異常値，特に5.1 mg/d*l*以上になった者に，瀕死重症例が多発していることがわかる（表1）。以下の分析は，すべて熱帯熱マラリアに関して行うことにする。

3）原虫数と総ビリルビン

ここでは，総ビリルビンが1 mg/d*l*以下の7人も加えて検討した。両者とも，最低値と最高値の開きが大きい。血液1 μl中の原虫数（pc）の対数

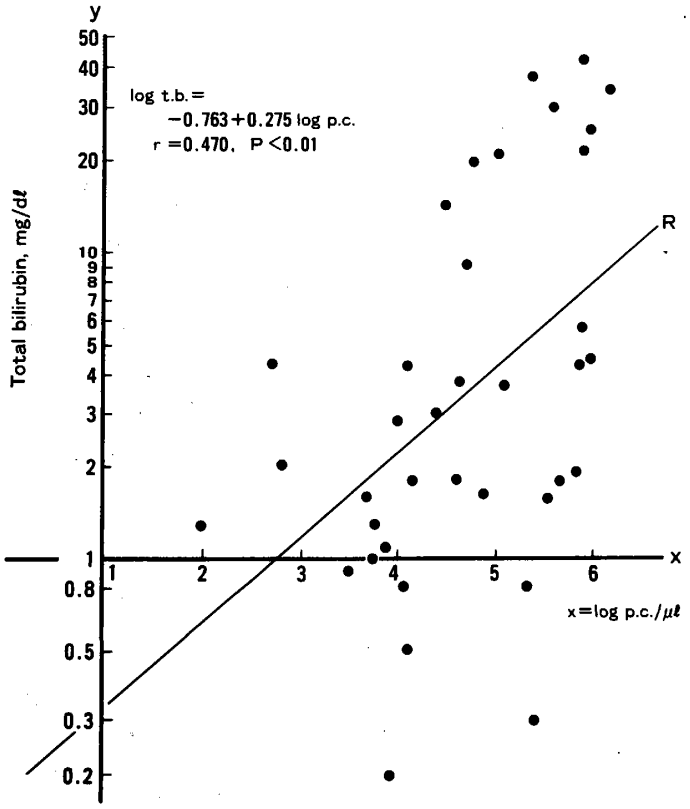

Figure 1 Maximum parasite count (x) *vs* serum total bilirubin (y) in falciparum malaria.
Regression line R indicates: $y=-0.763+0.275x$, $r=0.470$, $p<0.01$,
where x=log of parasite count/μl, and y=log of total bilirubin mg/d*l*.

x=log pcと総ビリルビン（tb）の対数y=log tbの相関関係を調べ，有意の相関関係が得られた（図1）。

log tb=−0.763+0.275 log pc, r=0.470, p<0.01

すなわち感染原虫数が多いほど，総ビリルビンの値も高くなる。例外的に原虫数が1 μl当たり250,900の高い値を示したのに，総ビリルビンは0.3 mg/dlに止まったものもある。しかし一般的には原虫数が多いほど予後は悪いので，総ビリルビンが高いものほど予後が悪かったこと（表1）と一致する所見である。

4）直接と間接ビリルビン，および直接/総ビリルビン比

マラリアにおける血清ビリルビンの増加は，急速な溶血によるもので，間接ビリルビンの増加が期待される。総ビリルビンが1 mg/dl以上で，直接ビリルビンが測定してあった34人の総，直接，間接ビリルビンおよび直接/総ビリルビン比を図2に箱ひげ図で示す。すなわち，中央の縦長の矩形で検査値の50%が入る区間を，上下に延びる直線で最高と最低値を含む範囲を，横に交差する線分で平均値を示した。データが正規分布するときは平均値は縦長の矩形のほぼ中央にくるので，平均値が上方にずれていることは，極端に高い値を示すものの影響を示している。

総，直接と間接ビリルビンの平均値は，それぞれ9.4, 5.4, 3.9 mg/dlで，直接/総ビリルビン比の平均値は0.5であった。直接と間接ビリルビンの平均値と分散には，有意差はなかった。すなわち，

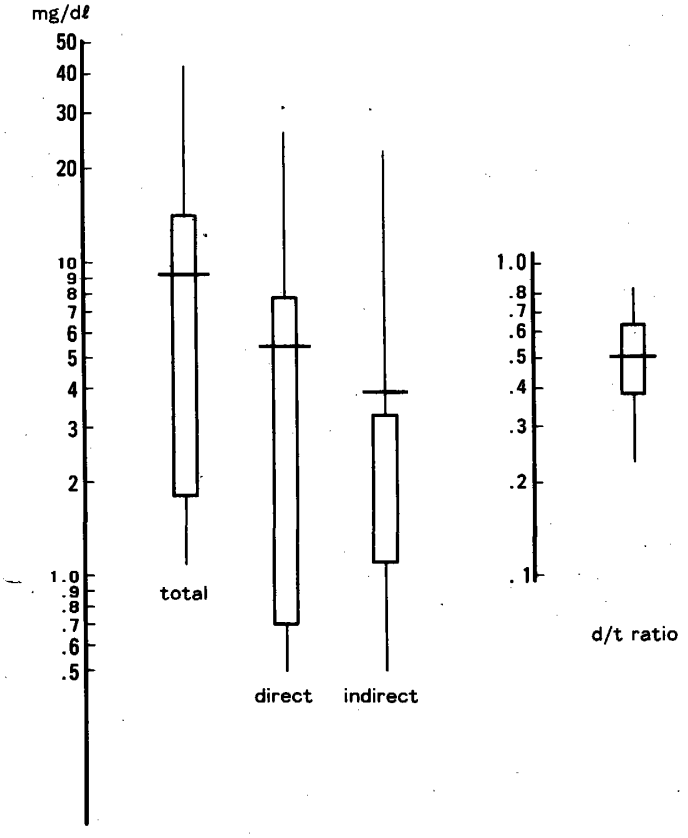

Figure 2 Serum total, direct and indirect bilirubin and d/t ratio (direct/total) of bilirubin in falciparum malaria.
An upright quadrangle indicates a range in which 50% of the data are found. Two lines above and below the quadrangle indicate ranges of maximum and minimum values. A transverse line indicates the mean value.

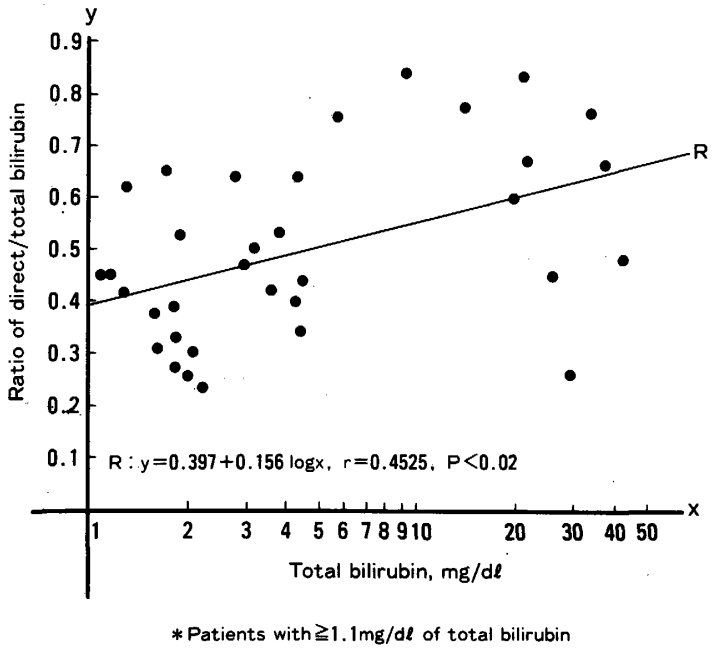

Figure 3 Correlation between serum total bilirubin (x) and ratio of direct/total bilirubin (y) in falciparum malaria.
Regression line R indicates: $y=0.397+0.156 \log x$, $r=0.4525$, $p<0.02$.

間接ビリルビンと平行して，直接ビリルビンも増加している所見である。

5）総ビリルビンと直接/総ビリルビン比

血清総ビリルビンは，1.1から42.3 mg/dlの広い範囲にわたるので，その対数（x）と直接/総ビリルビン比（y）の相関関係を調べた。両者の間に，有意な相関関係が認められた。

$y=0.397+0.156 \log x$, $r=0.4525$, $p<0.02$

すなわち，総ビリルビンが増加すると直接/総ビリルビン比も増加する（図3）。しかし中には，血清総ビリルビンが30 mg/dlに達したのに直接/総ビリルビン比が0.26の低値を示したものもあった。

6）その他の検査成績：SGPT, SGOT, γ-GTPおよびLDH

総ビリルビンが1.1 mg/dl以上の高値を示したものについて，肝機能に関係のあるSGPT, SGOT, γ-GTPおよびLDHを分析した。各項目について得られたデータを図2と同様に箱ひげ図によって，図4に示す。平均値はSGPT (70), SGOT (132), γ-GTP (74), LDH (1,112) で

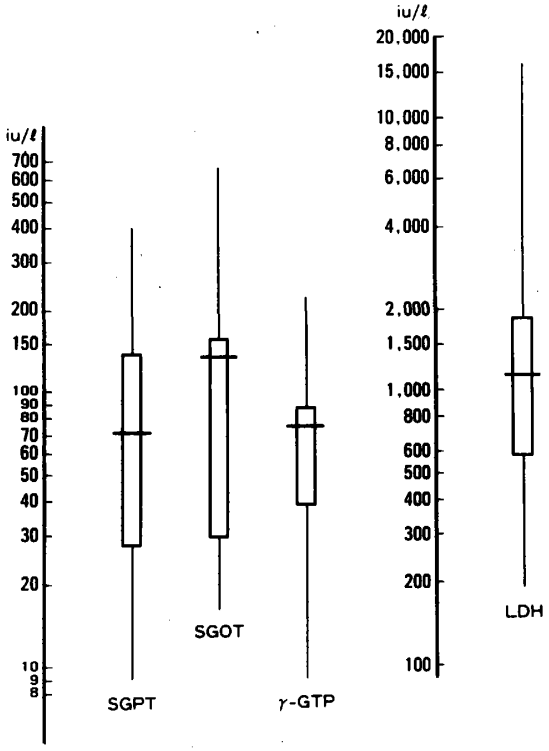

Figure 4 SGPT, SGOT, γ-GTP and LDH in falciparum malaria patients.

ある。SGPTよりもSGOTの方が高くなること，赤血球の崩壊を反映してLDHが最高15,140に達していることが特徴である。γ-GTPも高い者が多いが，これは飲酒癖の多いものがあることも考慮する必要があろう。

この他にアルカリフォスファターゼ（Al-P）も上昇した例があるが，問題になるようなことはないので省略する。総ビリルビン値が高くなった患者では，尿中にもビリルビンが検出され，同時にウロビリノーゲンも検出されている。また血清総，直接と間接ビリルビン，SGOT，SGPT，LDHいずれもマラリア原虫の消失とともに急速に，多くの場合3－4週間内に正常値にもどった。

考　察

図1と3に示したように，原虫数の増加→（赤血球崩壊の増加）→総ビリルビンの増加→直接/総ビリルビン比の増加という一般の図式が明らかになった。このことから原虫数が多くなる熱帯熱マラリアが，強い黄疸の出現で問題になることも推定できる。

1）熱帯熱と他のマラリアの差

先ず強調すべきことは，同じマラリアといっても熱帯熱マラリアは他のマラリアに比べて，血液内原虫数と貧血や血液生化学的異常所見を呈する者が多く（海老沢ら，1990a，b），かつ重症になりやすいことである。これは血清総ビリルビンの最高値が，熱帯熱マラリアでは42.3 mg/dl，三日熱マラリアでは6.4 mg/dlであったことからも明白である。

2）総ビリルビンと予後

熱帯熱マラリアで，総ビリルビンが5.1 mg/dl以上の14人が，すべて瀕死重症と判定された。これは，感染原虫数が多いことを反映するものである。予後判定の上からも，総ビリルビンの上昇は要注意の所見としてよいであろう。

3）直接と間接ビリルビンの動向

マラリアの基本的病態は，分裂体による毛細血管の一時的閉塞と，定期的に起こる溶血であるから，間接ビリルビンの増加が推定される。しかしこれとほぼ平行して，直接ビリルビンも上昇しており，直接/総ビリルビン比の平均値は0.5であった。さらに尿中にはビリルビンとウロビリノーゲンも出ているから，間接型ビリルビンが多少増加していても，閉塞性黄疸とは容易に区別できよう。

4）その他の血清酵素反応

熱帯熱マラリア患者ではSGPTだけでなく，SGOT，γ-GTPの上昇が見られたが，肝炎患者に見られるほど高いものではない（岩田ら，1982）。LDHはマラリアそのものの病態を反映して，極めて高い値を呈していた。

高熱と黄疸を伴う，重症熱帯熱マラリア患者を初めて見る医師にとって，マラリアを念頭におかないで診断すると，直接と間接ビリルビンが共に上昇しているので，診断が困難である。しかし3と4の所見を総合すれば，劇症肝炎，あるいは閉塞性肝炎とは，容易に鑑別できよう。

結　語

マラリア患者，特に熱帯熱マラリア患者の血清ビリルビンについて検討し，その黄疸が肝炎や閉塞性黄疸と異なることを示した。

1）血清総ビリルビンは，熱帯熱マラリアでは三日熱，および卵形マラリアに比べて，1.1 mg/dl以上の高値を示すものが多い（71%:49%，$p<0.01$）。熱帯熱マラリアでは，42.3 mg/dlの最高値を示したものがある。

以下の記載は，熱帯熱マラリアの血清ビリルビンに関するものである。

2）総ビリルビンが5.1 mg/dl以上になったものは，重症であった。

3）血液内原虫数と総ビリルビン値には，正の相関がある（$p<0.01$）。

4）総，直接，間接ビリルビン，および直接/総ビリルビン比の平均値は，それぞれ9.4，5.4，3.9 mg/dlと0.5であった。直接と間接ビリルビンの値には，分散と平均値に有意差はない。

5）総ビリルビンと直接/総ビリルビン比には，正の相関がある（$p<0.02$）。

5）肝機能および溶血に関したSGPT，SGOT，γ-GTP，およびLDHの平均値は，それぞれ70，132，74，1,112で特にLDHの最高値が15,140に達

したことは，溶血の強さをよく示している。

以上の所見を総合すると，急性肝炎は勿論，たとえ間接ビリルビンが増加していても，閉塞性黄疸は除外されよう。

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Clinical study

SERUM BILIRUBIN IN MALARIA PATIENTS

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The serum bilirubin of malaria patients was investigated in an effort to show that jaundice occuring in malaria patients differs from hepatocellular (hepatitis) and obstructive jaundice.

1. The incidence of abnormally high serum total bilirubin (≧1.1 mg/d*l*) was more frequent in cases of falciparum than in vivax and ovale malaria patients put together (71% *vs* 49%, $p<0.01$). The maximum value of a falciparum malaria patient was 42.3 mg/d*l*.

The following data were obtained concerning falciparum malaria:

2. All 14 patients showing ≧5.1 mg/d*l* of serum total bilirubin were moribund, and 7 of them died.

3. A positive correlation was noted between the maximum parasite count and serum total bilirubin ($p<0.01$).

4. The mean values of total, direct and indirect bilirubin and the ratio of direct/total (d/t) bilirubin were 9.4, 5.4, 3.9 mg/d*l* and 0.5, respectively. There were no significant differences in the mean and variance of direct and indirect bilirubin.

5. A positive correlation was found between serum total bilirubin and d/t ratio ($p<0.02$).

6. The mean values of SGOT, SGPT, γ-GTP and LDH were 70, 132, 74 and 1,110, respectively. The maximum value of LDH was 15,140. The above data may prove to be helpful in differentiating jaundice in malaria patients from hepatocellular and obstructive jaundice.

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Special lecture

1 THE INTERNATIONAL HEALTH AIDS OF JAPAN: ITS PAST AND FUTURE

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Honorable Director of TB Institute

Present world health services seem to be classified into 3 patterns. The first one is seen in the advanced countries in where infectious diseases are seldom and people are suffering from malignant tumors and adult diseases and their population patterns are shifted to aged people. In this group the geriatric diseases are the main target of health service of the society. The second one is seen in NIES or oil-producing countries in where the diseases patterns are just behind of the advanced countries and there are little serious problems in their health services. The third types are recognized among the most of the developing countries where many infectious and parasitic diseases are prevailing and infant mortalities are very high and the feasibilities of medical and sanitary services are very low due to the financial difficulties. These situations need rapid improvement. The populations proportions of people living in these three types against those of whole world are 19% in the first group, 46% in the second group and 35% in the third one. On the other hand the global spread of HIV infection and environmental hazards on human health are the common threat throughout the world. The most urgent problem to be solved is the unbalanced medical service in the world. The difficulties of improving the level of health services in the greater majority of the developing countries are derived from the delays of their economical development. In some developing countries their economical crisis resulted in big slow downs of health services. In those countries, populations are increasing rapidly and there are no hope of improving their health service by elevating people's living standards. Effective provisions of health and medical services in these countries are very difficult due to their scanty health budgets and poor educational and social services. The front health service systems are not be well organized and the developments of PHC are very slow. The systems of controlling diseases and managing health services are very poorly established. What is more, there are serious problems of the migration waves of farmers from their home villages into big urban area after natural disasters, severe drought and civil wars which make new slums in every big cities. HIV is prevailing in and out of Africa. As a natural course of event in those areas TB are much more furious than any past time.

