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ETHNOGEOGRAPHICAL COINCIDENCE OF ENDEMIC KAPOSI'S SARCOMA AND AFRICAN BURKITT'S LYMPHOMA IN WESTERN KENYA

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Abstract: Geopathological studies on endemic Kaposi's sarcoma (KS) and African Burkitt's lymphoma (BL) in western Kenya were performed, and revealed that KS and BL had relatively same geographical and ethnical distribution. The western region of Kenya stands almost exactly astride the equator. It accounts for almost one third of the whole country in area and about one half in population. Western Kenya is composed of three provinces; Nyanza Province, Western Province and Rift Valley Province. Out of 25,343 surgical pathological specimens at provincial hospitals in Nyanza, Western and Rift Valley for 8 years during 1979 to 1986, 124 and 135 cases were histologically diagnosed as KS and BL respectively. Frequency in all malignant tumors was 2.92% (KS) and 3.18% (BL). The high incidence of KS was found between the age of 50 and 59, while all BL cases were found under 22 years. The male to female ratio was 8.4:1.0 in KS and 1.2:1.0 in BL. The incidence of KS and BL per 100,000 population in each province is as follows: a) 2.12 (KS) and 3.54 (BL) in Nyanza Province, b) 1.80 (KS) and 1.20 (BL) in Western Province, and c) 1.11 (KS) and 0.68 (BL) in Rift Valley Province. Nyanza Province and Western Province are tropical savannah areas, whereas Rift Valley Province is a tropical highland. The incidence of KS and BL per 100,000 population among main ethnic groups in western Kenya is as follows: the Luo, the main inhabitants of Nyanza Province around Lake Victoria, showed the highest incidence of KS (2.56) and BL (4.35), followed by the Luhya, the main inhabitants of Western Province, the Kalenjin, the inhabitants of the tropical highland in Rift Valley Province, and the Kisii, the inhabitants of highland area of Nyanza Province. The Luo are descended from the Nilotic groups and the Luhya belong to the Bantu. No case of KS and only a few cases of BL were found among the inhabitants of desert or semi-desert areas. No other tumors showed above mentioned characteristics. The geographical and ethnical coincidence of KS and BL was more clear in the child population than in the adult. These results suggest that there is a geographical coincidence of KS and BL based on same etiological cofactors including high temperature, high humidity, unknown transmissible agents, and probably genetic factors and life styles. This was mainly demonstrated in Nyanza Province around Lake Victoria in western Kenya.

INTORODUCTION

Kaposi's sarcoma (KS; initially called Idiopathisches Multiples Pigment Sarkom der

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Haut) was first described in eastern Europe (Kaposi, 1872). Up to the present day, many cases of KS have been reported from European and North American countries and a relatively high incidence of KS has appeared among the inhabitants of eastern Europe and Mediterranean countries, as well as those of Jewish origin in central Europe (Bluefarb, 1957; Oettle, 1962; Rothman, 1962). The first report of African KS from French Cameroon was made by Jojot and Laigret in 1922. Nowadays, it is well known that endemic KS is more prevalent in African continent than any other part of the world (Oettle, 1962; Maclean, 1963). When the ratio of endemic KS to all malignant tumors was considered, the highest value was found in central Africa with the incidence of endemic KS decreasing with distance away from this area (Cox and Helwig, 1959; Cook, 1962; Davies and Lothe, 1962; Keen, 1962; Oettle, 1962; Maclean, 1963; Slavin *et al.*, 1969; Taylor *et al.*, 1971a; Schmid, 1973; Toriyama *et al.*, 1987a). It has been reported that cytomegalovirus (CMV) is etiologically concerned with KS in Africa (Burkes *et al.*, 1985). Since the occurrence of the first Aquired Immunodeficiency Syndrome (AIDS) case (Centers for Disease Control, 1981a), KS has been considered one of the main complications of AIDS (Centers for Disease Control, 1981b; Friedman-Kien *et al.*, 1981). Several cases of African endemic KS contained viral inclusions, and European and American patients with KS were found to possess increased serum-levels of antibody to CMV when compared with normal controls and patients with other malignancies (Giraldo *et al.*, 1972a, 1972b, 1975; Boldogh *et al.*, 1981). However, KS in AIDS (epidemic type) is a little different from endemic KS in Africa in its manifestations (Toriyama *et al.*, 1987b). In North America, the incidence of malignant B-cell lymphoma which is also related to AIDS has been increasing (Ziegler *et al.*, 1982, 1984; Levine *et al.*, 1984, 1985).

Burkitt's lymphoma (BL), a malignant B-cell lymphoma, is also endemic throughout most of tropical Africa (Burkitt, 1962). Epstein-Barr virus (EBV) is now known to be the causative agent of BL (Epstein *et al.*, 1964; Old *et al.*, 1966; de Schryver *et al.*, 1969; Henle *et al.*, 1969, 1973; Gunven *et al.*, 1970; zur Hausen *et al.*, 1970; Kaschka-Dierich *et al.*, 1976). It has been suggested that EBV may initiate a lymphoid tumor of a susceptible individual whose immunological response has been altered by malaria, especially *Plasmodium falciparum* (Hutt, 1970).

Across African continent between the latitudes of approximately 15° north and south of the Equator, BL appears to be highly prevalent (lymphoma belt) (Burkitt, 1962, 1966, 1969). Although it is supposed that the lymphoma belt and the areas with the high incidence of KS are overlapping (Burkitt, 1970), no reports clarifying the geographical and ethnical coincidence of endemic KS and African BL have been published up to the present day. It is the aim of this study to discuss in more detail the geographical and ethnical distribution as well as some etiological cofactors between endemic KS and African BL in western Kenya.

Materials and Methods

This study was based on the histopathological examinations of surgical specimens, almost all of which were performed in the Histology Departments of two hospitals, the Rift Valley Provincial General Hospital in Nakuru and the Nyanza Provincial General Hospital in Kisumu, Kenya. During the eight-year period between 1979 and 1986, a total of 25,343 surgical specimens from Nyanza, Western, and Rift Valley Provinces in western Kenya were examined histologically for KS and BL. Relevant information and clinical data were

collected as accurately as possible, with attention being paid to age, sex, ethnic group, place of residence, and macroscopic appearances. The diagnoses of KS and BL were based on clinical and histological grounds. Histological examinations were performed using H. E., periodic acid Schiff (P.A.S.), reticulum, elastic van Gieson and Azan Mallory stains. A demographic structure was obtained from the Kenya Population Census 1979 (Government of Kenya, 1979)

Table 1 Incidence of Kaposi's sarcoma (KS) and Burkitt's lymphoma (BL) amongst malignant tumors (1979-1986)

Year	No. of surg. specimen	No. of malig. tumor		No. of KS	KS / malig. tumor (%)	No. of BL	BL / malig. tumor (%)
1979	1,179	Male	102	2	1.96	4	3.92
		Female	76	0	—	1	1.32
		Unknown	7	0	—	0	—
		(Total)	185	2	1.08	5	2.70
1980	3,359	Male	262	12	4.58	4	1.53
		Female	318	1	0.31	0	—
		Unknown	3	0	—	0	—
		(Total)	583	13	2.23	4	0.69
1981	3,652	Male	336	22	6.55	9	2.68
		Female	368	5	1.36	9	2.54
		Unknown	5	1	20.00	0	—
		(Total)	709	28	3.95	18	2.54
1982	4,401	Male	213	24	11.27	6	1.98
		Female	315	2	1.36	7	2.22
		Unknown	25	1	4.00	0	—
		(Total)	553	27	4.88	13	2.35
1983	3,905	Male	303	8	2.64	6	1.98
		Female	253	0	—	15	5.93
		Unknown	19	0	—	0	—
		(Total)	575	8	1.39	21	3.65
1984	2,493	Male	198	8	4.04	15	7.58
		Female	216	0	—	3	1.39
		Unknown	1	0	—	1	100.00
		(Total)	415	8	1.93	19	4.58
1985	3,914	Male	323	18	5.57	17	5.26
		Female	424	3	0.70	16	3.77
		Unknown	9	0	—	0	—
		(Total)	756	21	2.65	33	4.37
1986	2,440	Male	180	15	8.33	12	6.67
		Female	271	2	0.74	10	3.69
		Unknown	16	0	—	0	—
		(Total)	467	17	3.64	22	4.71
Total	25,343	Male	1,917	109	5.69	73	3.80
		Female	2,241	13	0.58	61	2.72
		Unknown	85	2	2.35	1	1.18
		(Total)	4,243	124	2.92	135	3.18

Table 2 Age and sex distribution of 124 cases of Kaposi's sarcoma (KS)

Age Group	Sex			
	Male	Female	Unknown	Total
0 — 4	6	0	0	6
5 — 9	4	2	0	6
10—14	3	1	0	4
15—19	1	1	0	2
20—24	4	0	0	4
25—29	7	1	0	8
30—34	4	0	0	4
35—39	5	0	0	5
40—44	9	1	0	10
45—49	7	2	0	9
50—54	15	1	0	16
55—59	7	0	0	7
60 and over	17	0	0	17
"Child"*	1	0	0	1
"Adult"**	14	3	0	17
Unknown	5	1	2	8
Total	109	13	2	124

Table 3 Age and sex distribution of 135 cases of Burkitt's lymphoma (BL)

Age Group	Sex			
	Male	Female	Unknown	Total
0 — 4	18	2	0	20
5 — 9	30	29	1	60
10—14	14	17	0	31
15—19	1	6	0	7
20 and over	0	1	0	1
"Child"*	5	4	0	9
Unknown	5	2	0	7
Total	73	61	1	135

*Exact age is unknown, probably under 14 years of age.
**Exact age is unknown, probably over 15 years of age.

RESULTS

Incidence of KS and BL amongst malignant tumors

During the eight-year period between 1979 and 1986, out of 25,343 surgical specimens, 4,243 specimens were malignant tumors. Among them 124 cases were histologically diagnosed as KS and 135 cases as BL. Table 1 shows the ratio of KS and BL to all malignant tumors during the period; 1.08% and 2.70% in 1979, 2.23% and 0.69% in 1980, 3.95% and 2.54% in 1981, 4.88% and 2.35% in 1982, 1.39% and 3.65% in 1983, 1.93% and 4.58% in 1984, 2.65% and 4.37% in 1985, and 3.64% and 4.71% in 1986 respectively. The total incidence of KS and BL amongst all malignant tumors in western Kenya for the eight-year period was 2.92% and 3.18% respectively.

Age and sex distribution of KS and BL

Tables 2 and 3 indicate the age and sex distribution of KS and BL respectively. The high incidence of KS cases was found between the age of 50 and 59, while all BL cases were found under 22 years. The male to female ratio was 8.4:1.0 in KS and 1.2:1.0 in BL. The highest incidence of BL cases was found in the 5—9-year-old age group. The analyses of sex distribution revealed that males had significantly higher rates in KS ($p<0.001$) and BL ($p<0.05$) than females (by the Chi-square test, using 4,243 cases of malignant tumors).

Table 4 Anatomical distribution of 124 cases of Kaposi's sarcoma (KS)

Localization	Number of cases												Total
	Adult				Child				Unknown				
	Male	Female	Unknown	Total	Male	Female	Unknown	Total	Male	Female	Unknown	Total	
Foot	34	5	0	39	1	0	0	1	1	0	1	2	42
Leg	22	0	0	22	1	0	0	1	2	0	1	3	26
Lymph node(s)	3	1	0	4	11	2	0	13	1	1	0	2	19
Hand	16	1	0	17	0	0	0	0	0	0	1	1	18
Arm	10	0	1	11	0	0	0	0	1	0	0	1	12
Thigh	6	0	0	6	0	0	0	0	1	0	0	1	7
Knee	5	0	0	5	0	0	0	0	0	0	0	0	5
Ankle	4	0	0	4	0	0	0	0	0	0	0	0	4
Trunk	3	1	0	4	0	0	0	0	0	0	0	0	4
Orbit	1	0	0	1	0	0	0	0	0	0	0	0	1
Upper lid	0	0	0	1	0	0	1	0	0	0	0	0	1
Mandible	0	0	0	0	1	0	0	1	0	0	0	0	1
Axilla	1	0	0	1	0	0	0	0	0	0	0	0	1
Pharynx	1	0	0	1	0	0	0	0	0	0	0	0	1
Tonsil	0	1	0	1	0	0	0	0	0	0	0	0	1
Unknown	3	1	0	4	0	0	0	0	0	0	0	0	4
Total	109	10	1	120	15	2	0	17	6	1	2	9	146*

* including multiple lesions.

Table 5 Anatomical distribution of 135 cases of Burkitt's lymphoma (BL)

Localization	Number of cases			
	Male	Female	Unknown	Total
Oral cavity (includ. Nasal cavity)	17	12	0	29
Maxilla and Mandibula (includ. Cheek, Orbit and Eye)	19	9	1	29
Abdominal mass	8	7	0	15
Unknown lymph node	9	6	0	15
Ovary	0	14	0	14
Neck (includ. Cervical lymph node and Thyroid)	8	5	0	13
Inguinal lymph node and Groin	3	1	0	4
Kidney	1	2	0	3
Axillary lymph node	0	2	0	2
Mediastinum	0	1	0	1
Spleen	1	0	0	1
Head	1	0	0	1
Shoulder	0	1	0	1
Hand	1	0	0	1
Hip	1	0	0	1
Mesenteric lymph node	1	0	0	1
Unknown	4	0	0	4
Total	73	61	1	135

Table 6 Geographical and sex distribution of Kaposi's sarcoma (KS) and Burkitt's lymphoma (BL)

Province	District	Number of KS				Number of BL			
		Male	Female	Unknown	Total	Male	Female	Unknown	Total
Western	Bungoma	9	1	0	10	0	0	0	0
	Busia	5	0	0	5	1	0	0	1
	Kakamega	14	3	1	18	9	12	0	21
	(Total)	28	4	1	33	10	12	0	22
Nyanza	Siaya	8	2	0	10	3	2	0	5
	Kisumu	24	0	0	24	32	31	1	64
	South Nyanza	11	1	0	12	11	4	0	15
	Kisii	7	1	0	8	1	5	0	6
	(Total)	50	4	0	54	47	42	1	90
Rift Valley	Turkana	0	0	0	0	0	1	0	1
	West Pokot	3	0	0	3	0	1	0	1
	Trans Nzoia	2	0	0	2	2	1	0	3
	Uasin Gishu	4	0	0	4	0	0	0	0
	Elgeyo Marakwet	1	0	0	1	0	0	0	0
	Baringo	1	0	0	1	1	0	0	1
	Nandi	3	0	0	3	2	0	0	2
	Nakuru	13	2	1	16	6	3	0	9
	Kericho	3	1	0	4	4	1	0	5
	Narok	1	1	0	2	0	0	0	0
	Others	0	0	0	0	0	0	0	0
	(Total)	31	4	1	36	15	7	0	22
Unknown		0	1	0	1	1	0	0	1
Total		109	13	2	124	73	61	1	135

Anatomical distribution of KS and BL

The anatomical distribution of KS and BL are shown in Tables 4 and 5 respectively. The most common sites of primary lesion of KS in adults were the foot, followed by the leg, hand and arm. In children, the primary lesion of KS was predominantly of lymph node(s) origin. The most common sites of primary lesion of BL were the oral cavity, followed by the maxilla, mandibula, abdominal cavity, lymph nodes and ovary.

Geographical distribution of KS and BL

Tables 6 and 7 show the geographical distribution of KS and BL. Out of 124 collected cases of KS, 54 were from Nyanza Province, 36 from Rift Valley Province and 33 from Western Province (Table 6). The estimated incidence of KS per 100,000 population in each province and district for the eight-year period was as follows: Nyanza Province showed the highest incidence of 2.12, followed by 1.80 in Western Province and 1.11 in Rift Valley Province (Table 7). Kisumu District in Nyanza Province showed the highest incidence of 4.98, followed by Nakuru District of Rift Valley Province with 3.06 and Siaya District of Nyanza Province with 2.11 (Table 7).

Table 7 Geographical distribution of Kaposi's sarcoma (KS) and Burkitt's lymphoma (BL) in western Kenya (per 100,000 population)

Province	District	Population in '000s*	KS	KS 100,000 population	BL	BL 100,000 population
Western	Bungoma	503.9	10	1.98	0	0.00
	Busia	297.8	5	1.68	1	0.34
	Kakamega	1,030.9	18	1.75	21	2.04
	(Total)	1,832.7	33	1.80	22	1.20
Nyanza	Siaya	474.5	10	2.11	5	1.05
	Kisumu	482.3	24	4.98	64	13.27
	South Nyanza	817.6	12	1.47	15	1.83
	Kisii	869.5	8	0.92	6	0.69
	(Total)	2,544.0	54	2.12	90	3.54
Rift Valley	Turkana	142.7	0	0.00	1	0.70
	West Pokot	158.7	3	1.89	1	0.63
	Trans Nzoia	259.5	2	0.77	3	1.16
	Uasin Gishu	300.8	4	1.33	0	0.00
	Elgeyo Marakwet	148.9	1	0.67	0	0.00
	Baringo	203.8	1	0.49	1	0.49
	Nandi	299.3	3	1.00	2	0.67
	Nakuru	522.7	16	3.06	9	1.72
	Kericho	633.3	4	0.63	5	0.79
	Narok	210.3	2	0.96	0	0.00
	Others	211.4	0	0.00	0	0.00
	(Total)	3,240.4	36	1.11	22	0.68
Unknown		—	1	—	1	—
Total		7,617.0	124	1.63	135	1.77

*A demographic structure was obtained from the Kenya Population Census 1979 (Government of Kenya, 1979).

Table 8 Ethnical distribution of Kaposi's sarcoma (KS) and Burkitt's lymphoma (BL) (per 100,000 population)

Ethnic Group	Population in '000s (1979, estimated)	KS	KS 100,000 population	BL	BL 100,000 population
Luo	1,955.9	50	2.56	85	4.35
Luhya	2,119.7	35	1.65	22	1.04
Kalenjin	1,652.2	17	1.03	13	0.79
Kisii	944.1	9	0.95	11	1.17
Teso	132.7	1	0.75	0	—
Kikuyu	3,202.8*	11	0.34	0	—
Turkana	207.2	0	—	1	0.48
Arab	—	0	—	1	—
Unknown	—	1	—	2	—

*Exact number of their population in western Kenya is unclear.

Table 9 Ethnical incidence of Kaposi's sarcoma (KS) and Burkitt's lymphoma (BL) amongst malignant tumors

Ethnic Group		No. of malig. tumor	KS	KS / malig. tumor (%)	BL	BL / malig. tumor (%)
Luo	Male	625	46	7.36	48	7.68
	Female	765	4	0.52	36	4.70
	Unknown	6	0	—	1	16.67
	(Total)	1,396	50	3.58	85	6.09
Luhya	Male	347	30	8.65	10	2.88
	Female	391	1	0.26	12	3.07
	Unknown	9	4	44.44	0	—
	(Total)	747	35	4.69	22	2.95
Kalenjin	Male	402	15	3.73	8	1.99
	Female	472	2	0.42	5	1.06
	Unknown	22	0	—	0	—
	(Total)	896	17	1.90	13	1.45
Kisii	Male	165	8	4.85	5	3.03
	Female	153	1	0.65	6	3.92
	Unknown	1	0	—	0	—
	(Total)	319	9	2.82	11	3.45
Teso	Male	8	1	12.50	0	—
	Female	9	0	—	0	—
	Unknown	1	0	—	0	—
	(Total)	18	1	5.56	0	—
Kikuyu	Male	181	9	4.97	0	—
	Female	303	2	0.66	0	—
	Unknown	18	0	—	0	—
	(Total)	502	11	2.19	0	—
Others	(Total)	365	1	0.27	4	1.10
Total		4,243	124	2.92	135	3.18

Out of a total of 135 collected BL cases, 90 were from Nyanza Province, and 22 each from both Western Province and Rift Valley Province (Table 6). The estimated incidence of BL per 100,000 population in each province and district for the eight-year period was as follows: Nyanza Province showed the highest incidence of 3.54, followed by 1.20 in Western Province and 0.68 in Rift Valley Province (Table 7). Kisumu District in Nyanza Province showed the highest incidence of 13.27, followed by Kakamega District of Western Province with 2.04, South Nyanza District of Nyanza Province with 1.83, Nakuru District of Rift Valley Province with 1.72, Trans Nzoia District of Rift Valley Province with 1.16 and Siaya District of Nyanza Province with 1.05 (Table 7). High incidence rates of both diseases appeared in Kisumu, Siaya and South Nyanza Districts of Nyanza Province, in the tropical savannah around Lake Victoria, Nakuru District of Rift Valley Province, in the tropical highland, and Kakamega District of Western Province, in the tropical savannah. When the numbers of cases of KS and BL were compared by region, there was a significant positive

correlation ($r=0.819$, $p<0.001$), i. e., a high incidence of KS(BL) cases were found in regions which exhibited a high incidence of BL (KS) cases.

Figures 2, 3 and 4 are adapted from figures in Health and Disease in Kenya (Vogel *et al.*, 1974) showing these factors for references. The occurrence of KS and BL was analysed in relation to the altitude, mean annual temperature and mean annual rainfall in western Kenya. Among the factors the annnual rainfall correlated with the occurence of KS, but it was not significant statistically. As for BL, the temperature gave the highest correlation value, but again without significance.

Ethnical distribution

Table 8 shows the ethnical distribution of KS and BL per 100,000 population for the eight -year period between 1979 to 1986. In the case of KS, the Luo, the main inhabitants of Nyanza Province, around Lake Victoria showed the highest incidence with 2.56, followed by the Luhya, the main inhabitants of Western Province, with 1.65, the Kalenjin, the inhabitants of the tropical highland in Rift Valley Province, with 1.03, and the Kisii, the inhabitants of highland area of Nyanza Province, with 0.95. On the other hand, the highest incidence of BL was seen among the Luo with 4.35, followed by the Kisii with 1.17, the Luhya with 1.04, and the Kalenjin with 0.79. Table 9 shows the ethnical incidence of KS and BL amongst malignant tumors. The Luhya had a higher rate of KS than the Kisii ($p<0.05$), Kikuyu ($p<0.05$) and Kalenjin ($p<0.01$). There was no significant difference between the Luhya and the Luo (by the Chi-square test, using 4,243 cases of malignant tumors). The number of cases of Teso was so small that could not be analysed. The highest incidence of both KS and BL appeared in the Luo, Luhya and Kisii. The analysed results showed a clear association between the ethnic group and the occurrence rate of KS ($p<0.025$) or BL($p<0.001$).

Table 10 Child type Kaposi's sarcoma (KS) in western Kenya

Case	Age	Sex	Site of lesion	Ethnic group	District	Province
1	1	M	Inguinal lymph node	Luhya	Busia	Western
2	1y6m	M	Generalized lymph nodes	Luo	South Nyanza	Nyanza
3	1y8m	M	Generalized lymph nodes	Luo	Kisumu	Nyanza
4	1y9m	M	Generalized lymph nodes	Luhya	Kakamega	Western
5	1y9m	M	Generalized lymph nodes	Luo	Siaya	Nyanza
6	2y6m	M	Unknown lymph node	Luhya	Kisumu	Nyanza
7	5	F	Generalized lymph nodes	Luo	South Nyanza	Nyanza
8	6	M	Unknown lymph node	Luo	Kisumu	Nyanza
9	7	M	Generalized lymph nodes	Luo	Kakamega	Western
10	7	F	Mandibula	Luo	Siaya	Nyanza
11	8	M	Elbow lymph node	Kisii	Kisii	Nyanza
12	9	M	Foot	Luo	South Nyanza	Nyanza
13	10	F	Unknown lymph node	Luhya	Kakamega	Western
14	11	M	Leg	Luo	Kisumu	Nyanza
15	12	M	Upper lid	Luo	Busia	Western
16	12	M	Cervical lymph node	Luo	Kisumu	Nyanza
17	Child*	M	Generalized lymph nodes	Luo	Kisumu	Nyanza

*Exact age in unknown.

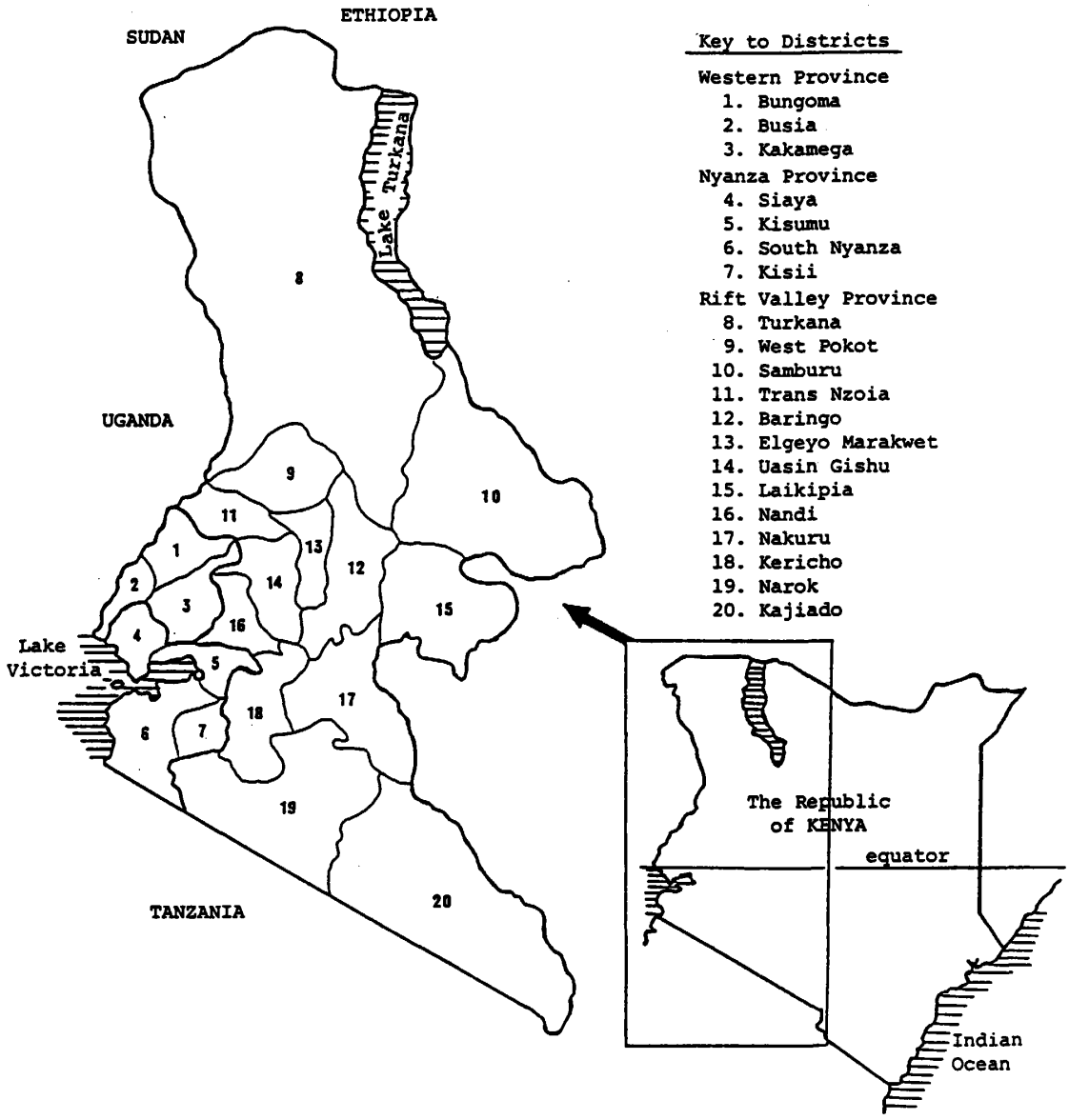

Figure 1 Map of western Kenya showing districts. (Adapted from figures in Vogel *et al.*: Health and Disease in Kenya. East African Literature Bureau, 1974)

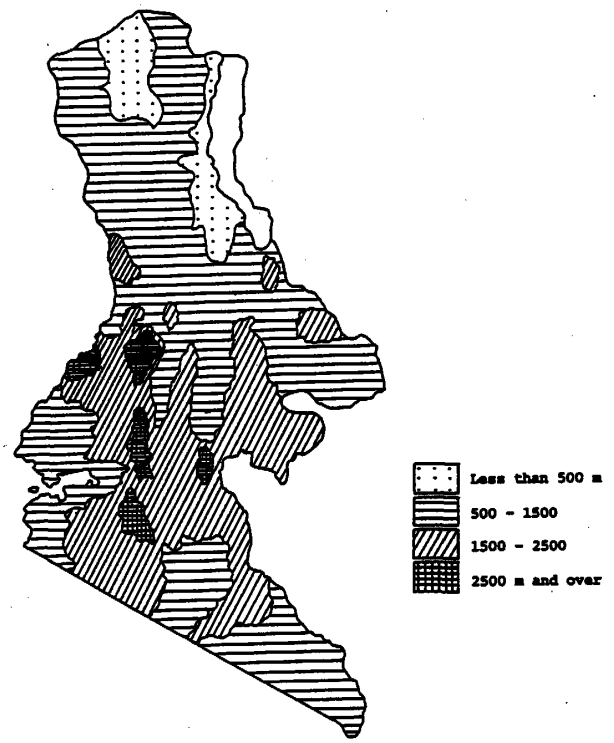

Figure 2 Map of western Kenya showing altitudes above sea level. (Adapted from figures in Vogel *et al.*: Health and Disease in Kenya. East African Literature Bureau, 1974)

