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CHARACTERISTICS OF ESCHERICHIA COLI ISOLATED IN BANGLADESH AND IN JAPAN

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Abstract: A comparative study of *Escherichia coli* strains isolated in Bangladesh and Japan was carried out. The frequency of intestinally pathogenic *E. coli* strains was 3 times higher in those from Bangladesh than from Japan. Drug sensitivity pattern showed no significant differences between isolates from the two countries, although those from Bangladesh were more resistant to ampicillin than those from Japan. The relationship between the appearance of drug resistant strains and antibiotic use was discussed.

INTRODUCTION

Infectious diseases due to bacteria are a big problem throughout the world despite widespread use of antibiotics over the past few decades. The pattern of bacterial infection varies from one region to another due to the presence of different pathogens and hosts in each environment. The natural and social environments are very different in between Bangladesh and Japan. These different environments might have brought about the dissimilar patterns of infectious diseases and of antibiotic consumption in both countries. A study of the universally present *Escherichia coli* in these 2 different environments would most likely be beneficial in understanding its ecology and the epidemiology of its diseases as well as other medical problems.

MATERIALS AND METHODS

Bacterial strains. E. coli isolated from diarrheal patients in Dhaka (Bangladesh) during the period from November 1985 to January 1986, and in Okinawa (Japan) throughout 1985 were used. Altogether there were 192 strains from Bangladesh and 157 from Japan. All isolates were collected from diarrheal stools regardless of etiology, and stored in a butt of nutrient agar (Eiken) until use.

Serotyping of enteropathogenic E. coli. Each isolate was subcultured on a nutrient agar plate, and agglutination against anti-sera (mixture of monovalent anti-O and corresponding anti-K antibody: Denkaseiken Co., Tokyo, Japan) was tested on a slide glass. After this

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screening test, the agglutinated strains (possible enteropathogenic *E. coli*=EPEC and enteroinvasive *E. coli*=EIEC) were suspended in normal saline solution and heated at 100°C for 1 hr. The heat-treated bacterial suspensions were adjusted to a turbidity of McFarland number 2, and agglutination was reexamined in a test tube against monovalent anti-O antibody (Denkaseiken Co., Tokyo, Japan). Possible enterohemorrhagic *E. coli* (EHEC) were examined with anti-serum kindly donated by Dr. Kudoh, Tokyo Metropolitan Institute of Health.

Toxin production. Production of heat labile enterotoxin (LT) was examined by a modified Elecktest (Biken test), as described by Honda *et al.* (1981), and by colony hybridization method using a ^{32}P labeled DNA probe for LT subunit A, as described by Kaper *et al.* (1981).

Drug Sensitivity. Certified original powder of ampicillin (ABPC), gentamicin (GM), minocycline (MINO), cefazoline (CEZ), and ceftizoxime (CZX) were used. The organisms were cultured overnight in heart infusion broth (Eiken) at 37°C, after which the culture fluid was diluted 10-fold with normal saline. The diluted culture fluid was inoculated on to 10 plates of heart infusion agar containing each drug in serial 2-fold dilutions ranging from 100 to 0.2 $\mu g/ml$ (0.0125 to 6.25 $\mu g/ml$ for ceftizoxime). The sensitivity of the *E. coli* isolates to each drug was expressed as a minimum inhibitory concentration (MIC).

Results

The isolation rates of enteropathogenic *E. coli* (including enterotoxigenic *E. coli*=ETEC, enteroinvasive *E. coli*=EIEC, enterohemorrhagic *E. coli*=EHEC, and traditionally so called enteropathogenic *E. coli*=EPEC) are shown in Table 1. Of 192 isolates from Bang-

Table 1 Isolation frequency of enteropathogenic *E. coli*

	Bangladesh	Japan
EPEC	20	5
EIEC	1	3
ETEC (LT)	7	0
EHEC (probable)	2	1
Total	30	9

EPEC: traditionally so called enteropathogenic *E. coli* as determined by O antigen, EIEC: specific serotype of EPEC, ETEC (LT): *E. coli* producing heat labile enterotoxin, EHEC (probable): *E. coli* with negative fermentation of sorbitol, negative ornithine-lysine decarboxylation, and bearing antigen O157.