The Japanese oversea health aids started in 1960. In those days it was just like big scale traveling clinics, however, it changed the styles as the medical aids projects in 1970. After the terminations of reparations of II world war in the latter half of the nineteen-seventies, the free medical aids started and increased yearly. In the nineteen-eighties, combination of free medical aids and technical cooperation became the major parts of oversea health aids of Japan.

Today Japan is the second biggest economical major power, and is responsible for supporting the developments of developing countries in cooperation with other advanced

countries. Amount of the Japanese ODA is now biggest in the world, however, the order of the rate of ODA against GNP among advanced countries has been as low as the 13th. This means the effort of Japanese Government to help retarded countries is not enough comparing with those of other advanced countries. From humanistic view point the medical service should be very important in ODA. The level of Japanese medicine is fairly high and the Japanese pharmaceutical industries and medical instrument industries are also in high level and they have enough abilities to support developing countries.

Japan possesses also enough knowledges and experiences in succeeding in control of parasitic and infectious diseases in the past and is able to offer them to oversea medical stuffs in developing countries by training them in Japan.

Dr. H. Nakashima has become the first Secretary General of WHO from Asia and the world is expecting Japan to play his big roll in the international health service.

But Japan has many disadvantage as follows in playing his rolls in international services. We have little experiences in international cooperations in health service. We are suffering the shortage of stuffs and specialists in this field. We don't have enough knowledges and ideas in international health service. We don't have the exact system of criteria on the evaluation of our foreign medical aids. The Japanese are in general very weak in foreign language. Very few Japanese are well versed in foreign languages besides English. Medical instrument and drugs produced in Japan are made only for Japanese use. Very little informations are available about WHO and JICA in Japan and people don't know how they can work in international fields. The communication of the Government and NGO is very poor.

Japanese oversea medical aids have been originally established only by the requests of the partners and as a rule Japanese officials have had no original active intention. This attitude resulted in tremendous wastes of medical instruments and facilities of aids projects. Medical aids from Japan in the future must be taken into account of the intornational unbalance of health service and also social unbalances within the countries.

Medically developing countries must be classified in 3 classes A. B. C and their geographical circumstance, cultural and linguistic situation should be cautiously studied. Before starting aids to some country, they must examine and evaluate whether the past aids to that country from Japan had been effective or not. Offer the most suitable medical aids project from Japan and if it seems to be more suitable to ask the aids from WHO do not hesitate to ask it to WHO. Regarding the maximum term of medical aid, in some project the limited 5 years is too short and much longer term should be considered case by case.

2 THE FUNDAMENTAL NATURE OF CHAGAS' DISEASE: A VIEW PROVIDED BY IMMUNE RESPONSES AND IDIOTYPES

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The etiologic agent of Chagas' disease is the protozoan *Trypanosoma cruzi*, which infects 10-15 million people in endemic areas throughout Latin America, and is naturally transmitted by insect vectors of the family Reduviidae. Infection can also occur by congenital passage, orally, by laboratory accident, and in organ transplants and blood transfusions. About 15,000 *T. cruzi* infections a year occur in Brazil by contaminated blood transfusions, and 5-51% of the blood units recently tested in Bolivia were serologically positive. Transfusion cases in the United States have been traced to immigrant Latin American blood donors. Recent immigrations of people of Japanese heritage from Brazil to Japan, might be something Japanese parasitologists and tropical medicine workers need to consider in the future.

There are 3 life-cycle forms of *T. cruzi*. Epimastigotes multiply in the midgut of the insect vector, then differentiate in the hind-gut into infectious trypomastigotes, which are excreted with the feces or urine after the insect takes a blood meal. They enter the body by mucous membranes or abraded skin, enter mammalian host cells, escape from the phagolysosomal vacuole, and differentiate into amastigotes. Intracellular amastigotes multiply in host cells, redifferentiate to trypomastigotes, to be released upon rupture of the cell.

Acute Chagas' disease can be a mild or severe illness of 1-2 months or asymptomatic. Morbidity can be localized (chagoma at the site of infection), and/or systemic (fever, edema, lymphadenopathy, enlargement of the heart, electrocardiographic changes). Acute disease is usually accompanied by general immunosuppression, blood and tissue parasitemia, and can be fatal. Usually, symptoms and parasitemia decrease in a few months, followed by lifelong chronic infection with little morbidity, in which parasites are difficult to demonstrate. Serologically-positive, chronic asymptomatic patients are termed indeterminate (I). Morbidity-associated lesions develop in 20-30% of chronic patients, after a variable number of years (usually 10-20). Severe disease often involves myocardiopathy (Cardiac disease; C), ranging from minor electrocardiographic changes to sudden death by heart failure. Severe "digestive" megasyndromes can also develop.

Chagasic myocardial lesions are widely distributed lymphocytic/histiocytic inflammatory infiltrates, often with multinucleated giant cells and plasma cells. Cardiac muscle and neuron destruction occurs, followed by progressive fibrotic replacement. The cellular participants in these lesions are only now being phenotypically defined. Asymptomatic I-patients who die of other, unrelated causes have identical (but less intense) lesions. Longitudinal studies in endemic areas estimate the risk of I-patients developing overt cardiopathy in the third decade of life is 2-5% per year.

The immunopathogenesis and immunoregulation of these cardiac lesions is of primary

interest in my laboratory and to our collaborators. This presentation will focus on the immunologic profiles which occur during the chronic stages of the infection, and the finding that both responses against *T. cruzi* antigens, and their immunoregulation, and responses against idiotypes associated with these anti-*T. cruzi* responses correlate with the presence of the different clinical forms of the infection.

The pathogenesis of chronic Chagas' disease is still in question. This is primarily because whether the patient has the I-, C-, or digestive-form, it is difficult to detect organisms, either as blood stage trypomastigotes or tissue-dwelling amastigotes. Because of this it is popular to consider that *T. cruzi* may induce an autoimmune state (anti-heart muscle; anti-nerve) that then perpetuates itself in the presence or absence of organisms. Circumstantial evidence for this theory is based on numerous findings in chagasic patients of lymphocytes and antibodies that respond to, or react with *T. cruzi* antigens and self antigens. Yet, as with any proposed autoimmune condition, it is difficult to prove a cause-and-effect relationship.

Chagasic patients' peripheral blood mononuclear cells (PBMC) respond to *T. cruzi* epimastigote antigenic extracts (EPI). If these responses are subdivided into levels of responsiveness and analyzed, almost all of the low responders are C-patients. The responses of these patients are also augmented the most by removal of adherent macrophage suppressor cells, and their responses are partly augmented by indomethacin. Chronic I-patients are medium or high responders, and their PBMC exhibit little or no adherent cell-mediated immunoregulation. Comparisons of patients' Ab responses by Western blotting against separated EPI and their cell-mediated responses by T cell-Western blotting have also shown some differences between C- and I-patients. PBMC from I-patients responded more often to high molecular weight components (100-150 kD), while both I-and C-patients responded well to moieties between 28-32 and 48-57 kD. The Ab reactivity profiles were similar to those of PBMC. All chagasic patients' sera had Abs against a *T. cruzi* GP57/51 antigen, and the level of all chronic patients' PBMC responses to crude EPI correlated strongly with their responses to highly purified GP57/51.

Patients with chronic Chagas' disease have peripheral blood anti-idiotype (anti-Id) T cells that respond to anti-EPI antibodies immunoaffinity-purified from the sera of patients. Some patients' PBMC anti-Id responses to anti-EPI Ids from C-patients (Id-C) are inhibited by chloroquine (Group 1), but some other patients' PBMC anti-Id responses to Id-C are not inhibited by chloroquine, anti-HLA Class II antigens, or sodium azide (Group 2). Almost 70% of the patients in Group 1 are asymptomatic, and 100% of those in Group 2 have severe disease. This is a striking clinical correlation with a given type of anti-Id responsiveness. The direct (non-processed; non-MHC-presented) stimulation of anti-Id T cells from C-patients by Ids expressed on anti-EPI antibodies from C-patients could provide an immunopathogenic basis for disease. Anti-Id specific rabbit sera can detect Id differences in the anti-EPI Abs from pooled or individual C *vs.* I-cases. Competitive ELISA assays and Western blot analyses of the Ids confirms that the Ids on I-patients' anti-EPI Abs are associated with the primary structure of Ig heavy and light chains, while the Ids on C-patients' anti-EPI Abs are defined by intact, non-denatured Ab molecules.

The chronic, endemic nature of Chagas' disease provides optimum opportunities for strong maternal/Kneonatal Id interactions that could influence subsequent immune response and immunoregulatory abilities of children born of infected mothers. Such interactions do occur naturally, because the cord blood mononuclear cells from chagasic mothers' children

respond to Ids on anti-EPI Abs. A hypothesis based on idiotype-induced pathology and immunoregulation will be described that could, through maternal/neonatal influences, account for many aspects of the immunology and pathology of Chagas' disease.

General presentation

1 WAYS OF THE JAPANESE SOCIETY OF TROPICAL MEDICINE AND THE JAPAN ASSOCIATION FOR INTERNATIONAL HEALTH

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The Japanese Society of Tropical Medicine was founded in 1959 and the First Annual Meeting was held in the autumn of that year under the presidency of Prof. Kaoru Morishita now deceased, of Osaka University. Since then all areas of tropical medicine, including parasitology, bacteriology, medical entomology, infectious diseases, public health, nutrition and acclimatization have been studied on the basis of factors such as climate, biology, culture (socioeconomic) and genetics. The results of the studies have contributed not only to science but also to the welfare of human beings. The achievements of these societies have improved the health of residents of the tropics, and also promoted international cooperation.

The very begining of tropical medicine as a separate field of medicine was probably the foundation of the Liverpool School of Tropical Diseases in 1898. Soon after, the name of the school was changed to the Liverpool School of Tropical Medicine. The first prospectus of the school, issued in 1900, gave as its objectives:

(1) The training of workers in the special subject of tropical medicine,
(2) The promotion of research into tropical diseases,
(3) The organization of measures for prevention of diseases in the tropics,
(4) The clinical care of patients suffering from tropical diseases (This objective of the school was omitted accidently in the first prospectus but added later).

These objectives have pointed out the direction of tropical medicine since then.

In Japan, international cooperation in the field of health was much encouraged by "Japan Salon for International Health" held in June, 1983, by medical doctors and persons in related fields. Various fields are part of the promotion of international health. It is necessary to enhance health of all persons everywhere; this concept was the starting point for the fouda-tion of the Japan Association for International Health in 1986. The First Annual Meeting was held in March 1986 under the guidance of Prof. Emeritus Naoichi Zuyama of Tokyo University. The members of this association are medical practitioners, including medical doctors, other workers in medical fields, and students. All want to contribute to the resolution of the problems of disease and to the establishment of health and welfare, through interna-tional cooperation in collaboration with the inhabitants of developing countries, in particular.

These two groups plan close cooperation in the future, both having as their goal the health of persons all over the world.

2 MEDICAL PROBLEMS OF THE JAPANESE STAYING AT THE OVERSEA DEVELOPMENT COUNTRIES

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We discuss here about following items.

1. Medical problems obtained from the medical check of the Japanese staying at the oversea development countries.
   a) Problems of tropical diseases or endemic diseases, such as viral hepatitis, dengue fever, typhoid fever, cholera and malaria.
   b) Inquiry of the reliable medical facility in the tropical countries.
   c) Inquiry of the medical check results by the tropical countries.
   d) Transportation system of the patient.
2. Diseases which Japanese in tropical countries are suffering from
   a) Water borne diseases—contamination of drinking water
   b) Mosquito borne diseases—malaria and dengue fever.

3 PRESENT STATUS OF THE MEDICAL CHECK OF THE JAPANESE STAYING AT THE OVERSEA DEVELOPMENT COUNTRIES

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We carried out medical check of the Japanese staying at the oversea development countries for 20 years. In 1988 we went to 7 countries in Souch East Asia, 4 countries in South West Asia, one in Oceania, 6 in Middle East and 7 in South America, and checked 2,573

persons there.

We discuss here the results obtained from medical check and analysis the infection routes of viral hepatitis of type A as well as type B.

4 MALARIA STUDIES IN BANGLADESH

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Malaria continues to be a major public health problem in Bangladesh, according to the Directorate of Health Services of the Bangladesh Government. Annual incidence of malaria cases reported in the the country for the past 10 years has remained more or less static with only slight annual fluctuations. The maximum number recorded was 45,902 in 1981, which was 0.42 per 1,000 population. The country has been divided into three strata in terms of malaria transmission potential and activities developed accordingly. Stratum-1 is first priority areas which have a total 10 million population, most of them in the eastern part of the country. The proportion of *Pl. falciparum* infection showed an increasing trend and constituted approximately 67% of all cases in 1989. The majority of these were from the Chittagong District and it was suspected there were cases scattered in other districts which had been brought in from the hilly areas. Some 34 species of anopheline mosquitoes have been recorded in the country, four of which, namely *An. dirus, An. philippinensis, An. minimus* and *An. sundaicus* are considered important vectors. *An. dirus* from hilly and forest areas, in particular, seems to be playing a very important role.

5 ASSESSMENT OF EPIDEMICS BY FREQUENCY DISTRIBUTION CURVE OF MALARIA ANTIBODY TITERS

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When a malaria control campaign comes to an advanced stage, microscopic observation of blood smears may miss lower parasitemias than microscopic threshold. On such an occasion, a suitable method for seroepidemiology is required in order to detect latent malarial foci in the controlled areas. A follow up surveys were conducted at two villages, Sennar and Mobi, in the Sudan Gezira in December 1987 and January 1989. In 1987, 80 school children were examined in Sennar and 5 children with parasitemia were detected, while, in Mobi, none out of 46 manifested parasitemia. The statistical analysis showed no significant difference of

endemicity between the two villages. Antibody positivity rates of Sennar and Mobi were 52.5% and 34.8% respectively. Again significant statistical difference was not noted between the villages. However notable difference of endemicity derived from the type of frequency distribution curves of malaria antibody titers was recognized: A bimodal distribution of titers was obtained with sera collected in Sennar, and antibody pattern characterized by a single low-titerd peak resembling an expotential curve was obtained with those collected in Mobi. The former type of curve suggests that the population sampled lived in an endemic area where transmission had recently occurred, and the latter shows that malaria was absent or transmission had been interrupted in the area. Thus a potential higher risk of malaria epidemics in Sennar than in Mobi was estimated in December 1987. In August 1988, a heavy rainfall caused a flood of the Nile in the Gezira area and a malaria epidemic did occur in Sennar triggered by the flood. In January 1989 in Sennar, 34 out of 72 examinees manifested parasitemia, positivity rate of antibody was 75.0%, and the second mode of the frequency distribution curve became higher than that obtained before the flood. While in Mobi, 7 out of 90 manifested parasitemia and antibody positivity rate was 29.0%, which was the same level of endemicity as obtained in 1987. However the antibody pattern changed into bimodal one, which suggested a small outbreak of malaria caused by the flood. It was affirmed in this survey that the endemicity of malaria can be precisely reflected in the shape of the frequency distribution curve of antibody titers. And, with this method, potential risk of malaria outbreak in Sennar was foreseen in 1987 before the flood that triggered the epidemic in 1988.

6 EPIDEMIOLOGY OF MALARIA THROUGH QUESTIONNARE CONDUCTED ON JICA EXPERTS WORKING IN THE TROPICAL AND TEMPERATE AREA

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A questionnaire was conducted on JICA experts living in various places on the situation, countermeasure and problems of malaria in their area in 1963. The objectives of the survey was to collect informations on

1) local situations of malarial transmission
2) distribution of drug resistant malaria
3) personal history of catching malaria
4) basis of diagnosis
5) actual conditions on prophylactic measure, treatment and means to obtain antimalarial drugs, and
6) health problems they are facing in general

Samples were selected ramdomly from 1,110 experts. 211 questionnaires were sent and 136 replies were collected. Results are largely as follows:

1) Malaria is endemic in most countries of their duty in Asia, Africa and Central-South America. And it is comfirmed that experts in Africa are exposed to higher risk of

infection than those in other regions from the view point of daily behavioral range.

2) There was considerable difference in the informations on the situation of drug resistant malaria between those obtained by the present study, and those reported by WHO, which might be due to unsatisfactory understanding of respondents regarding to the presence of resistant malaria parasite and its effect on the health problem in the countries in warm climates.

3) Incidence of malaria among Japanese experts was highest in Africa showing 22%, that in both Africa and Cental-South America was approximately 6%.

4) Diagnosis was mainly based on the clinical signs and symptoms, the confirmed cases by parasitological examination was only 27%.

5) Generally speaking, the experts possess proper knowledge about prophylaxis of malria.

Distribution of chloroquine resistant malaria is expanding recently. Moreover, multidrug resistance, resistance against newly developed mefloquine have been reported, causing some confusions in the countermeasure by the authorities.

It is suggested from the results that proper measure should be taken regarding the use of new drugs and health education as well as clinical diagnosis and treatment for Japanese working in malaria endemic areas most of whom is not immune against the disease.

7 A FOLLOW UP STUDY OF MALARIA INDIRECT FLUORESCENT ANTIBODY POSITIVITY RATES OF JAPANESE STAYED IN THE ENDEMIC COUNTRIES

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Since 1982, we have conducted malaria serological survey with indirect fluorescent antibody test on Japanese who had stayed in malarious areas for 2-3 years and returned home. Five hundred and eighty three females and 2,127 males of 1-71 years old with peak age at 25 were tested. The endemic countries they stayed are 11 African 13 Asian and 6 Latin American countries. The returner from Africa made the majority of the examinees. In Africa, it was noted that positivity rate in 1987-1990 increased comparing to that tested in 1982-4. In some countries, as high as 40% positivity rate was recorded in recent years. In Asia, stable positivity rate was noted in Papua New Guinea and in Solomon islands. Significant positivity rates were recorded in the Philippines and Malaysia in some years, however, positive cases turned to negligible rates or to null in the following years. Japanese are non-immune to malaria, and likely to take prophylactic drugs. This follow-up study will work as a parameter for malaria incidence and the prevalence of drug resistance malaria, particularly in Africa where malaria monitoring systems are still insufficient in general.