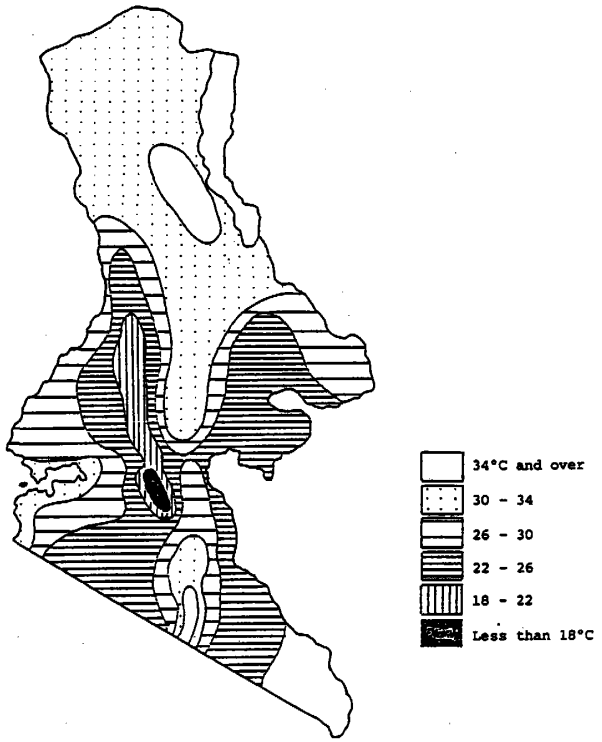

Figure 3 Map of western Kenya showing mean annual temperature. (Adapted from figures in Vogel *et al.*: Health and Disease in Kenya. East African Literature Bureau, 1974)

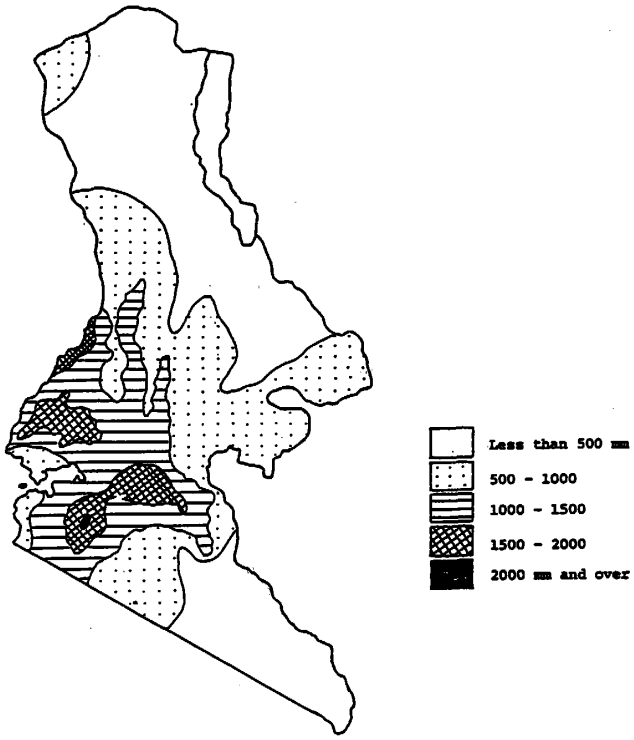

Figure 4 Map of western Kenya showing mean annual rain fall. (Adapted from figures in Vogel *et al.*: Health and Disease in Kenya. East African Literature Bureau, 1974)

KS in children and BL

Table 10 shows the 17 cases of KS found in children (under 14 years old) between 1979 and 1986. Twelve cases in all appeared in Nyanza Province, followed by 5 cases in Western Province, while no case was reported from Rift Valley Province. Kisumu District had the largest number with 6 cases, followed by South Nyanza District and Kakamega District with 3 cases each, Busia District and Siaya District with 2 cases each, and Kisii District with one case. Significantly more cases were found in Kisumu District than Busia District ($p<0.05$) or Kisii District ($p<0.01$). As for ethnic group, 12 cases of KS in children were from the the Luo, with 4 cases of the Luhya and one case of the Kisii. KS in children were more prevalent in the Luo than in the Luhya ($p<0.05$) or the Kisii ($p<0.05$). There was a significant level of association of KS in children with region ($p<0.05$) and ethnic group ($p<0.05$). Majority of BL cases were found in children under 14 years old (Table 3). Ethnically, the Luo had a higher rate of BL than the Kisii, Luhya, and Kalenjin ($p<0.01$) (Table 8). Geographically, Kisumu District had a higher rate of BL than Kakamega, South Nyanza, Siaya and Kisii Districts ($p<0.01$) (Table 7) (by the Chi-square test, using the number of KS in children and BL against the total populations in each ethnic group and region). The occurrence rate of BL in ethnic groups and regions showed a clear association of BL with ethnic group and with region. The geographical and ethnical distributions of KS and BL coincided far more in children than in the adult population.

Discussion

The Republic of Kenya stands almost exactly astride the equator; its area is 569, 137 km^2 and its population is 15,327,000 (Bhushan, 1982). Western Kenya (Western, Nyanza and Rift Valley Provinces) accounts for one third of the whole country, 189,578 km^2, in area and about one half, 7,617,000, in population (Bhushan, 1982). It is bounded by Sudan and Ethiopia on the north, Uganda on the west and Tanzania on the south, and consists of three provinces, namely, Western, Nyanza and Rift Valley Provinces. These provinces are divided into three, four and thirteen districts respectively (Figure 1). These areas experience a wide variation of climatic conditions (Figure 2, 3 and 4). The most northern part, Turkana District, and the most southern part, Kajiado District, are dry desert or dry tropical savannah and have mean annual rainfalls of 100 to 200 mm and 300 to 700 mm respectively, and mean annual temperatures of 33 to 36°C and 22 to 33°C respectively. The altitudes of these areas are 650 m and 650 to 1,000 m respectively. On the contrary, the central part of Rift Valley Province is tropical highland with an altitude of 1,500 to 2,000 m, a mean annual rainfall of 1,200 to 2,000 mm and a mean annual temperature of 18 to 22°C. Western Province is tropical savannah with an altitude of 1,200 to 2,000 m, a mean annual rainfall of 1,250 to 1,750 mm and a mean annual temperature of 26 to 30°C. Nyanza Province is tropical savannah situated around Lake Victoria with altitude of 1,100 to 1,800 m, a mean annual rainfall of 1,000 to 1,750 mm and a mean annual temperature of 26 to 34°C.

When the geographical distribution of endemic KS and African BL per 100,000 population and the incidence of both diseases amongst malignant tumors in western Kenya were considered, coincidences of both diseases were observed in Kisumu District around Lake Victoria in Nyanza Province, Kakamega District in Western Province, Siaya District in Nyanza Province and Nakuru District in Rift Valley Province. Kisumu and Siaya Districts around Lake Victoria and Kakamega District consist of relatively moist tropical savannah whereas Nakuru District is situated in tropical highland which has relatively moist climatic conditions. Although the author could not detect any apparent statistical significances, it is likely that some environmental factors, such as high temperature (mean annual temperature over 26°C) and humidity (annual rainfall over 1,000 mm), influence the causation of KS and BL in western Kenya. And ethnically, a high incidence of KS and BL appeared among the Luhya, descended from the Bantu, and the Luo, descended from the Nilotic groups. These ethnic groups are from different origins (Fedders and Salvadori, 1979), but are living in moist and high temperature areas. These findings suggest that some environmental factors and some transmissible agents influence more the causation of endemic KS and African BL rather than genetic factors.

Although EBV is now known as the causative agent of BL (Epstein *et al.*, 1964; Old *et al.*, 1966; Henle *et al.*, 1969, 1973; de Schryver *et al.*, 1969; Gunven *et al.*, 1970; Kaschka-Dierich *et al.*, 1976), most studies of American cases have failed to demonstrate an association between high titers of anti-EBV and BL, and it was suggested that, even if EBV is etiologic for BL, it is only one of several factors (Linder and Purtio, 1984). Also it has been suggested that EBV may initiate a lymphoid tumor if it infects a susceptible individual whose immunological response has been altered by malaria, especially *P. falciparum* (Hutt, 1970). As a result of similar etiological conditions, CMV has been linked with endemic KS in Africa (Burkes *et al.*, 1985).

With special reference to KS in children, it has been suggested that preceding persistent infection with malaria, especially *P. falciparum* before CMV infection may play a role as one of the etiological factors of KS (Safai *et al.*, 1980). It was recognized that continuous stimulation with foreign antigens might cause a relative immunodeficiency in Africa (Oettle, 1962; Master *et al.*, 1970; Taylor *et al.*, 1971b). Although the tropical savannah around Lake Victoria in western Kenya is a holoendemic area of *P. falciparum* (Vogel *et al.*, 1974), it is not known whether malaria infections could be an etiological cofactor of endemic KS and African BL or not. However, some environmental cofactors, including climatic conditions, life styles of the inhabitants and other unknown causative agents, might play a role in the causation of endemic KS and African BL. Furthermore, unknown transmissible agents might be one of the etiological cofactors of the both diseases, in a similar way to the relationship between epidemic KS and malignant B-cell lymphoma in AIDS.

According to this statistical study on the geographical distribution of endemic KS and African BL, a relalatively high temperature and moist climatic conditions were considered to be related to the high incidence of endemic KS and African BL in western Kenya. No case of KS and only a few cases of BL were found among the inhabitants of desert or semi-desert areas. No other tumors showed above mentioned tendencies. The geographical and ethnical coincidence of KS and BL was more clear in the child population than in the adult. These results suggest that there is a geographical coincidence of KS and BL based on same etiological cofactors including high temperature, high humidity, unknown transmissible agents, and probably genetic factors and life styles. This was mainly demonstrated in Nyanza Province around Lake Victoria in western Kenya.

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西ケニアにおける地方病型カポシ肉腫とアフリカ型バーキットリンパ腫の部族および地理的分布の一致性

鳥山　寛

アフリカ型（地方病型）カポシ肉腫（ＫＳ）はアフリカ中央部に多発地帯があり，その地域から東西南北に地理的に遠ざかるにつれてその頻度は減少すると言われており，今までに我々が西ケニアで行った調査結果もそれを裏付けている。これとは別に赤道をはさんで東アフリカから西アフリカへ帯状のリンパ腫の多発地帯（Lymphoma Belt）が存在する。1979年から1986年の８年間にわたって，ケニア西部においてカポシ肉腫とＢ細胞型悪性リンパ腫の一つであるアフリカ型バーキットリンパ腫（ＢＬ）の，病理組織学的診断を基礎とした疫学的調査を行い，それらの地理的および部族的分布を検討した。その結果，ＫＳとＢＬの相関性および自然環境や人的環境との関連性はつぎのようであった。１）ＫＳ，ＢＬともに高温で比較的湿潤な地域に多く見られた。２）部族的にはＫＳ，ＢＬともに主として熱帯サバンナに居住するルオー族およびルヒア族に多く見られた。３）乾燥した地域での発生頻度は非常に低かった。４）他の腫瘍では，このような地理的，部族的，および自然環境的な特徴は著明ではなかった。５）特にＢＬと小児型ＫＳでは地理的，部族的一致性が強く認められた。これらの結果より，両疾患の発生には遺伝的あるいは先天的要因というよりも，何らかの共通した環境因子の存在や生活様式のより強い関与があるものと思われる。

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輸入動物の寄生虫

IV. 輸入チンパンジーにおける寄生虫感染状況

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森　祐介[2]・笹岡　貞信[2]
昭和63年2月24日受付／昭和63年5月20日受理

先に影井，浅野（1980）は実験動物として輸入された霊長類の寄生虫について検査を行いその調査結果を報告したが，その報告の中では原虫類については触れておらず，また血液についての検査も行われていなかった。

そこで，今回は肝炎研究のためにアフリカのシエラ・レオーネより輸入し，東京大学医学部，ならびに同大同学部および化学及血清療法研究所等で研究のために用いられた後，三和化学研究所霊長類センターで飼育されているチンパンジーについて検査する機会を得たので，その検査結果について報告する。

材料と方法

対象としたチンパンジー（*Pan troglodytes*）は肝炎研究の為に西アフリカのシエラ・レオーネより輸入し，東京大学医学部ならびに同大同学部，および化学及血清療法研究所において研究に供された後，三和化学研究所霊長類センターで飼育されていた輸入後7年未満のものおよび日本で生まれたものを対象に糞便検査ならびに血液検査，skin-snipを行い虫卵・幼虫等の有無について検査した。

糞便検査はTween 80加クエン酸緩衝液遠心沈殿集卵法，直接塗抹ハイデンハイン鉄ヘマトキシリン染色法，ならびに試験管内濾紙培養法を行い，原虫嚢子・栄養型，ならびに虫卵または幼虫から種の同定を行った。

一方，血液検査は法のごとく採血後，薄層・厚層塗抹ギムザ染色標本を作成し，血液原虫，microfilariaの検査を行った。skin-snipも法のごとく皮膚採集を行い，ギムザ染色後microfilariaの有無を検索した。

結　果

糞便検査結果

糞便検査の結果は表1に見る様に，蠕虫類は4種類がみられたがすべて線虫類で，吸虫・条虫類は見出されなかった。中でも糞線虫（*Strongyloides fuelleborni*）は最も高率に感染をしており，検査時下痢の状態を示した便29個体中15（51.7%）から糞線虫卵並びに幼虫が見出され，下痢症の原因となっていることが示唆された。ついで鞭虫（*Trichuris trichiura*）の感染が見られた。また蟯虫（*Enterobius* sp.）卵は三和化学霊長類センター飼育のものからは全く見出されなかったが，東京大学で飼育・研究中のものから38.1%に未だ幼虫形成の見られない虫卵が多数認められた。

なお，東京大学で飼育されているものの1頭から線虫卵（Strongylid egg，大きさ74.0—87.1×37.8—39.5 μm）が見出されたが，培養による幼虫検出ができず属種を知ることは出来なかった。

本論文の要旨は第55回日本寄生虫学会大会（札幌，1986）において報告した。
1．国立予防衛生研究所寄生虫部
2．三和化学研究所霊長類センター

Table 1 Results of fecal examination for helminth-infection in chimpanzees, *Pan troglodytes*, imported from Africa

Breeding place	Chimpanzee Sex	No. exam.	Positive case of egg of *Strongyloides fuelleborni*	*Trichuris trichiura*	*Enterobius* spp.	Strongylid
Kaketsu-ken* and Sanwa Co.	M	8	4 (50.0)	0	0	0
	F	2	1 (50.0)	0	0	0
	T	10	5 (50.0)	0	0	0
Tokyo University and Sanwa Co.	M	10	4 (40.0)	1 (10.0)	0	0
	F	9	3 (33.3)	0	0	0
	T	19	7 (36.8)	1 (5.3)	0	0
Sanwa Co.	M	6	5 (83.3)	2 (33.3)	0	0
	F	4	3 (75.0)	2 (50.0)	0	0
	T	10	8 (80.0)	4 (40.0)	0	0
Subtotal in Sanwa Co.	M	24	13 (54.2)	3 (12.5)	0	0
	F	15	7 (46.7)	2 (13.3)	0	0
	T	39	20 (51.3)	5 (12.8)	0	0
Tokyo University	M	4	1 (25.0)	0	1 (25.0)	1 (25.0)
	F	9	2 (22.2)	0	6 (66.7)	1 (11.1)
	Mix	8	3 (37.5)	0	1 (12.5)	4 (50.0)
	T	21	6 (28.6)	0	8 (38.1)	6 (28.6)
Total		60	26 (43.3)	5 (8.3)	8 (13.3)	12 (20.0)

*：Japanese abbreviation of Chemico-Sero-Terapeutic Institute Inc.
The positive rate (percentage) is shown in parentheses.

一方，原虫類は表2に見る様に10種類（メニール鞭毛虫，ランブル鞭毛虫，赤痢アメーバ，大腸アメーバ，*Entamoeba hartmanni, E. polecki, E.* sp.，ヨードアメーバ，小形アメーバ，大腸バランチジウム）が高率に見られ，特に赤痢アメーバの寄生率が高いことは注目に値した。赤痢アメーバの感染率は霊長類センター飼育中の39頭では51.3%，東京大学医学部42.9%と極めて高率であったが，飼育場所別にみた場合，寄生率に大きな差は見られなかった。

また検査時下痢症状を呈した便29個体中9個（31.0%）に赤痢アメーバ嚢子が，12個（41.4%）にランブル鞭毛虫嚢子が見出された。

なお，表中には示されなかったが，日本で生まれた雌雄各1頭中の雌から赤痢アメーバ，大腸アメーバ，*E. polecki* 嚢子が見出された。

血液・skin-snip 検査の結果

血液並びに skin-snip 検査は霊長類センターで飼育中のチンパンジーについてのみ行ったが，血液原虫は全く見出されなかった。

フィラリアについては，表3には示していないが，日本で生まれた2頭の1歳児からは全く microfilaria を見出すことは出来なかったが，その他のチンパンジーの血液検査では表3に示すように被鞘がなく，体長160—200μmで核が尾部先端まで満たされ，先端が鈍に終わっている *Dipetalonema perstans* の microfilaria（写真1，Fros, 1956）が全体で39頭中15頭（38.5%）に見出された。一方，skin-snip 標本では2種類の microfilaria が主に霊長類センターで飼育されたチンパンジーのみに見出され，その1種は体長180—240 μm でやや体幅が広く，尾端が釣針状に強く曲がり，一列に並んだ核が尾端まで伸びた *D. streptocerca*（写真2，Orihel, 1970, 1984）が10.3%に，もう1種は体長が極めて長く（300—340 μm），その割りには体幅の細い *D. rodhaini*（写真3，Orihel, 1970）が12.8%に見出された。

Table 2 Results of fecal examination for Protozoa-infection in chimpanzees, *Pan troglodytes*, imported from Africa

Breeding place	Cmimpanzee Sex	No. exam.	Positive cases of *Chilomastix mesnili*	*Giardia lamblia*	*Entamoeba histolytica*	*Entamoeba coli*	*Entamoeba hartmanni*	*Entamoeba polecki*	*Entamoeba* sp.	*Iodamoeba buetschlii*	*Endolimax nana*	*Balantidium coli*
Kaketsu-ken*	M	8	2 (25.0)	4 (50.0)	4 (50.0)	2 (25.0)	2 (25.0)	1 (12.5)	1 (12.5)	3 (37.5)	1 (12.5)	1 (12.5)
and Sanwa	F	2	0	0	1 (50.0)	0	0	0	0	0	0	0
Co.	T	10	2 (20.0)	4 (40.0)	5 (50.0)	2 (20.0)	2 (20.0)	1 (10.0)	1 (10.0)	3 (30.0)	1 (10.0)	1 (10.0)
Tokyo Uni-	M	10	2 (20.0)	3 (30.0)	5 (50.0)	3 (30.0)	1 (10.0)	2 (20.0)	2 (20.0)	4 (40.0)	3 (30.0)	0
versity and	F	9	2 (22.2)	5 (66.7)	4 (44.4)	4 (44.4)	2 (22.2)	1 (11.1)	1 (11.1)	0	4 (44.4)	1 (11.1)
Sanwa Co.	T	19	4 (21.1)	8 (42.1)	9 (47.4)	7 (36.8)	3 (15.8)	3 (15.8)	3 (15.8)	4 (21.1)	7 (36.3)	1 (5.3)
Sanwa Co.	R	6	0	0	4 (66.7)	1 (16.7)	0	3 (50.0)	0	0	2 (50.0)	0
	F	4	1 (25.0)	1 (25.0)	2 (50.0)	1 (25.0)	0	2 (50.0)	0	1 (25.0)	1 (25.0)	0
	T	10	1 (10.0)	1 (10.0)	6 (60.0)	2 (20.0)	0	5 (50.0)	0	1 (10.0)	3 (30.0)	0
Subtotal in	M	24	4 (16.7)	7 (29.2)	13 (54.2)	6 (25.0)	3 (12.5)	6 (25.0)	3 (12.5)	7 (29.2)	6 (25.0)	1 (4.2)
Sanwa Co.	F	15	3 (20.0)	6 (40.0)	7 (46.7)	5 (33.3)	2 (13.3)	3 (20.0)	1 (6.7)	1 (6.7)	5 (33.3)	1 (6.7)
	T	39	7 (17.9)	13 (33.3)	20 (51.3)	11 (28.2)	5 (12.8)	9 (23.1)	4 (10.3)	8 (20.5)	11 (28.2)	2 (5.1)
Tokyo Uni-	M	4	1 (25.0)	0	3 (75.0)	4 (100.0)	0	0	0	1 (25.0)	1 (25.0)	0
versity	F	9	0	1 (11.1)	4 (44.4)	7 (77.8)	0	0	0	1 (11.1)	0	0
	Mix	8	1 (12.5)	0	2 (25.0)	4 (50.0)	0	0	0	0	0	0
	T	21	2 (9.5)	1 (4.8)	9 (42.9)	15 (71.4)	0	0	0	2 (9.5)	1 (4.8)	0
Total		60	9 (15.0)	14 (23.3)	29 (48.3)	26 (43.3)	5 (8.3)	9 (15.0)	4 (6.7)	10 (16.7)	12 (20.0)	2 (3.3)

* : Japanese abbreviation of Chemo-Sero-Therapeutic Institute Inc.
The positive rate (percentage) is shown in parentheses.

Table 3 Results of blood and skin-snip examination for microfilariae of chimpanzees, *Pan troglodytes*, imported from Africa

Breeding place	Chimpanzee Sex	No. exam.	Positive cases of microfilariae of *Dipetalonema perstans* from blood	*Dipetalonema streptocerca* from skin	*Dipetalonema rodhaini* from skin
Kaketsu-ken*	M	8	5 (62.5)	1 (12.5)	1 (12.5)
and Sanwa Co.	F	2	1 (50.0)	0	0
	T	10	6 (60.0)	1 (10.0)	1 (10.0)
Tokyo University	M	10	3 (30.0)	0	0
and Sanwa Co.	F	9	2 (22.2)	0	0
	T	19	5 (26.3)	0	0
Sanwa Co.	M	6	3 (50.0)	2 (33.3)	2 (33.3)
	F	4	1 (25.0)	1 (25.0)	2 (50.0)
	T	10	4 (40.0)	3 (30.0)	4 (40.0)
Total	M	24	11 (45.8)	3 (12.5)	3 (12.5)
	F	15	4 (26.7)	1 (6.7)	2 (13.3)
	T	39	15 (38.5)	4 (10.3)	5 (12.8)

* : Japanese abbreviation of Chemo-Sero-Therapeutic Institute Inc.
The positive rate (percentage) is shown in parentheses.

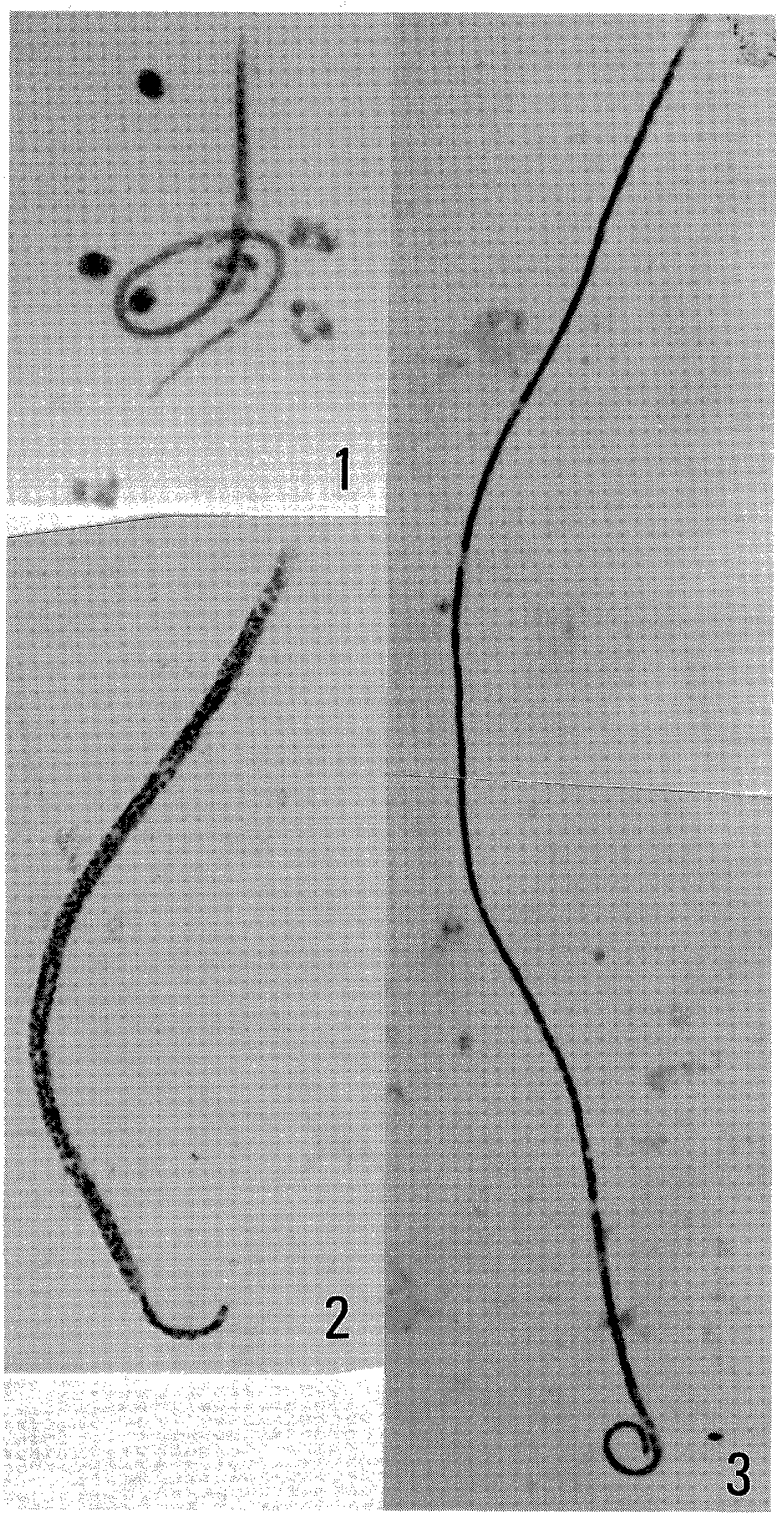

Photo. 1 A microfilaria of *Dipetalonema perstans* in blood of a chimapanzee (×500).
Photo. 2 A microfilaria of *D. streptocerca* found in skin of a chimpanzee by skin-snip method (×500).
Photo. 3 A microfilaria of *D. rodhaini* found in skin of a chimpanzee by skin-snip method (×700).

Table 4 Prevalence of parasite infection according to age of chimpanzees in Sanwa Kagaku Kenkyusho Co.

Chimpanzee Age	No. exam.	Positive Cases of parasites	Cases infected with Protozoa	Helminths	Microfilaria
1	2	1 (50.0)	1 (50.0)	0	0
3	3	3 (100.0)	1	3	1
4	5	5 (100.0)	3	3	4
5	2	2 (100.0)	2	2	1
	10	10 (100.0)	6 (60.0)	8 (80.0)	6 (60.0)
7	6	6 (100.0)	2	4	4
8	7	6 (85.7)	5	4	4
9	2	2 (100.0)	2	1	0
10	8	6 (75.0)	6	2	1
	23	20 (87.0)	15 (65.2)	11 (47.8)	9 (39.1)
12	5	4 (80.0)	4	1	2
15	1	1 (100.0)	1	1	0
	6	5 (83.3)	5 (83.3)	2 (33.3)	2 (33.3)

The positive rate (percentage) is shown in parentheses.

Table 5 Prevalence of parasite infection according to the duration of breeding period of chimpanzees in Sanwa Kagaku Kenkyusho Co.

Chimpanzee Breeding period (year)	No. exam.	Positive cases of parasite	Cases infected with Protozoa	Helminths	Microfilaria
1	12	11 (91.7)	7	8	6
2	2	2 (100.0)	2	0	2
	14	13 (92.9)	9 (64.3)	8 (57.1)	8 (57.1)
3	2	2 (100.0)	0	2	2
4	10	8 (80.0)	6	3	4
5	3	3 (100.0)	2	3	1
	15	13 (86.7)	8 (53.3)	8 (53.3)	7 (46.7)
6	3	3 (100.0)	3	2	0
7	9	7 (77.8)	7	3	2
	12	10 (83.3)	10 (83.3)	5 (41.7)	2 (16.7)

The positive rate (percentage) is shown in parentheses.