8 INVESTIGATION OF IMPORTED MALARIA CASES IN 1988-1989

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Since 1960, no indigenous malaria has been detected in Japan. In our country, however, increase in imported malaria is recently drawing attention again as the international travel is getting more active. The present report is to introduce the current status of imported malaria in Japan, which is based on the results of the investigation on the incidence of malaria, carried out by the authors for 2 years in 1988 and 1989 inclusive.

The total number of malaria cases detected for 2 years was 185 (83 in 1988 and 102 in 1989). All were imported. The patients were made up 132 Japanese (71.4%) and 53 foreigners (28.6%). The malaria cases consisted of 149 males (80.5%) and 36 females (19.5%). As a whole, the majority of the patients were males in the 20-40 year age group. Of the 185 cases, 56.8% were caused by *Plasmodium vivax*, 35.1% by *P. falciparum*, 2.2% by *P. malariae*, 1.6% by *P. ovale* and mixed infection respectively. The species was not determined in the remaining cases. In the majority of patients (64.1%) were acquired in the Southeast Asia and Western Pacific areas, in 30.9% the origin was Africa area. Malaria cases grouped according to the period of time between the date of arrival in Japan and the onset of illness was analysed. Falciparum malaria started within 1 month after arrival in Japan in all cases, while only 52.0% of the vivax malaria cases began to show its clinical symptoms in the same period of time and 28.0% took as long as 6 months to over a year untill the onset of the symptom. These results suggest that surveillance concerning malaria onset is required, in general, for at least 1 month after arrival for falciparum malaria, and 6 months to one year for vivax malaria. Regarding the prognosis of patients, relapse concerned in 8.9% of the vivax malaria, but no death was reported.

Malaria is endemic to a wide region of the torrid and subtropical zones and it is thought that as many as two hundred million people are infected with malaria and that one million die of the disease every year. Recentry, this disease has become a serious problem of public health due to increase in imported cases in Western countries and also Japan.

9 THE LOCALIZATION OF NEURAMINIDASE ON STAGES OF *TRYPANOSOMA CRUZI*

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A growing body of evidence indicates that neuraminidase is important in mediating interactions between *Trypanosoma cruzi* and host cells. This parasite has three developmental forms which differ in their expression of neuraminidase activity. *In vitro* studies have demonstrated that living trypomastigotes have the highest enzymatic activity. Neuraminidase was shown to be heterogeneously distributed among strains of *T. cruzi* with the highest level of activity being associated with the least virulent strains. Previous work using indirect immunofluorescence and immunoprecipitation assays suggested that neuraminidase is located on the surface membrane of trypomastigotes. In this study, we used immunoelectron microscopy to directly demonstrate the distribution and localization of neuraminidase in various stages of *T. cruzi*.

Cultures of the Sylvio X-10/4 strain of *T. cruzi* were used in all experiments. Immunoelectron microscopy using TCN-2, a monoclonal antibody specific for the neuraminidase, was performed to determine the precise localization of the parasite enzyme. In agreement with previous observations, TCN-2 reacted with tissue culture trypomastigotes, but not with epimastigotes, amastigotes or intracellular forms in intermediate stages of development.

Neuraminidase was localized on the surface of tissue culture trypomastigotes, in particular in the flagellar pocket region. Neuraminidase was also detected in the Golgi apparatus of the trypomastigotes, suggesting that the enzyme is modified post-translationally. In agreement with this suggestion, digestion of neuraminidase with N-glycanase, an enzyme that releases N-linked oligosaccharides, decreased the molecular weight of the polypeptides that make up neuraminidase. Neuraminidase was also found in intra- and extracellular trypomastigotes.

Immunoprecipitation by TCN-2 of metabolically labeled parasites revealed that neuraminidase of intracellular trypomastigotes is molecularly indistinguishable from the enzyme of extracellular parasites.

10 CHEMOTAXIS OF ELONGATED BLOODSTREAM FORMS OF *TRYPANOSOMA VIVAX* (IL 1392) AGAINST POLLEN GRAINS OF *FUCHSIA HYBRIDA* AND *SAINTPAULIA IONANTHA*

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Bloodstream forms (BSFs) of a West African stock of *Trypanosoma vivax* (one of three major pathogens of African trypanosomiasis) are infective for rodents and transform from actively dividing normal forms (N-BSFs) at the rising parasitemia to non-dividing elongated forms (E-BSFs) at the terminal stage of infection.

The present study revealed that these two forms showed different responses to pollen grains of *Fuchsia hybrida* as well as of *Saintpaulia ionantha* when the grains were mixed with the infected mouse blood. Although the N-BSFs showed no response to the grains, the E-BSFs showed rapid swarming around the grains within 30 seconds. The result demonstrated, for the first time, the existence of chemotaxis in the BSFs of a salivarian trypanosome against a natural substance.

This reaction was observed by placing 3-5 drops of tail blood of the infected mouse blood over an area where the grains were smeared on a microscope slide and immediately examining by phase-contrast microscopy. The test slides could be stored at least for two months indicating that the substance on the grains which attracted the E-BSFs, is highly stable at room temperature. Subsequent examinations of pollen grains of *Hemerocallis fulva* and two species of *Lilium* revealed the absence of the attractant on the pollens of these plants.

The findings suggest that (1) the chemotaxis of E-BSFs is highly stage specific, (2) the attractant is present on the pollens of specific plants, such as *Fuchsia* and *Saintpaulia*, and (3) this system may be useful for studying the mechanism underlying the stage transformation of *T. vivax*, particularly from the dividing stage to the non-dividing stage. Identification of the chemical nature of the attractant is presently underway.

11 LEISHMANIASIS AND ITS VECTOR SANDFLY IN PARAGUAY

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Cutaneous and mucocutaneous leishmaniasis are endemic in Paraguay; the disease occurs in a wide range of the country. It is, therefore, a considerable problem of public health, especially in the north-eastern area, where agricultural human population is very high, because of the suitable (fertile) terrain for cultivation. Little information, however, has been available on epidemiological features of the disease, such as infection rates of inhabitants,

sandflies and wild and domestic mammals with *Leishmania* in each endemic area of Paraguay.

Historical notes: Many clinical cases were reported in the country since Migone (1913) described a case of visceral leishmaniasis (it is considered, at present, to be an imported one). Recently, Yamasaki *et al.* (1988, 1989) briefly mentioned current status of the disease and schizodeme characterization of several *Leishmania* isolates in the country. As to the causative agent of leishmaniasis in Paraguay, only one species, *L. braziliensis*, has been recorded hitherto by Grimaldi *et al.* (1989) who examined *Leishmania* isolates from patients living in the south-eastern area of the country.

Object of the present survey: For a better understanding of the epidemiological features of leishmaniasis in Paraguay, a preliminary survey on natural infections of humans, wild and domestic mammals and vector sandflies was made.

Materials and methods: Examinations were performed in four endemic areas, Limoy, Departamento de Alto Parana, Brazilero-cue and Cantera-voca, Departamento de Caaguazu and Tavai, Departamento de Caazapa. Physical (dermatological) examinations of inhabitants were made; skin test (Montenegro reaction) was also employed by using antigens prepared from *L. panamensis* promastigotes (Furuya *et al.*, 1989)

Results: In the examination of 149 inhabitants (63 males and 86 females) in Limoy, Alto Parana, 66 (44.3%) were positive for dermal lesions, while 74 (49.7%), for intradermal reactions. These positivity rates had a tendency to be higher in males than in females; the rates increased with age in both sexes. Frequency distribution of dermal lesions in skin test-positive persons was 55%, lower extremities; 24%, upper extremities; 11%, trunk; and 10%, face. From these results, it was estimated that sandflies in the area preferably fed on lower parts of the body surface. Two of the 66 (3.0%) subjects with dermal lesions revealed severe mucocutaneous manifestations, losing nasal septum completely. Similar epidemiological data were also obtained in inhabitants of Tava-i, Departamento de Caazapa. In the present survey of sandflies, 5 species of the genus *Lutzomyia, intermedia, whitmanni, shannoni, migonei* and *fischeri*, were collected at different endemic areas. A total of 615 sandflies were dissected, of which only one (*Lu. whitmanni*) from Brazilero-cue was positive for leishmanial promastigotes, showing a very low rate of infection. At present, however, this species is estimated to be a potential vector of leishmaniasis in the country. To date, 12 species of *Lutzomyia* have been reported in Paraguay; the sandfly fauna, therefore, seems to be very poor (Alexander, pers. comm.). Although liver and spleen punctures of rats and wild deers were performed for *in vitro* culture, all were negative for the parasite.

12 THERAPEUTIC EFFECT OF SULFAMOYLDAPSONE (SDDS) IN MICE INFECTED WITH *LEISHMANIA DONOVANI*

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BALB/c mice infected with *Leishmania donovani* were treated with sulfamoyldapsone (SDDS) for appraising therapeutic effect. The mice were infected with 1×10^8 *L. donovani* promastigotes 2S-15M strain via tail vein. SDDS was administered five times by intramuscular (i. m.) or intraperitoneal (i. p.) injection on alternate day after infection of *L. donovani*. SDDS administered orally was done once a day for thirteen days after infection. In the second week after infection, impression smear of the liver was prepared to determine the parasite load which was expressed as Leishman Donovan unit (LDU) by 1,000 hepatic cell nuclei. In the mice infected with *L. donovani*, the group administered SDDS 100 mg/kg orally showed 69% inhibitive effect as compared with the group administered saline. This was confirmed by a repeated experiment. On the other hand, mice injected with Pentostam, a positive control drug, showed 79% inhibition. The groups administered SDDS 20 mg/kg orally, SDDS 20 mg/kg i. m. and SDDS 10 mg/kg i. p. showed 47%, 41% and 35% inhibitive effect respectively. The groups administered metronidazol 50 mg/kg orally and mebendazol 50 mg/kg orally showed 54% and 43% inhibitive effect respectively. SDDS 100 mg/kg orally can be useful for treatment of *L. donovani* in future because that had high inhibitive effect as well as Pentostam.

13 EPIDEMIOLOGICAL STUDIES ON VIRAL HEPATITIS AMONG LONG-TERM SOJOURNERS IN THE DEVELOPING COUNTRIES AND EVALUATION OF PREVENTIVE MEASURES

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It is known that acute viral hepatitis is very common among sojourners in developing countries. In order to conduct effective health control epidemiological studies were made on viral hepatitis which occurred among Japanese staying in developing countries, and evaluations were carried out on preventive measures against it.

The subjects of present study were a group of Japanese people staying in developing countries for two years. Mid year population of the group was 1,732 in 1988. Period of the present study is ten years from 1979 to 1988. The study was conducted based on the reports from offices in each country, survey trips and serological studies on the subjects.

In 1979 frequency of hepatitis A (HA) was very high showing 79% of total hepatitides. However after starting of inoculation of human immune serum globulin (ISG) the frequency of HA declined remarkably. Marked statistical significance was recognized in the efficacy of ISG.

Among 35 cases of hepatitis B (HB) (34 males and 1 female), 2 derived from HBe antigen carrier while the remaining 34 were regarded as infected during their stay in developing countries. No cases of HB were recognized among those who got injection of HB vaccine and statistical significance was recognized in the efficacy of HB vaccine. The rate of people whose HB marker terned positive during their stay is showing a tendency of increase (4.2% in 1987). The frequency of infection with HB virus is especially high in Asian and African countries where the carrier rates of native people were also high.

Ten cases of non-A non-B hepatitis was recognized. Among them 7 were infected in Asia, 1 in Africa and 1 in Middle America. The route of infection of the most of the cases were regarded as oral one.

When Japanese people stay in developing countries, the danger of contracting with acute viral hepatitis is high. Especially among younger generation, carelessness easily leads to contraction with HA. As preventive measures, inoculation of ISG is effective as well as paying attention to foods and drinks. The chances of infection with HB virus is also high. As routes of infection, sexual intercourse in considered to be important as well as medical practices. The efficacy of HB vaccine to those who stays in developing countries was confirmed.

14 INFECTION RATE OF INTESTINAL PARASITES IN JAPANESE INHABITING TROPICAL OVERSEA COUNTRIES —STUDY OF PAST 10 YEARS—

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Many Japanese inhabiting tropical oversea countries are suffering from intestinal protozoa and helminths diseases. We carried out to check intestinal parasites of Japanese in 8 countries of Asia and Oceania, 9 countries of Middle East and East Africa and 12 countries

of South America.

High positive infection rates were obtained from Japanese in these areas, such as 16.7% in Panama, 12.8% in Pakistan and 9.1% in Indonesia at the investigation in 1989. People in Asia and Oceania area were infected with highest of 5%, those in South America were of 4.5% and those in Middle East of 3.4%.

People in Asia, Oceania were apt to infect with helminth, on the other hand, those in Middle East were inclined to infect with protozoa.

15 BACTERIOLOGICAL AND CHEMICAL STUDY OF DRINKING WATER IN THE TROPICAL COUNTRIES —STUDY OF PAST 7 YEARS—

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Not a few Japanese living or traveling in tropical areas suffer from diarrhea and other intestinal infections, one of the major causes of which is considered contaminated drinking water.

Examination of drinking water used in houses of Japanese inhabiting in 8 countries of Asia and Oceania, in 9 countries of Middle East and East Africa and in 10 countries in South America were conducted as a past of the health management of Japanese in tropical developmental countries.

Results were obtained as follows 1) Most of tap water specimens collected contained no free available chlorine 2) Tap water specimens were obtain found contaminated with coliform bacilli and sometimes with *E. coli*. 3) In some countries of North Africa and Near East tap water was very high in hardness.

16 ESTABLISHMENT OF SPECIFIC T CELL LINES WHICH COULD RESPOND TO SOLUBLE EGG ANTIGENS OF *SCHISTOSOMA MANSONI*

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In schistosomiasis, the granuloma formation around the deposited eggs in portal vein is most important pathological phenomenon. It is also well known that cellular and/or humoral immune responses are correlated to regulate this disease. In schistosomiasis mansoni, cellular immune mechanisms are suspected to be more important than humoral immune system to modulate and regulate this disease. As T cell clones can be established to respond soluble egg antigens of *S. mansoni* (SEA), we will be able to understand more well the mechanisms of granuloma formation and the functions of T cells.

In this study, we tried to establish T cell lines or clones from CBA/J mice infected with *S. mansoni*. Spleens were aseptically removed from the 6 weeks infected CBA/J mice, which were infected with 30 cercariae of *S. mansoni* (Puerto Rican strain) with the tail exposure method. Homogenized spleen cells were cultured in 96 flat bottom wells with RPMI 1640, including 10% FCS, 3% Penicillin-Streptomycin (Gibco) and 5 μg/m*l* SEA. At 1 week later, culture supernatant was taken out and new complete medium, including 20% MLA-144 culture supernatant as IL-2, was added into each wells. As the cells in wells were multiplying, the supernatant were removed and new complete medium including SEA was put into each well with 5×10^5 irradiated feeder cells. After this, the supernatant was changed to new complete medium, including IL-2, or SEA with feeder cells as antigen presenting cells once a week. On the way, the multiplying cells were checked to respond to SEA antigen or IL-2 with ^{3}H-TdR up take test. In many cases, those T cell lines had lost to respond to SEA, but could respond only to IL-2. Only one T cell line could respond to SEA (10,865 c.p.m.: back ground 193 c.p.m.). As these cells were cultured with normal spleen cells in 24 wells including SEA-coated beads, these cells aggregated around beads at 5 days of cultivation. However, as these T cells were cultured with irradiated feeder cells, granuloma formation was not found around beads. The normal spleen cells could not aggregate around beads, as only those cells were cultured with beads. From this result, we may suspect that this T cell line be able to work as helper cells to make granuloma around eggs.

17 REGULATION OF THE T CELL RESPONSE TO *SCHISTOSOMA JAPONICUM* EGG ANTIGEN BY CONCOMITANT CELLULAR AND HUMORAL MECHANISMS

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Regulation system of *Schistosoma japonicum* egg antigen-specific T cell response was analyzed in humans. Individuals with previous infection of *S. japonicum* failed to show vigorous response of helper/inducer T cell response ($CD4^+$) to a particular fraction (18 kD) of soluble egg antigen (SEA), while strong response was observed for other SEA fractions. The diminished T cell response was restored when peripheral blood lymphocytes were pretreated with anti-HLA-DQ monoclonal antibody (mAb), or with anti-CD8 mAb. This suggested the existence of HLA-DQ-controlled, a SEA fraction (18 kD)-specific suppressor T cells ($CD8^+$).

On the other hand, serum-mediated regulation of SEA-specific T cell response was also tested. When we added autologous serum to SEA-specific human T cell lines ($DC3^+$, 4^+, 8^-), we observed suppression of T cell proliferation to the SEA fraction of 18 kD. This suppressive activity was detected in the IgG_2 subclass. Serum-mediated suppression was dose-dependent and antigen-specific. T cell response to the SEA fraction was modulated in the presence of 100 $\mu g/ml$ autologous as well as allogeneic infected IgG_2.