年齢別感染状況

これらの寄生虫感染を原虫類，蠕虫類，microfilariaと大きく分けて年齢別にみると表4に示すように霊長類センターで生まれた1歳のチンパンジー2頭中1頭に原虫類の感染が見られたが，蠕虫類の感染は見られなかった。

次いで3－5歳台では全てに寄生虫の感染が見られ，その後年齢の増加に伴って感染率が若干低下する傾向が見られたが，その原因は腸管蠕虫類並びにフィラリア類の感染率が低下するためで，原虫類の感染率には低下の傾向はみられず，むしろ上昇気味であった。

滞在年数からみた感染状況

更にチンパンジー輸入後のわが国における滞在期間と寄生虫感染率の関係をみると，表5に見るように原虫類の寄生率はあまり滞在年数に左右される事なく高率であるが，蠕虫類の感染は滞在年数が長くなるに従って寄生率が低下する傾向がみられ，特にmicrofilaria保有率は滞在年数6年以上では滞在年数1－2年のmicrofilaria保有率の1/3に減少していた。

考　察

チンパンジーは分類学上の位置からも人間に最も近い関係にある所から，解剖学的，行動学的，生態学的研究は勿論，医学の分野の実験動物としても，その価値は極めて高く，今回の肝炎研究には無くてはならない動物の1つである。従って逆にそのような類似性が人の感染症キャリアーにもなりかねず，人畜共通感染症にとっては詳細な資料を集めておく必要があるものと考えられる。

先に我々（影井・浅野，1980）が報告した輸入霊長類の寄生虫調査でもチンパンジーの寄生虫に関する若干の資料が得られたが，更に今回は腸管内および血液内寄生原虫相を追加することが出来，ついでmicrofilariaの検査からフィラリア感染の実態も知ることが出来た。

まず腸管寄生蠕虫類については先に我々（影井・浅野，1980）が報告した南米産チンパンジーの寄生虫相と殆んど変わらず，不明とした虫卵もおそらくは*Oesophagostomum*属あるいは*Trichostrongylus*属線虫と考えられたが，虫卵のみでは同定の根拠となるものがなく，属種名を書くのを差し控えた。ただ，東京大学医学部で飼育中のチンパンジーの糞便内に高率・多数の蟯虫卵がみられたことは，蟯虫の生活環から考えて奇異であるが，卵内容が未発育であることから，恐らく肛門周囲に出てきた雌虫を何等かの形で経口摂取したものか，あるいは糞便内に自然排虫されたものが破壊され虫卵が見られるに至ったものと考えられ，蟯虫寄生の実態を知るには再度肛門周囲検査によって確かめざるを得ないであろう。

Yamashita (1963) やMyers and Kuntz (1972) のチェックリストによるとチンパンジーには鉤頭虫類，条虫類，吸虫類の感染のあることも報告されているが，今回の調査ではこれら寄生蠕虫類は全く認められなかった。

いずれにしても，先に述べたごとく今回見出された蠕虫類は，全て人と動物間で共通に感染が起こり得るということから，極めて問題のあることで，現在各飼育施設でその対策を行っている。

次に腸管寄生原虫類についても，すべてMyers and Kunts（1972）のチンパンジーの寄生虫に関するチェックリストには登載されており，目新しいものではないが，ここでもまた人間に対して病原性の高い赤痢アメーバの嚢子が50%前後の高感染率を有していることは，人畜共通の寄生虫病として重要なことであり，また下痢便中の31%に赤痢アメーバ嚢子が見出されているなどチンパンジーの健康管理の上からも問題点のあるところである。その他，やや感染率は低下するがランブル鞭毛虫，大腸バランチジウムの感染のみられる事も重要なことであり，蠕虫類に対すると同様予防対策を施行中である。

チンパンジーからの血液寄生原虫類の報告も多くの*Trypanosoma*類，*Plasmodium*類，*Theileria*類等が報告されているが，今回の検査では全く見出す事は出来なかった。既にチンパンジーから報告されている血液寄生原虫の中には人体への感染の報告のみられるものもあり，熱帯熱マラリア原虫のように人に対して極めて重篤な症状をもたらすものも含まれているので，今後も観察を続けて

行くべきであろう。

フィラリア類についても今回見出された3種（*D. perstans, D. streptocerca, D. rodhaini*）はチンパンジーからはすでに見出され報告されており（Yamashita, 1963; Myers and Kuntz, 1972），新しい知見ではないが，前2種はアフリカ各地で人体感染者がかなり知られており，*D. perstans* は我が国への輸入症例もすでに報告されている（吉田ら，1982；大友・影井，未発表）。*D. perstans* ならびに *D. streptocerca* の伝播者は共に *Culicoides* 属のヌカカ類であることが知られているため，たとえ microfilaria を産出しているチンパンジーがわが国に輸入されてきていても，わが国での感染源となるか否かは，日本産ヌカカ類あるいはその他の吸血性昆虫での感受性試験を行っていない現在では不明であるが，今後まだまだ検疫の手を緩めることなく感受性試験と予防対策を行っていくべきであろう。

ただここで興味あることは，チンパンジー輸入後，年月を経るに従って腸管寄生蠕虫類ならびにフィラリア類の寄生率は減少していく傾向にあるが，消化管寄生原虫類は少なくとも飼育期間に関わりなく同じ感染率を有していたことである。

三和化学研究所霊長類センターにおけるチンパンジーの飼育環境をみると，2－3の例外はあるが夜間は個室に入れるものの昼間は前庭の広い運動場で，すべてのチンパンジーが共同で生活をしており，その運動場での生活の間に，個室の清掃を行っている。従ってチンパンジー同志は運動場という土壌を通して接触があり，その結果土壌伝播の寄生虫，特に原虫類，蠕虫類の中の糞線虫等の感染が容易に起こることになり，それも生活環の回転の早いものほど常時感染し根強く残るものと考えられた。

鞭虫はその卵が感染能力を持つに至るのに30数日を要するため感染はそれほど容易には起こらないし，一旦寄生すると，その寿命が極めて長いことから輸入時既に感染していたものはそれを保有し続け，表1に見られた飼育場別，即ちファーム別に感染率の違いが見られたものと考えられた。フィラリア類は中間宿主の存在なくして感染は起こらず，また中間宿主となりうるものが存在しても，感染率，血中あるいは皮下における microfilaria の分布状況によってかなり感染が抑制されること，年を経るにつれて自然に虫体が死滅するものも出現して寄生虫体が減少することなどがあり，飼育期間が長引くにつれてこれらの寄生虫の感染率の低下を惹起するものと考えられた。

ま と め

肝炎研究のためアフリカから輸入したチンパンジーについて寄生虫学的検索を行ったところ，次のような結果を得た。

1）検査の結果，蠕虫類4種類（*Strongyloides* fuelleborni, Trichuris trichiura, Enterobius sp. Strongylid線虫），腸管寄生原虫類10種類（*Chilomastix mesnili, Giardia lamblia, Entamoeba histolytica, E. coli, E. hartmanni, E.polecki, E.* sp., *Iodamoeba buetschlii, Endolimax nana, Balantidium* coli），フィラリア3種類（*Dipetalonemaperstans, D. streptocerca, D. rodhaini*）の感染が認められた。

2）以上の寄生虫類はチンパンジー自身の健康の上でも問題があるが，更にそれらの多くは人体への感染の上でも多くの問題を有しているため，その対策を必要とする。

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PARASITES OF ANIMALS IMPORTED TO JAPAN
IV. PARASITIC INFECTION OF CHIMPANZEES IMPORTED FROM AFRICA

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A survey of parasites among the chimpanzees, *Pan troglodytes*, imported from Africa as laboratory animals was undertaken by means of fecal examinations (HE staining method of direct smear, Tween 80 citric acid ether sedimentation procedure and filter-paper cultivation technique), blood smear technique and skin-snip method.

As a result, ova or larvae of 4 helminthic species (*Strongyloides fuelleborni, Enterobius* sp., *Trichuris trichiura* and a strongylid-nematode) and cysts or trophozoites of 10 protozoal species (*Chilomastix mesnili, Giardia lamblia, Entamoeba histolytica, E. coli, E. hartmanni, E. polecki, E.* sp., *Iodamoeba buetschlii, Endolimax nana* and *Balantidium coli*) were found in stool, and microfilariae of 3 filarial species (*Dipetalonema perstans, D. streptocerca* and *D. rodhaini*) were recovered from blood or skin-snips.

The fact was also shown that positiveness of helminthic ova and microfilariae in chimpanzees has a tendency to decrease when they are bred in laboratories, but that of protozoa is 83.3% even after the breeding of 6 to 7 years.

These parasites are important as the causative agents of sickness in chimpanzees. They are also, especially *Strongyloides fuelleborni* and *Entamoeba histolytica* from the primate, very important as causative agents of human sickness.

We think it is necessary to eradicate these parasites from chimpanzees bred in human society, because they are zoonotic pathogens and very dangerous to humans.

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CONTROL OF AEDES VECTORS OF DENGUE FEVER/DENGUE HAEMORRHAGIC FEVER IN SINGAPORE

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INTRODUCTION

In Singapore's early history and right up to 1960, malaria was the only mosquito-borne disease that claimed many lives from year to year. But, beginning in 1960, a second mosquito-borne disease, namely, dengue fever/dengue haemorrhagic fever (DF/DHF), began to afflict the nation as malaria had done. In that year, the first DF/DHF outbreak of 70 hospitalised cases occurred between June and October (Chew *et al.*, 1961). DF/DHF then became endemic and epidemics occurred periodimally in subsequent years (Chan *et al.*, 1966). In the outbreak years of 1966-8, respectively 24, 21 and 18 people, mostly children, died. In 1973, Singapore saw the largest DF/DHF outbreak of 1,187 cases with 27 deaths (Chan *et al.*, 1977). Malaria, by contrast, was killing only 1-3 people a year, although its incidence remained high, at about 200-400 cases a year. Thus, for about a decade, from the mid-1960s to the mid-1970s, DF/DHF replaced malaria as the most important mosquito-borne disease in Singapore.

Because of the appearance of DF/DHF in 1960 and its continuing medical importance in the subsequent years, and the resurgence of malaria in the early 1960s, a Vector Control Unit was set up in the Ministry of Health in June 1966, to deal with these two mosquito-borne diseases in particular. From June 1966 onwards, the control of DF/DHF had steadily increased until an effective integrated system was developed for controlling the *Aedes* vectors, and this eventually culminated in the successful control of the disease. Singapore's success story of DF/DHF control has been reported in detail in Chan (1985a). This paper serves to focus on, and summarize, the salient features of this control program, to which the interested reader is encouraged to refer.

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METHODS

Aedes Surveillance

Aedes breeding in relation to premises was by the method used in Chan *et al.* (1971). Essentially this involved collecting all the larvae and pupae within each breeding habitat in all accessible prerises and open spaces within the areas surveyed. The immature stages from each breeding habitat were collected with the aid of pipettes and ladles and placed in bottles. Information on each breeding habitat, such as collection area, type and location of habitat, were labelled on the bottles. The immature stages were then taken to the laboratory for identification and counting. Data were recorded on two types of prepared survey forms (Chan, 1985a).

The methods and indices used in the surveillance of larval and adult *Aedes* vectors of DF/DHF followed those given in Chan (1985a, b). Of three common larval indices, namely, House Index, Breteau Index, and Container Index, only the House Index was used although the Breteau Index is the best of these indices. This is because at low rates of infestation (i. e. below 5%) the House Index and the House Index and the Breteau Index are highly correlated and are essentially the same (Tinker, 1978).

Two adult indices were used to reflect the mosquito vector densities, namely, the House

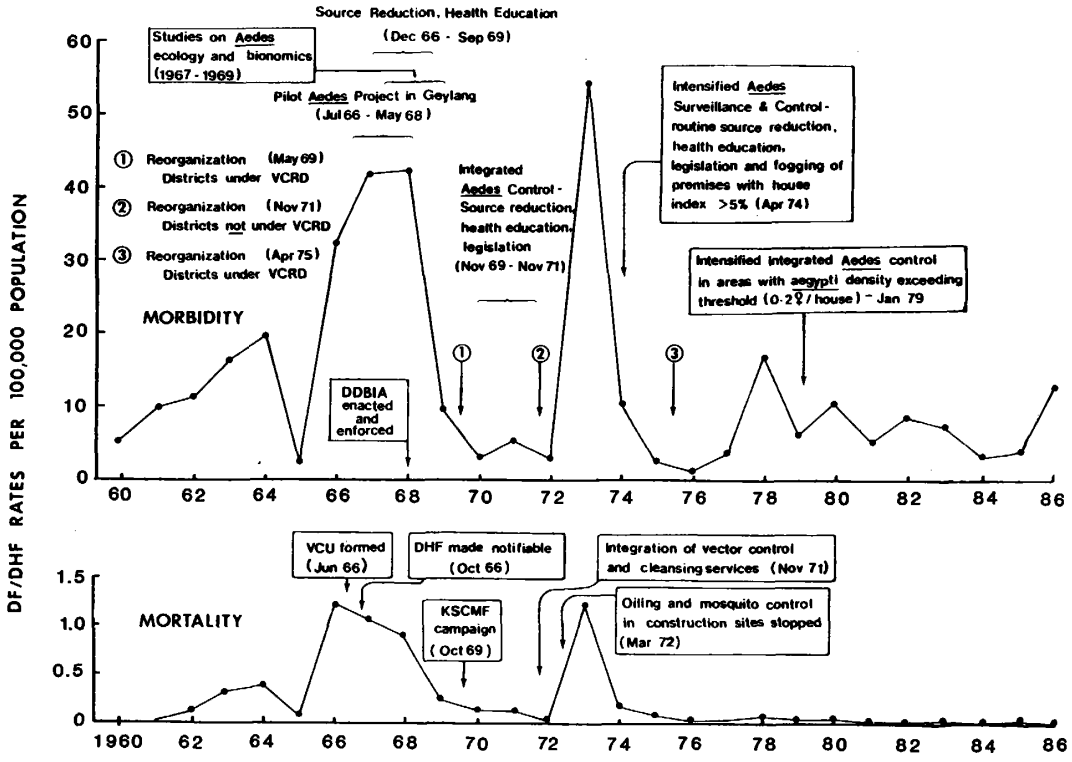

Figure 1 DF/DHF morbidity and mortality rates in Singapore, 1960-86, and key activities is *Aedes* vector control during the period.

Density Index for *Aedes aegypti* (number of females per house) and the Biting Rate Index for *Aedes albopictus* (number of females taken at bait per unit of time). These two density indices had been figured in relation to the incidence of DF/DHF in Chan (1985a, b). Their usefulness, together with the parous rate, in forecasting DF/DHA outbreaks, had also been illustrated in Chan (1985a, b).

Aedes Control

No attempt to control *Aedes* vectors of DF/DHF was ever carried out prior to the establishment of the Vector Control Unit in June 1966. With the setting up of the VCU, pilot control studies using source reduction, were initiated from July 1966 to May 1968 in the Geylang area, one of the highest endemic areas for DF/DHF in Singapore. At the same time (1967-1969) the ecology and bionomics of the two *Aedes* vectors, *Ae. aegypti* and *Ae. albopictus*, were also studied in detail. In the Geylang pilot study the initial control measures used were source reduction and health education (Figure 1). These measures proved so effective that after three round of control, the House Index fell from an original high mean of 16% to a low mean of 2% (Chan, 1967). However, in the absence of other measures, this level of achievement could not be sustained. It was immediately recognized that law

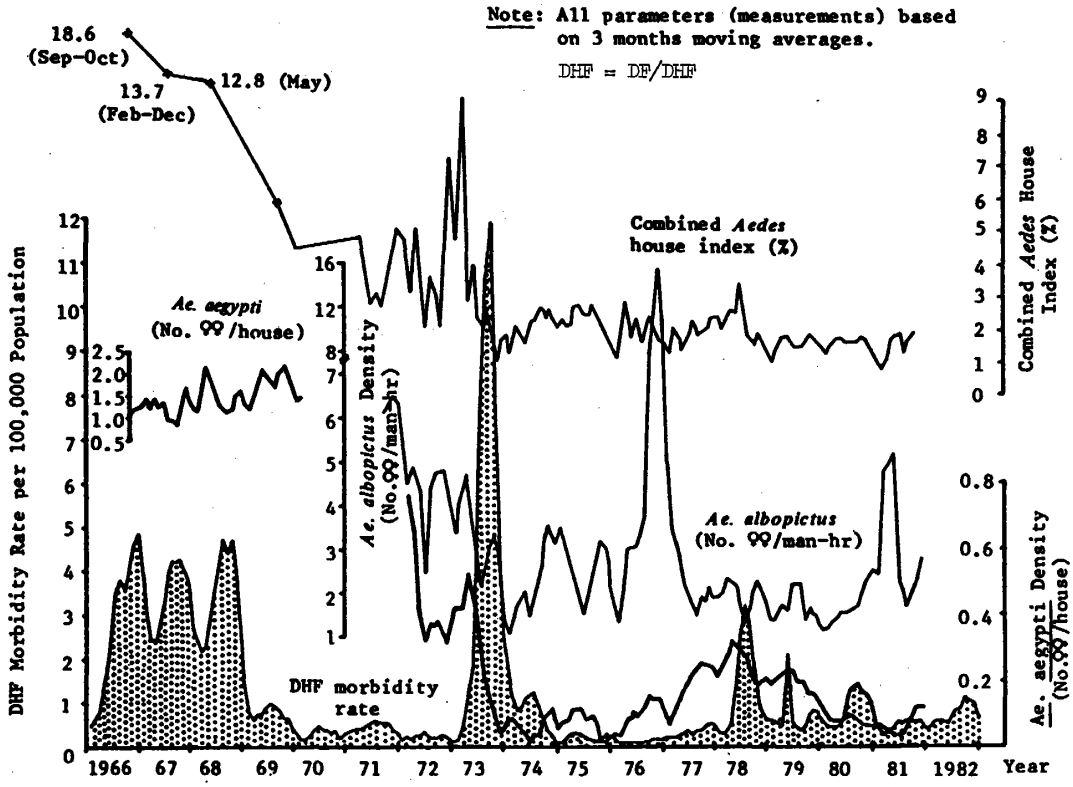

Figure 2 *Aedes* vector densities in relation to DHF morbidity rate per 100,000 population, 1966-82, Singapore.

enforcement (legislation) must be instituted to suppress mosquito breeding by the public. Thus, in 1968, the Destruction of Disease Bearing Insects Act (DDBIA) was passed to give power and teeth to enforcement actions by the inspectorate (vector control personnel). As the *Aedes* control measures, first implemented in 1966, were proceeding, it was noticeably observed that the incidence of DF/DHF began to fall sharply. However, a Keep Singapore Clean and Mosquito Free (KSCMF) campaign was carefully planned to be implemented in November 1969 in order to further reduce the density of the *Aedes* vectors. When this campaign was launched with the full backing and support of the Government as well as statutory boards and private companies, the results that followed were no less than expected. They were spectacular and dramatic. The morbidity rate of DF/DHF per 100,000 population fell from a high 42.15 in 1968 to a low of 9.25 by the end of 1969, to 3.42 in 1970 and 2.98 in 1972 . The *Aedes* house index also fell dramatically from a high 25% in highly endemic areas before the campaign to less than 5% after the campaign in the following four years. This level of achievement was maintained for the next three years, i.e. until 1973. Complacency in *Aedes* control set in as no outbreaks of DF/DHF occurred for four years. As a result, the largest DF/DHF outbreak occurred in 1973. In this outbreak it was necessary to use chemical control to quickly terminate transmisson, as children were dying in large numbers and the mosquito vector density remained high. It was after this outbreak that a fully integrated system of *Aedes* control, using source reduction, health education, law enforcement and chemical control, was developed to effectively terminate transmission of the disease and to prevent epidemics through vigilance in surveillance of both the disease and the vectors. Although two smaller outbreaks occurred in 1978 and 1986, and refinements of the four integrated measures were developed subsequent to the 1973 outbreak, these two outbreaks were quickly controlled using the same four integrated measures.

A fifth measure, that of slum clearance by the Singapore Housing and Development, Board (HDB) and urban redevelopment (which was also essentially slum clearance) by the Urban Redevelopment Authority (URA), which began in about 1960 when DF/DHF was first introduced into Singapore, contributed further and significantly to the reduction of *Aedes* breeding sources (Chan and Counsilman, 1985), for, whereas there were many slums in the 1960s, there are virtually none today. Slumhouses, it must be mentioned, had the highest *Aedes* house index while flats had the lowest (Chan *et al.*, 1971). With flats gradually replacing slumhouses, the *Aedes* density had to gradually decline (Chan and Counsilman, 1985).

Thus, the integrated measures implemented may be summarized as follows:

a) Source reduction+health education —1966-69 (Geylang Pilot Study)

b) Source reduction+health education+law enforcement —1969-72 (KSCMF Campaign)

c) Source reduction+health education+law enforcement+chemical control —1973

onwards

d) Slum clearance by HDB and URA—1960 onwards

RESULTS

Disease Incidence

In the 1960s and right up to the mid-1970s the morbidity and mortality rates per 100,000 population were high, especially in outbreak years (d. g. 1966-8, 1973) when they exceeded 40 and 50 respectively (Figure 1). From the mid-1970s onwards, these were low, not exceeding 20, even in outbreak years (1978, 1986) (Figure 1).

The most important factor in evaluating success of a control program is low or absence of the disease. Since the integrated measures had resulted in low or absence of fatal cases from 1974 onwards, they must be regarded as effective.

Mosquito Density

The absolute density of *Ae. aegypti* fluctuated between 0.8 ♀♀/house and 2.6 ♀♀/house, with a mean of about 1.5 ♀♀/house during the period 1967-1970 (Figure 2). Its density did not immediately fall following launching of the 1969 KSCMF campaign but it did eventually fall and, by 1972, its mean density was about 0.77 ♀♀/house. But this density (0.77 ♀♀/house) was later found to be able to support transmission of DF/DHF at epidemic level. It was the density occurring in April 1973, just before the largest DF/DHF outbreak occurred (Chan, 1985a). Following the control of the 1973 outbreak, the *Ae. aegypti* density fell to below its threshold density of 0.2 ♀♀/house, only to climb back to this level in 1978 (Figure 2). Thus, the integrated measures impremented had effective in suppressing the principal vector to a very low level.

Likewise, the combined *Aedes* house index (a measure of larval density) fell sharply following implementation of integrated control measures, especially source reduction, from a mean of 18.8% in 1966 to a mean of 4.5% in 1970, and subsequently to a mean not exceeding 2% after 1978 (Figure 2). Thus, again, the integrated control measures had proven highly effective in controlling the DF/DHF vectors.

DISCUSSION

Three years of intensive entomological and epidemiological studies from 1966 to 1969 provided the foundation for using an integrated system (based on an ecological approach) of controlling the *Aedes* vectors in Singapore (Chan, 1973). This system initially used only two integrated control measures, namely source reduction and health education. When the excellent results obtained could not be sustained, for lack of power to control or prevent the

public from breeding and/or harbouring mosquitoes, law enforcement was the logical consideration and outcome. Thus, the DDBIA was enacted and enforced on a small scale in 1968, and implemented on a countrywide scale following the launching of the KSCMF campaign in 1969 (Chan *et al.*, 1970; Chan, 1985a).

A fourth integrated measure, adulticiding (chemical control), was not used until the 1973 outbreak. A post-mortem of the 1973 outbreak showed these measures to be highly effective and revealed that transmission of DF/DHF at epidemic level had occurred when the combined *Aedes* house index had exceeded 5%, and that this probably corresponded with the threshold density of the vectors (Chan *et al.*, 1977). A new strategy was therefore developed to keep the combined *Aedes* house index in all built-up areas with high *Ae. aegypti* breeding down to below 5% at all times. Every area having a house index exceeding 5% would be immediately fogged to kill the adults, and cleared of breeding habitats. Thus, beginning in March 1974, a system of routine vector surveillance, year-round source reduction, health education and legislation, and fogging of premises with more than 5% house index was implemented (Chan, 1977). This system (strategy) was effective in achieving year-round control of the vectors, and was also successful in preventing two epidemics of DF/DHF which swept through the region, one in early 1976 and the other in early 1977. The results of control by this system were evident. The incidence of DF/DHF dropped from 229 cases in 1974 to 59 cases in 1975 and to a record low of 30 cases in 1976. In 1977 the incidence increased slightly, to 92 cases. In 1978 an outbreak of 384 cases with 2 deaths occurred. This was quickly controlled using the same integrated measures, thus proving again that they were highly effective (Chan, 1985a). In 1986, another outbreak of 260 cases with 1 death occurred between April 27 and September 13. This was again rapidly controlled using the same integrated measures (Goh, 1986).

A detailed discussion of the methods used for control of dengue vectors has been given in Chan (1978). Methods that are likely to be effective against especially *Ae. aegypti* breeding have also been discussed (Chan, 1985a). It leaves now only to discuss why the Singapore program had succeeded when many other programs throughout the world, even in the same region, had not succeeded. Obviously, it is not for the lack of methods or techniques in *Aedes* control that many control programs had failed. Neither is it the lack of organizational skills in many governmental programs that *Aedes* control had failed. What, then, is the trouble with so many national programs? It is my conviction after reviewing some country programs and attending a meeting on *Ae. aegypti* control in June 1987 at Parson's Island, that one or the following ingredients which ensure success in *Aedes* control, are missing in many national programs:

1) The approach to, and emphasis on, *Aedes* control should be source reduction and not chemical control (too many countries emphasise and depend on chemical control).
2) There must be a resolve and commitment to control *Aedes* vectors to a satisfactory

level, e.g. to below threshold density for transmission of DF/DHF.

3) The *Aedes* control program must be government-sponsored, and therefore well staffed and equipped under all conditions (routine and emergency) and fully supported and backed and emergency) and fully supported and backed by all authorities concerned.
4) There must be a strong leadership and good management of the *Aedes* control program.
5) There must be a multimedia health education program directed against the *Aedes* vectors, especially *aegypti*, i.e. the public must be educated and motivated to prevent and control *Aedes* breeding in their premises.
6) There must be community participation, i.e. the public must be successfully motivated to participate in preventing and controlling *Aedes* breeding in their own premises.
7) There must be law enforcement measures, i.e. legal sanctions that make the breeding and harbouring of mosquitoes in premises a crime and therefore punishable by fines and/or imprisonment.

All these ingredients were present in the classic program and model for *Ae. aegypti* control and eradication in Brazil (Soper *et al.*, 1943) and the Singapore program. Many of these had been recently re-emphasised by Halstead (1984), and by various others at the Parson's Island June 1987 meeting, in addition to my own statements made repeatedly in several publications (Chan, 1972, 1973, 1975, 1977, 1978, 1984, 1985a). The importance of thoroughness in the implemention of control measures, the need to countercheck scrupulously every control action undertaken in the field, and the keeping of detailed records of all activities carried out, cannot be over emphasized. Constant supervision of vector control personnel at all levels and assessment of the effectiveness of measures implemented, form the necessary foundation for a successful program. It must never be forgotten that among Dr. Soper's well-tested methods, the following were emphasised by Dr. Hermelino Gusmao who delivered the Fourth Annual Soper Lecture to the American Society of Tropical Medicine and Hygiene a few years ago:

1) Undivided and well-defined responsibility of each man in the field.
2) Delegation of authority matching the corresponding level of responsibility.
3) Complete and meticulous system of daily written reports of the work done by each man at every level of responsibility.
4) Careful independent checking, rechecking and counterchecking of results.
5) Forceful and unrelenting supervision.

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MANAGEMENT OF MALARIA WITH SPECIAL REFERENCE TO DRUG RESISTANCE

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Abstract: The management of acute malaria consists of chemotherapy to destroy asexual forms of malaria parasites, and amelioration of the pathophysiologic changes to restore the normal function of all organs. In choosing appropriate drug for the treatment of malaria, the sensitivity of the parasites to the drug must be considered. The regimen recommended are as follows: 1) in areas where *P. falciparum* is sensitive to 4-aminoquinolines, for the non-immunes without complications, chloroquine or amodiaquine 300 mg base t.i.d. on the first day then once daily for 2-4 days; for semi-immunes, chloroquine or amodiaquine 600 mg base single dose. 2) in 4-aminoquinoline resistant areas but sensitive to antifol/sulfa combinations, sulfadoxine or sulfalene 1,500 mg plus pyrimethamine 75 mg single dose (Fansidar or Metakelfin 3 tab.); for non-immunes without complications, quinine or quinidine 600 mg every 8 hr for 2-3 days will accelerate the clearance of fever and parasitaemia. 3) in areas with resistance to both 4-aminoquinolines and antifol/sulfa combinations, quinine or quinidine 600 mg every 8 hr for 7 days. 4) in areas with resistance to 4-aminoquinolines, antifol/sulfa combinations and quinine

4. 1 quinine 600 mg every 8 hr plus tetracycline 250 mg b. i. d. for 500 mg b. i. d. for 7 days.
4. 2 mefloquine 750-1,000 mg single dose.
4. 3 MSP (mefloquine 250 mg, sulfadoxine 500 mg+pyrimethamine 25 mg) 3 tablets single dose;

in areas with natural transmission of malaria, primaquine 30-45 mg single dose to eradicate gametocytes and interrupt transmission.

Severe malaria

If the patients is seriously ill or has over 5% parasitaemia chloroquine or quinine should be given paresterally as follows: -

chloroquine 2.5 mg/kg subcutaneously or intramuscularly every 4 hr or 3.5 mg/kg every 6 hr or continuous intravenous of 10 mg/kg in 8 hr followed by 5 mg/kg in 8 hr then 2 doses of 5 mg/kg every 12 hr to a total dose of 25 mg/kg.

quinine hydrochloride 20 mg/kg in 500 m*l* of 5% dextrose or normal saline solution intraveneous drip in 4 hr followed by 10 mg/kg every 8 hr until oral therapy can be administered. A total dose for an adult weighing 60 kg is 13,000 mg in 7 days.