Considering our finding that the $CD8^+$ suppressor T cells had no effect on established T cell lines, the suppressor T cells and infected IgG_2 had distinct regulatory mechanisms. Those results suggest that T cell response to a particular component(s) of SEA is strictly regulated through both cellular and humoral mechanisms in human chronic schistosomiasis japonica.

18 CROSS-REACTIVE IDS ON RABBIT ANTI-SEA ANTIBODIES STIMULATE ANTI-ID LYMPHOCYTES RESPONSES BY CELLS FROM CBA/J MICE INFECTED WITH *SCHISTOSOMA MANSONI*

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Many recent studies have investigated the possible roles of idiotypic/anti-idiotypic interactions in schistosomiasis mansoni and japonica. In those works, syngenic idiotypic antibodies were used in human and experimental animals. In this study, we described a system in which spleen cells from *S. mansoni*-infected mice proliferated *in vitro* upon exposure to blastogenic anti-SEA antibodies from rabbits immunized with soluble eggs

antigen of *S. mansoni* (SEA), but those of normal mice did not.

Male CBA/J mice (obtained from Charles River Japan, Inc.) were infected with tail exposure method of 30-45 cercaria of Puerto Rican strain of *S. mansoni*.

Polyclonal anti-SEA antibodies were prepared from individual serum of rabbits immunized with SEA by affinity chromatography on a SEA-Sepharose column. Normal rabbits IgG (NRIgG) was prepared from the pooled sera of healthy rabbits by the method of ammonium sulfated precipitation.

Polyclonal purified anti-SEA antibodies were separated in 10% SDS-polyacryl-amide gel electrophoresis and stained with Coomassie blue stain. The visible bands of purified anti-SEA antibodies were those that correspond to immunogloblin heavy and light chains and no SEA components. The individual purified anti-SEA antibodies had showed different IgG bands by Western-immunoblotting method.

Lymph node and spleen cells (5×10^5 cells/well) were cultured for 3 days in 96 well microtiter plates in 200 μl RPMI 1640, containing 10% heat-inactivated fetal calf serum, 3% penicillin-streptomycin (Gibco) and either 4 $\mu g/ml$ SEA or 40 $\mu g/ml$ anti-SEA antibodies. Cells were plused for the final 8 hr of culture with 0.5 μCi ^{3}H-TdR, harvested on glass fiber filters and incorporated radioactivity was counted in liquid scintillation counter.

Until 6 weeks after infection, spleen cells did not respond to purified anti-SEA antibodies from immunized rabbits. At 8 weeks after infection, spleen cells responded storongly to anti-SEA antibodies. However, the individual purified anti-SEA antibodies had induced different responses from 8 weeks infected mice. The specificity of the responses was demonstrated by the inability of cells of all-stage infected mice to heterogenic NRIgG. Furthermore, spleen and lymph node cells from individual uninfected mice did not respond to anti-SEA antibodies nor to NRIgG. Similary, cells from uninfected mice did not respond to SEA, while cells from 6 to 12 weeks infected mice responded strongly.

These data demonstrated that T lymphocytes from *S. mansoni*-infected CBA/J mice can be stimulated to proliferate by exposure to heterogenic antibodies with specificity for schistosomal antigens.

19 IMMUNOGENICITY OF ULTRAVIOLET-ATTENUATED *SCHISTOSOMA MANSONI* CERCARIAE

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It has been well demonstrated that highly gamma irradiated cercariae of *Schistosoma mansoni* elicit the high level of immunity against the challenge infection (Smithers and Doenhoff, 1982; Dean, 1983; Kamiya, 1986). Even though the nature of this immunity has been mainly analyzed in mouse model, the newer vaccinated laboratory animal model may provide the new facets of immunity to elucidate its mechanisms. And also, the nature of stimulus has a profound influence on immune expression in laboratoy animals.

In these aspects, we investigated the immunogenicity of ultraviolet (UV)-attenuated

cercariae of *S. mansoni*. Cercariae of *S. mansoni* were irradiated at 254 nm with UV lamp. The parasites exposed by 15 or 18 mJ/cm^2 of irradiation were not recovered by portal perfusion at 21 days post infection. This fact was confirmed according to chase of the irradiated parasite migration by means of tissue mincing method and also indirect immunohistochemical staining.

Moreover, Hartley guinea pigs, ICR mice, male and female Mongolian gerbils vaccinated with cercariae attenuated by 18 mJ/cm^2 UV-irradiation showed 44, 51, 13 and 22% immunity against challenge infection, respectively. This revealed that different kinds of vaccinated animals exhibit different level of resistance against the challenge infection.

To investigate the mechanisms of protective immunity induced by UV-attenuated cercaria vaccination, we examined the peripheral eosinophil kinetics of vaccinated guinea pigs.

Vaccinated guinea pigs did not show any peripheral eosinophilia until the end of experiment at 12 weeks post vaccination, although peripheral eosinophil number in guinea pigs infected with 500 normal cercariae significantly increased after 8 weeks post infection. However, when vaccinated guinea pigs were challenged with normal cercariae at 5 weeks of vaccination, peripheral eosinophila was evoked at 3 weeks of post-challenge.

We also mentioned the significant role of epidermal Langerhans cells and lymphocyte proliferation in lymph node of guinea pigs vaccinated with UV-attenuated cercariae.

Therefore, vaccine model using UV-attenuated cercariae will provide precious informations on the mechanisms of protective immunity.

20 A COMMUNITY BASED STUDY ON MEASLES EPIDEMICS I. FACTORS AFFECTING SPREAD OF MEASLES VACCINE

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Previous studies conducted by Takasu and Ahemed (1987) disclosed two remarkable characteristics of SSPE in Karachi; (1) High incidence of the disease in the area, and (2) relatively older contraction of preceding measles infection in many of the cases. In relation to these findings, we conducted a preliminary investigation on clinical epidemiology of measles in children in the city. In order to clarify the incidence and clinical pictures of measles, mothers and grandmothers were interviewed asking for their concepts on clinical pictures of the disease and history of measles attack in their children. Interviewers were also asked questions on factors affecting acceptance of immunizations, *e. g.* information resources and motivation to attend immunizations, key person to take children to clinic, and reasons of absence of immunizations.

During a period from 16th to 24th November 1988, and 28th October to 24th November 1989, 126 mothers, grandmothers and fathers who accompanied their children to Civil Hospital Karachi (Hospital group) and 50 families in Neelam Colony in Karachi (Community group) were interviewed.

(1) Clinical manifestations of measles, "khasera" in Urdo usually developed high fever for more than seven days with generalized exanthema and severe cough. Once the disease attacked a child, he/she had got long lasting immunity. These findings may indicate that "khasera" has an entity very similar to measles and it has not been confused with other viral exanthematous diseases such as rubella and enterovirus infections. As to complications, many of the interviewers talked of many kind of serious complications of measles. Importance of immunizations are well recognized to the interviewers. (2) The interviewers had a total of 814 children. All but 5 had no repeated episode of measles. This finding again supports the idea that "khasera" gives long lasting immunity, same as measles. Age of the contraction of measles distributed widely from infancy to 12 years old but most of them were attacked before 6 years of age, mainly between 9 and 18 months. Relationship between measles vaccination and measles attack was not clearly defined. However, there were many vaccine failures according to their immunization records. A survey on seroconversion of children after the measles immunization has now been in progress. (3) There was no relation between the age, occupational and educational backgrounds and the answers of the interviewers. There was no difference between the two groups, Hospital and Community. (4) Most of the mothers felt responsible to take their children to immunization but in some combined families with a large number in a household the decision makers to take children to immunization have still been grandparents. (5) Clinical staff and local health educators and mass media such as TV have been well utilized for information resources of immunization. (6) Distribution of medical facilities was one of the most urgent factors to spread the immunization.

21 STUDY ON THE MECHANISM OF THERMAL ACCLIMATIZATION IN THE PIKA (WHISTLE RABBIT)

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The aim of this study is to identify the inductions of HSPs (heat shock proteins) and CSPs (cold shock proteins) in weak heat- and strong cold-tolerant pika in order to clarify the mechanism of its character of weak heat-tolerance and to examine the physiological correlation between thermo-tolerance and HSPs or CSPs. (1) HSP 70 in rats and HSP 68 in rabbits were induced by the heat-shock. In pika rabbits, the induction of HSP by the heat-shock was not detected by 10% SDS-PAGE not only in liver tissue but also in those tissues such as kidney, adrenal gland, spleen, brain and skeletal muscle. According to previous research, tissues of liver, kidney, adrenal gland and spleen have relatively large ability of inducing HSP 70 than other tissues in hyperthermia of rat. In further study, Western blotting method with anti-HSP 70 antibody was performed. HSP 70 was detected in liver tissue of all rats under heat shock, but only one showed positive in all pikas. These results suggest that

the difficulty of induction of HSPs somewhat relate to the characteristic of weak heat-tolerance in pika rabbits. (2) Induction of HSP 68 by chronic heat load instead of heat-shock was observed on 10% SDS-PAGE. In the experiment of Western blotting method with anti-HSP 70 antibody, the 68 kD protein responded to anti-HSP 70 antibody but the spot of staining showed smaller M. W. size. This discrepancy may be explained as a gradation of proteins including 68 kD protein. (3) Contrarily in cold-shock experiments, induction of CSP was not identified in rat, rabbit or pika. But one result was found that 32 kD protein in cytosol fraction vanished in one rat after cold-shock. Four kinds of plasma proteins (55, 27, 25, 20 kD) vanished in chipmunk, a hybernator, during and after the period of hybernation has been reported in previous paper. Therefore, the present finding showed the similarity to the previous report of chipmunk and suggests that not only hybernation but also short term of cold-shock may lead to the similar result. Furthermore, physiological significance of HSP and CSP was discussed from the viewpoint of thermal acclimation.

22 CONTINUED LOW MICROFILARIA PREVALENCE IN AMERICAN SAMOA IN THE PAST 17 YEARS

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American Samoa which consists of 4 islands with the total population of 35,000 is the US territory in the South Pacific. The climate is hot and wet through the year. Most inhabitants are ethnic Samoan and there is a considerable amount of population movement between the territory and the neighboring independent country of Western Samoa. Filariasis in this area is caused by diurnally subperiodic type of *Wuchereria bancrofti* which is transmitted by two main vectors, *Aedes polynesiensis* and *Ae. samoanus*.

The yearly change of microfilaria (mf) prevalence rates was studied in the years from 1974 to 1990 (up to July), in order to know if filariasis has been increasing or decreasing after two island-wide mass treatments with diethylcarbamazine (DEC) in 1963 and 1965, which successfully reduced the prevalence rate from 20.4% in 1962 to 0.36% in 1967. Subjects included in the study were food handlers, applicants for government jobs, visa applicants and drivers of commercial vehicles, who were legally requested to have a physical checkup including a mf test.

As a whole, the mf prevalence was found to have been kept at a low level (0.8-2.6%) in the past 17 years. The rates were maintained particularly low in 1982-1987. This period coincided with the DEC mass drug administrations (MDAs) carried out in Western Samoa in 1982, 1983 and 1986. The continued low prevalence in American Samoa seemed to have reduced clinical filariasis significantly in the past 8 years. Possible factors to keep the low prevalence in American Samoa are;

1. improvement of living conditions,
2. reduced mosquito breeding sites, and
3. the MDAs in Western Samoa.

23 PRESENT CONDITION OF CHAGAS' DISEASE IN NORTHEAST BRAZIL: ELECTROCARDIOGRAPHIC ABNORMALITIES AND SERODIAGNOSIS

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Antibodies against *T. cruzi* of out patients at Hospital Oswald Cruz in Recife, were detected by IFA and ELISA. Examinations were performed on men and women aged 0 to 85 years with an average age of 47.5 years. The patients were divided into 3 groups. A group, 54 cases (38.8%) who had already been diagnosed as Chagas' disease. B group, 55 cases (39. 6%) who were diagnosed some heart diseases. Group C, there were 30 cases (21.6%) who were going to the ambulatory butits and clinical diagnosis remained unclear.

Of 54 patients of Group A, complete ECG records were available for 47 as follows: 24 (51.5%) with CRBBB, 12 (25.5%) LAH, 9 (19.1%) PVC, 7 (14.9%) AV-block, 8 (17%) LPH, 4 (8.5%) RBBB, 1 (2.1%) LBBB, including plural findings, wheres 7 of these had normal ECG findings with positive IFA and ELISA titers for anti-*T. cruzi* antibodies in these cases. B group, complete ECG records were available for 20. Three of ten were serological positive for *T. cruzi* infection, the first one whose chief symptom was dyspnea showed AV-block, the second one with pericarditis showed LAH. The third, 51-years-old house wife who lived in Nazaré da Mata where about 130 km northeast from Recife had been bitten by a *Triatoma*, vector of *T. cruzi*. Her ECG showed PVC though she had no symptom. In group C, both IFA and ELISA gave a positive result in 13 (43.3%) out of 30. Complete ECG records were available for 7 of 30, as follows: 3 CRBBB, 2 PVC, 2 AV-block, 1 RBBB. Our serodiagnosis gave a positive result for *T. cruzi* antibodies in 85 (61.2%) with an average of 47.8 years. Especially in this inspection, it is of interest that 3 (2%) out of 85 had been infected by transfusion. Moreover there were two probable congenital cases. Concerning of them a newborn baby, X-ray diagnosis gave a cardiomegaly and both IFA and ELISA were positive. The other one is 22-years-old man whose mother had a diagnosis of Chagas' disease. He was born in Recife and has never been lived in endemic area of Chagas' disease, but both IFA and ELISA were positive. Most of the patients who were serologically positive were from surrounding zone of Recife (Nazaré da Mata, Limoeiro, etc.). Follow-up on congenital or transfusion infections of *T. cruzi* should be paid more attention in this city.

24 REEVALUATION OF THE INSECTICIDE RESIDUAL SPRAYING AS A CONTROL MEASURE OF MALARIA IN GUATEMALA

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In order to reevaluate the effectiveness and to improve the measure of residual spraying, the village scale experiment was carried out in La Avellana, Guatemala, during the period from March 1988 to October 1990, on *Anopheles albimanus* of main vector of malaria in this country. As the results of preliminary observations in 1988, it was revealed that the vector bit man evenly indoors and outdoors and got away outdoors shortly after sucking blood and the resting mosquitoes were found out predominantly on roof of shed. From the results of insecticide studies, propoxur was the choice selection by reasons of the high insecticidal activity especially on short time contact, the high residual effect and strong vapor action.

In 1989, propoxur was treated 3 cycles by the improved method in the village, covering about 2,400 houses and the area of about 200 km^2. In 1990, deltamethrin, which is used currently on a nationwide scale, was tested. As the results, the vector density did not remarkably decreased, in spite of that the residual activity on the surface of the houses lasted for long time and the mosquitoes rested on the insecticide residues without notable avoidance. However, the reduction of the parous rate and the remarkable change of the endophagus behavior were observed. After the application of the insecticide, an endophagus rate fell down from about 50% to 30-40%. By contraries, the number of cases by an active case detection decreased obviously, namely 1.55% and 1.43% of positive rates before and shortly after the launching of the spraying fell down to 0, 0 and 0.27% after 13, 15 and 17 months respectively. But the decrease of number of cases by passive case detection was not remarkable. As a conclusion, it seemed that the decrease of malaria cases might occur presumably although the vector density did not always fall. It is likely presumed that the impact of the insecticiding was not so strong due to the change of an endophagus behavior. According to another results, which investigated a relation between an insecticide spraying and the incidence of malaria in considerable villages in the southern region, the positive correlation was approved to exist between both ones.

The residual spraying of insecticide seemed to be effective to some extent to control malaria in Guatemala. But, it was assumed that the effectiveness decreases considerably compared with one in the past, due to the change of the behavior principally.

25 MALARIA CONTROL PROGRAMME IN DAR ES SALAAM AND TANGA, TANZANIA: ACTIVITIES AND RESULTS ON THE FIRST YEAR (1988/89)

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Dar es Salaam and Tanga are port towns situated on the east coastal Tanzania, where the temperature range is 24-28°C, the humidity high comparatively in all seasons. Malaria is endemic in the both towns with high degree of the transmission caused by the principal vectors of *Anopheles gambiae* and *An. funestus*. Although the transmission may not be perennial, varying to some extent with the season, no season is consistently free from malaria resulting in a considerable degree of immune response in all age groups of inhabitants, particularly in the adults.

The activities of the urban malaria control programme in Dar es Salaam and Tanga have practically started in 1988 through integrated vector mosquito control measures. Namely, larviciding and environmental management are applied in the central area of the cities, and residual house spraying with fenitrothion in the urban-rural fringe areas. The larviciding area in Dar es Salaam is 200 km^2, and the residual spraying covers 50,000 houses twice a year on the plan.

The parasite rate is surveyed among 2,000 and 800 apparently healthy schoolchildren aged 6-8, once a month and once two month in Dar es Salaam and Tanga, respectively. In addition to the blood examination, data of clinical suspected malaria cases are collected regularly from 48 dispensaries in Dar es Salaam, and from 9 in Tanga for epidemiological evaluation.

Entomological evaluation is based on systematic field sampling of malaria vector population from geographical representative locations within the insecticiding operation area and non operation area. For this purpose 60 catching sites in 12 areas of Dar es Salaam and 12 catching sites in 4 areas of Tanga were selected.

Before the control operation in 1988, the parasite rate among schoolchildren proved to be 34% (October, ranged 5.5-73.5% among 6 schools surveyed) in Dar es Salaam, and 42% (May-June, 25.6-64.2, 4 schools) in Tanga. *Plasmodium falciparum* was found in more than 99% of the positive films. Other malaria species were found as a mixed infection with *P. falciparum*, *P. malariae* being dominant comparatively in Dar es Salaam and Tanga, but *P. ovale* or *P. vivax* was not detected in each area, respectively.