Complications or pathophysiologic changes e. g. coma, convulsions, hypoglycaemia, hyperpyrexia, anaemia, dehydration, renal failure, pulmonary oedema, jaundice, electrolytes and acid-base disturbances etc. must be corrected by effective measures.

Steroids, mannitol, dextran, heparin and adrenaline are not recommended as they

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seem to do more harm than good.

Vivax malaria

Chloroquine 600 mg single dose or 300 mg twice 6 hr apart followed by primaquine 15 mg daily for 14 days.

Ovale malaria

Chloroquine 600 mg, followed by 300 mg after 6 hr then 300 mg daily for 2 days and primaquine as in vivax malaria.

Malariae malaria

Chloroquine as in ovale malaria.

The aim of treating a patient is to free him from suffering and to restore his health so that he would be able to do his work. The physician should always bear in mind that he is treating the patient not disease alone.

In malaria the patient suffers from fever and dysfunctions of the various organs e.g. the brain, the liver, the kidneys etc. These changes are more pronounced in *P. falciparum* infection with pernicious manifestation (cerebral symptoms, hypoglycaemia, jaundice, dehydration, renal failure, hyperpyrexia, etc.), therefore, in the management of falciparum malaria with severe manifestations we must take all of these into consideration.

The pathophysiologic changes or the complications and the infection must be treated simultaneously. Theatment of the infection alone would be ineffectual when the complications have already set in and it is equally ineffective to remedy the complication without dealing with the infection. Many patients have died in spite of the clearance of parasitaemia because of the failure of the organs to revive their functions back to normal. The treatment therefore consists of : -

- Specific treatment or chemotherapy to eradicate the asexual erythrocytic forms of the parasites.

- General or supportive treatment in order to correct the pathophysiologic changes and to restore the functions of the organs.

Antimalarial chemotherapy

Antimalarial drugs in the treatment of patients suffering from clinical malaria are blood schizontocides acting on asexual erythrocytic stages of malaria parasites. They include 4-aminoquinolines (chloquine, amodiaquine), quinine and quinidine, sulfonamides and sulfones, dihydrofolate reductase inhibitors (pyrimethamine), antibiotics (tetracycline) and mefloquine.

Chloroquine

Since after the World War II chloroquine has been the drug of choice in treating malaria; it is a powerful schizontocide and the action is rapid. In early sixties *Plasmodium falciparum* in Colombia, South America (Young, 1962) and in Thailand, Southeast Asia (Harinasuta *et al.*, 1962) developed resistance to this antimalarial. The resistant strains from the two foci spread in all directions; all countries in Southeast Asia, Southern Asia and South China. East Africa became affected in 1978 starting with Kenya and Tanzania. The area most serious affected in Africa is south of the Sahara, as chloroquine has lost its usefulness in combating their major illness (Campbell *et al.*, 1979). In South America all malarious countries are

affected with the exception of Argentina, Paraguay and Peru, which have practically no falciparum malaria. Since 1969, Panama, south of the canal, has been maintained to be the northern limit of distribution. The prevelence and degree of resistance of *P. falciparum* to chloroquine is greater in Southeast Asia than in South America.

At prenent, the only species of human malaria parasite that has developed resistance to chloroquine is *P. falciparum*. Thus, chloroquine is still the drug of choice in the treatment of other plasmodial infections. Vivax malaria is very sensitive to chloroquine even at a single dose of 450 mg base (Harinasuta *et al.*, 1980a). In addition, although chloroquine resistance is so widespread, it is still valuable for the treatment and control of malaria in areas such as West Africa, part of India, Indonesia and the Philippines where drug resistance does not yet exist to any significant extent. It is well tolerated, safe, inexpensive and fast acting. The dose should be adjusted for non-immune and semi-immune patients. Resistance must be suspected if the patient fails to respond to the standard dosage.

Sulfadoxine and pyrimethamine

A combination of sulfadoxine and pyrimethamine (S/P) is the first line alternative antimalarial which was introduced in the late 1960s with a satisfactory cure rate of approximately 90% (Harinasuta *et al.*, 1967). Currently, its cure rate has declined significantly in many parts of the world, e. g. Brazil, Colombia, Vietnam, Kampuchea, etc. In Thailand it has decreesed from 80-83% in 1975-76 to 50-56% in 1978-79 and to 10% in 1980-81 (Harinasuta *et al.*, 1980b). The frequency of S/P resistance is high enough to necessitate the increasing use of alternative drugs.

Quinine

Quinine has been one of the most effective drugs for the treatment of malaria since it was introduced at the beginning of the 17th century, However, it was replaced by the synthetic antimalarials, mepacrine in the 1930s and chloroquine in the late 1940s. With the increasing occurrence of resistance of some malaria parasites to many available synthetic commpounds in the early 1969s, quinine regained its value (Harinasuta *et al.*, 1965). Acute falciparum infection responds well to quinine even though the drug does not always produce a radical cure. Although the efficacy of quinine has been reduced in various parts of the tropics it has not become a worldwide problem. At times, it is difficult to distinguish between strain variation and true resistance. Quinine failure is mostly the rerult of too short a course of treatment and/or inadequate dose. Some strains of *P. falciparum* highly resistant to chloroquine show cross resistance to quinine, but the combination of tetracycline with quinine increases its cure rate from 70-80% to almost 100% (Bunnag and Harinasuta, 1986). It is now the best regimen in treating falciparum malaria in Thailand.

Quinidine

Quinidine has been found to be more potent than quinine in treating falciparum malaria. *In vitro* minimum inhibitory concentrations for quinidine were consistently two to three times lower than those for quinine (White *et al.*, 1984).

Mefloquine

Mefloquine, the most promising antimalarial in this era, has been introduced recently

with a cure rate of about 95% (Harinasuta, 1983). A few cases of primary drug resistance have been observed in Thailand (Boudreau *et al.*, 1982) and also in a patient who contracted the infection in East Africa. (Bygbjerg *et al.*, 1983) Reduced sensitivity was also found among *P. falciparum* isolates tested in Thailand and the Philippines (Smrkovski *et al.*, 1982). The risk of selecting out primary resistant strains of *P. falciparum* exists when mefloquine is used alone because such strains are known to have been present in some areas even before the drug was deployed. These observations have convinced many malariologists that it would be unwise to encourage widespread use of mefloquine alone and that a rationel combination should be developed that will delay the emergence of mefloquine resistance (Merkli *et al.*, 1980; Peters *et al.*, 1977, 1984). Combination of mefloquine with sulfadoxine and pyrimethamine (MSP) in the ratio of 10:20:1 is recommended. Unfortunately, R I and RII response of resistance to such a combination has already emerged in Indonesia and Thailand (WHO, 1984). There is also *in vitro* evidence which shows MSP has no advantage over mefloquine.

Antimalarials regimen recommended in treating acute falciparum malaria

Chemotherapy of acute falciparum malaria varies according to the status of drug resistance in the area where the infection has been acquired, as well as to the immune status of the patients. In 4-aminoquinoline sensitive areas, for non-immunes-uncomplicated cases, chloroquine or amodiaquine 300 mg base thrice should be given on the first day then once daily for 2-4 days, while a single dose of 600 mg base is adequate for the semi-immunes. In 4-aminoquinoline resistance areas but sensitive to antifol/sulfa combinations, a single dose of sulfadoxine or sulfalene 1,500 mg plus pyrimethamine 75 mg should be given. When oral therapy is not possible, a parenteral preparation is available. In non-immune, uncomplicated cases quinine 600 mg every 8 hr for 2-3 days will accelerate the clearance of fever and parasitaemia. In areas with resistance to both 4-aminoquinolines and antifol/sulfa combinations, quinine 600 mg should be given every 8 hr for 7 days. In area with resistance to 4-aminoquinolines, antifol/sulfa combinations and quinine; quinine 600 mg should be given every 8 hr together with tetracycline 250 mg q. i. d. or 500 mg b. i. d. for 7 days. In the area where natural transmission of malaria occurs, a single dose of primaquine 30-45 mg should be given to destroy the gametocytes and interrupt transmission. In case of severe manifestations or has over 5% parasitaemia, quinine, or chloroquine should be given parenterally followed by oral therapy as above as soon as the patient can take it. The drug must be given slowly and well diluted. A loading dose is recommended: - quinine dihydrochloride 20 mg/kg body weight in 500 m*l* of 5% dextrose intravenous infusion over a period of 4 hr followed by 10 mg/kg body weight every 8 hr to a total dose of 13,200 mg in 7 days for an adult weighing 60 kg (White *et al.*, 1983a). However, an equivalent dose of quinidine base can be used instead of quinine. Chloroquine can be given parenterally. Subcutaneous or intramuscular injection at a dosage of 2.5 mg base/kg every 4 hr or 3.5 mg base/kg every 6 hr or continuous intravenous infusion of 5 mg base every 6 hr or with an initial dose of 10 mg base over 8 hr followed by 5 mg base every 8 hr is recommended. The total dese is 25 mg base/kg (White *et al.*, 1987).

Mefloquine is the most effective antimalarial with only few cases of resistance reported so far : - a single dose of 750-1,000 mg mefloquine (Harinasuta *et al.*, 1983) or of 3 tablets

of MSP (1 tablet contains 250 mg mefloquine, 500 mg sulfadoxine and 25 mg pyrimethamine) is recommended (Harinasuta *et al.*, 1987).

Combination of cinchona alkaloids (quinine, quinidine and cinchonine) proves effective in the treatment of resistance falciparum malaria (Bunnag, D., personal communication) and is under investigation at the Bangkok Hospital for Tropical Diseases. In China Qinghaosu was found to be affective in treating chloroquine resistance falciparum malaria.

MANAGEMENT OF COMPLICATIONS OR SUPPORTIVE MEASURES
(Harinasuta *et al.*, 1985)

1. Hyperpyrexia

High fever may lead to convulsions in children and some adults and to fetal distress in pregnant women. Tepid sponging, fanning, exposure to cool air, cooling blanket and antipyretic drugs usually produce a good effect. Rectal temperature should be monitored regularly and should not be allowed to exceed 39.0°C.

2. Convulsions

Convulsions are a common manifestation, found in one third of cerebral malaria patients. The level of consciousness may deteriorate after a convulsion. It often leads to aspirasion pneumonia, asphyxia and death. Anticonvulsant such as diazepam (10-20 mg or 0.2-0.4 mg/kg in children) should be given intravenously and should be repeated every 5-15 min until the convulsions are controlled. Good nursing care will minimize other complications which usually follow convulsions.

3. Hypoglycaemia

Hypoglyhaemia has been observed frequently in patients with severe falciparum malaria and appears to be most common in pregnant women suffering from severe malaria. It may contribute to or cause the neurological disturbances attributed to cerebral malaria. Hypoglycaemia may be present during early convalescence, and can occur even while the patients is receiving 5% dextrose infusion. Possible explanations of this condition include glucose consumption by the malaria parasites and stimulation of insulin secretion by quinine (White *et al.*, 1983b).

The possibility of hypoglycaemia should always be considered in any patient with the appropriate symptoms (anxiety, breathlessness, sweating, convulsion, impaired consciousness, or severe neurological deficits) (Harinasuta *et al.*, 1982). Blood examination for glucose should be performed to support the diagnosis. One simple method is the dextrostix test; the result can be obtained in 1 min. Prompt administration of intravenous glucose is essential and life-saving in these cases. The infusion of 50% glucose 50 m*l* or injection of 1 mg glucagon intramuscularly every 30 min should be sufficient for most of the patients. If excessive concentrated glucose is given, fluid overload and pulmonary oedema may result. Thus, glucose should be administered with care. Hypoglycaemia may recur despite repeated doses of intravenous glucose.

4. Severe Anaemia and Haemoglobinuria

Severe progressive anaemia in falciparum malaria causing reduced blood viscosity and oxygen carriage with increased cardiac output may pose a major clinical proglem. Anaemia is caused by rupture of parasitised red blood cells as well as nonparasitised red blood cells. Bone marrow dysfunction and increased phagocytosis by the reticuloendothelial system play some role in later stages (Weatherell and Abdalla, 1982). Haemolysis is related both to

malaria and, separately, to oxidant antimalarial drugs. Deficiency in G-6-PD and other red cell enzymes together with abnormal haemoglobins may increase susceptibility to oxidant-induced haemolysis. With antimalarials such as primaquine, haemolysis may be aggravated. When haemolysis occurs haemoglobin appears in the plasma and is excreted in the urine. In severe cases this is usually preceeded by a state of shock with a sudden drop in the temperature. In mind cases the haemolytic crisis abates and the urine becomes clear after a few hours or a day. The volume and character of the urine and the fluid chart recording the intake and output suggest the prognosis and indicate the treatment. Each urine specimen should be kept in a separate bottle, the excretion time labelled, the specific gravity measured and the colour observed. In severe cases, the skin appears very pale. Haematocrit should be measured twice daily. Sudden drop of falling to below 20% require fresh blood transfusion or packed red cells. Another dangerous condition is renal insufficiency due to the mechanical blockage of tubules by haemoglobin pigments precipitated by acid urine. Alkalinization of urine and parenteral administration of saline and glucose will help the excretion but excessive transfusion and overloading of the circulation with intravenous infusions must be avoided.

5. Dehydration

Severe malaria is often associated with dehydration as a result of fever, profound sweating and inadequate oral fluid intake. Dryness of the mouth and tongue, loss of skin turgor, ocular hypotention, postural hypotension, lowering of jugular venous or central venous pressure indicate dehydration. A fluid chart should be posted and fluids given by mouth as soon as possible. In cases when intravenous fluid is required the daily amount of an adult patient of average weight is about 2-3 litres starting with 500-1,000 m*l* of 5% glucose or NSS within 2-4 hr which may be repeated if necessary until the CVP returns to normal (at 5 cm water) and urine output more than 30 m*l* per hr. Be sure not to prescribe 2,000-3,000 m*l* intravenous drip continuously at a single order; 500-1,000 m*l* should be given at a time and evaluate patients condition at regular intervals. An excess of intravenous infusion often precipitates pulmonary oedema.

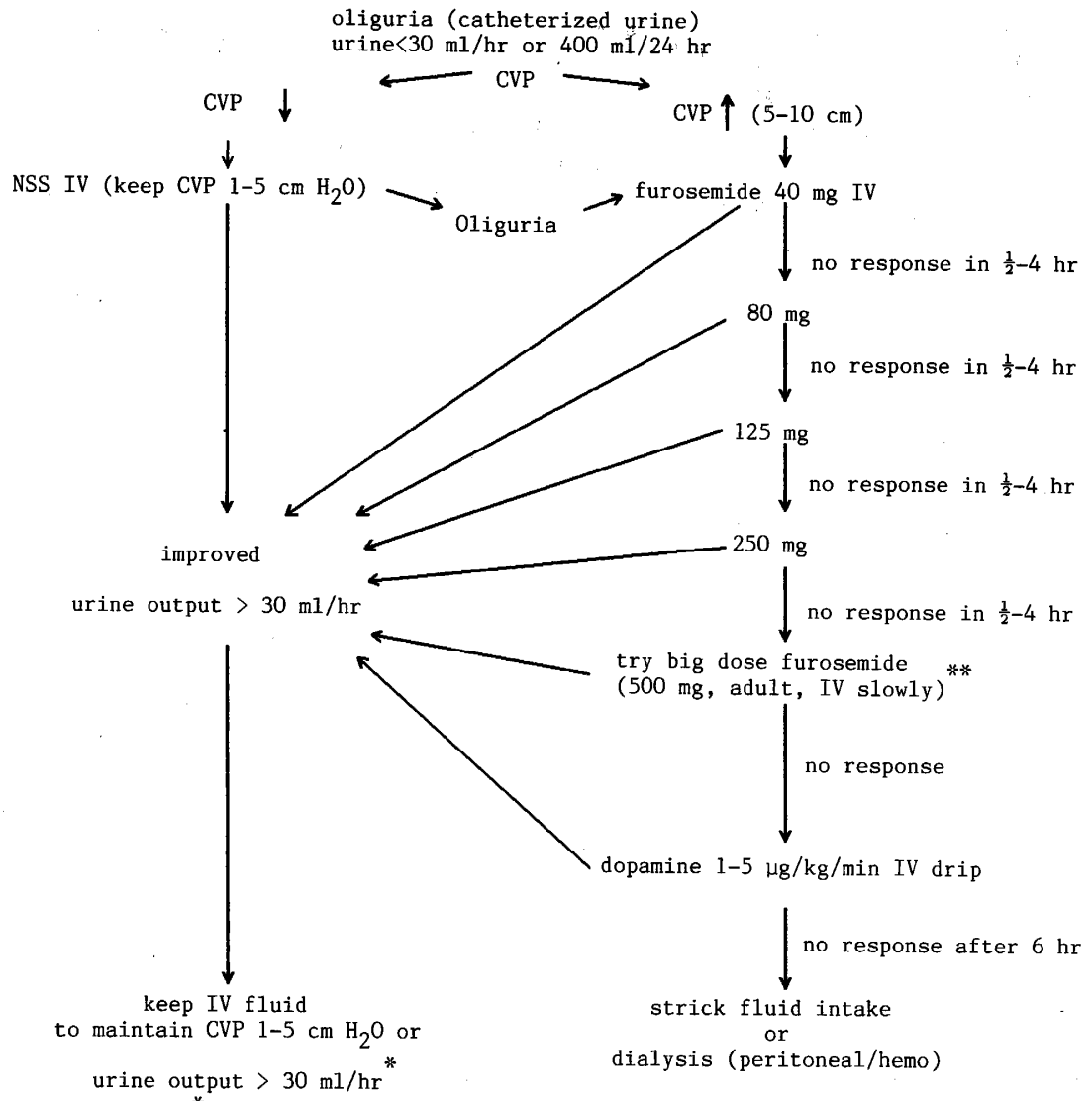
6. Renal Failure

Renal impairment is common in severe malaria mostly due to dehydration and early correction of electrolyte and water deficits is usually followed by improvement. Dehydrated patients with low urine output (less than 30 m*l*/hr) should be managed with careful fluid replacement until the central venous pressure (CVP) is up to 5 cm water or until the jugular venous pulse becomes visible when the patient at 45 degree. Delayed correction of dehydration may be followed by persistent oliguria. Urine output is best monitored by inserting a urethral catheter under sterile conditions, and recording of urine volumes hourly.

If the urine output remains low (less than 30 m*l*/hr) after rehydration, a potent loop diuretic such as furosemide should be given in an initial dose of 40 mg intravenously (see diagram). If there is no response after 30 min and the patient has pulmonary oedema, the dose should be doubled every 30 min until a total of one gram has been given. In not critically ill cases the interval between each dose may be lengthened to 1-4 hr. Oliguria which is unresponsive to one gram of furosemide usually reflects established acute renal failure. In occasional cases dopamine given as a carefully controlled intravenous infusion of 2.5-5.0 mcg/kg/min may increase the urine output. If dopamine fails after 6 hr the infusion should be stopped.

In oliguric patients it is essential to maintain fluid balance but when the blood urea

Management of Oliguria



*Until the patient improved and can take oral fluid.

**A total dose of furosemide must not exceed 1 g; it is toxic and may cause deafness.

nitrogen exceeds 60 mg/d*l*; when the serum creatinine is greater than 6 mg/d*l*; when the serum potassium is greater than 5.5 mEq/*l* or there is tall, peaked T waves on an ECG tracing; or there is metabolic acidosis or pulmonary oedema then dialysis is needed urgently and this may have to be continued for many days or even weeks because of persistent oliguria. Peritoneal dialysis is often adequate to control uraemia in renal failure due to falciparum malaria but peritonitis is a frequent complication necessitating antibiotic treatment.

As renal function improves, a diuretic phase may ensue with daily urine volumes exceeding 3 litres. This loss must be replaced and if patient is unable to drink to restore this large deficit, intravenous fluids will be necessary.

In the first three days quinine dosage should not be reduced in renal failure, but in case

of no improvement or deterioration the dose of quinine should then be reduced after the third day by one half.

7. Pulmonary Oedema

One of the most serious complication in severe malaria is pulmonary oedema. It occurs in 10% of cerebral malaria patients and the outcome is usually fatal. Pregnant and post partum women, patients with high parasitaemia and patients in renal failure are among the high risk groups. Most of these cases are precipitated by overhydration. However pulmonary oedema resembling adult respiratory distress syndrome may develop in cases not associated with overhydration. This may be due to increases in pulmonary capillary permeability. The treatment of this condition is seldom successful, therefore careful fluid replacement in order to avoid overload is essential. The daily intake of normal saline solution should not exceed 1 *l* (150 mEq sodium) and even less when sodium in any other from is prescribed such as penicillin G sodium or blood transfution. Five per cent dextrose in water should be given if more fluid is required.

Patient with pulmonary oedema should be nursed propped up, given oxygen to breathe, and their fluid balance should be monitored by central venous pressure. Diuretics as mentioned earlier should be tried. Sodium nitroprusside or dopamine should be given carefully. Venesection and the use of mechanical ventilation with positive end expiratory pressure should be considered. Pulmonary oedema is often confused with bronchitis and pneumonitis; chest X-ray is helpful for differential diagnosis.

8. Jaundice

Jaundice, as a result of red blood cells haemolysis and cholestasis is common in severe falciparum malaria but hepatic failure has not yet been observed. Generally symptomatic treatment is all that is required; uneventful recovery takes 1 to 3 weeks.

9. Acidosis

Acidosis occasionally follows hyperparasitaemia, shock, renal failure and hypoglycaemia. It has a fatal outcome. Tissue hypoxia and anaerobic metabolism are belived to be the factors. High serum lactate has been detected in these cases. The treatment of this condition is to correct the associated factors.

10. Bleeding Disorders

Low platelet count (less than 80,000/mm^3) is not an uncommon finding in falciparum malaria but spontaneous bleeding and coagulopathy (DIC) are rarely seen (Looareesuwan *et al.*, 1983a). Therefore, the use of heparin is either unhelpful or positively dangerous. Systemic bleeing is a grave prognosis. Fresh blood transfusion (in large amount) is essential.

11. Hyperparasitaemia

Immediate treatment must not be delayed in patients with parasitaemia greater than 5%. The clinical state may rapidly deteriorate. Cerebral, renal, hepatic and pulmonary involvement may follow within a few hours. Retinal haemorrhage, a prognostic sign indicating poor outcome in cerebral malaria is not uncommon (Looareesuwan *et al.*, 1983a). Blood transfusion should be given to replace the expected rapid drop in haematocrit. In a few cases with 30% or more parasitaeria, exchange transfusion has proved helpful.

DRUGS NOT RECOMMENDED

1. Steroids eg. prednisolone, dexamethasone

Results from a carefully designed study of 100 cases of cerebral malaria carried out at Pra Pok Klao Hospital, Chantaburi province, Thailand proved that dexamethasone did not reduce the mortality rate but prolonged coma and increased the incidence of complications such as pneumonia and gastrointestinal bleeding. Therefore steroids are contraisdicated in cerebral malaria (Warrell *et al.*, 1982).

2. Osmotic diuretics for reducing cerebral oedema eg. urea and mannitol

It has been shown that significant cerebral oedema does not occur in cerebral malaria (Looareesuwas *et al.*, 1983b) thus osmotic diuretics are not necessary.

3. Plasma volume expanders eg. dextran

Plasma volume expanders are unnecessary as blood viscosity in cerebral malaria is already reduced as a result of the low haematocrit.

4. Heparin

Heparin had been used on the misunderstanding that disseminated intravascular coagulation (DIC) was the pathogenesis of cerebral malaria. This complication is fortunately very rare. Heparin produced serious side effects with further bleeding which may be fatal. Therefore it should not be used.

5. Adrenaline

Adrenaline was thought to expel the parasitised red cells from the capillaries. There is no evidence to support this and adrenaline seems likely to do more harm than good.

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会　場：横浜市技能文化会館
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—国際医療協力部の活動を通じて—

我妻　堯
（国立病院医療センター・国際医療協力部）

最近の日本政府の対外援助は対GNP比で先進国の中でも上位にはいっており，医療協力に関しては，多数の発展途上国に病院を建設したり，最新の医療機器を寄付するなど，かなりの程度まで援助が行われている。しかし技術協力の面では充分な数の医師を長期間海外に派遣することが困難で，発展途上国からの要請に充分対応することが出来ない。最新設備の病院の管理運営方法，或いは超音波診断装置，CTスキャン，内視鏡など最新の医療機器を応用した診断技術などについて，発展途上国の医師にノウハウを指導教育する人的交流の面では，未だに他の先進国に遅れをとっている。長期間，派遣可能な医師を得難い理由については，多くの因子が考えられるが，一般に日本の病院は人手不足で他の国で技術指導が出来る程に優秀な医師が1－2年の長期間に亙って病院を離れることについては，病院の了解が得られないことが多い。熱意のある医師が自分の希望で海外に長期出張しようとすれば，病院を退職させられることになり，任務を終って帰国した際の再就職が困難になる。政府はこのような状態を改善するひとつの手段として，昭和61年10月1日から国立病院医療センターに国際医療協力部を新設した。当面は協力部の中に派遣協力課がひとつで，スタッフも，部長，課長各1名，課員は小児科1名，内科2名，外科3名という小さな所帯である。設立以降の活動は主として国際協力事業団（JICA）による途上国援助に専門家集団として協力する方式で行っている。即ち，南米のボリビア，パプア・ニューギニア，フィリピン，象牙海岸等に対する無償資金援助計画の基本設計調査団にそれぞれ課員を派遣した。技術協力の面では，ボリビア国サンタクルス市に，昨年わが国が建設した総合病院に技術協力を行うことを決定し既に専門家を派遣した。協力部としては当面，この病院に対する技術協力に，相当のエネルギーを費やすことが予想される。

今後は海外派遣ばかりでなく，途上国からの研修員を病院，研究部に受けいれて技術指導を行うことも計画している。これらの国際協力計画に参加した経験を通じて，われわれが学び得た医療協力の実状や問題点について，批判，反省を加え今後の抱負について述べてみたい。

2　私達の医動物学研究国際交流

松村　武男　（神戸大・医・医動物）

本講座の研究と教育活動は，1975年9月から発足した。すでに，JICA後援による本学部を中心とした外国人受託研修コース（第1回から第5回まで「熱学疫学」，第6回から第10回まで「医科学技術」研修コースと改称）が，1973年より開始され，46名の開発途上国からの研修員を迎え，研究指導を行った。本講座の活動の一端として，私共は，全面的にこの研修コースを支援・協力し，受皿である本学部内教官で組織されているインドネシア委員会にも積極的に参加してきた。本講座で受け入れた個別研修員は，46名中3名に過ぎないが，個別研修の前に行われる集団研修の講座・実習などで全研修員の指導に参加した。

さて，1964年インドネシアとの医学交流に端を発して積み重ねられてきた本学部での国際交流の機運は，前述のJICA研修コースの外に，その後文部省学術審議会の建議（1977年9月）に基づいて神戸大学医学部附属医学研究国際交流センター（ICMRと略：岩井誠三センター長）設置として結実し，医学研究国際交流の理念が具体的に機構化されてきているのが現状である。日本の学術振興会の指導の下に3つの柱に基づいて活動がなされている。

(1)若年研究者の招へい，(2)共同研究の実施，(3)特定のテーマについての討議会の開催である。対

象国は主として，インドネシア・フィリピン・タイ・シンガポールである。本学部では，1979年4月より1986年3月の7年間に延べ人数185名の研究者招へい，本学部を中心とした研究者などの派遣280名，共同研究プロジェクト10課題，討議会7回などが持たれた。本講座でも，延べ人数25名以上の招へい研究者と，延べ人数5名以上の派遣研究者があった。

このICMRの活動と併行して開始されたのが，論文博士制度による対象国研究者の研究指導と育成である。本活動は1979年よりICMRが窓口であったが，1985年4月より直接日本学術振興会が担当し，対象が全国医学研究機関に広げられた。本講座ではこの制度を通して，インドネシアの2名の研究者が，すでに学位を取得しており，現在母国にて活躍中である。これまでの本講座での医学研究国際交流の経過と諸問題を述べると共に，今後の展望と期待について考察を試みたい。

3　プライマリ・ヘルス・ケア（PHC）協力 —過去，現在，未来の状況—

小野寺伸夫　　（国立公衆衛生院）

近年，国際社会においてPHCの発展は，世界の人々の生命と健康を守る重要な保健戦略の1つとして登場している。PHCは生命と健康を巡る様々な課題に関し，地域において，より包括された機能でシステム化されることが期待されている。

1978年，アルマ・アタにおいてPHCの国際会議が開催され，紀元2000年までに世界のすべての人々が健康を享受しうる水準を達成する社会目標を達成しうる鍵であるとされた。この基本戦略は，1951年の世界保健総会において，保健計画の課題が取り上げられ，1966年には発展途上国における国家保健計画が論じられた。そのため，1968年から1969年にかけて，保健計画の課題として地域保健サービスの組織化に関する国際的研究がなされ，1973年には保健デリバリー・システムが論じられた。これらの経過をふまえ，1975年，第55回WHO執行理事会はPHCについての決議がなされた。PHCについてのわが国の対応であるが，1978年3月，アルマ・アタ宣言に先立ち，第5回SEAMIC（東南アジア医療情報センター）のワーク・ショップに際し関係各国でPHCを積極的に推進する企画のあることが確認された。さらに，1985年4月「21世紀への保健医療とマンパワー開発」会議でPHCと社会的公正を目ざすための施策等に関する東京宣言がなされた。

PHCに関する国際協力は協力の基本理念をふまえ，わが国との2国間技術協力がタイおよびユーゴスラビアで展開されている。タイについてはASEAN, Training Center for PHC Developmentがマヒドール大学および公衆衛生省の協力により進められ，ユーゴスラビアについては，ザグレブ大学A. Stamper School of Public HealthにおいてThe Continuing Education for PHCが進められている。さらに国際研修として多年にわたって進められている国家衛生行政セミナー（National Health Administration Seminar）の意義は今後とも大である。

講　演

1　輸入感染症の実態と対応

安方　魁人（厚生省・成田空港検疫所）

近年，国際交通の発展は航空機の大型化，スピード化に加え各航空会社のサービス競争より利用者も年々大幅に増加し，まことに目覚しいものがある。その半面，外国で各種の伝染病に感染してわが国に入国する患者も年を追って増加している。当検疫所も検疫伝染病をはじめ国際伝染病の国内侵入の防止，輸入食品等の安全衛生の確保，空港区域（航空機を含む）の衛生管理の保持等に日夜努力している。

今回は対人検疫を中心として，その実態を以下の通り説明する。

1　海外旅行とその背景
　① 海外旅行の実態
　　年齢別，行先別，目的別の実態から
　② 検疫行政は，空港検疫は，成田及び羽田では
　③ 世界と日本
2　対人検疫のモデル
　① 通常の検疫
　② 不明疾患に対する検疫
　　特に国際伝染病に対して
3　実績
　別添資料を中心として
4　新しい時代に対応して

2　ウイルス・リケッチア症の輸入と流行

北村　敬，小松　俊彦
（予研・腸内ウイルス）

わが国に常在せず，国外から導入されて流行を起こす疾患として，厚生省は国際伝染病と輸入感染症を分けている。前者は，Lassa 熱，Marburg 病，Ebola 出血熱など，外国で人獣共通伝染病として常在し，わが国などに侵入した場合，ヒトからヒトへの流行を起こし得るものを指し，何れもクラス—4 病原体に指定されて，厚生省の国際伝染病予防対策要綱（案）に基づいて，患者は東京都立荏原病院の特殊感染症病棟に収容され，ウイルス学的検査は，国立予防衛生研究所高度安全実験室で行われる。

輸入感染症は，過去に於いて日本にも常在したが，公衆衛生対策が成功して，非常在となり，外国からの侵入により再流行の可能性の残っているものをいい，ウイルス病としては急性灰白髄炎（ポリオ）がその代表である。わが国では，生ワクチンによる予防接種が成功して，1960年代後半以後，ポリオ流行は存在しないが，年間数例見られるワクチン関連麻痺症例の他に，数年に 1 例の頻度で野生型ポリオウイルスによる麻痺例が発生する。これに関連して，成田空港の到着便のトイレ汚水および入国者の検便材料から，野生型ポリオウイルスを含めた多くのエンテロウイルスが分離され，健康ウイルス保有者を介してのウイルス持ち込みが疑われている。

主として，外国に常在するウイルスがわが国にも常在している事が発見されて，その疫学的位置づけが決まっていない例もある。わが国で発生した実験用ラット関連腎症候性出血熱（HFRS）は，中国や韓国で，1967年以後，出現した都市型，軽症型 HFRS ウイルスに依って起こったものであり，また，東京湾を含めた多くの港湾区域でドブネズミから病原性のない HFRS 関連ウイルスが分離され，一定の率でヒトに感染している事も証明されている。

同じような状況がリケッチア症でも見られている。1984年，徳島県で発生した熱性疾患は，ロッキー山紅斑熱リケッチアに近縁のリケッチアに依って起こったものであり，新しい紅斑熱リケッチアの発見か，病原体の輸入侵入に依るものであるか，興味を持たれている。

ヒト免疫不全ウイルス（HIV）の感染で起こる AIDS は完全に外来性感染症で，わが国で発症している AIDS 患者および HIV 感染者は，外国より輸入した血液または血液製剤の投与，または間

接，直接に，外人と連なる性的接触に依って感染したものである。特にわが国では，国外での異性間接触に依る感染者の比率が増大しつつある。

3 High Risk Group における *Chlamydia trachomatis* の検出について

和田　明，斎藤　充司（川崎市衛研）

川崎市内の繁華街近くの産婦人科医院と同市内住宅地区にある産婦人科医院を訪れたソープ・ランド嬢（98例），会社員（34例），主婦（100例），計232名の女性の子宮頸管スメアについて，免疫螢光体抗体直接法による *Chlamydia trachomatis*（*C. trachomatis*）の疫学調査を行った。

職業別による *C. trachomatis* 陽性率はソープランド嬢（31.6%），会社員（5.9%），主婦（4.0%）であった。ソープランド嬢の住所別陽性率は東京（42.9%），川崎（29.4%），横浜（13.8%）であった。陽性率を年齢別にみると，加齢に従って減少傾向がみられ，20—29歳，30—39歳で，それぞれ66.7%，31.3%，30.0%であった。

主婦については，繁華街近くの居住者は7.1%，住宅地区居住者では2.8%と，前者は後者の約2.5倍高い値を示した。

C. trachomatis 感染症は非淋菌性尿道炎，子宮外妊娠，不妊症，それに垂直感染による新生児の結膜炎および肺炎等との関連性が知られている。ピルの普及によって今後，性の解放が *C. trachomatis* を含むSTDの増加をもたらすことが予想されるため，本症の予防対策が必要と考える。

4 クラミジア感染症の臨床

長田　尚夫
（聖マリアンナ医大・泌尿器科）

クラミジア感染症が話題となったのは，世界的なSTDの流行のためである。男子尿道炎の原因微生物として *C. trachomatis* が注目されている。以下，聖マリアンナ医大病院泌尿器科を受診した非淋菌性尿道炎患者について，*C. trachomatis* 感染症の臨床的研究成績を述べる。

(1) 男子尿道炎は，淋菌性尿道炎よりも非淋菌性尿道炎の方が多く，1：4の割合であった。*C. trachomatis* 性尿道炎は男子尿道炎の36.1%を占めていた。そして淋菌性尿道炎の17.5%に *C. trachomatis* 感染が合併していた。

(2) *C. trachomatis* 性尿道炎は淋菌性尿道炎にくらべ，症状が軽微で，分泌物排出も軽度のものが多く，尿道スミアや初尿の白血球数も少ないものが多いが，両者は症状だけでは区別できない。

(3) *C. trachomatis* 感染症の診断は，細胞培養法に代って，螢光抗体法を用いた直接塗抹法（Micro Trak 法）と酵素免疫法（Chlamydiazyme）が簡単で迅速なルーチン検査法として，臨床応用が可能である。

(4) 泌尿器性器系の *C. trachomatis* 感染症は，テトラサイクリン系薬剤の2週間投与がすすめられた。その他にもマクロライド系薬剤，新ピリドンカルボン酸系薬剤のなかにも有効なものがあり，完全な治療が可能である。

(5) *C. trachomatis* 性子宮頸管炎は自覚症状が乏しいため，男性に *C. trachomatis* 感染症が証明された場合には，女性パートナーが無症状であっても，男女カップルとして同時治療を行うことが必要である。

5 腎症候性出血熱　ラット型ウイルスの疫学

森田　千春　（予研・ウイルス）

腎症候性出血（流行性出血熱）はユーラシア大陸に広く分布する出血熱であり，腎障害を伴うことを特徴としている。原因ウイルスはブニヤウイルス科に属しているが，他のブニヤウイルス科のウイルスが節足動物をベクターとしているのに対し，本ウイルスについては現在まで節足動物のベクターは知られていない。種々のネズミより，直接ヒトに感染する。現在本ウイルスは血清学的に4つの型に分類される。この血清型は，その由来動物と密接に関係している。すなわち極東のアポデムスに由来するウイルス，ヨーロッパのヤチネズミに由来するウイルス，および北米で発見されたハタネズミに由来するウイルスはその地域のネズミに限定されており，世界的に分布を拡げてゆくものとは考えられない。しかしながらラット型のウイルスはその分布は広く，現在までヒトの疾病の報告されていない北米，南米，東南アジア，

エジプト等からもネズミの陽性例が報告されている。

ラット型ウイルスによるヒトの疾病は，疫学的に都市型と実験室型に分類される。都市型の最初の発生は1960年—1970年にかけて大阪梅田地区において発生したもので，10年間に100人以上の患者が報告されている。汚染源となったネズミの種類は同定されていないが，疫学的にはドブネズミと考えられている。同様の発生がその後，中国，韓国より報告されている。実験室型は1970年以後，日本の関西地域を中心に発生したものと同様の発生が，ヨーロッパからも報告されている。

我々は1982年に札幌の流行例より分離したウイルスを用い，東京湾埋立地のドブネズミの調査を行ったところ，27.9%が抗体を保有することを見出した。また，この埋立地の従業員の4.5%が抗体を保有し，一般住民の0.9%と比較し高いことが証明されたが，発病者は認められなかった。最近，我々は大阪での流行が続いていた1963年に，東京湾で採取されたドブネズミの血清50例中8例の抗体陽性であることを証明した。このことは東京においても，大阪と同じ時期に本ウイルスの侵入のあったことを示している。東京湾岸の3地点において長期間にわたり抗体調査を行うと，ネズミの生息密度とは無関係に抗体陽性率に差が認められ，ネズミ間におけるウイルスの伝播様式を規制している因子が，複雑であることが予想される。

6　ウイルス性出血熱—特にラッサ熱の現状

倉田　毅

（予研・病理）

ラッサ熱は，西アフリカ一帯の風土病的疾患であり，ウイルス性出血熱の代表的なものである。1969年に極めて劇的にナイジェリアのラッサ村に登場した。フラーの“熱病 Fever”（1974）は，“みえない恐怖”を世界中に広めるのに貢献した。1970年代前半での院内感染その他による報告の合計は，118名の発症中48名死亡（致死率40.7%）というものであった。1975年に自然界の宿主がマシトミスであり，ラッサウイルスがアレナウイルス科に属することも続けて判明した。1976年以降は，米国防疫センターが，シェラレオーネに実験室をおき，臨床，疫学，マストミスの実験，治療等の研究を系統的に行っており，その中で，ラッサ熱の実態が明らかにされてきている。

ラッサウイルスは表面に不規則突起を有する多形性を示し，内に砂状顆粒がみられる。1本鎖RNAをもち，ビリオンの径は110～130 nmである。媒介動物は *Mastomys natalensis* で，ウイルスは全臓器から検出されるがマストミスは症状を示さない。ヒトへの感染は創傷から，あるいはマストミスの糞尿に汚染された食物から経口的に，また患者の吐物，血液，排泄物との接触により成立する。過去10年間に，シェラレオーネの病室，実験室で重症患者に接した医師，看護婦，技師等は100人以上にのぼるが感染者は全く出ていない。マスクとゴム手袋で防止できている。患者との濃厚接触（性交等）による二次，三次感染例はある。

ラッサ熱は現在西アフリカ一帯に存在し，毎年の感染者は20—30万人と推定されている。各村落での抗体検査の結果からみて，大部分は不顕性感染である。死亡率は，発症して病院を訪れた中・重症例中の約17%で，全感染者の1～2%以内である。マストミスは患者発生地域の家に住みついており，生活様式が変らぬ限り，患者は0にはならない。

臨床的には，発熱，咽頭痛，筋肉痛等のインフルエンザ様症状ではじまる。重症化すると皮膚点状出血，消化管出血，胸膜炎，心のう炎がみられる。肝障害は軽度みられ，GOT値と血中ウイルス量値は予後判定推重要である。迅速診断法は免疫螢光法しかない。補体結合反応の安定した陽性値は発症1カ月以上しないと得られない。抗原検索は眼結膜で，ウイルス分離は尿，血液で行う。治療法として，リバビリンと回復期患者血清の併用が有効とされている。

7　ウイルス肝炎：輸入および流行の現状

飯野　四郎　　（東大・医・一内科）

肝炎ウイルスとしてはA型肝炎ウイルス，B型肝炎ウイルス，デルタ肝炎ウイルス，非A非B型（流行型・非流行型）肝炎ウイルスが知られているが，いずれのウイルスにも海外で感染し，肝炎を発症する可能性がある。それぞれの肝炎につい

て疫学的な面から現状を述べたい。

1．経口感染型肝炎

散発性あるいは流行性（集団発生）にみられる。一過性感染のみで，慢性化はない。

(1) A型肝炎　北米・ヨーロッパ（地中海沿岸を除く）・日本を除いた世界各国はウイルスが常在していると考えられる。日本人では40歳以上では抗体保有率が高いが，30歳以下では低率である。抗体非保有者では常に感染の危険性がある。免疫グロブリンにより一時的に予防が可能である。

(2) 流行型非A非B型肝炎　ビルマ・インドから中東を経て，アフリカまで，この肝炎の流行が知られている（水系感染による集団発生）。妊婦での死亡率が高い。日本での存在は知られていない。ウイルスが同定されていないので，詳細は不明である。

2．非経口感染型肝炎

血液を介して感染するもので，輸血および性行為に関連した感染が重要である。慢性化・キャリア化が問題となる。

(1) B型肝炎　感染源は血中ウイルス量の多い，HBe抗原陽性のHBs抗原キャリアである。キャリア率は，約6％とアジア・アフリカ諸国で多い。感染は医療行為を除けば性行為に関連して起こる。日本人における抗体保有率は，約20％である。ワクチンにより予防が可能である。

(2) デルタ肝炎　B型肝炎ウイルスの存在下でのみ感染が成立する。地中海沿岸から中東に多い。日本人では稀であるが，日本人のHBs抗原キャリアが流行地で感染する可能性がある。

(3) 非A非B型肝炎　ウイルスが同定されておらず詳細は不明である。輸血後肝炎の主体をなす。慢性化しやすい。ヨーロッパでは少ないと考えられる。

8 AIDS

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AIDSは，1981年アメリカで発見された，人類にとって全く新しい疾患である。そして1983年，病原ウイルスであるHIV（Human lmmunodeficiency Virus）が見出されている。

AIDSは急速にアメリカ，ヨーロッパに拡がった。やがて，アフリカに多数の患者がいることが分った。健康献血者におけるHIV抗体の陽性率は，中央アフリカでは5～8％，西アフリカでは1％である。

これよりアフリカ全体には2,500万人のキャリアがおり，50万人のAIDS患者がいる可能性がある。

アフリカのAIDSは，男女同数であり，主として異性間性交によって感染するなど，欧米のAIDSとは様相が異なっている。

更に，AIDSの多発地帯にいるサルより，HIVと類似したサルAIDSウイルス（SIV）が見出された。そして，ヒトのHIVの起源は，サルのSIVであるという見解が有力になっている。

東南アジア地域では，日本を含めてAIDSの蔓延は著しくない。これは，日本と同様にこの地域では，東洋的な道徳が支配していることによると考える。

日本では，1985年3月，始めてＡＩＤＳの第1例が報告され，1987年6月現在43例に達した。その他に臨床症接を伴わないＨＩＶ感染者（いわゆるキャリア）が255人報告されている。

ＨＩＶはヘルパーＴリンパ球と強い親和性があり，したがって感染により細胞性免疫を中心とする免疫不全を起こす。その結果，日和見感染や悪性腫瘍が発生し，死に至る日和見感染として，日本では*Pneumocystis carinii*肺炎，*Candida albicans*症が多く見られている。

最近，アフリカのAIDS患者から，SIVに近いHIV－2が分離された。すでにヨーロッパ，アメリカにもHIV－2によるAIDSの患者が見付かっている。こうして，AIDSの原因は決して1種のウイルスではないことが解明されつつある。

AIDS予防の目的で，ワクチンの開発が急がれている。最近，フランスのDaniel Zaguryはアフリカにおいて，ワクチニアを使用して作成したワクチンを，自身を含めて数人に接種し，中和抗体の産生を認めた。また副作用はなかった。

9 後天性免疫不全症候群(AIDS)の剖検例に見られた*Pneumocystis carinii*の全身播種

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岡田　三徳，中村　早人，北岡　利雄
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（京都府医大・一病理）

Pneumocystis carinii (Pc)は，免疫抑制下に於いて，肺炎を引き起こしてくる日和見感染の1病原体であるが，稀に肺以外にも感染病巣の見られた症例や全身に播種した例が報告されている。今回我々は血友病Bを基礎疾患に持ち，第IX因子製剤の投与を受けていた患者で，TBLBにてPc肺炎が証明され，HIV抗体陽性でAIDSと診断された症例の剖検病理切片を検鏡する機会を与えられた。組織切片上，メテナミン銀染色にてPcの嚢子が肺以外の広範な臓器にも確認された。それらは，胃，空腸，回腸，結腸，虫垂，横隔膜，甲状腺，腸間膜リンパ節，腹腔リンパ節，膵周囲リンパ節の計10種の臓器であった。肺では特に著明な線維増加と，その中に綴込められたように散在するPc感染巣が，特徴的であった。空腸には粘膜にfocusがあり，回腸と共に筋層から漿膜下層にかけてのPcによる浸潤性の病巣が認められた。リンパ節では，濾胞の消失と石灰化を伴なう壊死の周りにfociがあり，輸入リンパ管の中にもPcの集塊を認めた。全体的に細胞性の反応は乏しかったが，Mϕが各所に目立った。病理学的には，Pcの血行性およびリンパ行性の播種経路が共に考えられた。Pcの肺外播種を引き起こす要因については，目下のところ全く解っていない。Pcによる感染病巣は，決して肺のみに限られているのではないということを念頭に置き，少しでも疑わしい時には，積極的にメテナミン銀染色やギムザ染色を試みてみるべきである。また，過去の剖検例を洗い直してみれば，見逃していた肺外Pnuemocystosisがもっと見つかってくる可能性は決して少なくないと思われる。

10 日本とタイのインフルエンザ

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インフルエンザウイルスは，不連続抗原変異が起きた際には，外国から輸入されることは，過去の記録にも明らかである。連続抗原変異ウイルスについても，ウイルスの国際的伝播は考え易いのであるが，antigemic driftと呼ばれる現象の本態から考えると，世界の異なった地域において，同様の突然変異ウイルスが選択されていると考えることもできる。

私共は1977年より，タイ・ウイルス研究所，チェンマイ大学，シリラー病院，ソンクラ大学その他との共同調査により，次の成績を得た。

1）タイでは，インフルエンザは雨季を中心とする6—11月に多いが，年間を通じて常在する。

2）タイ人の血中インフルエンザHI抗体価は，日本人のそれに比べて低い。

3）A型の各亜型(H1N1，H3N2)，およびB型の流行状況を日本とタイとで比較すると，A型各亜型の場合には，日本の流行の3—6カ月前に，タイで同一亜型の流行がみられた。

4）両地域よりの各亜型内のHI抗原変異ウイルスを比較すると，H1N1の変異ウイルスの出現は，1981年まではタイでは日本より遅かった。それ以後のH1H1，およびH3N2ウイルスでは，HI変異ウイルスの出現は，一般に日本よりタイで先行していた。

5）ウイルス全RNFのoligonucleotide finger print mappingおよび，HA遺伝子のnucleotide sequenceに基づくウイルスの進化図の所見は，変異ウイルスはそれぞれの地域で独立して出現しているとの考えを支持した。

11 Influenza in Southeast Asia with special reference to Malaysia

Dora Tan (WHO Influenza National Centre, IMR, Malaysia)

（和文抄録なし）

12 熱帯医学ウイルス学分野における分子生物学

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アルボウイルス（特に日本脳炎ウイルス）と狂犬病ウイルスの2種にしぼり，近年のバイオテクノロジーを用いた諸研究，および分子生物学的研究の成果を紹介し，最後に現在私共が行っている狂犬病ウイルスに関する研究を紹介した。これらの研究は，(1) 単クローン抗体の作成とその応用に関する研究，(2) フィンガープリント法によるウイルスRNA遺伝子の解析，(3) ウイルスゲノムの塩基配列の決定，(4) 遺伝子組換えによる特定遺伝子の発現とワクチンへの応用に大別されるが，(1)は更に，世界各地で分離されるウイルス株間の抗原性の違いの検索への応用，患者血液や蚊体内におけるウイルス抗原の迅速検出への応用，ウイルスの感染防御抗原の微細構造の解析への応用，更には抗体存在下でのウイルスの培養による種々の変異株の選択などへの応用に分けることができた。

これらの研究により，今後，ウイルス遺伝子の構造，ウイルスの複製，ウイルス遺伝子の発現調節機構などウイルスの本質にせまる基本的諸問題から，ウイルス感染病の発病病理，ウイルスの生態学の新たな展開，そして，遺伝子組換えによる安全で安価な成分ワクチンやリコンビナント生ワクチンへの応用まで，広範囲にわたる活発な研究が期待できるが，これらの先端技術を使用しての研究がより実り多いものになるためには，従来行われてきた個体レベル，あるいは細胞レベルでの生物学的研究と同時に平行して行われるべきであることを述べた。

13 フラビウイルスの分子生物学

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現在，熱帯地方において年間患者発生数および死亡者数の多いウイルス疾患として，フラビウイルスによるウイルス疾患が上げられる。フラビウイルスには日本脳炎ウイルス，黄熱ウイルスやデングウイルスなどがあげられ，それぞれ日本脳炎，黄熱病，デング出血熱を引き起こすことが知られている。黄熱病は，野口英世博士により研究されたことで有名であり，また日本脳炎は，その名が示すとおり病原ウイルスが1935年に日本で最初に分離されたウイルスであり，考えているとフラビウイルスは日本人に取って馴染み深いウイルスのようである。特に日本脳炎ウイルスは，1920年代から多くの研究者により疫学的，病理学的，免疫学的研究が脈々と成され，現在もそれは続けられている。近年における分子生物学の進歩は目ざましいものであるが，フラビウイルスの解析にもこれら手法が用いられ，刻一刻とその神秘のベールが剝されつつある。我々は日本脳炎ウイルス，およびデングウイルスIII型の遺伝子RNAの塩基配列の解析を行ってきたが，世界に先駆けてそれら遺伝子の全構造を明らかにすることに成功した。本学会では，これら塩基配列をもとにした遺伝子の構造や機能の話題を中心に，フラビウイルスの分子生物学的研究の現況，これからの課題，果たす役割に付いて述べてみたい。

14 単クローン抗体を用いたデングウイルスE蛋白質の抗原分析

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デングウイルス（DV）は熱帯地域においては今なお多数の患者が出ており，重要なウイルス感染症となっている。抗体による増殖促進等のDV特異的な抗原体反応を理解するためには，DV物異蛋白質の解析が必須である。この目的で我々はデングI型（D1）望月株に対するいくつかの単クローン抗体を調製した。

ELISAによって4種のDV血清タイプおよび日本脳炎ウイルス（JEV）に対する反応性を調査した。14種の単クローン抗体の中で4種（A群）はDVおよびJEVに対するELISA反応性が同程度にあり，6種（B群）はDVとの反応性がより高く，4種はDVと強く反応することが解った。この傾向はD1，およびJEVを抗原とするHI反応の活性にも当てはまった。しかしながら，B，C群の中でD1にのみ特異的に反応するという単クロー

ン抗体はなかった。間接螢光抗体法によって，A，B，C群の単クローン抗体を調べたが，いずれもD1感染細胞の核膜周辺を中心とする細胞質に螢光が認められた。ウイルス中和活性は弱いながら2-4D-1(A)，2-3G-1(B)に認められた。ウェスタン・ブロット法による調査ではA群とB，C群とで反応性にいくらか差異があったが，明確ではない。以上の諸結果は，DVに共通して反応する単クローン抗体が多く得られること示している。D1望月株E蛋白質ではDV共通抗原部位の方が，強い抗原性を有する可能性が示唆される。

15 チクングニアウイルスに対する牛黄の不活化作用

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チクングニアウイルスに対する牛黄の作用を検討した。

材料と方法

(1) 牛黄：オースオラリア産玉牛黄

(2) 基源：*Bos taurus*の胆のう，または胆管中に生じた結石

(3) 組織培養細胞：BHK21細胞

(4) ウイルス：チクングニアウイルス

(5) ウイルス阻害テスト：牛黄をPBSに溶解し，その遠心上清とウイルス浮遊液を保温。BHK21細胞に接種し，プラク数を計測した。

結果と考察

牛黄溶解液，およびそれのウイルス浮遊液との混合液のpHは，7.2—7.7だった。チクングニアウイルスに対する影響はほとんどないと考えられる。牛黄濃度，保温時間を種々に変えて，それぞれの時のプラク数を求めた。

その結果を対照に対してのプラク減少率：

（対照のプラク数－牛黄溶解液のプラク数)／(対照のプラク数)×100（%）　を求めた。

牛黄濃度および保温時間が増加する程，プラク減少率は増加した。牛黄濃度≧10 mg/m*l*では，プラク形成は，100%阻止された。

結論

牛黄は，チクングニアウイルスに対して不活化作用を示した。この不活化作用は，牛黄濃度および保温時間に比例をした。

16 ニワトリ胚およびヒト二倍体細胞狂犬病ワクチンによる中和抗体産生について

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1980年に認可されたニワトリ胚細胞（CEC）ワクチンは現在，おもにアフリカ，インド，東南アジア等の狂犬病流行国に滞在，または旅行する人に予防用，治療用として使用されている。この市販CECワクチンの投与を受けた日本人の血清と，ヒト二倍体細胞狂犬病ワクチン（HDCV）の投与を受けたフィリピン人の血清の中和抗体価を迅速螢光フォーカス抑制法を調べた。CECワクチンは皮下に1 m*l*ずつ，1週間隔（0，7日）2回の接種群（グループI）さらに8カ月から14カ月後にブースターを受けた群(グループII)。HDCVは筋肉内に1 m*l*ずつ28日間隔(0，28)，2回の接種群（グループIII）と皮内に0.1 m*l*，0，7，28日の3回接種群（グループIV）の血清が得られた。その結果，グループIの接種後11—12カ月後の血清では，33例中25例（76%）がに40以上の中和抗体価を示した。グループIIの6—12カ月後の血清では30例中27例（90%）が1：40以上の中和抗体価を示した。グループIIの6—12カ月後の血清では，30例中27例（90%）が，1：40以上の中和抗体価を示した。グループIII，グループIVの接種後12カ月の血清では，24例中16例(67%)，19例中13例(18%)が1：40以上の中和抗体価をそれぞれ示した。さらにこのCECワクチンとHDCVとの間で，狂犬病ウイルス構成蛋白以外の蛋白混入について，SDS-PAGEとイムノブロッティング法等で比較した。その結果，CECワクチンはHDCVと比較して，総蛋白が少ないにもかかわらず，接種後1年での中和抗体価の接続があった。しかしCECワクチンを3回接種された人の内，3人は1：40以下の抗体価を示した。このことから，予防用ワクチンを受けた場合，中和抗体価のチェックが必要とされる。

17 デング出血熱における α_2-Plasmin Inhibitor-Plasmin-Complexの変動と臨床症状

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〈目的〉我々はデング出血熱（以下DHF）患者の凝血学的異常は急性DICによることを明らかにしたが，今回DHF患者の臨床症状発現に対する線溶系の関与を明らかにするために，血漿中のα_2-Plasmin Inhibitor-Plasmin-Complex（以正複合体）を経時的に測定し，興味ある成績を得たので報告する。

〈対象および方法〉インドネシア大学病院小児科に入院したDHFのうち，臨床症状からgradeIIあるいはIIIと判定された患者を対象として，血小板数，フィブリノゲン，FDP(D-dimer)，複合体(帝人株式会社製キット）などを測定した。

〈結果〉複合体はgradeII患児では二相性の増加を，gradeIII患児では一過性の急激な増加を示した。すなわち，前者では発熱3日目に軽度の増加を示したあと，一時減少し，6日目以降漸増した。一方，後者は6日目以降増加し，発熱10日目には平均14.2 μg/mlと著しい増加を示した。複合体の増加と併行して，回復期にFDPが増加する傾向が認められた。臨床症状の指標のひとつとして，胸部X線により胸水貯留の所見を観察したところ，DHFの発病初期の線溶系の活性化の低下はフィリプリン血栓除去不全をきたし，胸水貯留をはじめとするDHF重症化の原因となる可能性が示唆された。また，発熱後期の線溶系の亢進は，この時期の異常出血に関与する可能性が示唆された。

18 ツツガムシの宿主としての鼠類の生理・生態

矢部　辰男　　(神奈川県衛研)

神奈川県における近年のつつが虫病流行は，当初はタテツツガムシによると推定された。すなわち1983年から85年までは，10月から1月に患者が発生し(計26名)，これは発生季節から考え，主にタテツツガムシによる感染と思われる。しかも感染場所は，ほとんどが県西部の東名高速道沿いであり，タテツツガムシによるつつが虫病が知られる東富士山麓に接している。ただし86年の患者(計15名）の感染地は分散拡大し，かつ春（2月から5月）にも5名の患者が発生し，フトゲツツガムシによる感染が加わったことを示した。