The population densities of the vector mosquitoes decreased after the residual house spraying for 1-2 months at least. The parasite rate among children was also on the decrease in the operation areas, reducing the rate by at least 70% for a period of 6 months following completion of spraying, thus achieving the first year objective.

26 GEOPATHOLOGICAL STUDY ON MALIGNANT TUMORS IN WESTERN KENYA, EAST AFRICA

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We performed the geopathological study on malignant tumors (MT) in western Kenya, East Africa. The western region of Kenya stands almost exactly astride the equator. It accounts for almost one thirds of the whole country in area and about one half in population. Natural environments show various features, such as, hot and dry desert areas, cool and moist tropical highlands and hot and dry tropical savannahs. Also various ethnic groups inhabit in this area. Western Kenya is composed of three provinces; Western, Nyanza and Rift Valley Province.

The results were as follows: 1) Out of 30,452 surgical pathological specimens at provincial and district hospitals in western Kenya during 10 year-period between 1979 and 1988, 5,386 cases were histologically diagnosed as MTs. 2) The most common MT in western Kenya was cervical cancer (CC, 1,104 cases, 20.5%), followed by malignant lymphoma (ML, including leukemia, 794, 14.7%), skin cancer (SC, 777, 14.4%), malignant soft tissue tumor (MST, 223, 4.1%), esophageal cancer (EC, 221, 3.9%), breast cancer (BC, 189, 3.5%) and Kaposi's sarcoma (KS, 150, 2.8%). 3) The most common MT in male was ML (including leukemia, 477, 20.8%), followed by SC (399, 17.4%), EC (170, 7.4%), KS (128, 5.6%), MST (128, 5.6%), prostatic cancer (97, 4.2%) and hepatocellular carcinoma (78, 8.4%). 4) The most common MT in female was CC (1,104, 37.1%), followed by SC(376, 12.7%), ML (including leukemia, 277, 9.3%), BC (164, 5.5%), ovarian cancer (99, 3.3%) and MST (95, 3.2%). 4) CC, ML and SC were equally distributed in western Kenya ethnogeographically. 5) On the other hands, KS, Burkitt's lymphoma (BL), fibrosarcoma (FS), penile cancer (PC) and EC showed specific ethnogeographical distributions in western Kenya. These findings suggest that some etiological factors, such as, natural environments, life styles and probably in some part, genetic factors might have some relations with oncogenicities of KS, BL, FS, PC and EC.

27 ENTEROPATHOGENIC *E. COLI* (EPEC) AND ENTEROTOXIGENIC *E. COLI* (ETEC) ISOLATED IN NORTHEAST THAILAND AND THEIR RESISTANCE TO ANTIBIOTICS

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Escherichia coli samples from Northeast Thailand were examined the pathogenicity. To determine the enterotoxigenic *E. coli* (ETEC), which is one of the most predominant diarrhoeal pathogen in tropical developing countries, twenty three specimens from stool of both asymptomatic (9 samples) and diarrhoeal patients (14 samples) from children under 5 years old were collected from Srinagarin Hospital, Khon-kaen University in Thailand. Those diarrhoeal samples have no information of another pathogen such as *Vibrio, Shigella* or *Salmonella*. The heat-labile (LT) enterotoxin of *E. coli* was detected by both Biken test (Modified Elek method) and latex agglutination method (Denka Seiken). Heat-stable (ST) enterotoxin of *E. coli* was detected by suckling mice method. Enteropathogenic *Escherichia coli* (EPEC) were detected with antisera (Denka Seiken), then antibiotic susceptibility of these *E. coli* samples were also tested (BBL Kirby-Bauer method) because increasing of antibiotic resistance bacteria also becoming problem in tropical developing countries such as Thailand.

Though none of LT enterotoxigenic *E. coli* was detected by both Biken test and latex agglutination method, a ST enterotoxigenic strain was detected from a diarhoea sample. This samples have reconfirmed by ELISA (Denka Seiken method). Three samples from asymptomatic and four samples from diarrhoea were ETEC serotype. Both asymptomatic and diarrhoea samples included EPEC and ETEC showed antibiotics resistance to such as streptomycin, ampicillin, tetracycline, chloramphenicol, kanamycin or sulfathoxazole-trimethoprim.

Though the purpose of this study was to detect enterotoxigenicity of *E. coli*, healthy carriers of EPEC and resistance of antibiotics of *E. coli* were also pointed out.

28 THE RELATION BETWEEN PROTEIN AND TOXIN PRODUCTION IN *VIBRIO CHOLERAE*

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Relation between cholera toxin (CT) and protein production was tested for develop the better detection of CT. Five *V. cholerae* samples were inoculated to the syncase broth. These

samples were incubated by both shaking and static condition under 30°C. Cholera toxin was detected by RPLA (Denka Seiken) method. Cell protein of *V. cholerae* was measured by the Lowry's method. Protein which produced in the supernatant was determined by means of Bio-Rad method. The shaking incubation was superior to the static incubation for both total protein production and CT production by *V. cholerae*. Though CT detection became positive after 15 hrs passed and protein production was rapidly under the shaking incubation, it took 48 hrs by means of static incubation to get both CT positive result goes and enough protein production. But as far as the result of amount of the protein produced in supernatant after 48 hrs incubation, *V. cholerae* strains showed higher protein production by the static incubation method than the shaking incubation.

This study showed that amount of protein produced by *V. cholerea* reflected the CT detection and also showed that by 48 hrs incubation without shaking, the amount of protein production increased and also detection of CT became possible.

29 CAUSATIVE ORGANISMS OF RESPIRATORY INFECTIONS —COMMUNITY AQUIRED INFECTION— IN NORTHERN THAILAND

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Bacterial respiratory infections are one of the important disease in tropical area. It is not known which pathogenic organisms are predominate in Thailand. Therefore, this study was carried out to identify the causative agents.

Purpose : 1) To identify the pathogenic organisms responsible for bacterial respiratory infections. 2) To identify suitable antibiotic available in Thailand after discussing with physicians of Mae Sot General Hospital. 3) Compared the result of Thailand with those in Nagasaki.

Material and Methods : Out patient and in-patient of Mae Sot General Hospital during 1989. 11.—1990. 1. and 1990. 7.—1990. 8. To determine the causative bacteria, sputum were collected from patients. Gram-stain and acid fast stain were done to observe the inflammatory cytology and bacteria. Sputum culture was also done. Identification of isolated bacteria was done at the Laboratory of Chiang Mai University.

Results : Rate of causative organisms in Mae Sot area. 1989. 11.—1990. 1. (n=48): *H. influenzae* 41.7%, *S. pneumoniae* 27.1%, *B. catarrhalis* 20.8%, *S. aureus* 4.2%, *K. pnemoniae* 4.2%, *E. cloaca* 2.1%, 1990. 7.—1990. 8. (n=24): *H. inf.* 41.7%, *S. pne.* 37.5%, *B. cata.* 4.2%, *K. pne.* 16.7%,

Discussion : 1) We found that *H. inf.*, *S. pne.* and *B. cat.* were main causative agents of

respiratory infections in Mae Sot area. The occurence of respiratory infections by *B. cat.* decreased in summer and increased in winter. These results are similar to that of in Nagasaki. 2) By MIC_{90} , we found that TC is unsuitable for *H. inf.*, *S. pne.* and *B. cat.* as first choice of antibiotic, with the cooperation of pysicians from Mae Sot General Hospital, we must determine the first choice of antibiotic more suitable than TC.

30 THE POSSIBILITY TO AQUIRE *STRONGYLOIDES STERCORALIS* INFECTION AMONG PUPILS IN THE ENDEMIC AREAS OF STRONGYLOIDIASIS IN OKINAWA, JAPAN

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In Okinawa Prefecture, Japan, high prevalence of *Strongyloides stercoralis* infection has been demonstrated among adult inhabitants by agar-plate culture of feces. However, its prevalence among young people and the possibility to acquire new infections have not been adequately known. An epidemiological survey with questionnaires and fecal examinations was made to evaluate the possibility to acquire *S. stercoralis* infection among pupils in Miwa and Takamine areas, Itoman City, where the positive rate of *S. stercoralis* infection among adult inhabitants was proved to be 20.5% and 15.8%, respectively. In Miwa and Takamine areas, 96.1% and 81.5%, respectively, of the adult inhabitants with *Strongyloides* infection are farmers or engaged in farming partly, 53.3% and 33.3%, respectively, used human feces as manure before 1972, the year of the retrocession of Okinawa to Japan, and 33.3% and 14.8%, respectively, continued to fertilize with human feces after 1972. Moreover, 6 farmers still apply their stools on the fields, and 1 inhabitant offers his family's excreta to a farmer as manure. Among the primary school and junior high school pupils in Miwa area, 23.5% and 41.5%, respectively, participate in farming, and 2.6% and 10.1%, respectively, are often barefooted at farming. In Takamine area, 19.7% and 27.9% of the primary and junior high school pupils, respectively, also participate in farming, and 2.2% and 1.9%, respectively, are often barefooted at farming. The usage of human feces as manure and the participation of barefooted pupils in farming at the present time may cause infections with *S. stercoralis* among the pupils. Actually, *S. stercoralis* was detected in each one among 782 primary school and 240 junior high school pupils of Miwa area by the agar-plate culture of the feces, although *Strongyloides* infection was not detected among 455 pupils of Takamine area. An educational campaign on hygiene for the inhabitants may be necessary to prevent *Strongyloides* infection among the pupils.

31 A COMPARATIVE STUDY ON AN IMPROVEMENT OF THE AGAR PLATE METHOD REGARDING SAFETY (SEALING) IN NORTHERN THAILAND

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The agar plate method, a superior method for the detection of *Strongyloides stercoralis* from stool, has been improved regarding safety standards in this study. Agar dishes are sealed with adhesive tape to prevent filariform larvae from crawling out of the dishes. Out of 54 specimens collected from children in Chiang Mai (Thailand), the coincidence rate (positive number in both methods+negative number in both methods divided by the total number) between the sealing method and the customary method is 94.4%. Sealing depresses neither the detection rate nor the development of worms. This improvement not only improves safety, but it also helps to eliminate such cumbersome problems as offensive smell of the cultured stool and break out of maggots, as pointed out in previous studies.

32 HOW SENSITIVE IS THE AGAR PLATE METHOD FOR *STRONGYLOIDES STERCORALIS*?

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We have been reported that the agar plate method is much more reliable to detect *Strongyloides* than other traditional stool examination methods.

This method is, in short, when a fecal sample is placed at the center of an agar plate, worms crawl out onto the agar media during incubation (27°C, 2 days), and then characteristic tracks become detectable.

The difficulty in detecting *Strongyloides* primarily depends on a small number of larvae in the feces, because of a small number of eggs produced by one female parasite, and in case few worms parasitizes. And appearance of larvae into the feces may be often intermittent.

We have studied how sensitive the agar plate method was when few worms were in the samples. We made several kinds of feces mixture with various larvae density, and tried the method.

The feces from a patient was diluted serially with water at first, and then mixed with feces from healthy persons. Five kinds of mixture with 4×10^3, 4×10^2, 40, 4, 0.4 larvae/2 g, respectively (We used 2 g mixture per one agar plate.) were made. We placed 2 g mixture to each agar plate, and incubated at 27°C for 2, 5, 8 and 11 days respectively (5 plates each).

On the second day, all plates were positisve in the groups of 4×10^3 to 4 larvae/plate, and

3/5 were positive in the group of 0.4 larvae/plate. On the 5th, 8th and 11th day, similar results were obtained.

The conclusion is that we can detect only one alive *Strongyloides* larva using the agar plate method. This method was very sensitive to *Strongyloides*.

33 FOUR ADDITIONAL CASES OF GNATHOSTOMIASIS DOLORESI AND APPLICABILITY OF ELISA DIAGNOSIS

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Genus *Gnathostoma* is naturally a parasite of wild animals. Although this genus includes more than 10 species, only one species, namely *G. spinigerum*, had long been thought to cause human gnathostomiasis. Recently, however, confirmed human gnathostomiasis cases caused by *G. hispidum, G. nipponicum*, or *G. doloresi* were reported. Thus, all *Gnathostoma* spp. seem to cause the larva migrans in humans. Since human gnathostomiasis is caused by ingesting the third stage larvae in fishes, amphibians or reptiles, this disease is one of the important food-born parasitic diseases. In Miyazaki Prefecture, Kyushu, Japan, we have already reported a total of 10 (three confirmed and seven suspected) cases of gnathostomiasis doloresi during 1985-88. Although the additional case was not found in 1989, four cases including one confirmed case newly appeared in 1990, indicating that *G. doloresi* infection is still endemic in this area.

The new confirmed case was a 55-years old house-wife having past history of having eaten raw slices of the flesh of freshwater fishes which were caught by her husband. Since 6 April 1990, she had been suffering with right-lower abdominal pain and had an appendectomy on the 12th April in the local hospital. On 13 April, a rapidly extending creeping eruption with pain and itch developed just above the site of appendectomy. She admitted to the Department of Dermatology because gnathostomiasis was strongly suspected. At the time of admission, laboratory examination data related to parasitic diseases were as follows; WBC 9,500, Eo 13.9%, IgE 50.5 IU/L, CRP (+), immediate type skintest against *G. doloresi* antigen (−). Since linear vesicle formation was noticed at the top of the creeping eruption, this part was biopsied. When the biopsied specimen was carefully examined under a dissecting microscope, a parasite-like creature was moving inside the vesicle. An intact living worm of approximately 3 mm length was dissected out, and, this worm was identified as the juvenile adult of *G. doloresi* because it had 7 lines of hooklets on the head-bulb; the 2nd and 7th rows were incomplete.

Although her husband also noticed a mobile indulation with mild pain and itch on his right lumbar region on 20 May 1990, the indulation was too large to be extirpated. His laboratory data was; WBC 4,800, Eo 25.5%, IgE 10,833 U/L, CRP (−), immediate type skin test for *G. doloresi* (+). About one month later, the indulation spontaneously disappeared.

Since the skin test of this couple gave an opposite result, we carried out specific antibody

detection by IgG-ELISA for these and other gnathostomiasis cases to test the applicability for the diagnosis. The results show that 3 out of 4 confirmed cases were positive, whereas 2 out of 5 suspected cases were positive by the ELISA. Our results were markedly different from those previously reported high positivity of ELISA diagnosis for gnathostomiasis spinigerum in Thailand or in Guatemala. This discrepancy seems to be due to, at least in part, the intensity and the duration of infection. Further study is required to clarify this point.

34 AN INTEGRATED APPLICATION OF AN INSECTGROWTH REGULATOR, PYRIPROXYEN AND SOUND TRAP SYSTEM FOR THE CONTROL OF THE VECTOR OF JAPANESE ENCEPHALITIS IN AN OPEN PADDY FIELD IN THAILAND

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Realization of integrated application of sound trap system and an insectgrowth regulator, pyriproxyfen (IGR) was successfully proven at an open paddy field in Sai Noi District, Nontaburi, Thailand. A blocked site with 2,900 m^2 was treated with pyriproxyfen (IGR) by the concentration of 5 ppb of granule in 5% of chemical. The depth of the water was kept about 5 cm and the length of the rice plant was 25 to 30 cm during the experiment. The mosquitoes collected were mono-specific *Culex tritaeniorhynchus*. The mosquito population at the site dominantly responded to 350 and 1,000 Hz in the comparison among sound frequency responses. After application of the chemical during 6 days consequent overnight trapping (18:00 pm to 6:00 am) was performed by emitting these two frequencies from 16 traps. Then the trappings were performed to monitor the population of the vector on 23rd and 33rd day after the application of the IGR by using 10 traps. The number of the vector trapped decreased from 2,347.7 on the commencement day to 971.9 1st day. A slight increasing was proven to 1,990.3 on the 2nd. This level of the trapping numbers was kept till 33 days, though some decreasing were proven on 15th and 23rd.

These results mint that the integrated control method had enough ability to decrease the number of the vector at the study site, however, the increasing of the number on the 2nd day suggested the migration of the vector from neighboring area. The continuation of the overnight-trapping for five nights strikingly proved significant decreasing of the population of the vector before the effect of IGR.

The investigation of the parity of the vectors collected by sweeping also proved significant decreasing due to the function of IGR, being nulliparous rate of 6.0% on the 15th from 46.0% on 6th, whereas trapped number of the vectors was not much changed. This phenomena can be suggested because of migration of parous vectors from neighboring area not treated. Thus in open field the utility of combination of these two control method were

proven to contribute effectively for interruption of the transmission of Japanese encephalitis by extreme reduction of risky infected vector and to keep lower vector density in such endemic open field.

35 COMPARISONS OF GENETIC MAPS OF TWO *AEDES* MOSQUITO SPECIES —WITH SPECIAL EMPHASIS ON FILARIAL SUSCEPTIBILITY LOCI—

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Genetic maps of two mosquito species, *Aedes* (*Stegomyia*) *albopictus*, *Ae.* (*Finlaya*) *togoi*, have been constructed in our laboratory. These maps can be utilized for genetic analysis of filarial susceptibilities of *Brugia malayi*, *B. pahangi*, or *Dirofilaria immitis*.

Comparisons of genetic maps of these two species and *Ae.* (*Stegomyia*) *aegypti* suggest that chromosomal translocations or inversions have occurred among the three linkage groups (2N=6) of the three *Aedes* species during the evolutionary time. But there is a good indication that the region surrounding the sex-determining locus (*M/m*) is similar in the gene order to each other in the three species and so this region has been conserved during the evolution.