近年の調査では，県西部の東名高速道沿いに，タテツツガムシがごく普通に認められたが，1950年代の調査ではこの地域にタテツツガムシは全く認められていない。したがって，近年になって何らかの原因でタテツツガムシが侵入したのではなかろうか。

タテツツガムシは日当たりのよい草やぶを好むことが知られている。また，その主要宿主であるアカネズミも草やぶを好む。渇きに対する強さの指標となる腎臓の構造を見ると，アカネズミは比較的乾燥した環境に生息可能なことがわかる（腎臓の皮質に対する髄質の相対的厚さは7.3±1.8となり，これはクマネズミの70.1，ドブネズミの69.8などより大きく，渇きに強いことを示す)。したがってアカネズミは，水場から離れた，日当たりのよい草やぶにも住めるであろう。そこで神奈川県では近年，西部の東名高速道沿いを中心に，タテツツガムシとアカネズミの好む草やぶが増えたのではないか。たとえば東名高速道の斜面は，人手が加わらずに放置され，草やぶになっていた。これらの地域に，多数建設されたゴルフ場の周りにも草やぶは多い。

19 ツツガムシ病の1症例

中村　眞人，関根　秀夫，安田　　隆
前波　輝彦，石田　尚志
(聖マリアンナ医大・一内科)

今回我々は静岡県富士市岩本山山中において発症したと思われる，典型的臨床症状を呈したツツガムシ病を経験した。患者は36歳男性で昭和61年11月下旬より12月中旬にかけ，頻回に岩本山を散策していた。12月17日頃より発熱，頭痛，全身倦怠感出現し近医を受診。感冒の診断でペニシリン系抗生剤の投与を受けたが症状改善せず。さらに前胸部，軀幹に皮診が出現したため12月20日に共立蒲原総合病院受診入院となった。入院時眼球結膜充血，両腋窩リンパ節腫脹，丘疹状発疹を認め

た。臨床検査では，白血球数2,100/㎣，血小板数9,900/㎣と減少，GOT621U，GPT461Uと軽度の肝機能障害，CRP2+，ワイル・フェリックス反応陰性を示した。入院後発疹は，軀幹から四肢に広がり，左上腕屈側に潰瘍化したツツガムシによるものと思われる刺口を認めた。直ちにドキシサイクリン200㎎/日を投与。投与後2病日にて下熱し，以後徐々に臨床症状の改善を認めた。同時に検討したツツガムシ抗体価はGilliam，Kato，Karp法とも抗体価上昇を呈し，1カ月，さらには2カ月後と徐々に低下傾向を認めた。

わが国におけるツツガムシ病の発生は，昭和38年以後急激に減少したが，昭和51年頃より増加傾向が指摘されている。ペニシリン系さらにセフェム系抗生剤の台頭している現況で，急性熱性疾患に対して一考を要する症例と思われる。

20 Recent advances on molecular biology of malaria research

Chariya R. Brockelman (Dep. Microbiol., Fac. Sci., Mahidol Univ. Thailand)

(和文抄録なし)

21 高IFA値を有する輸入熱帯熱マラリア症例に見られたcrisis formのDNA量

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西山　利正，高橋　優三，荒木　恒治
(奈良医大・寄生虫)
鈴木　　守　(群馬大・医・寄生虫)

Tariaferro & Tariaferro (1944) は猿マラリアにおいてparasitemiaの増加が自然経過中に抑制されることを観察し，分裂体中の娘細胞数が減じ，かつ形態的に変形した娘細胞の出現を認めた。彼らはこの変性娘細胞をcrisis formと名付け，獲得免疫の関与を想定したが，以後，この名称は，広く何等かの免疫機序による変性赤血球内寄生マラリア原虫にも使用されるようになった。我々は高IFA値を示した輸入熱帯熱マラリア症例で，低いparasitemiaと変性環状体を多く認め報告した(天野ら，1985，第27回日熱医会総会)。今回は，タンザニアからの輸入熱帯熱マラリア症例（症例1：29歳日本人女性，parasitemia 0.012%，IFA値1,024倍，症例2：35歳日本人男性，parasitemia 0.77%，IFA値256倍)の血液標本に見られる変性環状体をcrisis formとして，そのDNA量と形態との関係を検討した。相対DNA量の測定には，DAPI染色（1 μg/ml）による螢光測光法を用い，タイ症例（男児，parasitemia 4.82%）のマラリア原虫を対称とした。crisis formは赤血球内寄生原虫中，症例1で38.8%に，症例2で51.7%に認められ，その核は，核内空胞を持って環状，V字型，棒状など種々に変性していた。期待に反して，crisis formの変性した核の各形態と，そのDNA量との間には有意の差を認めなかった。標準型のマラリア原虫核DNA量の平均値は，3例とも，ほぼ近似した値を示すと考えられた。症例2では，標準型とcrisis formの平均核DNA量は，ほぼ同レベルであったが，一方，症例1では，crisis formのそれ（33.5±6.9FU）が，標準型のそれ（40.7±6.2FU）よりも低かった（$p<0.001$)。crisis formのDNA量の変化は，赤血球内寄生原虫発育抑制の結果を物語っているのかも知れない。

22 熱帯熱マラリア原虫メロゾイト表面抗原遺伝子の株間における構造変異

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J. G. Scaife
(エジンバラ大・分子生物)

熱帯熱マラリア原虫のメロゾイト表面には，免疫防御を付与する抗原が存在する。この抗原はその前駆体が分子量19万から22万の糖タンパクとして分裂期に合成され，メロゾイト形成時に断片化を受けてメロゾイト表面に局在する。この表面抗原は多型的で，分離株により抗原性が異なる。この抗原はマラリアワクチンの有内候補であるので，多型性の遺伝子レベルでの解析は重要である。私達はパプア・ニューギニア分離のMAD20株からメロゾイト表面抗原前駆体の遺伝子をクローニングし，全塩基配列を決定したので，既に報告されている他株の遺伝子と比較検討してみた。

その結果，遺伝子中には7つの変異領域が分散し，約4割を構成していた。Kl・Wellcome株では各所に欠失があり，遺伝子中程から3′側にかけ

て30—60塩基もの欠失が4カ所存在した。また，3′側の変異領域には欠失によるフレームシフトも約30塩基認められた。しかし，最も驚くべきことに，各変異領域はただ2つの異なるタイプのみに現われていることで，MADはK1とどの変異領域においても異なっていた。Wellcome株は第1番目の変異領域ではMADタイプ，第2番目以降はK1と同一であり，CAMP株では第1と第2番目ではK1タイプ，その他はMADと同一であった。このことはメロゾイト表面抗原遺伝子が2つの異なる原型対立遺伝子により表現され，遺伝子の変異はこれら対立遺伝子の有性生殖体接合時における遺伝子内組み換えから生じ，その結果として抗原の多型性が現われることを示唆する。事実，Wellcome株とCAMP株の遺伝子塩基配列中にはMADとK1遺伝子の組み換え部位と考えられる配列が存在していた。遺伝子内組み換えによるマラリア原虫表面抗原の多様性の出現は，マラリア原虫の免疫宿主体内での生存，また，原虫の遺伝・進化を考える上で重要な問題を投げかけると思える。

23 熱帯熱マラリア原虫における生殖母体形成の誘導(II) 種々な株における生殖母体形成の誘導

小野　忠相，中林　敏夫
(阪大・微研・原虫)

従来，人為的にマラリア原虫の生殖母体を確実に誘発する方法がなく，生殖母体形成に関する研究はこれが自然に誘発される原虫株を用いて行われている。先に私達はRPMI1640倍地の粉末を抗熱帯熱マラリア原虫抗体産生hybridoma細胞の培養上清液に溶解し，調整した液を使用することにより，生殖母体形成が誘導されることを報告した。その時用いた原虫株はFVO株とFUP株であり，その後，FUP株は生殖母体形成が誘導されなくなった。生殖母体形成の誘導は原虫側の要因としても調べる必要があり，そこで今回は他の株について誘発実験を試みた。用いた株は0662，FCB1，FCR-3およびR・FCR-3であり，いずれも群馬大・医・鈴木　守教授より，長崎大・熱帯医研・原虫学部門を経て恵与されたものである。その結果，上記4株のいずれにおいても生殖母体形成が認められ，誘導液の効果がFVO株以外の原虫株に対しても有効であることがわかった。

次に生殖母体形成率すなわち，無性生殖期原虫から有性生殖期原虫である生殖母体に転換する原虫の率を高める試みを種々行った。その結果，原虫の培養はRPMI1640培地にhypoxanthine (50 mg/l)，glutamine (500 mg/l) を加えた培地を用いて行い，培養5－6日目に培養液を加える生殖母体形成誘導液もこれらの薬剤を加えて作製したものを用いると，生殖母体形成率が著しく高まることが見出された。

24 マラリア流行地におけるELISAの疫学的応用

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石井　明　(岡山大・医・寄生虫)
渋谷　敏朗　(帝京大・医・寄生虫)
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(Regional Leallh Loboratory of Medan)

マラリアの免疫診断法には螢光抗体法(IFAT)，Radioimmunoassay (RIA)，ELISA (Enzymeimmunoassay) などがある。疫学調査の一手段としての免疫診断法は，簡便かつ感度が高く特異性のあるものが望まれるが，IFATは検査に要する時間，螢光顕微鏡の使用など制約があり，必ずしも最適のものとはいえない。近年ELISAが手技が簡便で感度が高く，かつ少量の血清，試薬で検査ができ，一回に大量の検体が処理でき，主観の入らない方法として開発された。我々はインドネシア北スマトラ州における，北スマトラ地域保健対策プロジェクトのひとつとして，プロジェクト地域内のマラリア患者についてELISAでマラリア抗体を測定する機会を得たので，報告する。

20μlのヘマトクリット管で収集した血清を100倍希釈し，アルカリフォスファターゼ標識IgG抗体を用いてELISAを行ったところPCDで約52%，小学校学童で約38%と高い抗体価が認められた。対照として非流行地であるメダン市内の学校，衛生研究所職員，小児病院外来患者，日本人を調べたところ，全てELISA陰性で，流行地住民との間に明らかな差がみられた。ELISAと脾腫の関係，

年齢別抗体価の分布，ELISAと螢光抗体法の関係などについても検討した結果，ELISAは急性マラリアの診断に関しては顕微鏡学的検査に一歩ゆずるにしても，疫学的調査の手段として有用な方法と考えられる。

25 高濃度クロロキン一回投与の *Plasmodium yoelii nigeriensis* (N67)の *Anopheles stephensi* に対する感染性

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ネズミマラリア (*Plasmodium yoelii nigeriensis* N67) に感染したマウスに100，10，1 mg/kgのクロロキンを投与し，その後12時間，または24時間後に蚊に吸血させた。吸血直後マウスの血液をとりELISA法により血液中のクロロキン量を測り，またヘマトクリット法により貧血の程度を測定した。一方，吸血した蚊を解剖し，オーシストの数を数え，ウィリアムの平均値を算出し感染性の度合とした。

マウスは血中のクロロキン量が多いほどヘマトクリット値も上がり正常に近くなった。投与したクロロキン量が多いほど，12時間あるいは24時間後のParasitaemiaは減少を示した。Gametocytaemiaも減少し，特に100 mg/kg投与後12時間，または10 mg/kg，100 mg/kg投与後24時間で赤血球10,000中gametocytesは0となった。ところが，その血液を吸血した蚊ではオーシストが形成された。100 mg/kg投与後12時間の場合では，得られたオーシストの数は特に多くコントロールとほぼ同じで中腸全体を覆うものもみられた。

二項分布より信頼限界を求めると，観察値0の場合でもgametocytesは3/10,000RBCsの比で存在しうる。その3コの存在をこれまで得られた結果を使い計算すると，血液標本中，gametocytesが0であっても1匹の蚊が7,000以上のgametocytesを取り入れる可能性があり，それは蚊の中腸全体を覆ってしまうほどの，多数のオーシストを産生する可能性であることがわかった。

26 アマゾン地域トメアス日系移住地域におけるマラリアの急増
＝1976・86・87年血清疫学調査の結果＝

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鈴木　守，脇　誠治，小杉留美子
(群馬大・医・寄生虫)
M. M. Hida　(USESP,São Paulo)
J. J. Ferraroni　(INPA, Manaus)
R. Brawn　(SUCAM, Belém)

アマゾン河口Belém市の南約250kmに位置し1962年より開発された日系移住地Daini Tomé-AÇu(250 km²) に住む日系農民(J)70家族350人および日系農家に雇用されている現地人季節労働者(B)を対象として問診および採血を実施し，間接螢光抗体法(IFAT)による熱帯熱マラリア抗原および三日熱マラリア抗原に対する抗体価を検査した。その結果1976年の抗体陽性者はJ 145名(平均年齢29.8歳) 中2.8%，B461名 (平均33.6歳) 中16.5%であり，1986年ではJ 107名 (平均33.7歳) 中23.4%，B 20名 (平均21.5歳) 中60.0%であり，1987年ではJ 144名，(平均29.5歳) 中21.5%，B 60名 (平均32.9歳) 中28.3%であった。また1976年調査のJのうち10年後に再調査した49名 (平均28.2歳) について見ると1976年2名 (4.1%)，1986年12名 (24.5%) で現地労働者のレベルに近づいた。我々は1976年の調査で血液塗抹標本の直接検鏡ではマラリア原虫の検出はなかったがIFATによるJ－B間の抗体陽性率の差から季節労働者の流入によって誘発されるマラリア流行の危険を予測した。1986年と1987年の調査により抗体陽性者が約8倍に増加していることが判明した。一方SUCAM (マラリア対策保健所) によるTomé-AÇu郡における直接検鏡による原虫の検出はこの10年間に15.5倍に増加している。この原因として①現地労働者の流入，②マラリア流行危険度のより高い新開発地との交流などが示唆された。

以上の10年におよぶ調査結果によりIFATは低流行地においての危険を予測する上で有用な一手段であることが示唆された。

27 グアテマラ共和国の6カ村におけるマラリア，G6PD欠之症，Duffy血液型の調査

松岡　裕之，長谷川　恩，石井　明
（岡山大・医・寄生虫）

グアテマラ共和国（人口800万人）では，マラリア患者は全国6,000名余のボランティアによりpassive case detectionで把握されている。年間40—50万件の厚層標本が中央の保健省マラリア局に寄せられ，5.5〜7.7万人のマラリア原虫陽性者が検出されている。陽性者にはクロロキンとプリマキンが投与されている。我々はこの記録をもとにマラリアが比較的多く検出されている村落6カ所を選び，雨期にあたる1986年11月，全年齢層を対象に血液検査を行った。10歳未満の小児には腹部触診も加えた。血液検査はマラリア診断のための厚層標本のほか，Duffyの血液型，glucose-6-phosphate dehydrogenase(G6PD)欠之者のスクリーニングを行った。

1,510名から採血を行い，31名に原虫陽性者を得た(2.1%)。*P. falciparum* 3例，*P. vivax*28例であった。村落別では原虫陽性率は最高6.3%であり，2カ村で0%であった。新生児原虫陽性率は1.2%であった。年齢層別では小児•成人とも原虫陽性率に差を認めなかった。血液中の原虫濃度の高い者は若年層で多くみられた。脾腫は10歳未満の小児759名中1例のみであった(0.1%)。G6PD欠乏者は男子567名中3名（0.5%）にみられたが，女子には認めなかった。Duffy血液型はFy(a+b−)55.3%，Fy(a+b+)18.0%，Fy(a−b+)11.7%，Fy(a−b−)15.0%であった。gene frequencyは，Fy^a47%，Fy^b16%，fy37%であった。三日熱マラリア陽性者28名は全員Duffy血液型陽性であり，これは統計的に有意であった($p<0.025$)。

28 マラリアの臨床管理，特に薬剤耐性問題

中林　敏夫　　（阪大・微研・原虫）

本シンポジウムはキーノート演題として学会長，神田教授により準備されたもので，主として総合司会者である中林とProf. Danai Bunnag (Mahidol Univ., Thailand) が組織した。演者としてProf. M. Fernex (Univ. of Basle, Switzerland), Prof. K. T. Harinasuta (Mahidol Univ., Thailand)，海老沢教授(東邦大)および中林が選択され，司会者として大友教授（岐阜大）および天野博士（天理病院）が会の運営に当たった。

1955年以来，DDTの残留噴霧とChloroquine投与を中心としたWHO主導によるマラリア根絶計画は，一旦は顕著な成果を挙げてきたが，やがて，殺虫剤耐性蚊や薬剤耐性原虫の出現とその分布の拡大，当事国の経済的理由などから，継続的実施が困難となった。現在では，対策生認としてPHCの一環として当事国の自主的運営にゆだねられるに至った。現在マラリア発生は年間約2億にも達し，地球上総人口の約半数が発生地域に居住している。

急性熱帯熱マラリア患者の臨床管理は，速かな診断確定と，適切な抗マラリア薬投与および対症療法の施行にあるといえる。しかしChloroquine耐性熱帯熱はアフリカを含めほとんど全発生地に分布し，一部地域ではFansidar耐性が，またQuinine低感受性原虫の報告がみられる。新薬剤としてのMefloquineも主としてFansidarとの合剤としてタイなどで使用されるが，本薬に対する耐性発現も予測されつつある。Quinghaosuは脳症併発例に著効が知られ，またQuinidineの治効も認識されつつある。他方Fansidarの副作用による死亡例も見出され，マラリアの予防内服，治療は少なからぬ混乱状態にあるものとみてよい。

かかる現況に即して，熱帯患者の臨床管理，特に薬剤耐性問題を中心とした講演，討議においては，最新の知見，治療経験が披露されるものとして期待される。

29 Treatment and prevention of malaria with Fansidar

Michel Fornex (Fac. Med., Univ. Basle, Switzerland)

（和文抄録なし）

30 Management of malaria with special reference to drug resistance

Khunig, T. Harinasuta (Bangkok Hospital for Tropical Diseases)
Danai Bunnag (Mahidol Univ., Thailand)

(和文抄録なし)

31 マラリア治療と予防上の問題点

海老沢 功 (東邦大・医・公衆衛生)

マラリア治療上問題となるのは熱帯熱マラリアにおける薬剤耐性，治療開始時期の遅延による重症化と瀕死重症例の治療である。

1）1966—1985年の20年間に経験した熱帯熱マラリア患者のべ144人における治療成績を見ると，クロロキン耐性例はクロロキンによる治療例64人中19例，約30%である。再燃した症例はMP錠，ファンシダール（SP錠），キニーネ単独，またはMP或いはSP錠とキニーネを併用して2例を除き全治した。タイ・カンボジア国境とタイ・ビルマ国境地域で感染した2名はクロロキン，ファンシダール，キニーネ，ファンシダールとキニーネの併用，テトラサイクリンいずれを用いても全治せず，R1耐性を示して再燃した。

2）腎不全，脳症，DICなどを併発し集中治療を必要とした重症例，あるいは未治療のまま死亡した熱帯熱マラリア症例は総て5病日以後に脳症，腎不全，DICなどを併発している。発病後5日以内に適切な抗マラリア薬を用いて治療を開始されたものは総て重篤な合併症を起こさずに治療している。しかし中には発病後2週或いは3週間以上たってから治療を開始されても，特に合併症を起こさないで治癒した例もある。マラリア感染の危険に曝されたものが発熱したらなるべく速やかに医師の診察を受けるように指示しているが，その限界が5日以内ということである。上記重症例の治療の実際に関しては速やかにキニーネの点滴注射を主体として治療した例では成功したが，同時に腹膜灌流，血液透析，全血交換なども同時に行った。肺水腫を合併したものには，気管内挿管による人工呼吸が奏功した。経口的にのみ抗マラリア薬を与えて治療した例では，治癒が長引くか不成功に終った。その中には副腎内に出血し，急激な血圧降下を起こして死亡したものがある。

2）マラリア予防薬の選択に関しては，クロロキン耐性熱帯熱マラリア流行地に出向するものに対して何を処方すべきか問題がある。1987年4月WHO専門家の集りではクロロキンを主体とし，毎日プログアニル200mgを内服することが推奨された。三日熱マラリア予防に帰国後クロロキンを4週間内服した後，プリマキンを14日間内服することを日本人には推奨する。

32 40余年を経て再発したと思われる四日熱マラリア症例

中林 敏夫，小野 忠相
(阪大・微研・原虫)
藤川 潤，小橋 裕司，友田伊一郎，
江口 忠，南方 保，小中 義照，
久保 勝彦 (北野病院・内科)

四日熱マラリアは長年月後の再発（再燃）を来すことが知られている。本症例は昭和15年(1940)に中国，蘇州でマラリア（虫種不明）に感染して以来，現在まで時々発熱を繰り返したが，マラリアの診断，治療を受けることなく経過したものである。本年，腎障害で入院，免疫異常が疑われ，4月23日よりプレドニゾロン40mg/日の投与を受けた。5月16日胃角に小潰瘍が認められ20mg/日に減量したが，5月28日，投与28日目に悪寒，発熱を来し，その後，患者からの情報もあって末梢血検査を施行したところ，四日熱原虫を検出(parasitemia 0.03%) したものである。治療はFansidar（2，1，1）の他に，原虫種未確定の段階でもあって，primaquine 15mg base/日，14日間投与を行い現在まで発熱はない。海外渡航歴，輸血歴はなく，患者住居の近辺にマラリア患者発生もなく，おそらくは47年前に中国で罹患した四日熱マラリアの再燃であろうと推測された。わが国では，海老沢により，36年を経て脾摘出手術後に発症した四日熱マラリアの再燃例が報告されている。本症例は47年後の四日熱マラリア再燃例であるが，免疫抑制剤のステロイド投与が，再燃の誘

発に関係したものと注目される。

33 アメーバ症を中心とした原虫感染症に対する化学療法：新しい薬剤の開発，およびdrug delivery systemの応用

竹内　勤
（慶応大・医・寄生虫）

アメーバ症に関する化学療法は現在の第1選択薬剤であるメトロニダゾール，ティニダゾールなどのニトロイミダゾール系薬剤に発ガン性，変異原性があるため必ずしも安全とはいえず，また最近はニトロイミダゾール系薬剤が奏効しない例が散見されている。従って，ニトロイミダゾール系薬剤にかわり得る薬剤の開発，あるいは副作用を軽減するための投与法の改良は緊急に必要とされている。本報告では最近の当研究室における抗アメーバ作用を示す薬剤の開発の現況，および薬剤効果増強のためのdrug delivery systemの応用を中心として報告したい。

1．ハロゲン化ビスフェノール，およびクワシノイドの殺アメーバ作用

ジクロロフェンなどのハロゲン化ビスフェノール誘導体は*in vitro, in vivo*にて強い抗アメーバ作用を示すことはすでに報告した。今回は同系の化合物のうちブロム置換体，および植物由来のクワシノイドのうち何種かを更に構造を修飾した誘導体に関し抗アメーバ作用を調べた。その結果，ブロム置換体もBI-S-33培地中で強い抗アメーバ作用を示すことが判り，またクワシノイドの中にも強力な同様の作用を呈するものが見出された。

2．Drug delivery systemの応用

現在種々の物質がdrug delivery systemのキャリアーとして応用されているが，我々の研究室ではメタクリル酸より作成したラテックス粒子を用い薬剤の固定化がどのような効果を示すのかについてアメーバを用いて*in vitro*の系において検討した。これは赤痢アメーバの活発なphagocytosisの能力を利用したものである。その結果ラテックス粒子の生分解性，あるいは薬剤の放出はまだ充分とはいえないが，固定化されたハロゲン化ビスフェノールは特に培地内の濃度が低い場合，freeのものより高い抗アメーバ作用を示した。現在さらに薬剤放出速度の速い粒子を作成するべく種々改良を試みており，あわせて報告したい。

3．その他の原虫に対する薬剤の開発

上述の抗アメーバ作用を示す薬剤以外にもマラリア，トリパノソーマ，リーシュマニアに対するものの中で興味あるものが見出されており，あわせて一端を報告したい。

34 シストキャリアーにおける抗赤痢アメーバ抗体

奥沢　英一，野崎　智義，竹内　勤
（慶大・医・寄生虫）

我々はすでにシストキャリアーのスクリーニングにmicro-ELISAが利用できる可能性があることを報告した。(87年日本寄生虫学会大会)。これが特異的な反応であるか否か，認識されている抗原がいかなるものかに関して検討を行ったので，これを報告する。

まず，これが特異的な反応であるか否かを確認するため，沈降反応陰性でmicro-ELISA陽性の血清を用いて，以正の2つの実験を行った。

1）被検血清と反応させる前に，抗原を異種抗体（免疫家兎血清）でブロックする実験。対照として正常免疫家兎血清を用いた。

2）吸収試験：虫体のホモジネートで吸収した血清と無操作の血清の比較。

これら2つの実験からキャリアーにおける発色は特異的な反応と考えられた。

次にこの反応はmicro-ELISAの抗原（可溶性分画）に特異なるものか，アメーバ膜表面等にも存在するのかを調べる目的で，形態を保った虫体そのもの（シャウジン固定）を抗原として酵素抗体法を行った。この結果，虫体そのものを抗原として用いても，キャリアー血中の抗体と反応していると考えられた。

最後にimmunoblottingの手法を用いて，キャリアー血中の抗体がアメーバ性肝膿瘍やアメーバ性腸炎患者の血中抗体と同一のものか否かを検討した。その結果，キャリアー血清を用いた場合もバンドが確認された。バンドのパターンでは，キャリアーの場合はアメーバ性肝膿瘍の場合より，ア

メーバ性腸炎の場合とバンドパターンに類似性があると考えられたが，キャリアー同士で比較してもバリエーションが大きく，バンドの詳細を議論できるまでには至らなかった。

35 エクアドル国におけるリーシュマニア皮膚潰瘍の細菌叢

川端　真人　　（日大・医・臨床病理）
三森　龍之　（熊本大・医・寄生虫病）
古谷　正人，橋口　義久
（高知医大・寄生虫）

エクアドル国を含む中南米各国に分布する皮膚粘膜リーシュマニア症には種々の病型が知られている。これらは病原体である *Leishmania blaziliensis* の亜種または株間にみられる病原性の差によるものとされているが，宿主側の要因や環境による要因も考えられている。一方，ペルー国の山岳地域には浅い潰瘍を形成し，自然治癒傾向を特徴とするアンデス型リーシュマニア症があり，この病型がエクアドル国にも存在することが確認されている。今回我々は，リーシュマニア皮膚潰瘍から細菌を分離，同定し，エクアドル国のアンデス型リーシュマニア症（高地型）と皮膚粘膜型リーシュマニア症（低地型）の細菌叢を比較検討した。

調査対象は臨床的および免疫学的にリーシュマニア皮膚潰瘍と診断された51例（高地型11例，低地型40例）で，46例から細菌が検出され，高地型は81.1%，低地型は90.5%であった。菌種別にみると，グラム陽性球菌，嫌気性菌は地域間に差はみられないが，グラム陰性桿菌は高地型が18.2%に対し，低地型では37.5%と高頻度であった。検出されたグラム陰性桿菌は腸内細菌科を主体とするグループであり，これらの細菌叢と潰瘍病変形成との因果関係は今後検討を要する。

本研究はエクアドル国立熱帯医学研究所，A. Davila, V. Coronel, E. Gomezとの共同研究で行われた。

36 *Leishmania donovani* amastigoteの *in vitro* cultivation systemの確立

谷藤　久人，佐竹　弘靖，山根　和子
綿矢　有佑　（岡山大・薬・薬品化学）

（目的）寄生原虫の核酸代謝系は宿主と異なっており，プリンヌクレオチドの新生合成系が欠損し，かつ寄生虫特有のSalvage系路を有している。我々はこのことに着目し，nucleoside analogの抗寄生原虫作用の検索を行った。その結果，*L. donovani* promastigoteに対し，有効なinosine analogを見い出した。

今回我々はマウス・マクロファージ系J 774・1細胞を用い，*L. donovani* amastigoteの *in vivo* like cultivation systemを確立したので，この詳細について報告する。又，このsystemを用いて，inosine analogの効果を検討したので，あわせて報告する。

（方法）J 774・1細胞をlipopolysaccharide（1 μg/m*l*，Difco，*E. coli* O172：B8）および，hemin（4 μg/m*l*）を含む無血清培地GITで一日培養し，これに*L. donovani* promastigoteを加え，さらに一日培養した。培地中の原虫を除いた後，一定時間毎に染色し，J 774・1細胞内の原虫を数えた。又，24時間毎にPercollを用いて原虫を単離し，原虫の形態変化を観察した。

（結果）*L. donovani* promastigoteは数時間で，J 774・1細胞に侵入した。原虫感染後，5日間で，ほぼ100%の原虫がamastigote型に形態変化した。inosine analogである3′-deoxyinosineおよびcarbocyclic inosineは，J 774・1細胞内に存在する原虫に対しても抗原虫作用を持つことがわかった。

37 *Leishmania donovani* 並びに *Trypanosoma gambiense* 感染マウスに対するinosine analogの薬物効果について

森重　和久，安治　敏樹，J. 木村，
石井　明　　（岡山大・医・寄生虫）
綿矢　有佑　（岡山大・薬・薬品化学）

綿矢らは先に *in vitro* において，carbocyclic inosineと3′-deoxyinosineが*L. donovani*に対

し毒性効果を有し，carbocyclic inosineは*Crithidia fasciculata*に対しても有効であると報告した。今回は*L. donovani*感染マウス，*T. gambiense*感染マウスを用いて2つの薬剤の治療効果を調べた成績を報告する。