In the genetic map of *Ae. aegypti* (Munstermann and Craig, 1987), linkage group I (sex chromosome) contains the following segment; f^{m} ($\sim f^{m4}$)—(2-3 map units)—*Me* (malic enzyme)—(5 units)—*M/m* (sex)—(3 units)—*Acp* (acid phosphatase). The above-mentioned $f^{m}\sim f^{m4}$ are the locus (or loci) for susceptibilities to four filarial species, *B.* malayi, Waltonella flexicauda, D. repens, and *D. immitis*, respectively. Although any filarial susceptibility locus has not been located in the genetic map of either *Ae. togoi* or *Ae. albopictus*, linkage group I of both species includes a gene order very similar to the above segment in *Ae. aegypti*: i. e. *Me*—(ca. 5 units)—*M/m*—(3-5 units)—*Acp* in *Ae. togoi*; also, *Me*—(5 units)—*M/m*—in *Ae. albopictus*. Such tracing of gene loci in linkage group I gives an assumption that the filarial susceptibility locus may be close to *Me* in both *Ae. togoi* and *Ae. albopictus*, although cross experiments remain to be done to verify this assumption.

36 NON-MENDELIAN POPULATION OF A *TRIATOMA INFESTANS* STRAIN, A MAIN VECTOR INSECT OF CHAGAS' DISEASE

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The cytogenetic informations have often contributed to the control programmes for

discriminating insect vectors of endemic diseases. Chromosome observation of triatomine bugs, medically important vectors of American trypanosomiasis (Chagas' disease), was attempted. We demonstrated by C-banding technique that a Brazilian strain of *Triatoma infestans* (2n=22) included at least three cytotypes in terms of constitutive heterochromatin polymorphism. The first type designated as cytotype A has six chromosomes (chromosomes 1-3) with enlarged C-heterochromatin blocks at both terminals. Likewise, cytotypes B and C possess seven and eight such chromosomes, respectively. The chromosomes characterized by the C-block were numbered by the order of size from large to small. Chromosomes 1 and 2 had prominent C-bands at the both terminal regions one side of which is larger than the other. Chromosomes 3 and 4 were characterized by a unilateral heterochromatin block. The homology of these chromosome pairs was confirmed based on pairing patterns at metaphase I of males. A total of 85 bugs examined, included 39 cytotypes A, 26 B and 20 C, respectively. Cytotype B seems to be the hybrid between cytotypes A and C, because chromosome 4 of cytotype B is heteromorphic for terminal C-band (−/+), whereas it is homomorphic (−/−) in cytotype A and (+/+) in cytotype C. The frequency of cytotype B was significantly low from the expected value (A, 31.8; B, 40.4; and C, 12.9) by Hardy-Weinberg expectation test, suggesting non-Mendelian population in mating. We propose an assumption that there are some reproductive barriers between cytotypes A and C, and thus the viability of their F1 (cytotype B) is reduced significantly. Although we need further genetic experiments among the various cytotypes, the terminal C-bands in chromosomes 1-3 and heteromorphism in chromosome 4 mentioned above would potential markers for discriminating vector individuals.

37 PHYLOGENETIC ANALYSIS OF THREE SPECIES OF *PARAGONIMUS*, HUMAN INCIDENTAL PARASITES IN THAILAND AND JAPAN 1. ISOZYMES ANALYSIS

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According to an occurrence of human incidental parasites, *Paragonimus miyazakii*, one of the harmful zoonotic agents causing Paragonimiasis among man and animals were reported in many cases in Japan as well as in Thailand due to *Paragonimus heterotremus*. These parasites whose natural definitive hosts as animals were considered to be related species because their pathogenicity and clinical manifestation in human were very much similar such as high frequency of plueural effusion, high eosinophilia of the blood, pleural exudate and spontaneous pneumothorax.

Another species from Thailand, *P. siamensis* was also considered to relate with *P. miyazakii* and *P. heterotremus* as well. Epidemiological studies found that there was no report of *P. siamensis* infect man in this country inspite of the crab intermediate host of this parasite, the inhabitant were fond of eating raw crab.

Although, the life cycle and morphological characters among 3 species of *Paragonimus*

were discriminated on each other. An attempt to determine the genetic relationships among all of them was performed. However, cytological qualification were very difficult to see especially euchromatin and heterochromatin.

Thus, emphasis on the genetic differentiation among 3 related species *P. miyazakii* from Japan, *P. heterotremus* and *P. siamensis* from Thailand were assessed by electrophoretic analysis of allozymes. Twenty one loci of sixteen enzymes examined; Acid phosphatase, Adenylate kinase, Aldehyde oxidase, Esterase, General protien, Glucose phosphateisomerase, Glucose 6 phosphate dehydrogenase, Alpha glycerophosphate dehydrogenase, Hydroxybutylate dehydrogenase, Hexokinase, Malic dehydrogenase, Malic enzymes, Octanal dehydrogenase, Phosphoglucomutase, 6 phosphogluconate dehydrogenase and Tetrazolium exidase were analysed by using polyacrylamide vertical slab gel electrophoresis and estimated the genetic drifts between the phenotypic frequencies of respective pair combinations among the 3 species of *Paragonimus*. The genetic distance between *P. miyazakii* and *P. heterotremus*, between *P. miyazakii* and *P. siamensis* and between *P. heterotremus* and *P. siamensis* were 1.0036, 1.2942 and 1.4297 respectively. Comparing these values with standard genetic distance described by Ayala and Kiger (1980), these suggested that the 3 *Paragonimus* could be classified into an independent species from each other, and also indicated that *P. miyazakii* was genetically closest to *P. heterotremus* than *P. siamensis*.

However, *P. ohirai* is another one of interesting species in Japan. Epidemiology, life history and pathogenicity of this parasite are similar to *P. siamensis* from Thailand. Therefore, the phylogenic relationships between these 2 parasites will be studied further.

38 TWO-DIMENTIONAL ANALYSIS OF A PUTATIVE EGGSHELL PRECURSOR PROTEIN OF THE FEMALE *SCHISTOSOMA JAPONICUM*

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Production of eggs in schistosomes is dependent upon the integrated functioning of two different cell types- the ovum and the vitelline cells. The parasite egg is formed from approximately 30 vitelline cells and one fertilized ovum which become encapsulated by eggshell within the ootype. It has been suggested that the formation of the eggshell in the ootype occurs via the rapid enzyme-dependent cross-linking of precursor proteins provided by the vitelline cells. This may occur by the oxidation of tyrosine residues of the precursor protein but has never been adequately investigated. In adult worms of *Schistosoma japonicum*, we have obtained experimental results that a major female-specific protein with apparent molecular weight of 34 kD, as determined by SDS-PAGE, was suggested to be a precursor of the eggshell proteins. It has proved difficult to detect major female-specific proteins by direct staining of SDS gels. This 34 kD protein was mainly identified by radiolabelling of parasites *in vitro* with [^{14}C]tyrosine and fluorography of SDS gels (Kawanaka, 1990).

On the other hand, it is known that eggshell hydrolysates of *S. japonicum* are very rich in glycine and poor in methionine (Byram and Senft, 1979). In *in vitro* experiments, we have

observed in autoradiograms that the synthesized protein with [^{14}C] glycine in vitelline cells was utilized in formation of the shells of newly formed eggs. As may be expected, a [^{14}C]glycine-labelled female-specific protein was demonstrated at 34 kD in the parasite protein on fluorography of SDS gels. A single band in the one-dimentional gel, however, is usually composed of several polypeptide species having different isoelectric points. In order to determine whether the identical polypeptide species were being detected in the region 34 kD of the female protein, we performed two-dimentional gel electrophoresis of [^{14}C]tyrosine-, [^{14}C]glycine-, and [^{35}S]methionine-labelled parasite proteins.

In [^{14}C]tyrosine-labelled proteins, at least five spots with apparent molecular weight of 34 kD region were exclusively observed in the female and these pI values were 6.0, 5.8, 5.5, 4.8 and 4.6. By labelling the female protein with [^{14}C]glycine followed by separation by two-dimentional electrophoresis, three spots with pI values 6.0, 5.8 and 5.5 were identified and they were thoroughly corresponded to [^{14}C]tyrosine-labelled spots. Any female-specific polypeptide was not detected at 34 kD region by labelling the parasites with [^{35}S]methionine. The results were suggested that a putative eggshell precursor protein of *S. japonicum* (34 kD) was resolved into three polypeptide species with different pI values.

39 *FASCIOLA* CYSTEIN PROTEASE: SPECIES SPECIFICITY AND AVAILABILITY AS AN ANTIGEN FOR ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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Previous work in our laboratory indicated the presence of a cysteine protease in the intestinal epithelial cells of *Fasciola* adult worms. We believe that the enzyme is excreted into the intestinal lumen as a digestive enzyme and induces the IgG antibody production in the host animal. In this study, we have examined the species specificity and availability of the enzyme as an antigen for ELISA in the diagnosis of human fascioliasis.

The *Fasciola* protease fraction, partially purified by amonium sulfate precipitation and Sephadex G-75 column chromatography, contains a major protein with a molecular weight of 27 kD that was identified as the enzyme by specific monoclonal antibodies in an immunoblot analysis. For comparison, a partially purified *Schistosoma mansoni* cysteine protease containing a major protein with a molecular weight of 31 kD was also used. In our ELISA system, we used the following conditions: (1) the concentration of the enzyme antigen used to sensitize the microtiter-plate wells was 5 $\mu g/ml$; (2) the dilution with PBS of the sera examined was 1:1,000; (3) peroxidase-labeled anti-human IgG conjugate (1:1,000) and (4) the substrate ABTS for color development were used; (5) absorbance at 405 nm was measured.

When the *Fasciola* cysteine protease was used as the antigen for ELISA, the sera from patients with fascioliasis (N=13) gave the highest absorbance of 1.603 ± 0.394 (mean$\pm$SD). For the active fascioliasis sera, no false negative case was observed. However, the heterologous reactions between the *Fasciola* enzyme with the schistsomiasis japonica sera (Philippines, N=13) and with the schistosomiasis mansoni sera (Brazil, N=10) yielded the

lower absorbance of 0.511±0.278 and 0.573±0.168, respectively, statistically significant comparing with the value of fascioliasis sera at the $p<0.001$ level. Similarly, the absorbance values for the sera from patients with paragonimiasis (N=3), angiostrongylosis (N=1), gnathostomiasis (N=1), sparganosis (N=1), and amebiasis (N=1) were 0.425±0.024, 0.307, 0.278, 0.179, and 0.309, respectively, and close to the values for the normal human sera (0.228± 0.095).

When the *S. mansoni* cysteine protease and the patient sera (homologous combinations) were examined in ELISA, an absorbance of 1.248±0.573 was obtained. Cross-reactions with the schistosomiasis japonica sera were observed, but the average absorbance values (0.792) were lower than that of schistosomiasis mansoni sera (1.248). No cross-reaction between the *Schistosoma* protease and the fascioliasis sera was observed.

The results obtained in this study indicate that the *Fasciola* and *Schistosoma* cysteine proteases, even at their partially purified state, are highly species-specific as the sensitive ELISA antigens for the immunodiagnosis of human parasitic diseases. Therefore, it is predicted that *Fasciola* protease antigen will serve as a valuable ELISA antigen in epidemiological surveys of human fascioliasis, including the heterotopic parasitism and immature liver-fluke's migration.

40 AVIDIN BINDING COMPONENTS IN PARASITES

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Strong and specific affinity of avidin for biotin has been widely utilized for enzyme immunoassay. Biotin is also known as vitamin H and certain growth factors.

We recently found that some parasites contain avidin binding components (ABC) in their extracts. In this study, we first investigated ABC in extract of various parasites. The extracts were dotted on a membrane and ABC were detected by peroxidase conjugated avidin-D. Then, ABC positive extracts were applied to SDS-PAGE, electrically transferred to a membrane and ABC patterns were observed on the membrane by treatment with peroxidase conjugated avidin-D and a substrate. Parasites used are as follows. Trematode: *Paragonimus miyazakii, P. westermani, P. ohirai, Fasciola* sp., *Clonorchis sinensis* and *Schistosoma japonicum*; Nematode: *Gnathostoma doloresi, Toxocara canis, Dirofilaria immitis, Trichuris vulpis* and *Angiostrongylus cantonensis*; Cestode: *Diphyllobothrium latum, Spirometra erinacei, Hymenolepis diminuta, H. nana, Taenia saginata* and *Dipylidium caninum*.

Among the extracts studied, those from *T. canis, G. doloresi, D. latum, S. erinacei* and all the trematode except *S. japonicum* contained much ABC. A little amount of ABC were detected in extracts of *S. japonicum* and *A. cantonensis* and no ABC was detected in those of other parasites with the method used. ABC patterns of the extracts studied differed each other. Even those of close related *Paragonimus* species, *P. miyazakii, P. westermani* and *P. ohirai*, could be discriminated by their ABC patterns. No individual variation was observed among flukes of the same species. These observations suggest that ABC pattern can be one of the markers for identification of parasites.

41 EFFECT OF CHEMOTHERAPY WITH PIQUITONG (PRAZIQUANTEL) ON MURINE SCHISTOSOMIASIS MANSONI AND CHANGES OF SERUM ANTIBODY LEVELS

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In mice experimentally infected with *Schistosoma mansoni*, piquitong (Paraziquantel) that synthesized in Shanghai, China, administered orally at the levels of 750 mg/kg (Group-1), 150 mg/kg (Group-2) or medium alone (Group-3) for two consecutive days of two months after infection. Mice were autopsied and examined one, two, four, eight, 12, 16 and 20 weeks after treatment.

The results of the piquitong treatment with doses of 750 mg/kg for two consecutive days showed that none of worms recovered from the mice within least 7 days after treatment.

The mature schistosome eggs in the liver and intestine disappeared and the number of the calcified eggs became to increase in the mice of Group-1 from eight weeks after treatment.

High levels of IgG and IgM antibody titers against adult and egg antigens by ELISA were observed in sera of all mice groups until 20 weeks after treatment. On the other hand lower titer of IgA antibody against egg antigen was observed from eight weeks after treatment in the group of cured mice.

42 DETECTION AND QUANTITATIVE ASSAY OF MAMUSHI SNAKE VENOM AND ANTIVENOM BY ELISA —WITH SPECIAL REFERENCE TO *IN VIVO* EFFECT OF THE ANTIVENOM

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Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods are used for detection of snake venom in patients to identify the biting snakes and assessment of antivenom potency. There are few report of the venom movement in patients and animals and the *in vivo* effect of antivenom. It is suggested that the venom is absorbed at least partialy via the lymphatics and some is excreted in the urine from the result that the high levels of the venom were detected from lymph node, kidneys and blood in the snake bite victim by Australian elapid snake. And it is reported that Crotalus venom showed strong localization to renal tubules and blood vessel walls in rats injected intraperitoneally.

We investigated the potency of the antivenom by monitoring the Mamushi venom in the serum and injection site of rats using ELISA. The venom concentration in the serum of rats injected intramusculary reached a maximum at 3-6 hrs after the injecton, while in the

injection site it decreased rapidly during 6 hrs. However, the venom could be detected in the serum and injection site even at 48 hrs after injection.

The venom levels in rats injected with the antivenom intravenously or intramuscularly 30 min after the venom injection were measured. Only 50% of the venom was detected at 30 min after i. m. injection of the antivenom, whereas no venom was detected after i. v. injection of the antivenom. The venom in the injection site also decreased by the antivenom injection. However, the venom was detected in the injection site during 24 hrs after i. m. injection of the antivenom, whereas no venom was detected at 6 hrs after i. v. injection of the antivenom.

Further investigation was carried out for monitoring the antivenom concentration in the sera of rats and rabbits following i. v. and i. m. injection of the antivenom. The antivenom concentration in the serum of rats injected intravenously with 50 units of the antivenom showed 6 units/m*l* at 1 hr, while it reached a maximum (1 unit/m*l*) in the serum of rats injected intramuscularly 6 hrs after injection, and it needed two days to reach a maximum (1.1 unit/m*l*) in rabbit injected intramuscularly with the antivenom (500 units).

These results showed that the patients in snake bites must be administrated with the antivenom by i. v. injection.

43 SEA SNAKE BITES IN OKINAWA

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We have a very limited knowledge about the realities of sea snake bites in Japan. The only record we have is a report made by Mishima (1963) on a death case caused by *Hydrophis cyanocinctus* in Kagoshima Prefecture. Yet the report was not detailed enough to clarify the nature of the bites. In January 1989, an injury case by *H. cyanocinctus* occurred in Naha, Okinawa. The victim narrowly survived the accident after one month's hospitalization, though he received no treatment by sea snake antivenin.

We conducted on-the-spot surveys of the sea snake bites during 18 months from February 1989 through July 1990. Of the 7 wounded persons, 6 (85.7%) died. The elapsed time between injuries to consequent deaths were 2 to 5 hrs for 4 persons, and 15 to 20 hrs for 2 persons. Our surveys include a death case by *Laticauda semifasciata* which we presume is the first record ever reported in the world. The following circumstances are the main reasons why we have been unable to clarify the sea snake bites in Okinawa.

1: The Prefectural Government has been without an adequate information system due to the absense of sea snake antivenin that is supposed to deal with the sea snake bites.
2: When bitten, the victims fall into a critical condition so quickly that they usually die or become fatal before hospitalized. This fact has been preventing doctors from obtaining full knowledge about the sea snake bites.

44 IXODID TICKS AND INCIDENCE OF *BORRELIA BURDORFERI* IN NAGANO PREFECTURE, JAPAN (PRELIMINARY REPORT)

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Lyme disease described as a separate entity in 1977 in USA has also been reported sporadically from Hokkaido, Kagoshima and Nagano Prefectures since 1987. It is of prior importance to have a thorough investigation of the epidemiology of this tick-born disease in Japan. Since the disease is a typical zoonosis, we have various means to realize incidences of spirochetal infection in given areas. We think, however, it is most efficient to demonstrate spirochetae in unfed ticks. So we collected ticks by flagging the vesitation at 20 sites located mostly in the national or prefectural parks in Nagano Prefecture in May and June, 1990. *Ixodes persulcatus* and *I. ovatus* were most abundant in grassy and low-shrub habitats on and along lanes and tracks in forests. *Borrelia burgdorferi*, the etiologic agent of Lyme disease, was detected with IFA adopting the monoclonal antibody H5332 and/or isolated from the above 2 ticks from 18 sites, indicating a wide dissemination of the pathogen throughout the whole prefecture. The infection rate was unexpectedly high and more than 20% on an average in the adults of both species. Similar prevalence levels may be recorded in adults of common ixodid ticks in some of the 8 prefectures that neighbor Nagano Prefecture.

45 RECURRENT OUTBREAKS OF TSUTSUGAMUSHI DISEASE AND FLUCTUATION OF VECTOR POPULATION DENSITIES

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The recurrent outbreaks of tsutsugamushi disease were noticed in the middle of the 1970's. The causes of the rapid increase of patients have been discussed in conection with the changes in usage of both antibiotics and pesticides, remarkable improvements in diagnostic techniques and some other artificial factors from the beginning of the 1980's, ignoring possible chages in vector population densities. We intended to supplement the shortage in the consideration of causality with ecological studies of the main vectors, *Leptotrombidium pallidum* and *L. scutellare*. *L. pallidum* was densely populated on grasslands newly formed on abandoned farms and on larger paddy banks that have recently been made during the paddy zone reconstruction program. Environmental chages brought about by the abandonment of farms in depopulated areas and the construction of many larger banks in paddy zones are thought to have provided suitable habitats for *L. pallidum* and are probably responsible for the recurrent outbreaks of tsutsugamushi disease transmitted by this chigger. *L. scutella-*

re was shown to be populated at well-drained sites on the eastern slope of Mt. Fuji, Shizuoka Prefecture, and the specific habitat for this vector was the sparse *Miscanthus* grassland developed on the sunny granular soilbed. However, we can not evaluate these findings in relation to the recent outbreaks of the disease transmitted by *L. scutellare* yet.

46 EPIDEMIOLOGICAL STUDY ON SCHISTOSOMIASIS MANSONI IN RURAL AREAS AROUND RECIFE, BRAZIL

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Parasitological, clinical, ultrasonographic and clinico-biochemical studies have been carried out on 485 inhabitants in São Lourenço, 25 km northwest of Recife, and on 619 inhabitants in Cabo, 50 km southeast of Recife, Pernambuco, Brazil. Stool examination demonstrated a high prevalence of intestinal parasitic infections. Moreover, the prevalence of *Schistosoma mansoni* in the inhabitants of São Lourenço and Cabo were 82.1 and 9.8%, respectively.

Ultrasonographic examination of the subjects of São Lourenço demonstrated a frequent abnormarity such as hepatic peri-portal fibrosis, splenomegaly and visualization of splenic vein. There was a positive correlation between the degree of splenomegaly and these hepatosplenic abnormarities and between the degree of hepatic peri-portal fibrosis and splenic vein, whereas there seems little correlation between the degree of these hepatosplenic abnormarities and the intensity of infection as monitored with EPG.

While six enzymatic activities in the sera (GOT, GPT, γ-GTP, MAO, OCT and ALPase), which reflect a liver function, were determined on 334 sera of the subjects in São Lourenço, only ALPase activity significantly increased in the sera of the subjects infected with *S. mansoni*.

The concentration of three serum markers of liver fibrosis [P(III)P-NP, laminin and IRβPH] were determined by radioimmunoassay or enzymeimmunoassay. There was no significant increase in P(III)P-NP concentration in the sera of *S. mansoni*-infected subjects, whereas a significant elevation was observed in the laminin and IRβPH concentrations in the sera of egg-positive subjects. Moreover, we also demonstrated a significant elevation of hydroxyproline concentration in the urine of egg-positive subjects.

These findings probably suggest cross-sectional examinations using ultrasonography, and quantitative analysis of serum ALPase activity, serum marker of liver fibrosis (laminin and IRβPH) and urinary hydroxyproline concentration provide a more accurate informations on morbidity and pathologic state of the subjects infected with *S. mansoni*.

47 CHARACTERISTICS OF ULTRASONOGRAPHIC FINDINGS AND LIVER DYSFUNCTION OF *SCHISTOSOMA JAPONICUM* INFECTION IN LEYTE, PHILIPPINES

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Ultrasonographic (US) examination was performed on 45 newly infected cases and 45 reinfected cases with schistosomiasis japonica, in Schistosomiasis Hospital, Palo, Leyte, the Philippines. US liver images were classified into 4 patterns (type 0: normal pattern, type 1: linear pattern, type 2: nodule pattern, type 3: network or fishscale pattern). Correlation between US patterns and the liver functions was studied. In the chronic cases in Japan, cases of type 3 were common, while those of types 1 and 2 were rare. In Leyte, however, patients of type 3 were less than those of types 0 and 1. Types 0 and 1 were commonly found by US in newly infected cases, and type 3 in long-term infected cases. Big splenomegaly with collateral vessels was also found in long-term infected cases. There was no correlation between US findings and spleen index. No correlation was found between US findings and the levels of aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase and lactate dehydrogenaze. The level of alkaline phosphatase in all groups of the infected patients showed significant elevation over the healthy controls. Significant elevation of the level of total bile acid was also observed in the patients with types 2, 3 and liver cirrhotic pattern.

48 ULTRASONOGRAPHIC AND PATHOPHYSIOLOGICAL CHANGES AFTER TREATMENT WITH PRAZIQUANTEL IN *SCHISTOSOMA JAPONICUM* INFECTION IN LEYTE, PHILIPPINES

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Ultrasonographic (US) examination was performed on 32 newly infected cases and 20 reinfected cases of schistosomiasis japonica, every 3 or 6 months in Schistosomiasis Hospital, Palo, Leyte, Philippines. An improvement of the thickening echogenecity of the wall of portal vein and echogenic bands in the liver lobes was found by US examination 6 months after PZQ treatment. Typical network pattern or fishscale pattern did not change after the treatment. Splenomegaly decreased in 42 patients without collateral vessels, but not in 10 patients with

collateral vessels. There was no significant difference in results of liver function tests such as the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, choline-esterase and lactate dehydrogenase between before and after PZQ treatment. Significant decrease of the level of peripheral total bile acid (TBA) was observed in patients with and without periportal fibrosis after PZQ treatment. In cases with network pattern, the level of procollagen-III-peptide tended to decline. US examination was useful for evaluating the portal hypertention and for comparing the changes in the US images of liver fibrosis before and after PZQ treatment. As a parameter for evaluating the liver dysfunction and fibrosis of hepatosplenic schistosomiasis japonica, TBA appeared to be more reliable than the other liver function tests.

49 BASIC STUDIES ON MONGOLIAN GERBILS AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (5) SERUM LIPID

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The Mongolian gerbil is used as laboratory animal in the fields of virology, cerebral neurology, endocrinology and parasitology. Especially, in the parasitology, the Mongolian gerbil is very useful as the susceptible host to filarial infection. But, there is almost no report about biological characters in this animal. In order to know biological characteristics of the gerbils including the coat color mutants, we made comparative study on biochemical assay among coat color mutants of gerbils and Wister rats. In this examination, we studied on the serum lipid components in agouti (wild) gerbil compared with those in the coat color mutants such as white spotted agouti, albino, black and white spotted black type maintained in our laboratory. Five adult gerbils of each sex or each coat color which are 14 to 15 weeks old, totalling 50 gerbils were used. Gerbils and rats were kept in conventional condition at 24 ± 2°C room temperature and 65 ± 5% relative humidity. Blood samples were obtained from their hearts under anesthetization with ether. Sera were separated and stored at -20°C until examination. Serum lipid components were analyzed by Hitachi 736 autoanalyzer.

Total cholesterol level of each coat color gerbil was within the range of 67-171 mg/d*l*. Other lipid component values were 14-39 mg/d*l* in free-cholesterol, 50-135 mg/d*l* in ester-choresterol, 40-219 mg/d*l* in triglyceride, 91-190 mg/d*l* in phospholipid, 0.27-0.88 mEq/*l* in NEFA and 65-314 mg/d*l* in β-lipoprotein, respectively. There were not significant difference on the serum lipid component levels among coat color mutants or sexes of the gerbils. All lipid component levels were higher than rats.

50 BASIC STUDIES ON MONGOLIAN GERBILS AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (6) BIOCHEMICAL ASSAY

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Mogolian gerbil has been used very widely as a experimental animal. Especially in the parasitology, this animal has been very useful as a susceptible host of experimental filariasis. But there are few reports on basic characteristics of Mongolian gerbil and it is recognized to be necessary in recent years. In this experiment, we made comparative study on biochemical assay among coat color mutants of Mongolian gerbil and Wistar rats.

Animals were kept under conventional condition at 24 ± 2°C room temperature and 65 ± 5% relative humidity. Five adult animals of each sex or each coat color were used in this study. Blood samples were obtained from the retro-orbital venous plexus of the anesthetized animal with ether by heparinized capillary pipettes. Sera were separated and were kept under −20°C until assay. Biochemical assay was made by using Hitachi 736 autoanalyzer.

GOT, GPT, LDH, ALP and CPK levels of gerbils were very higher than rats and they were most highest levels in the rodents according to the past reports. Ch-E levels of gerbils had no sex difference and that is different from rats. AMY, UA, BUN, CRE data showed almost same level as rats. Na, K and Cl levels of gerbils were higher than rats and other rodents. There were no significant difference between sexes or coat color mutants of gerbils.

51 BASIC STUDIES ON MONGOLIAN GERBILS AS A SUSCEPTIBLE HOST TO FILARIAL INFECTION (7) SERUM PROTEIN

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In order to know biological characteristics of the gerbils including the coat color mutants, in this experiment, we studied on the serum protein components of agouti (wild) gerbil compared with those of the coat color mutants which are white spotted agouti, albino, black and white spotted black type maintained in our laboratory.

Five animals of each sex or each coat color which are 14 to 15 weeks old, totalling 50 gerbils were used. Blood samples were obtained from their hearts under anesthetization with ether. Sera were separated and stored at −20°C until examinations. Total protein was analyzed by Hitachi 736 autoanalyzer and the serum protein fractions were examined by the

electrophoretic method.

Average total protein value of each coat color gerbil was within the range of 5.2-6.8 g/d*l*. A/G ratio of each coat color gerbil were around 1.5. The typical pattern of serum protein fractions of gerbils had 5 peaks. Those were 57.8-61.2% in albumin, 1.9-2.7% in α_1-globulin, 8.8-11.2% in α_2-globulin, 4.4-9.2% in β-globulin and 18.1-23.6% in γ-globulin, respectively. There was not significant difference on the serum protein components among coat color mutants of the gerbil.

Our examination appeared that A/G ratios of the conventional Mongolian gerbils including coat color mutants are almost same value as that of human, 1.5.

52 COMPARATIVE STUDIES ON SENSITIVITY TO *BRUGIA PAHANGI* AMONG COAT COLOR MUTANTS OF MONGOLIAN GERBILS

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In this oxperiment, we made comparative study on sensitivity to *Brugia pahangi* among coat color mutants of Mongoloan gerbil which widely used as a susceptible host to filarial infection.

Coat color mutants of gerbils such as agouti, white spotted agouti, albino, black and white spotted black type were kept under conventional condition and 8-9 weeks old, male and female animals were used. Infective larvae (L_3) of *Brugia pahangi* were obtained from *Aedes aegypti* mosquitoes infected 14 days previously by blood meals on infected gerbils. Gerbils were infected by subcutaneous injection of 100 L_3 at inguinal region. Blood samples were taken from the retro-orbital venous plexus every week and percentage of eosinophils and microfilaria counts per 30 μl blood were determined. After observation of 25 weeks, animals were bled and their organ weights were measured.

As mf appearance rates in each coat color of male gerbils were 100% except black type and female gerbils also showed 67-100% positive rate, it appeared that all coat color mutants of Mongolian gerbils also have sensitivity to *B. pahangi*.

In each coat color of male gerbils, microfilariae began to appear at 8-9th week after infection and attained to peak level of 100-300 counts at 15-20th week. In only albino and black type gerbils, mf counts continued to rise until 25th week. In female gerbils, microfilariae also began to appear at 9-12th week and attained to peak level at 15th week. But mf counts were very lower than male gerbils and highest count in female gerbils were only 65 per 30 μl blood.

Eosinophil responses were quite different betweeen males and females or between mf positive gerbils and mf negative them. Namely, mf positive male gerbils showed two distinct phases of eosinophil responses, one in the prepatent phase at 3rd week and another in the patent phase at 9-10th week, in contrast to only former response in mf negative gerbils. In

mf positive female gerbils, eosinophil responses did not show clear phase. On the other hand, eosinophil responses showed some difference among coat color mutants. Mf positive female gerbils showed almost low response but only albino female were observed 11.5% at 7th week and continued to show 3.5-5.5% until 18th week.

In organ weight examination after observation, weight of spleen showed remarkable difference between control group and infection group.

53 FALCIPARUM MALARIA WITH BONE MARROW ABNORMALITIES RESEMBLING MALIGNANT HISTIOCYTOSIS

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We recently examined a patient with *Plasmodium falciparum* malaria, whose bone marrow transiently showed a similar finding of malignant histiocytosis.

A 24 year old Japanese businessman was admitted to our hospital with 4 days history of high fever and chill. He had been in Madras, India for one month just before this admission but had never been in malaria endemic area before this trip. On admission, high fever, jaundice and hepatosplenomegaly were observed and laboratory examination showed marked anemia and thrombocytopenia. The patient was diagnosed as *Plasmodium falciparum* malaria by blood smear (parasite density: 140,000 ring form/mm^3), and he was treated with quinine hydrochloride (1.5 g/day for 7 day). After 3 days, fever disappeared and no ring form was detected on the blood smear. A bone marrow aspiration was performed on admission and revealed a normally cellular marrow with increased histiocytes (8.5%). The majority of the increased histiocytes showed intense hemophagocytosis and atypical histiocytes were also apparent. These bone marrow findings were consistent with malignant histiocytosis. Two months after the malaria treatment, the bone marrow was reexamined. The number of histiocytes was normal and neither hemophagocytic nor atypical histiocytes were observed.

Our present report is of the first malarial case to show malignant histiocytosis-like syndrome. Epidemiological studies have shown that the occurence of malignant histiocytosis was significantly higher in tropical area than in temperate zone. It has been hypothesized that this high incidence might be related to repeated infections of tropical infectious organisms, particularly malaria. Our case apperars to support a role for malaria infection in the etiology of malignant histiocytosis.

54 USE OF MEFLOQUINE IN TREATMENT OF IMPORTED FALCIPARUM AND VIVAX MALARIA

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Mefloquine (a 4-quinolinemethanol derivative, structual analogue of quinine) is usually used in combination with sulfadoxine/pyrimethamine, however, antimalarial treatment with mefloquine alone has not been performed because of unavailable in Japan. This report deals with our clinical experience of mefloquine alone therapy on two cases with imported *Plasmodium falciparum* and *P. vivax* malaria.

The first case, a 2-year-old Japanese girl, had the febrile attack on her way home from lake-side area of Tanganyika, Zaire in March, 1990. Although treatment with chloroquine in Kenya eliminated her malarial symptoms, 7 days after discharge she had fever in Japan and visited our hospital 5 days after the recrudescence. On admission, her temperature was 38.2°C by axilla. Anemia and hepatomegaly were found. The important laboratory findings were; hemoglobin 8.1 g/dl, platelet $11.2\times10^4/\mu l$, CRP 4.5 mg/dl, FDP$<$10 μg/ml, creatinine 0.3 mg/dl, glucose 100 mg/dl, total cholesterol 133 mg/dl and LDH 2,268 IU. The thin blood smear revealed ring forms of *Plasmodium falciparum* and parasitemia was 11,925/μl. The treatment with mefloquine (375 mg$\times$2 days) was started on the first hospital day and parasitemia completely disappear. The clearance of fever and parasitemia was 12 hrs and 60 hrs, respectively. She had been displeased, irritable and insomnia since her admission, and these symptoms were continued 3 days after administration of mefloquine. And addition to loss of appetite, she complained of transient nausea and abdominal pain after chemotherapy. The second case was a 26-year-old Japanese male, had the febrile attack on his way home from Papua New Guinea in June, 1990. He was seen in our hospital 7 days after onset of the disease. On admission, the patient had clear consciousness although complained of severe fatigue. The important laboratory findings were; hemoglobin 14.3 g/dl, platelet $5.7\times10^4/\mu l$, CRP 7.4 mg/dl, FDP$<$10 μg/ml, creatinine 1.0 mg/dl, glucose 121 mg/dl, total cholesterol 99 mg/dl and LDH 673 IU. Abdominal ultrasonicated echography showed splenomegaly. The thin blood smear revealed trophozoites and gametocytes of *Plasmodium vivax* and parasitemia was 6,266/μl. The treatment with mefloquine (total 1,500 mg for 24 hrs) was started on the second hospital day and parasitemia completely disappear. The clearance of fever and parasitemia was 36 hrs and 60 hrs, respectively. No side effects was found.