（材料と方法）

1．*L. donovani* 治療実験ではBALB/cマウス，8週齢，雄を用い，*L. donovani* promastigote 1×10^8をi. v. 注射し，その後1日おきに5回3′-deoxyinosine. carbocyclic inosine投与を行い4週後解剖した。肝臓の割面のstamp smearをGiemsa染色し，1,000肝細胞中のamastigote（LDV）を数え，薬効を評価した。

2．*T. gambiense*治療実験ではddYマウス，6週齢，雄を用い，*T. gambiense* trypomastigote 1×10^4をi. p. 注射し，前日又は同日から1日1回毎日carbocyclic inosine, 3′-deoxyinosineを投与し，毎日の血液塗抹（Giemsa染色）での虫体数と，マウスのsurvival rateによって薬効を評価した。

（結果）

*L. donovani*治療実験において，LDVから評価すると，生食投与群が平均48に対し，carbocyclic inosine 100 mg/kg投与群は平均4で，約92%の減少効果を見せたが，強い副作用が見られた。3′-deoxyinosineは生食投与群に比し，約63%の減少効果を見せた。*T. gambiense*治療実験ではcarbocyclic inosineは効果は見られなかったが，3′-deoxyinosine 200 mg/kg（前日投与）において，9日目までの血液塗抹での虫体数は中程度以下で，マウスの半数は生存し，若干の有効性を見た。

38 超高圧電顕による*Trypanosoma evansi*の観察，特に膜下微小管の分布の様式について

比留木武雄（島根医大・微生物・免疫）
有井　達夫　（生理研・超高圧電顕室）

ある種の微生物の超微構造を研究する場合に，研究者がその微生物の三次元構造を念頭に置いているか否かは極めて大事なことと考えられる。しかしながら，微生物の表面構造のみを取り扱う走査電顕を除くと，一般の透過電顕を使用した研究の大半は，二次元の所見の記載にとどまっており，微生物の三次元構造までを考慮する研究者は少ない。

その原因は，ダイアモンドナイフを使ってさえ，連続切片が得がたいことにあるであろう（連続切片は，微生物の三次元構造を再構築するために必要である）。

今，超高圧電顕，H-1250（国立生理研）の使用は，微生物の“直接の”即ち“実際の”立体構築を観察することを可能にする。

この様な訳で，演者らはH-1250を用いて，*Trypanosoma evansi*台湾株の超微構造を研究し，普通の透過型電顕で今日まで，見逃されてきたいくつかの新しい知見を得ている。

本学会において，演者らは膜下微小管が2つの異なった方向に走り，それらは互いに紡ぎ合って，布の外観を呈する事を明らかにした。演者らはまた，reservoirから一旦出た鞭毛が，再び細胞質に入り込む立体像を示した。細胞内鞭毛の横断面と縦断面は，第26回本総会（鹿児島市，1985）で示した。どんな理由で，このような奇妙な鞭毛が生じるのか分からない。

もし，paraxial rodを伴うが，flagellar membraneを持たないaxonemeの存在が本質的なものであるならば，このトリパノゾーマの分類を改定することは妥当であろう。

39 TLA感作マウス脾臓細胞に及ぼすTLA添加培養の影響

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（帯広大・獣医生理）
尾崎　文雄　（八木病院）

BALB/c系6週齢の雌マウスにトキソプラズマ溶解抗原（TLA）100 μg加生理食塩水溶液を2週間隔で2回，大腿部筋肉内に投与してTLA感作マウスとした。初回投与後4週目に，TLA感作マウスおよび無処置マウスの脾臓細胞をConray-Ficoll法で分離調整し，これらの細胞をTLA100 μg/ml培養液で3日間培養した。培養後，細胞の形態および大きさをGiemsa染色塗抹標本あるいはアクリジンオレンジ染色浮遊細胞像を，Nikon

-Microphot FX顕微鏡自動画像解析装置（Luzex 500, Nireko, Co.）を用いて測定した。これら細胞のYAC-1, P-815あるいはS-180細胞に対する細胞傷害能を^{51}Cr遊出法およびレーザーフローサイトメトリー法（CS-20, Showadenko Co., Tokyo）で測定した。その結果，TLA感作マウス脾臓細胞をTLAで培養すると，顆粒を有する大型細胞が出現した。その培養脾臓細胞はYAC-1およびP-815細胞に対して細胞傷害性を有し，S-180細胞の増殖を抑制した。

40 長崎県の2島におけるトキソプラズマに関する血清疫学的研究

力富 直人，鈴木 寛，山本由美子
松本 慶蔵（長崎大・熱帯医研・内科）
麻生 貞郎（有川医師会）

トキソプラズマ抗体陽性率は地域により異なるが，加齢と共に上昇することは指摘されている。長崎県においても都市部と郡部の抗体陽性率は異なっていたが，老齢者の陽性率は同等であること，しかし，その抗体レベルは郡部の老齢者において高いことを既に報告した。そこで，今回は生活並びに経済環境が極めて類似した，長崎県の2つの島の住民を対象として研究を行った。

長崎県の平島と江ノ島の，それぞれの住民の約55%に相当する283名と270名の計553名を対象として，トキソプラズマ抗体をELISA法により測定した。

抗体陽性率は平島（44%）が，江ノ島（33%）より有意に高値であった。しかし，抗体レベルは江ノ島（2.8±0.6）が平島（2.5±0.6）より高値であった。年齢別陽性率は，江ノ島では年0.7%の感染率で，加齢と共に直線的に上昇していた。しかし，平島での年齢別陽性率は年0.85%で上昇していたが，これは階段状上昇を示した。1年間における抗体陽転者は平島（0.9%）でのみ検出された。抗体陽性者の1年間における4倍以上の抗体上昇は，平島で5.8%，江ノ島で2.6%に認められた。

以上のように，生活環境および経済環境が類似した同一県内の2つの島においても，トキソプラズマ感染の実態は異なった様体を示していた。

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Genaral presentation No. 41 to No. 82 will be appeared in the next issue

Forum

THE INTERNATIONAL RESEARCH EXCHANGE AND MEDICAL CARE

Chaired by Y. HAMASHIMA

(English abstracts not received in time)

General presentation

1 ACTUAL CONDITIONS AND ACCOMODATION AGAINST IMPORTED INFECTIOUS DISEASES

SAKITO YASUKATA

Narita Airport Quarantine Station

(Abstract not received in time)

2 VIRAL AND RICKETTSIAL DISEASES EXOGENOUS IN JAPAN

TAKASHI KITAMURA AND TOSHIHIKO KOMATSU

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Exogeneous infectious diseases which may become public health problems in Japan have been devided into two categories: i. e. A. international diseases (IND) (e. g. Lassa fever, Marburg virus disease and Ebola hemorrhagic fever), which are zoonotic in other areas in the world but have the possibility of man-to-man epidemic after in introduction to the non-endemic area, and B. importable diseases (ITD), which had been eradicated in Japan but are still endemic outside and have the possibility of importation and secondary epidemics.

IND agents have been designated as the class-4 pathogens and the handling of patients and virological specimens should follow the "Guidelines of Prevention and Control of International Diseases" (Draft) issued by the Ministry of Health and Welfare, in which the patients are treated in the Maximum Containment Ward of the Tokyo Metropolitan Ebara Hospital and the virological diagnostic tests are carried out in the Maximum Safety Laboratory of the National Institute of Health, Tokyo.

The best example of ITD may be the poliomylitis. Poliomyelitis has been eradicated in Japan in early sixties but there persists a few paralytic polio cases annually, most of those are vaccine-compatible, but there occurred few cases which excreted wild type polioviruses. We checked the enteroviruses brought by passengers entering by air route from Southeast Asia at the Narita Airport Quarantine Station. Several strains of poliovirus of wild type markers have been isolated from healthy passengers. These findings clearly suggest the importation of wild type poliovirus to cause paralytic polio among non-vaccinated infants.

Hemorrhagic fever with renal syndrome (HFRS) may be another case of ITD. It had

been endemic in Osaka during 1960's and expanded to medical institutions through experimental rats in 1970's. We isolated rat-adapted HFRS virus from experimental rats at one epidemic of laboratory cases. The virus was similar to HFRS virus of new, urban type HFRS prevalent in China and Korea.

In 1984, from a Tsutsugamushi-like febrile disease, a new rickettsia was isolated, which was later identified with that of Rocky mountain spotted fever (RMSF). Whether this isolate of RMSF is imported or indigenous may be decided by further studies.

HIV which causes AIDS is quite exogenous in Japan. HIV prevalence has been demonstrated only among hemophiliac received imported clotting agents or homo- or heterosexuals having contacts with foreigners.

Prevention and control of importable diseases should be also systematized to avoid the transfer or recovery of endemic status.

3 DETECTION OF CHLAMYDIA TRACHOMATIS FROM FEMALE GENITAL IN HIGH RISK GROUPS

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Genital tract smears of 232 female (98 of soup-land workers, 34 of office workers and 100 of house wives) were collected at two clinics of obstetrics and gynecology located at near a red-light district and a residential one in Kawasaki, and the tests to detect *Chlamydia trachomatis* (*C. trachomatis*) by the direct immunofluorescent test (Micro Trak™) were attempted for epidemiological study.

The positive rates of *C. trachomatis* according to their occupations were as follows; soap-land workers (31.6%), office workers (5.9%) and house wives (4.0%). On the positive rates of soup-land workers according to their place of residence, the rates of a groups lived in Tokyo showed 42.9%, and other groups lived in Kawasaki and Yokohama were 29.4% and 13.8% respectively. The positive rates of soup-land workers to *C. trachomatis* showed a tendency to decrease with age, and it revealed that the rates in the groups less than 20, 20-29, and 30-39 years old were 66.7%, 31.1% and 30.0% respectively.

The positive rates on groups of house wives lived near a red-light district and a residential area were 7.1% and 2.8% respectively, and the rates of the former groups showed 2.5 times higher than those of latter one.

It had been known that *C. trachomatis* infectious diseases are related to nongonococcal urithritis, extrauterine pregnancy and sterility in adults, and pneumonia and conjunctivitis in infants by maternal vertical infections. Sexual liberation promoted by prevalence of pills will bring about increase of STD including *C. trachomatis* in future. It will be nesessary to consider a counterplan of preventive control on these diseases.

4 A CLINICAL STUDY OF *CHLAMYDIA TRACHOMATIS* (*C. TRACHOMATIS*) INFECTION

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Clinical observations were made on male patients with urethritis in St. Marianna University Hospital.

(1) When urethritis was devided into gonorrhoeal and non-gonorrhoeal urethritis, the former occupied 20% and the latter 80%. In 36.1% of patients with urethritis, *C. trachomatis* was detected. 17.5% of patients with gonorrhoeal urethritis had with *C. trachomatis*, too.

(2) Clinical manifestation of *C. trachomatis* urethritis is usually more mild than that of gonococceal urethritis. However, we can not distinguish *C. trachomatis* from gonorrhoeal urethritis by their own symptoms.

(3) A more useful advance has been the development of techniques that will allow rapid detection of chlamydial antigen in urogenital secretion without resorting to cultures: that is Micro Trak method using FITC-labeled monoclonal antibody and Chlamydiazyme using enzyme immunoassay.

(4) We recommend treatment with tetracycline to *C. trachomatis* urethritis for 2 weeks. In addition, some of microlides and quinolincarboxylic acids were microbiologically and clinically effective with treatment of *C. trachomatis* urethritis.

(5) We suggest the necessity of treating female sexual partners of the *C. trachomatis* urethritis patients in spite of their symptoms. Because subjective complaints of *C. trachomatis* cervicitis patients may be minimal.

5 HEMORRHAGIC FEVER WITH RENAL SYNDROME —EPIDEMIOLOGY OF RAT TYPE VIRUS—

CHIHARU MORITA
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Causative agent of HFRS belongs to Bunyaviridae HFRS virus(es) was classified to 4 serotypes. Only rats type virus, main reservoir of which is *R. norvegicus*, is revealed to be globally spreading.

In Japan, urban type outbreak and also laboratory type outbreak were reported in 1960's and 1970's respectively. In 1982, SR-11 strain was isolated from a laboratory outbreak in Sapporo and used as antigen for surveillance of wild rodents. About 28% of *R. norvegicus* in a reclaimed ground area of Tokyo Bay and 4.5% workers in the area had antibodies against HFRS virus. Although the prevalence of the antibody in the workers significantly higher than that of age-matched control group, any kinds of symptoms related to HFRS were not recorded among the workers.

Recently, we obtained *R. norvegicus* sera collected in a shore of Tokyo Bay in 1963 when

urban-type outbreak in Osaka was occured. While 9 out of 50 sera showed positive, there was no record of human case in the area.

We continuously surveied *R. norvegicus* in 3 areas of Tokyo Bay for more than 5 years. The positive ratio of rats in 3 areas were quitely different among the areas and were not depend on the population density of *R. norvegicus*. The transmission of the virus vithin rats might be regulated by unknown factors.

6 VIRAL HEMORRHAGIC FEVER —PRESENT SITUATION OF LASSA FEVER—

TAKESHI KURATA
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(Abstract not received in time)

7 EPIDEMIOLOGICAL ASPECTS OF VIRAL HEPATITIS IN JAPAN AND FOREIGN COUNTRIES

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Viral hepatitis is a common infectious disease through out the world. Three viruses and two putative viruses are known; hepatitis A, hapatitis B, delta hetatitis, epidemic non-A, non-B hepatitis and blood-borne non-A, non-B hepatisis, respectively.

1. Enterically-transmitted viral hepatitis

There are two types of enterically transmitted viral hepatitis, hepatitis A and epidemic non-A, non-B hepatitis. These types of hepatitis are only acute diseases and are not chronic.

(1) Hepatitis A

Hepatitis A is an endemic disease in various countries except North America, Middle and North Europe and Japan. In Japan, people over 40 years old have anti-HA, but almost all younger persons below 30 years old have no antibody. Therefore, young Japanese abroad are always susceptible to hepatitis A infection. Passive immunization using immunoglobulin is effective in preventing infection for several months.

(2) Epidemic non-A, non-B hepatitis

This epidemic hepatitis has been reported from tropical and subtropical countries. Usually, there were water-borne outbreaks. A high-mortality rate in the pregnant woman has been confirmed. This type of hepatitis has not been reported in Japan.

2. Parenteral-transmitted viral hepatitis

There are three kinds of parenterally transmitted hepatitis, hepatitis B, delta hepatitis, and blood-borne non-A, non-B hepatitis. Both acute and chronic hepatitis due to these viruses is seen and carrier states exist.

(1) Hepatitis B

Two per cent of Japanese are carriers of HBsAg and 20% are positive for anti-HBs. In Asian and African countries, the carrier rate reaches 6 to 10%.

The carrier with HBeAg becomes an infectious source. Infection with hepatitis B virus is mainly related to sexual performance in Japan. Vaccine is available.

(2) Delta hepatitis

The delta hepatitis virus is defective, and it needs hepatitis B virus as a helper virus. This infection is rare in Japan.

(3) Blood-borne non-A, non-B hepatitis

This is the most important viral hepatitis in Japan now, because 95% of posttransfusion hepatitis and 40% of sporadic acute hepatitis is related to this agent. Unfortunately, we know few details because the virus has not been isolated.

8 AIDS

YUICHI SHIOKAWA
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(Abstract not received in time)

9 DISSEMINATION OF *PNEUMOCYSTIS CARINII* IN A CASE OF AIDS

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Pneumocystis carinii is an opportunistic pulmonary pathogen in immuno-compromised host and extrapulmonary spread has been rarely documented. We report here a widespread case of *P. carinii* infection seen in a 24-year-old Japanese man with AIDS. He had been administered concentrated factor IX for 7 years for hemophilia B. Diagnosis of AIDS was based on *P. carinii* pneumonia (PCP) by TBLB and on HIV antibody detected in his serum by ELISA and IFA. He died on the 70th day of hospitalization and autopsy was performed. We found *P. carinii* cysts other than the lungs with Gomori's methenamine silver nitrate stain (GMS) in stomach, jejunum, ileum, colon, mesoappendix, diaphragm, thyroid gland, mesenteric lymph node, celiac lymph node, and pancreatic lymph node. *P. carinii* foci within marked fibrosis were observed in the lungs. Such lesions were seen sporadically in the sections and the lungs did not show "full-blown" PCP. In the small intestine, severe infiltrative lesions of the organism were observed in the muscle layer to the subserosa and a mucosal lesion was found in the jejunum, also. The lymph nodes in the peritoneal cavity

showed "lymphoid depletion" and necrotic lesions with calcification were seen. *P. carinii* foci were observed in and around these lesions. Some afferent lymphatic vessels contained *P. carinii* masses in the lumen. Macrophage reaction was prominent though other cellular reactions were weak on the whole. Both hematogenous spread and lymphatic spread were inferred as the mode of dissemination. We can not draw any conclusions about factors which cause dissemination of the oganism. It should be kept in mind that *P. carinii* infection is never confined to lungs, so GMS stain or Giemsa stain should be performed if there are any suspicion. More cases of extrapulmonary pneumocystosis may be confirmed, if autopsized materials of other cases of AIDS and even other immunodeficiency cases of the past are reexamined.

10 INFLUENZA IN JAPAN AND THAILAND

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Past epidemiologic records show that the influenza virus which underwent the antigenic shift was imported to Japan from foreign countries. Virus which underwent the antigenic drift may be transmitted among different countries. However, a drifted virus with a similar antigenic character of HA could be selected out in different areas in the world.

In the joint work with Virus Res. Inst. Bangkok, Siriraj Hosp., Chiang Mai Univ., and Prince Song Khla Univ. during past 10 years, following results were obtained.

1) In Thailand, although it prevails during the period of June to November centered by the rainy season, sporadics of influenza continuously develops all year round.

2) The HI antibody titer to influenza is lower in Thai people than in Japanese.

3) Each of USSR and Hong Kong subtype of type A virus prevaild in Thailand three to six months earlier than in Japan.

4) Data of HI tests with reference antisera showed that antigenic drifts of USSR subtype proceded slower in Thailand than in Japan before 1981, but drifted mutant viruses of Hong Kong subtype developed in Thailand earlier than in Japan.

5) Results of the oligonucleotide finger print mapping of whole viral RNAs and the evolutional map of virus obtained with nucleotide sequencing of the viral HA genes favored the idea that drifted mutant viruses developed independently in different parts of the world.

11 INFLUENZA IN SOUTHEAST ASIA WITH SPECIAL REFERENCE TO MALAYSIA

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The Southeast Asian countries include Thailand, Singapore, Philippines, Indonesia and Malaysia. Information on influenza outbreaks in the tropical areas had previously been scarce, mainly because laboratory facilities for the diagnosis of influenza and detection of outbreaks had been limited in these developing countries. However, with the assistance of the WHO Influenza Programme through which laboratory support and guidance have been obtained, most of these countries are now able to carry out influenza surveillance and to suppy detailed epidemiological data for general information.

This paper aims at presenting an account of the status of influenza in the Southeast Aisan countries, comparing their patterns of influenza outbreaks with one another. The possible influence of environmental and physical factors (seasons, climatic conditions, urban/rural settings, overcrowding, pollution, age, status of health, nutrition, etc.) on the frequency, spread and severity of outbreaks is noted.

The influenza situation in Malaysia from 1954 to 1987 (33 years) is reviewed and the pattern of outbreaks compared with other countries. The need or otherwise for influenza immunization in the tropics is discussed.

12 MOLECULAR BIOLOGY IN THE VIROLOGICAL FIELD OF TROPICAL MEDICINE

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Recent progress in the molecular biology and biotechnology in the studies of arboviruses (especially Japanese encephalitis virus) and rabies virus was reviewed. These studies include 1. production of monoclonal antibodies and its application 2. analysis of viral RNA genome by fingerprinting 3. nucleotide sequencing of viral genome and 4. expression of particular viral gene and its application for vaccine production.

Extensive progress can be expected in the near future from these studies in various fields from basic problems of virus such as structure of viral gene, replication of virus and regulatory mechanisms of viral gene expression to the pathogenesis of viral diseases and the production of new vaccines by gene engineering, however biological studies in a host and cellular levels should be accompanied with these studies of molecular level to be more fruitful.

13 MOLECULAR BIOLOGY OF FLAVIVIRUS

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(Abstract not received in time)

14 ANTIGENIC ANALYSIS OF DENGUE VIRUS E PROTEIN BY USING MONOCLONAL ANTIBODIES

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MIYAKO TAKEGAMI AND SUSUMU HOTTA
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We have prepared various monoclonal antibodies (MAbs) raised against dengue virus type 1(D-1) by using hybridoma technology. The biological properties of 14 MAbs were examined by enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition (HI) test, indirected immunofluorescent assay (IFA), Western blot assay and so on.

In ELISA, 4 MAbs (A group) could react with not only 4 different serotypes of dengue virus (DV) but also Japanese encephalitis virus (JEV). Six MAbs (B) could react with DV better than JEV. The reactivities of other 4 MAbs with DV were much higher than those with JEV. HI titer of each MAb to D-1 or JEV was similar to the reactivity in ELISA. A MAb of B group, 2-3G-1 showed high HI titer (1 : 800) to D-1 but low titer to JEV (1 : $\leqq$100). Neutralizing activities of 2-4D-1(A) and 2-3G-1(B) were detected but the level of activity was low. The fluorescence in D-1 infected Vero cells was mainly observed in perinuclear site, in IFA using several MAbs.

The data of ELISA and HI show that MAbs of A and C groups are flavivirus group-specific and DV complex-specific, respectively. So far, serotype-specific MAb has not been obtained. These results combined with the data of the immunological analysis using mice and polyclonal antibodies to D-1 Mochizuki suggest that the antigenicity of D-1 Mochizuki E protein may be different from other D-1 strains.

15 EFFECTS OF GALL BLADDER STONE OF BOS TAURAS UPON CHIKUNGUNYA VIRUS

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Materials and Methods:

(1) A gall bladder stone of Bos Taurus
(2) Virus. Chikungunya virus, African strain.
(3) Cells. BHK-21 cells.
(4) Virus-inhibiting test. Two-tenths m*l* of a Chikungunya Virus suspension was mixed with an equal volume of a gall bladder stone solution at 37°C. A gall bladder stone were dissolved in PBS and sterilized by filtration through membrane filters. The mixture was immediately tested for viral infectivity. Viral infectivity was titrated by the plaque method on BHK monolayer cultures under a methyl cellulose overlay medium.

A gall bladder stone consisted mainly with bilirubin calcium, colic acids and amino acids.

Results and Discussion:

pH values of a gall bladder stone solution is 7.2~7.7. A gall bladder stone solution showed no cytotoxic effect on the cells and no morphological changes could be observed under an ordinary light microscope. The virus-inhibiting rate increased gradually with increase in the dose of gall bladder stone concentration and in the period of incubate time. The viral-inhigiting rate was 100% when the concentration of gall bladder stone solution was more than 10 mg/m*l*. It was shown that the infectivity of Chikungunya virus was inhibited by being mixed with gall bladder stone and that the degree of inhibition paralleled the amount of gall bladder stone in the mixture.

The present date indicate that a gall bladder stone inactives Chikungunya virus in vitro.

16 ANTIBODY RESPONSES TO CHICK EMBRYO CELL AND HUMAN DIPLOID CELL RABIES VACCINES

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In Japan chick embyo cell (CEC) rabies vaccine was licenced to use for human in 1980. Since then the vaccine has been used for pre-and post exposure immunization mainly to high risk persons visiting or staying in countries such as Africa, India and Southeast Asia where rabies is endemic.

Neutralizing antibody responses in Japanese vaccinated with commercial CEC vaccines were compared with those in Philippine who were given commercial human diploid cell rabies vaccine (HDCV) by rapid fluorescent focus inhibition test (RFFIT). The vaccinees divided into four groups as follows: Group I consisted of Japanese who had each 1.0 m*l* of CEC vaccine subcutaneously on days 0 and 7. The persons in Group II had a booster injection of CEC rabies vaccine between 8 and 14 months in addition to primary immunization as same as Group I. Group III included Philippine who had each 1.0 m*l* of HDCV intramuscularly on days 0 and 28. The persons in Group IV had each 0.1 m*l* of HDCV intradermally on days 0, 7 and 28. At the month between 11 and 12 after the last immunization 25 (76%) in Group I (33 vaccinees) showed neutralizing antibody levels exceeding 1 : 40. Between 6 and 12

months after the last immunization, 27 (90%) in Group II (30 vaccinees) showed antibody levels exceeding 1 : 40. At the month of 12 after the last immunization, who showed the antibody levels exceeding 1 : 40 were 16 (67%) and 13 (68%) in Group III (24 vaccinees) and in Group IV (19 vaccinees), respectively.

Fewer total protein contents were shown in CEC vaccine than in HDCV by SDS-PAGE and immunoblotting tests. Despite fewer total protein contents in the CEC rabies vaccine, persistence of neutralizing antibody levels had been kept when tested at about one year after the last immunization. However, 3 vaccinees given the CEC vaccine showed neutralizing antibody titers less than 1 : 40 even after three doses vaccination.

This is the reason why serological determination for neutralizing antibody level should be made to vaccinees with preexposure vaccination of CEC vaccine.

17 CHANGE OF α_2-PLASMIN INHIBITOR-PLASMIN-COMPLEX IN PATIENTS WITH DHF

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(Abstract not received in time)

18 PHYSIOLOGY AND ECOLOGY OF RODENTS AS CHIGGER HOST

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This study aims to discuss a cause of recent resurgence of scrub-typhus in Kanagawa Prefecture. In the 1950s the chigger *Leptotrombidium pallidum* was found throughout the prefecture, and some patients ascribed to the chigger were recorded. A total of 36 patients of the disease were found from 1983 to 1986, after years of virtual absence.

The recent disease is transmitted mainly by *L. scutellare*, because 31 patients were infected limitedly from October to January, the active season of larvae of *L. scutellare*. The other five infected from Feburuary to May are attributable to *L. pallidum*.

L. scutellare is popular in the main infection spots, the western area of the prefecture bordering upon the foothills of Mt. Fuji, arround the Tomei expressway. But there in the 1950s, no researchers found the chigger despite the efforts. Therefore *L. scutellare* was introduced probably in recent years.

The expressway was opened in 1969 joining the area to the foothills of Mt. Fuji, an endemic focus of the disease transmitted mainly by *L. scutellare*. The major slopes along the expressway are covered with grass-scrub and inhabited by *L. scutellare* and the main host mouse *Apodemus speciosus*. Percent medullary thickness (M/M+C/100, M=medulla, C=

cortex) of the kidney, 72.3±1.8 (mean±S. D., n=18), for *A. speciosus* hints efficient kidneys of the mouse to thrive in arid grass-scrub areas such as the slopes.

Therefore, the slopes of the expressway expanded probably *L. scutellare* helped by the mouse. But arround the focus, there are many grass-scrub areas such as golf course periphery and abandoned farmland other than the slopes. Further investigation is needed, therefore, to understand which grass-scrub area expanded primarily the chigger and the disease.

19 A CLINICAL CASE OF SCRUB TYPHUS

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20 RECENT ADVANCES ON MOLECULAR BIOLOGY OF MALARIA RESEARCH

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A review is given on recent achievements in malaria research with special reference to knowledge gained at the molecular level but limited to topics concerning malaria chromosomes and genome organization, regulation of gene expression, and applications of molecular biology in the diagnosis and prevention of the infection.

A novel technique of pulsed-field gradient (PFG) gel electrophoresis for karyotic and linkage analysis revealed that the chromosomal DNA molecules of *Plasmodium falciparum* are composed of at least seven discrete species which vary in size. A comparison of independent cultures and fresh isolates indicated that while chromosome number is constant, the sizes of analogous chromosomes vary widely, or are polymorphic. Experiments using whole *P. falciparum* chromosomes as hybridization probes to examine polymorphism within two independent parasite populations suggested that one mode of generating polymorphisms results from deletion or duplication. Studies in progress point to the possibility that chromosomal rearrangements also occur. These observations are concordant with the phenotypic changes which take place spontaneously among cloned *P. falciparum* in continuous cultures.

Regulation of gene expression in *Plasmodium* is still little understood. The hypothesis that gametocytogenesis is related to the presence of high percentage of repetitive DNA has proven true only in *P. berghei*. In *P. falciparum*, there is a significant difference in the organization of genomes and their expression between the gametocyte-forming and non-gametocyte-forming clones. The "signals" for the genes to switch on or off still remain to

be determined. The gene for glucose 6-phosphate dehydrogenase (G6PD) in *P. falciparum* is believed not to operate in asexual erythrocytic stages when these inhabit healthy erythrocytes. In G6PD-deficient hosts, however, parasite enzymes can be detected.

Techniques developed in the field of molecular biology and biotechnology have applied fruitfully to malaria research in recent years. The most successful application is in the development of anti-sporozoite vaccine which is entering a phase III trial in Thailand. Another example is the development of specific DNA probes for the diagnosis of *P. falciparum* infection. Genetically engineered probes developed at the Faculty of Science, Mahidol University are capable of detecting parasitemia of 0.001% from as little as 30 μl of blood sample. In addition, these probes are feasible in distinguishing between different parasite isolates. Synthetic oligonucleotides to be used as probes for *P. falciparum* are also available, but still await evaluation in the malaria endemic areas.