Mefloquine has nearly same antimalarial actions as quinine, being effective against both *Plasmodium falciparum* and *vivax*. Its half time *in vivo* (T$=$14 ds) is so long that the single dose treatment is recommended. In this study we followed the drug information given by the company. Its side effects including dizziness, vomiting, diarrhea, bradicardia and neuropsychiatric changes are usually mild, transient and tolerable, which was reconfirmed by the present cases.

55 INCIDENCES OF DIC COMPLICATION IN JAPANESE PATIENTS WITH MALARIA

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Forty eight patients with falciparum malaria (14) and vivax malaria (34) were evaluated retrospectively as to whether DIC (disseminated intravascular coagulation) had been complicated or not. For this purpose, four kinds of laboratory data in coagulation test, that is, serum fibrin-degradation products (FDP), platelets, fibrinogen, and prothrombin time were estimated according to the diagnostic criteria for DIC complication which was reported at 1988 by the Study Group for DIC complication which had been organized by the Ministry of Health and Welfare in Japan. Serum concentration of FDP was elevated in 8 cases (57%) of falciparum malaria and in 3 cases (9%) of vivax malaria. Thrombocytopenia was seen in 12 cases (88%) of falciparum malaria and in 30 cases (86%) of vivax malaria. Prothrombin time elongated in 4 cases (8%) and plasma concentration of fibrinogen decreased in 3 cases (17%). Among the coagulation tests, abnormality grades in FDP concentration had ciosest association with DIC in malaria cases, therefore FDP test is regarded as one of the most important and indispensable test in malignant malaria for checking the DIC complication.

According to incidences of DIC complication among these 48 cases, only 4 patients met the criteria for the diagnosis (the score must be over 7 points). All patients with DIC complication were infected with falciparum malaria and three of them developed to cerebral disturbance (cerebral malaria). One case of vivax malaria was suspected of the DIC complication (6 points).

As to therapy for DIC, heparin-sulfate was administered as an anti-coagulant therapy in 3 out of 4 cases with the DIC complication. The result of the treatment indicated that 3 patients, all of them had manifested cerebral disorders, were cured without complication of renal disturbance, whreas one patient escaped the anti-coagulant therapy developed renal insufficiency (serum concentrations of BUN and creatinine elevated at the peak values of 92 and 17 mg/dl, respectively). These clinical data suggest that the anti-coagulant therapy may be necessary for treating patients with DIC complication even when the DIC is caused by malarial infection.

56 THE SIGNIFICANCE OF TIME LAG IN THE TREATMENT OF FALCIPARUM MALARIA

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We have previously reported that there exists a time limit for effective treatment of falciparum malaria. In short, 4 days after the onset of the illness was found to be the time limit beyond which severe, life-threatening complications may develop. In order to elucidate the level of laboratory variables beyond which the patient's prognosis is poor, we examined the correlation between time lag in days *vs* maximum parasite count, minimum red blood cell (r.b.c.) count, minimum hemoglobin (Hb), maximum blood urea nitrogen (BUN) and creatinine concentration of each patient during the period of admission. A linear correlation was found between time lag (x) and the maximum parasite count in log units (y), $y=4.031+0.124x$, $r=0.3579$. All variables among patients whose treatment was started after 5 to 11 days of time lag (6th to 12th day of illness) were significantly worse than the variables of patients whose treatment was started after only 1 to 4 days of lag time. However, the most conspicuous change was observed in the abrupt increase of BUN and creatinine in the members of the latetreatment group: the mean BUN and creatinine levels in the early and late treatment groups were 16.0 *vs* 57.1 and 1.2 *vs* 3.4 mg/d*l* ($p<0.01$), respectively. The mean parasite counts of the early and late treatment groups were 23,400 *vs* 69,200/μl of blood ($p<0.05$), while the mean r. b. c. count and Hb concentration of the two groups were 3.81 *vs* 3.28 million per μl and 12.4 *vs* 10.0 g/d*l* ($p<0.01$), respectively.

In addition to the level consciousness of the patient, special attention should be paid to kidney function in the treatment of falciparum malaria patients whose treatment is delayed beyond the 5th day of illness.

57 DNA DIAGNOSIS OF FALCIPURUM MALARIA

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Techniques utilizing DNA hybridization probes for the rapid detection of *Plasmodium falciparum* had been reported. However, these methods had many problems about the sensitivity and the stability of these probes. We have developed two types of plasmid-born DNA probes: One represents the junction DNA sequence (410 bp) of the DHFR (dihydrofolate reductase)-TS (thymidylate synthase) gene. The other represents the DHFR DNA (790 bp). When these probes were used, the target sequence was amplified by PCR

(polymerase chain reaction) to increase the sensitivity. We used the Universal Probe System {Yamane *et al.* (1988): Nucleic Acid Res., Symposium series, 19, 93-95}. This system consists of two probes; a primary probe prepared from a chimeric phage-plasmid vector (pUCf1) containing a sequence complementary to a target, and a biotin-labeled secondary probe complementary to a portion of the primary probe, which is detected by the BCIP/NBT method. This system has a high sensitivity to the target DNA because the secondary probe is labeled with many biotin molecules.

We showed that the junction DNA was more sensitive than the DHFR DNA as target to detect of *P. falciparum* and the limit of detection was 10^2 parasites in 100 μl human blood with DHFR-TS junction probe.

58 SEROLOGIC EVIDENCE OF TOXOCARIASIS IN NORTHEAST BRAZIL

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Tropical eosinophilia syndrome (TES) is found most commonly in areas where filariasis occurs, notably South-East Asia and the east coasts of Africa and Brazil. The salient features of TES are asthma-like symptoms associated with hypereosinophilia. However, the same symptoms can be caused not only by infection with filarial parasites, but also by bacteria, fungi and other helminth parasites such as *Ascaris, Ancylostoma, Strongyloides, Schistosoma* and *Toxocara* spp. (Manson's Trop. Med.). Although *Toxocara* is known to contribute to TES, few cases have been reported from tropical regions (Lynch, 1988). In Brazil, *T. canis* infection in dogs is relatively common; the rate was 59.8% in São Paulo during 1980-1985 (Cortes, 1988). Moreover, in humans, two cases of infant toxocariasis (visceral larva migrans) were reported in São Paulo, in 1984 (Kawakami, 1984). In order to determine the incidence and status of toxocariasis in northeast Brazil, where TES is very common, 54 sera from children with a history of asthma and hepatomegaly (eosinophilia: >740 cells/μl) were screened serologically.

The enzyme-linked immunosorbent assay detected 21 positive sera (40%). Many of these cases (15 of 21) were positive for *Dilofilaria immitis* as well. Six of the *Toxocara*-positive were detected, in some children, were symptomatic. Their sera were further examined by the *Toxocara* species-specific Ouchterlony's diffusion-in-gel test (Nagakura, 1990) and one *Toxocara canis* and one *T. cati* infection were identified. This is the first report of serologically defined toxocariasis in Brazil, and the status of the disease in the tropics was discussed.

59 SERO-EPIDEMIOLOGICAL SURVEY OF HUMAN TOXOCARIASIS IN TROPICAL ZONE

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Sero-epidemiological survey of toxocariasis was carried out on 241 individuals (8 areas in 5 countries) lived in tropical zone. The serum samples were collected from 41 in Brazil (Recife), 49 in Taiwan (Taichung and Kaoshiung), 57 in Thailand (Bangkok), 21 in Indonesia (Napu in Sulawesi Island), and 73 in India (Darbhanga, New Delhi and Bombay). In this survey, we performed ELISA using *Toxocara canis* larval ES (TcnLES) as antigen, and calculated the positive rates and mean antibody titers against TcnLES antigen.

The inhabitants in Napu showed the highest positive rate (14.2%) and mean antibody titer (1.52 ± 0.35) among the eight areas. The people who possessed positive antibody titers for TcnLES had also high antibody titers for *Schistosoma japonicum* and *Dirofilaria immitis* antigens. Napu is known to the endemic area of schistosomiasis and filariasis. Therefore, we considered that mixed infection or previous sensitization had occurred in this area. In Recife, the positive rate and mean antibody titer were 7.32% and 1.49 ± 0.44, respectively, those figures were secondarily among 8 areas. Although the place was endemic area of filariasis, we could not detected the antibodies against another four helminth antigens including dirofilaria worm extracts.

There were no significant differences among the positive rate for TcnLES antigen in Taiwan (4.08%), Thailand (1.75%) and India (1.37%) as compared with those in Japan (1.37%). However, the mean antibody titers for TcnLES antigen in 4 countries were distributed 1.13 ± 0.39 to 1.23 ± 0.43, which were significantly higher than that in Japan. These results indicated that geographic and ecological factors were essential for understanding of etiology of toxocariasis.

60 SEROLOGIC TYPING OF CLINICAL CASES WITH AMEBIASIS BY GEL DIFFUSION PREECIPITIN TEST

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Although gel diffusion precipitin test (GDP) is a standard serodiagnostic method for invasive amebiasis, its detailed characterization has not been attempted. In this study, we analyzed the precipitin bands pattern using the fractionated antigens. The result from approximately 300 sera suggests that the patterns change reflecting clinical course.

Through this analysis, five groups of precipitation bands were recognized and designated as P, Q, PAI (greek), R, S bands respectively, and each sera could be classified into 4 serotypes based on the GDP patterns as follows.

1) Acute serotype, defined by presence of Q or S in the absence of typical P nor R, contained approximately 50 cases. Clinical feature of these cases was variable; from asymptomatic cases to liver abscess. However, in symptomatic cases, virtually all of these cases were fresh. It is interesting that cyst passing cases with liver abscess were involved into this type. With some pair sera, this serotype seems to develop from seronegative cases and change to chronic serotype in 1 month.

2) Chronic serotype, defined by presence of predominant R bands to P bands, contained about 70 cases. Clinical feature of this type is chronic colitis. Eighty percent of chronic colitis cases showed this serotype. Liver abscess cases with chronic colitis seem to be exceptional. This type seems to develop from the acute serotype approximately 2 month later from onset (bloody stool).

3) Advanced type, defined by presence of strong P bands, contained 25 cases. Clinical feature of this type is liver abscess with chronic colitis, which have past history of bloody stool, present colitis and no cyst passing in stool. This serotype seems to develop from chronic serotype in the case of liver abscess, and change to chronic serotype about 2 month after metastatic sign (fever over 38°C).

4) Negative or weak pattern: cases difficult to identify.

This serologic procedure is useful for not only clinical follow-up, but for pathophysiological study of amebiasis.

It would be of a great value to distinguish acute and chronic amebiasis, because the former has a variety of clinical symptoms and sometimes very severe status. On the other hand the latter shows characteristic serological response. It is likely that the amebiasis is a tremendously heterogeneous syndrome clinically judging from serologic aspects.

61 THE DISTRIBUTION OF *NAEGLERIA* SP. AND *ACANTHAMOEBA* SP. IN KANTO AREA OF JAPAN

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The isolation and culture of free living parasitic amoeba has been reported elsewhere. For the present study, author surveyed pond and river waters in Kanto area of Japan. Author isolated 6 strains of *Naegleria* and 3 strains of *Acanthamoeba*. The one strain of *Naegleria* proved pathogenic to mice.

The isolated strain of *Naegleria* was observed by electron microscopy. In these experiments, lamellar structures were found in the cytoplasm of *Amoeba*. Author reported before, these lamellar structures concerned with the virulence of *Naegleria* sp. Further pathogenic studies of these organelles are necessary to clarify their functions.

62 PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION AMONG HOUSE RATS, *RATTUS RATTUS* AND *R. NORVEGICUS* AND THE EXPERIMENTAL INFECTION IN *R. RATTUS*

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An epidemiological survey for *Cryptosporidium* infection was carried out on house rats. *Rattus rattus* (roof rats) and *R. norvegicus* (brown rats), captured in the Tokyo Metropolitan District. Of a total of 231 house rats, consistiong of 175 roof rats. 48 brown rats, and 8 species-indeterminate rats. 32 (13.9%) were found to be positive for *Cryptosporidium* oocysts in their feces. The incidence of the infection was 17.7% for roof rats and 2.1% for brown rat, respectively. In roof rat groups classified by body weight, the incidence was consistently high (13.8%-25.0%). There was no significant difference between the incidence of infection and the sexes. The size of the oocysts of the isolates from roof rats measurd $(3.7\pm0.22)\times(4.8\pm 0.3)$ μm. Roof rats experimentally inoculated with *Cryptosporidium* oocysts from naturally infected rats began to shed oocysts on days 2 to 3 post inoculation (PI). The number of oocysts in feces peaked on days 5 to 8 PI, declined rapidly, and thereafter markedly small numbers of oocysts were detected intermittently until day 60 PI. None of the roof rats showed any apparent clinical symptoms such as diarrhea in experimental infection.

63 THE FUNCTION OF DENGUE VIRUS SPECIFIC PROTEINS IN VIRUS REPRODUCTION: BIOLOGICAL ACTIVITIES OF NS5

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Dengue virus (DV) infection makes serious problem in tropical society. However, the mechanism of DV replication and functions of nonstructural proteins in viral life cycle are unknown. It is speculated from amino acid sequence analysis that NS5 may be viral RNA polymerase.

To clarify the function of NS5, we used a monospecific antiserum against Japanese encephalitis virus (JEV) NS5. Antiserum against JEV-NS5 (anti-NS5) detected DV-1 specific NS5 by Western blot (WB) and immunofluorescence antibody (IFA) analyses. The results indicated that DV-1 NS5 was cross-reactive with JEV-NS5 and localized at the perinuclear and membrane fractions in the infected cells. On the other hand, activity of *in vitro* RNA synthesis of DV-1 infected cell extracts was much more lower that of JEV infected ones and this might be related with the big difference in viral growth. Taken together with the data using anti-NS5, ability of DV-NS5 seems to be different from JEV NS5.

Next we compared virus growth of 3 kinds of newly isolated DVs from Indonesia (Fujita *et al.*, 1898). In the early stage of virus reproduction, *i. e.* until 2 days after virus infection, 3 strains grew more rapidly than that of Mochizuki strains. However, anti-NS5 could detect NS5 from only Mochizuki-infected cells. This result suggests that the immunogenicity of NS5 from newly isolated strains differs from that of Mochizuki. This immunogenic discrepancy seems to be related with biological activities of NS5. The comparison of amino acid sequence indicated that NS5 contains a variable region at the upstream of GDD sequence. This might be virus strain specific and has some roles in the function of NS5.

64 MECHANISM OF ENHANCEMENT OF ARBOVIRUS MULTIPLICATION IN MOSQUITOES INGESTING MICROFILARIAE OF *DIROFILARIA IMMITIS* BY CISTOPATHOLOGY AND ELECTRON MICROSCOPY

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Aedes albopictus mosquitoes of the Miki strain did not support the multiplication of chikungunya virus after oral infection. We investigated whether simultaneous ingestion of the virus and microfilariae of Dirofilaria spp. would encourage viral infection and dissemination throughout these mosquitoes. We allowed the mosquitoes to ingest difibrinated sheep

blood mixed with 3×10^8 PFU of virus per milliliter and 15 microfilariae per mosquito by an artificial feeding technique and compared the infection rate in the midgut and salivary gland with those of control mosquitoes ingesting the virus without microfilariae. The mosquitoes ingesting both agents were infected at a high rate. The virus titer of separate homogenates of mosquitoes' bodies and legs was significantly higher in these mosquitoes than in the controls. In four repeated experiments, the viral infection rates were higher as the dose of virus increased provided that microfilariae were also ingested. Microfilariae seem to enable the virus to enter the hemocoel through the holes they punctured in the midgut. We studied this histopathologically.

Fourteen days after infection, the salivary gland of the mosquitoes was examined under an electron microscope. Some viral particles were found scattered in the salivary gland. Probably when this virus multiplies in the hemocoel and salivary gland of a mosquito, it can be more effectively transmitted to human beings when they are bitten.

65 DEVELOPMENT OF MICROFILARIAE OF DIROFILARIA SPP. STUDIED BY *IN VITRO* USE OF HEMOLYMPH OF *AEDES ALBOPICTUS* MOSQUITOES

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Earl (1959) first attempted to cultivate microfilariae of *Dirofilaria immitis in vitro,* and Weinstein (1963) observed the development of microfilariae up to larvae the late first stage (sausage form) with various supplements to their media. There are few reports of development *in vitro* beyond this stage. Here, we studied whether microfilariae of Dirofilaria spp. could develop in organ culture and how the culture method could be simplified. Up to now, the development of microfilariae has been studied in a variety of tissue culture media through the alternating gas phase of the cultured cells. Microfilariae were obtained aseptically from the blood of dogs/a dog with microfilarimic blood (55,000-60,000 microfilariae/m*l*). The density of the microfilariae was adjusted to 2×10^2 per milliliter of the medium to be tested. The media used were NCTC 109, Leibovitze L-15, RPMI 1640, Tr 17, and MEM. We added cells of the *Aedes albopictus* cell line C6/36, 40% heat-inactivated fetal calf serum, 0.1% sodium casinate, and 0.5% *A. albopictus* hemolymph. The media were tested with and without supplements. The supplements supported larval development to the late first stage and, at a lower percentage, to the second stage, in NCTC 109 and Leibovitze L-15 media. Control media without supplements supported survival for only a few days, and the microfilariae did not develop.

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