21 DNA CONTENT OF CRISIS FORMS IN *PLASMODIUM FALCIPARUM* MALARIA CASES WITH HIGH IFA TITERS

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"Crisis forms" is a morphological term proposed by Taliaferro and Taliaferro (1944) to describe the degenerated intraerythrocytic malarial parasites caused by a host defense mechasism. The present contribution is designed to afford informations about the shape of nucleus and relative DNA content in crisis forms.

〈Sample〉 Blood with high IFA value were collected from 2 cases with imported *Plasmodium falciparum* malaria, and thin blood smears were stained with DAPI (1 $\mu g/ml$) and observed fluoromicroscopically.

〈Results〉 Crisis forms were detected in 38.8% (case 1) and 51.7% (case 2) of intraerythrocytic parasites. There was a diversity in the shape of nucleus, including ring-like, V-like and stick-like shape. Some had intranuclear vacuoles. In the case 2, there was no statistical difference in the amount of DNA between standard and crisis forms, while the case 1 had lower DNA content in crisis forms than in standard forms (33.5 ± 6.9 FU and 40.7 ± 6.2 FU respectively, $p < 0.001$).

It appears that the variation of DNA content in crisis forms may result from the inhibition of the intraerythrocytic development of parasites.

22 VARIATIONS IN A SURFACE ANTIGEN GENE OF *P. FALCIPARUM* MEROZOITE: COMPARISONS OF FOUR ISOLATES

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The malignant tertian malaria parasite, *Plasmodium falciparum*, has an antigen on the surface of merozoites. The precursor of the antigen with Mr 190 kD (p190) is synthesized at schizont stage and is processed into fragments during merozoite maturation. One of the features of p190 is antigenic diversity among parasite isolates (polymorphism). Since p190 is a candidate for a malaria vaccine, it is important to understand the polymorphic nature at the genetic level. We have determined the complete nucleotide sequence of the p190 gene from the MAD20 strain (a Papua New Guinea isolate). Comparison of the gene with that from other strains revealed that the gene consists of seven variable blocks flanked by conserved or semi-conserved blocks. Variable blocks occupying 40% of the gene showed extensive variations. Homology of sequences is between 10 and 30%. There are four 30-60 bp deletions/insertions from the middle of the gene through the edn. A shift in reading frame also occurred at the 3' variable block. What is more important is that variable sequences are not widely polymorphic but fall into two distinct types. Sequences of MAD and K1 differed at any variable block. Sequence of the first variable block of Wellcome is the same as MAD sequences but is same as K1 sequence from the second variable block down to the end. CAMP sequence is composed of MAD sequence at the 5' part and of K1 sequence at the 3' part. Together with results from Southern blots, these suggest that the p190 gene is dimorphic alleles and that reciprocal recombination within the gene, which is taken place at the sexual stage creates variations in the p190 antigen. In-depth analyses pointed out possible points at the nucleotide level of cross-over between K1 and MAD sequences to have CAMP and Wellcome sequences.

23 INDUCTION OF GAMETOCYTOGENESIS IN CULTURED *PLASMODIUM FALCIPARUM* (II). INDUCTION OF GAMETOCYTOGENESIS IN VARIOUS STRAINS

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Many unsuccessful attempts have been reported regarding the induction of gametocytogenesis in culture of *P. falciparum*. In the previous paper, however, we reported that the special RPMI 1640 medium which were prepared by dissolving powdered RPMI 1640 medium in a mixture of culture supernatants of anti-*P. falciparum* antibody producing hybridoma cells and lysate of hybridoma cells, induced gametocytogenesis in cultured *P.*

falciparum. Gametocytogenesis was consistently observed from 3 days after addition of this medium. But, induction of gametocytogenesis could not be observed only with the culture supernatant of anti-*P. falciparum* antibody producing hybridoma cells.

P. falciparum strain FVO which was isolated from a patient in 1967, was used in the previous study. Therefore, the present experiment was tried to answer a question whether induction of gametocytogenesis was demonstrated in other strains than FVO or not. Three strains, 0662, FCB1, FCR-3, and one clone, R FCR-3, of *P. falciparum* which were provided by courtesy of Dr. Mamoru Suzuki, Gunma University School of Medicine, were used in the present experiment. The result indicated that induction of gametocytogenesis was observed in all of them.

Nextly, various culture mediums were examined for their capability to enhance the induction rate of gametocytogenesis. It was found that addition of 50 μg/m*l* hypoxanthine and 500 μg/m*l* glutamine to the gametocytogenesis induction medium (the medium consists of 4 m*l* of regular RPMI 1640 medium and 4 m*l* of special RPMI 1640 medium) enhanced the induction rate of gametocytogenesis.

24 IMMUNODIAGNOSIS FOR MALARIA

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Antibodies to malaria parasite are demonstrable by any conventional technique, but their detection has little importance in clinical work, because film examination is still superior to other methods for diagnosising acutal infection. The main application of malaria serology is screeing blood donors and as epidemiological tools. The diagnosis of malaria is based on the identification of the parasites in stained blood films by light microscopy. The technique is too slow and too insensitive for use in large scale epidemiological investigations and efforts are made to develop new and more sensitive methods suitable for mass screening. The fluorescnce test has become widely use for those purposes. Recentry, the ELISA, being less time-consuming for testing numerous samples, has been applied in epidemiological survey. ELISA used antigen-coated plete which were prepared with *in vitro* infected, sonicated red blood cells. Antibodies to *Plasmodium falciparum* antigens were detected by reacting the immune sera with the antigen-coated plate, after which remaining bound to the plate was assayed by means of enzyme. We have standardised the ELISA method and more than 1,500 sera from our project area in North Sumatra, Indonesia were examined for malaria-IgG antibodies by ELISA. ELISA is also very sensitive and requires small amount of reagents, including *Plasmodium* antigen.

Summary: The ELISA could be applied for large-scale screening in antibody detection of malaria. It has several advantagen: 1) only 3μ*l* of serum is enough for the detection, 2) the results are obtainable through single reading with the least personal error, 3) the assay

does not require any particular experience, and 4) the antigen-coated plates could be used for a long time, at least one year.

25 INFECTIVITY TO *ANOPHELES STEPHENSI* OF *PLASMODIUM YOELII NIGERIENSIS* (N67) EXPOSED TO A SINGLE LARGE DOSE OF CHLOROQUINE

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Mice infected with *Plasmodium yoelii nigeriensis* were treated with 100, 10 or 1 mg/kg chloroquine on day 3 inoculation. Twelve or twenty-four hours after treatment, these mice were fed to mosquitoes and then killed to measure the chloroquine concentration in blood (by ELISA) and the degree of anaemia (by haematocrit). A week later, the fed mosquitoes were dissected and the numbers of oocysts in the mid-gut were counted.

Parasitaemias of the mice given the high dose of chloroquine decreased and the haematocrit increased with increasing chloroquine in the blood. The mean number of oocysts per mosquito fed on mice 12 hours after treatment with 100 mg/kg chloroquine was almost the same as the level in mosquitoes fed on control (untreated) mice despite the fact that no gametocytes were seen in an examination of 10,000 RBCs in thin blood films (50 fields). Though no gametocytes were seen calculated based on 95% coinfidence limits from the binomial distribution give the posibility of up to 3 gametocytes in a sample of 10,000 RBCs. This is equivalent to about 7,326 gametocytes per blood meal which would be enough to saturate the stomach wall with oocysts.

26 LONGITUDINAL SEROEPIDEMIOLOGICAL MALARIA SURVEY IN AN AMAZONOUS HYPOENDEMIC COLONY

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In 1976, and 10 years later, in 1986 and 1987, malaria field surveys were undertaken at Tomé-AÇu, Pará state, in Brazil. The place locates in an Amazon basin some 250 km south from Belém. We intended to make research to apply malaria indirect fluorescent antibody test to assess future risk of malaria epidemics in the region. In August 1976, 145 Japanese immigrant farmers and 461 normadic seasonal labourers were examined. Both groups of

people lived together in the same premises although the residences were separated. Blood specimens from each group were collected by filter paper method. Smears were also prepared but none got parasitemia. Four out of 145 Japanese settlers manifested low positive titers, while, 71 out of 461 immigrant labourers showed positive titers and 5 among the positive labourers gave the titer at 1 : 256. In 1986 and 1987, people lived in the same place were again examined. Positive cases were greatly increased in both surveys. In 1986, 25 in 107 resident Japanese farmers were positive and 9 people manifested high positive titers ($\geqq$1 : 160); 12 out of 20 examined immigrant laboures were positive and 5 among the positive labourers gave high positive titers. Fourty-nine settlers were examined in both surveys worked in 1976 and in 1986. Twelve among the 49 settlers showed positive titers reflecting recent past infections which completely coinceded with questionaire examinations. In 1987, 144 resident farmers were tested, and 31 gave positive results, however, positive case with high titer was not found. Whereas, 17 out of 60 examined immigrant laborours were positive, and 3 manifested high titers. The results indicated that epidemics during 1986-1987 was not so active as that shown in 1986. In the survey carried out in 1976, parasites were not detected in any examined inhabitat. However, IFAT results suggested latent present parasitemia or recent past infections especially among the migrant labourers. Thus, future risk of malaria epidemics was estimated only by means of IFAT but not by microscopic examination on smeared blood. During the following 10 years, malaria IFAT positivity rate of Japanese settlers have elevated to the close level shown by immigrant labourers living in the same place, which had been warned in the survey worked 10 years ago. The study has proved that IFAT works as one of the useful tool for the measurement of future risk of malaria epidemics in hypoendemic localities.

27 A SURVEY OF MALARIA, GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND DUFFY BLOOD GROUP IN SIX LOCALITIES IN GUATEMALA

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The first survey of glucose-6-phosphate dehydrogenase (G6PD) deficiency and the Duffy blood group was carried out in connection with a malariometric survey in November 1986 in the rainy season in Republic of Guatemala. In 1,510 persons, 28 patients with *Plasmodium vivax* and three patients with *P. falciparum* were found. The parasite rate was 2.1% in all age groups. The infant parasite rate was 1.2%. There was no difference in the parasite rates between the age groups. The spleen rate in children under nine years old was 0.1%. In 567 males, three persons were found to be G6PD deficient (0.5%), but no person was G6PD deficient in 943 females. Six hundred persons were examined for the Duffy blood group: 85.0% were Duffy positive and 15.0% were Duffy negative. All the patients infected with *P. vivax* were Duffy positive, which was statistically significant ($p<0.025$).

28 MANAGEMENT OF MALARIA WITH SPECIAL REFERENCE TO DRUG RESISTANCE

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This symposium is arranged by Prof. T. Kanda, President (St. Marianna University) in accordance to a key-note title and organized mainly by Prof. T. Nakabayashi (Osaka University) and Prof. Danai Bunnag (Mahidol University, Thailand) as chief-chairpersons. Prof. M. Fernex (University of Basle, Switzerland), Prof. K. T. Harinasuta (Mahidol University, Thailand), Prof. I. Ebisawa (Toho University) and Prof. Nakabayashi are designated as speakers and Prof. H. Ohtomo (Gifu University) and Dr. H. Amano (Tenri Hospital) are requested to proceed the symposium as co-chairpersons.

Since 1955, the malaria eradication program under WHO guidance on the basis of DDT residual spray and chloroquine administration had been produced remarkable effects in some areas but widly spreading of insecticide-resistant mosquitoes and drug-resistant parasites in the malarious area has badly interrupted the continuation of the program for the past decade. The malaria control program which would be integrated into PHC and executed voluntarily by each country was newly started several years ago. At present, malaria incidence rises to as many as nearly 200 millions in number in a year and almost half of the total population are residing in malarious areas. The early confirmed diagnosis, the immediate administration of proper antimalarials and the adequate symptomatic therapy to patients are equally of most importance in the clinical management of acute falciparum malaria. Chloroquine-resistant falciparum strains have already distributed in almost all malarious areas including Africa, and Fansider-resistant ones and quinine low-sensitive ones are also reported in some areas. Fansimef, MSP, (Fansidar plus mefloquine) has been widely used mainly in Thailand where the resistance problem is becoming more serious, but MSP resistance is also anticipated to occur in the near future. Quinghaosu is reported to produce a notable effect upon cerebral malaria and therapeutic effect of quinidine is also proved, whereas some lethal cases due to adverse effects of Fansieer have been known. Chemoprophylaxis and chemotherapy in malaria is now in great confusion under these condition of malaria problem. The newest knowledge and therapeutic experiences will be reported in this symposium and the following discussion related to the clinical management of acute falciparum infection, particularly topics focused on the drug-resistance problem.

29 TREATMENT AND PREVENTION OF MALARIA WITH FANSIDER

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The potentiating synergy between sulfonamides and dihydrofolate reductase inhibitor

against malaria parasites has been demonstrated by Greenberg *et al.* in 1948. They were testing sulfadiazine and chlorguanide against *Plasmodium gallinaceum* malaria in chicken. Rollo (1955) suggested an explanation for this potentiation. The sequential blockage of 2 enzymes responsible for the biosynthesis of folinic acid in the parasite.

Pyrimethamine is the most potent available antiparasitic dihydrofolate reductase inhibitor. Its plasma halflife ($t_{\frac{1}{2}}$) in man of about 100 hours. Sulfadoxine is a long-acting sulfonamide with a $t_{\frac{1}{2}}$ of about 150 hours which matches fairly well regarding the pharmacokinetics with pyrimethamine.

The potentiating synergy on several stages of development of the parasite between sulfadoxine and pyrimethamine was shown by Peters and his collaborators (1968). Richards (1986) demonstrated that sulfadoxine given concomitantly with pyrimethamine in *P. berghei* model, considerably delayed the development of resistance to both components, in an experiment lasting 52 weeks.

A fixed combination of sulfadoxine and pyrimethamine with a ratio of 20 : 1 has been developed under the trade name of 'Fansidar'. It has been marketed in European and tropical countries for the curative and suppressive treatment of malaria since 1971. It was mainly used in areas where *P. falciparum* was resistant to chloroquine.

For the treatment of severe malaria, quinine (or quinidine) has to be given (10 mg/kg in very slow infusions three times daily) followed by a single administration of 2-3 tablets of 'Fansidar'. This corresponds to 1,000 to 1,500 mg sulfadoxine and 50 or 75 mg pyrimethamine.

In 1973 4,855 patients treated with Fansidar were described in the literature and assessed by Havas *et al.* (1973). R. Leimer analyzed in 1982 73 clinical publications describing 6,581 treated subjects. In 2,576 cases of acute falciparum malaria a single dose of 2 or 3 tablets in adults gave an overall cure rate of 95%. In vivax malaria the cure rate was 95% (171 cases) and 37 patients with *Plasmodium malariae* were cured. In 15 publications 4,005 recipients of 'Fansidar' prophylaxis are described. The protection rate was 98%. For malaria suppression 'Fansidar' was normally given in a dosage of 1 tablet/week (adult dose) starting 1 week before entering the transmission area and the last dose had to be taken 4 weeks after returning.

In certain areas (Amazonas, Thai-Cambodian border) the incidence of resistance of *P. falciparum* strains to sulfadoxine-pyrimethamine increased in the early eighties. This resistance rate seems to stabilize and even decrease when the selection pressure diminishes.

'Fansider' contains a sulfonamide, sulfadoxine, and is therefore contraindicated in patients with allergy to sulfonamides. If a skin reaction or other serious side effects occur, the drug has to be stopped immediately.

The tolerability of 'Fansidar' given for curative purpose in malaria and toxoplasmosis was satisfactory, no severe adverse events being reported.

When used prophylactically, severe skin reactions of the Stevens-Johnson type have been observed. In Switzerland such reactions occurred in one out of 125,000 treated subjects. No fatalities occurred (Steffen 1986). When chloroquine or amodiaquine is associated with 'Fansidar' for malaria suppression very severe adverse events may occur including multivisceral syndromes. Therefore, such ad hoc combinations are no more recommended.

Steffen and his collaborators performed a prospective study in 1986/87 among 26,021 tourists travelling in East and West Africa. Proven malaria was assessed in 94 cases (0.4%). In those taking no antimalarial drugs, acute proven malaria occurred in 2.2%. In the 6,350

chloroquine-treated subjects the infection rate was 0.6%; in the 7,700 'Fansidar' treated 0.1%. These data include patients with good and poor compliance. Only one patient died of malaria (in the chloroquine treated group). Adverse events were reported by about 23% of the subjects receiving chloroquine and in 16.6% of those receiving 'Fansidar'. These adverse events include nausea, skin reactions and others. They were milder in the 'Fansidar' treated group than among subjects receiving chloroquine.

Wiedermann *et al.* (1984) studied the long-term tolerability of 'Fansidar' in a double-blind study versus chloroquine among 173 Austrians working in Nigeria. Except for 3 gastro-intestinal complaints in the 'Fansidar' group and 3 cases of insomnia in the chloroquine group, the long-term tolerability (6 to 24 months), including haematology and blood chemistry, was good.

When used according to the recommendations 'Fansidar' appears to be a useful drug for prevention and treatment of malaria.

30 MANAGEMENT OF MALARIA WITH SPECIAL REFERENCE TO DRUG RESISTANCE

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The details of this presentation are appeared in this journal at pp. 121-130.

31 PROBLEMS ASSOCIATED WITH TREATMENT AND PRVENTION OF MALARIA

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Drug-resistance and delay in the initiation of treatment were the major problems in 144 falciparum malaria patients who came to my attention during the period of 1966-1985. They were treated with chloroquine, Fansidar, MP (sulfamonomethoxine-pyrimethamine) tablets or quinine. Thirty percent (19 to 64 patients) of those who were treated with chloroquine were resistant to the drug but were successfully treated with either Fansidar, MP tablets or quinine. However, two patients infected in Thai-Cambodian and Thai-Burma borders were resistant to all these drugs and tetracycline.

The development of severe complication of falciparum malaria in primary infection cases, i. e. loss of consciousness, renal failure, hemorrhagic diathesis or death was related to the day of initiation of treatment. It developed in 1 of 46 cases whose treatment was started within the fifth day of illness, in 16 of 41 cases within 6-10th days of illness, in 6 of 17 cases within 11-15 days of illness, and in 2 of 12 cases in whom treatment was started on the 16th to the 67th day of illness. Intravenous quinine, peritoneal or haemodialysis for patients with

renal failure and mechanical ventilation for a pulmonary edema patient were lifesaving.

The drug(s) or drug-combination to be taken by travelers to malaria endemic areas where chloroquine-resistance is a problem is under serious consideration. We prescribe primaquine to prevent vivax malaria after 4 weeks of supressive tratment with chloroquine, although there is a controversy about the prescription.

32 A PRESUMED RELAPSE CASE OF QUARTAN MALARIA AFTER MORE THAN 40 YEARS

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It in known that quartan malaria has sometimes relapses (recrudescences) after many years of latent period. A patient, 67y., male, resident in Osaka, was infected with malaria (parasite unclear) in 1940, in Su-Chou of China and treated with quinine. Since then, the patient has fever attacks usually 2-3 times in a year, although diagnosis for malaria has not been made. In 1987, he was hospitalized for renal disorder and since April 23, Predonisolon, 40 mg/day, has been administered, as a sort of autoimmune disease was suspected. On May 16, a small ulcer was found at the gastric angle and Predonisolon dosage was reduced to 20 mg/day. He had fever attack (38.8°C) with chill on May 20, the 28th day of Predonisolon treatment. According to his report on the past infection of malaria, blood examination was done and *P. malariae* (parasitemia 0.03%) was detected on the blood smear of May 20. A radical treatment was given him with Fansidar, (2, 1, 1) and Primaquine (15 mg base/day, for 14 days), because the exact identification of parasite species was uncertain at the beginning of the treatment. Since he has no experience of oversea travel and of receiving blood transfusion, and no malaria patient has not been known in his neighborhood, this quartan malaria is presumed to be recrudescence of the quartan malaria with which he was infected in 1940, 47 years ago, in China. Recently, Ebisawa reported a recrudescence case of quartan malaria after 36 years which was probably induced by splenectomy operation. It is noticed that the recrudescence case reported here might be closely related to steroid treatment.

33 CHEMOTHERAPY OF PROTOZOAN INFECTIONS WITH PARTICULAR REFERENCE TO AMOEBIASIS: DEVELOPMENT OF NEW DRUGS AND DRUG DELIVERY SYSTEM

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(Abstract not received in time)

34 ANTI-*ENTAMOEBA HISTOLYTICA* ANTIBODY OF CYST CARRIERS

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We have already reported the possibility that micro-ELISA might be useful for screening of cyst carriers. On the basis of these previous findings it was examined if the reaction is specific, and what kind of antigen is recognized by the carriers' sera.

First, to prove that the reaction is specific, we tested the carriers' sera (negative by precipitin test) in two kinds of experimental protocols as follows.

1) Antigen blocking: Before the first antibody reaction (human test sample), epitope was covered by immunized rabbit serum. Normal rabbit serum was also used as the control.
2) absorption test: The carriers' sera incubated with whole homogenate of *E. histolytica* were compared with non-operated sera.

These lead us to judge the ELISA for cyst carriers was specific.

Next we use whole protozoa (fixed by Schaudine's) as antigen, and tried to do exzyme immunoassay. The protozoa were stained by diaminobenzidine with the carriers sera. This suggests existence of antibodies other than these detected by the ELISA. Since the reaction product was located on the amoeba cellular membrane.

By immunoblotting analysis we found a few bands. The carrier's sera formed similar bands to those by the sera from patients with amebic colitis.

35 BACTERIAL FLORA ISOLATED FROM LEISHMANIAL ULCER IN ECUADOR

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It has been well documented that clinical features of cutaneous leishmaniasis tend to differ between endemic regions in Latin America. Although various clinical pictures reflect the different species or subspecies of *Leishmania*, the genetically determined host responses to the parasite and the modification by environmental factors should be also considered. In Ecuador, the extensive epidemics of cutaneous and mucocutaneous leishmaniasis caused by *Leishmania braziliensis braziliensis* occur in low land of bilateral regions of the Andes mountains. Besides, sporatic cases of Andean leishmaniasis or "Uta" has been recently reported from mountainous regions of south-western part of Ecuador near the boundary of Peru. This type of lesions cause single or few painless lesion, and usually heal spontaneously in a short term. The causative agent is considered to be *L. b. peruviana*. In the present study, bacterial flora was isolated from Andean (high land) and mucocutaneous (low land) ulcer in an atterpt to know the effect of bacterial concomitant infection on the development of these distinct skin manifestations.

Of 51 leishmanial ulcers (11 from high land, and 40 from low land) based on the parasitological and/or immunological examinations, microogranisms were detected in 46 cases (90.2%). The overall detection rate was 81.1% in high land, and 90.5% in low land. The prevelence rate of Gram-negative rods, but not Gram-positive cocci or anaerobic bacilli was apparently different between two types of ulcer, and 18.2% in high land as opposed to 37.5% in low land. Gram-negative rods were composed of such Enterobacteriaceae as *Escherichia, Serratia, Klebsiella* and *Enterobacter*. On the other hand, histological examination showed inflammatory cell infiltrations mostly composed of small lymphocyte throughout the dermis in high land sample, while restricted to deep part of dermis in low land one.

36 *IN VIVO*-LIKE CULTIVATION OF *LEISHMANIA DONOVANI* AMASTIGOTES *IN VITRO*: USE FOR ASSAYING ANTIPARASITE AGENTS

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A mouse macrophage line, J774.1, supports *in vivo*-like cultivation of *Leishmania*

donovani in vitro. J774.1 was cultured in GIT medium containing lipopolysaccharide (1 μg/m*l*) and hemin (4 μg/m*l*), and the cells that adhered to the culture plates were exposed to prorastigotes of *L. donovani* (2S-15M) at a parasite to J774.1 ratio of 3 to 1 at 37°C and 5% CO_2 for 6 days. To examine whether the infection was successful, the cells were removed from culture plates once a day and the intracellular parasites were counted under a microscope. The infectivity was approximately 90% on day 1 through day 6.

The J774.1 cells were exposed to parasites for 1 day, and treated with a potential antiparasitic agent, carbocyclic inosine. The antiparasitic activity was then evaluated by measuring the decrease in number of host cells that remained infected. The results indicate that carbocyclic inosine is strongly toxic against intracellular *L. donovani*, without affecting the viability of cells.

37 THERAPEUTIC EFFECT OF INOSINE ANALOG IN MICE INFECTED WITH *LEISHMANIA DONOVANI* OR *TRYPANOSOMA GAMBIENSE*

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The BALB/c mice infected with *L. donovani* and the ddY mice infected with *T. gambiense* were treated with inosine analogs (carbocyclic inosine and 3'-deoxyinosine) for appraising those therapeutic effects. The mice infected with *L. donovani* promastigotes were treated with 5 different doses of each drug administered on alternative days. Four weeks after the infection, impression smear of the liver was prepared to determine the parasite load which was expressed as LDU by 1,000 hepatic cell nuclei. In the mice infected with *L. donovani*, 3'-deoxyinosine 100 mg/kg i. v. showed about 63% effect as compaired with the group of mice administered saline, and carbocyclic inosine 100 mg/kg i. v. showed about 92% effect compaired with saline. The mice infected with intraperitoneal injection of *T. gambiense* trypomastigotes were treated with drugs once a day for 4-8 days. The effect of drugs was measured by counting number of parasite on blood smear stained with Giemsa solution and survival rates of the mice. The group infected with *T. gambiense* and treated with carbocyclic inosine, all died on the fourth day as the mice treated with saline. On the other hand, 3'-deoxyinosine showed an effect to some extent. The mice treated with 3'-deoxyinosine showed twice longer survival time than the mice administered saline.

38 ULTRA-HIGH VOLTAGE ELECTRON MICROSCOPY OF *TRYPANOSOMA EVANSI*, WITH THE SPECIAL REFERENCE OF THE PATTERN OF DISTRIBUTION OF THE SUBPELLICULAR MICROTUBULES

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When an investigator examines the ultrastructure of a certain microorganism, it seems very important whether he may take the reconstitution of three dimentional structure of the organism into his consideration or not.

However, excluding of the scanning electron microscopical studies which can treat only the surface morphology of the microorganism, most of the studies related with the conventional electron microscopy remain the description of two dimentional findings, and few investigators gave their thought into three dimentional architecture of the microorganism.

The cause may be attributed to the difficulty in obtaining the sequential sections, even utilizing a diamond knife, which are necessary for the reconstitution of three dimentional architecture of the microorganism.

Now, use of the ultra-high voltage electron microscope, H-1250 (Nat. Inst. Physiol. Sci.) enables us to observe "direct" or "actual" streovisual architecture of the microorganism.

Thus, we examined on the ultrastructure of *Trypanosoma evansi*, Taiwan strain with the microscope and got some new findings which have been so far overlooking in the common transmission electron microscopy.

At this meeting, we indicated that the subpellicular microtubules ran two different directions and spun each other and took the appearance of a cloth.

We also showed in the streovisual image that the flagellum, once comming out from the reservoir, entered again into the cytoplasm. The transverse and longitudinal cutting-faces of the intracytoplasmic flagellum were already shown at the 26th meeting of this association (held at Kagoshima, 1985). It is unknown by what reason such an occult flagellum may occur.

If the existence of the axoneme associated with a paraxial rod, but having no flagellar membrane is intrinsic, then taxonomical revition of this tripanosome seems to be reasonable.

39 INFLUENCE ON THE TLA-TREATED MOUSE SPLEEN CELLS BY INCUBATION WITH THE SPECIFIC ANTIGEN IN VITRO

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(Abstract not received in time)

40 SEROEPIDEMIOLOGY OF *TOXOPLASMA* INFECTION IN 2 ISLANDS OF NAGASAKI BY ELISA

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In Hirashima Island and Enoshima Island in Nagasaki Prefecture, where human ecology and environmental conditions are the same, *toxoplasma* infection of apparently healthy inhabitants aged from 6 to 79 years was investigated by determining specific IgG antibody to *T. gondii* by ELISA. In 1985 serum specimens were collected from 283 and 270 inhabitants in Hirashima and Enoshima Islands, respectively. In 1986 serum samples were again collected from 196 and 205 inhabitants whose sera were collected in 1985 in the same islands. The overall positive rate of *toxoplasma* infection was significantly higher in Hirashima Island (44.2%) thas in Enoshima Island (34.1%). The positive rates at 7 areas in Hirashima Island were different from area to area, while at 3 areas in Enoshima Island the positive rates wer similar. The calculated annual incidence rates were similar in Hirashima Island (0.9%) and in Enoshima Island (0.8%). The positive rate increased with a linear fashion with advancing age in Enoshima Island and in a stepwise fashion in Hirashima Island. Furthermore, the calculated annual new infection risk was high at the age of 15 to 35 years (2.8%) in Hirashima Island and at the age of 65 to 75 years (3.8%) in Enoshima Island. At an interval of 1 year seroconversion (0.9%) was detected among inhabitants in Hirashima Island only. Rising rate of the antibody level was 5.8% in Hirashima Island and 2.6% in Enoshima Island. These results indicate that the prevalence pattern of *T. gondii* is different between the islands.

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