Table 2 Serotypes of EPEC and (EIEC) detected

Antigens	Bangladesh	Japan
O1	0	1
O25	4	2
O26	4	0
O44	1	0
O86	1	0
O86 a	0	1
O111	2	0
(O124)	(1)	(0)
O126	1	1
O127 a	3	0
O128	3	0
(O136)	(0)	(1)
O142	1	0
(O144)	(0)	(1)
(O164)	(0)	(1)
Total	20 (1)	5 (3)

ladesh, 20 strains of EPEC (10.4%) and 7 strains of LT producing ETEC (3.6%) were detected. On the other hand, in 157 isolates from Japan, 5 strains of EPEC (3.2%) and none of ETEC were found. A small number of EIEC and probable EHEC were detected from both countries. In total, the isolation rates of enteropathogenic *E. coli* were 15.6% (30/192) and 5.7% (9/157) of the total isolates in Bangladesh and Japan, respectively. Detected serotypes of EPEC and EIEC are shown in Table 2.

Drug sensitivity patterns are shown in Tables 3 and 4. And in Figures 1 to 5, the sensitivities of the isolates to each drug are expressed as cumulative MIC distribution curves, compared in both countries. In general, there were no significant differences between the isolates of the two countries, except that isolates from Bangladesh had moderately higher ampicillin MICs than those from Japan.

Discussion

Among the many factors influencing the incidence of diarrheal diseases, distribution of pathogens and susceptible hosts should be noted from an epidemiological point of view. Furthermore, the mode of interaction between the pathogen and its host (Host-parasite relationship) is important in pathological conditions. The incidence of diarrheal diseases due to bacteria appeared to be much higher in Bangladesh than in Japan, and indeed in the present study the isolation rate of intestinally pathogenic *E. coli* was 3 times higher in strains of Bangladesh origin than those obtained in Japan. These results agreed well with the incidence

Table 3 Drug sensitivity of *E. coli* isolated in Bangladesh

Concentration ($\mu g/ml$)	Drugs				
	ABPC	GM	MINO	CEZ	CZX
100<	105	0	0	1	×
100	4	0	1	1	×
50	1	0	1	1	×
25	1	1	7	7	×
12.5	3	1	31	27	×
6.25	18	0	50	26	×
3.13	39	9	20	19	0
1.56	16	38	27	61	0
0.78	5	123	39	45	0
0.39	0	11	13	3	0
0.20(≧)	(0)	(9)	(3)	(1)	3
0.10	×	×	×	×	11
0.05	×	×	×	×	60
0.025	×	×	×	×	87
0.0125	×	×	×	×	27
0.0063≧	×	×	×	×	4

Table 4 Drug sensitivity of *E. coli* isolated in Japan

Concentration ($\mu g/ml$)	Drugs				
	ABPC	GM	MINO	CEZ	CZX
100<	34	0	0	4	×
100	1	0	1	0	×
50	1	0	3	3	×
25	3	0	6	1	×
12.5	6	0	8	2	×
6.25	43	9	10	8	×
3.13	42	22	24	24	0
1.56	16	110	47	71	0
0.78	11	15	55	44	0
0.39	0	1	3	0	0
0.20(≧)	(0)	(0)	(0)	(0)	0
0.10	×	×	×	×	10
0.05	×	×	×	×	50
0.025	×	×	×	×	63
0.0125	×	×	×	×	29
0.0063≧	×	×	×	×	5

Values indicate the number of strains whose growth was inhibited at a given concentration of drug. ABPC : ampicillin, GM : gentamycin, MINO : minocycline, CEZ : cefazorine, CZX : ceftizoxime, ×: not examined at these concentrations.

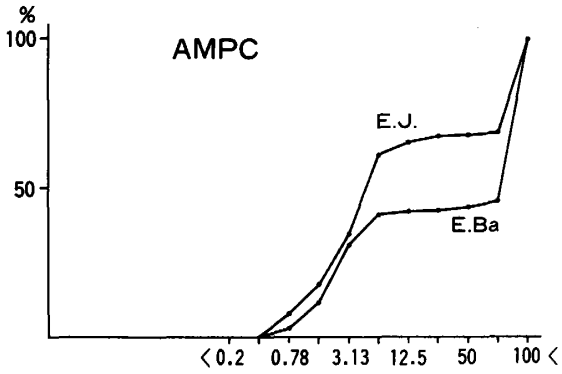

Figure 1

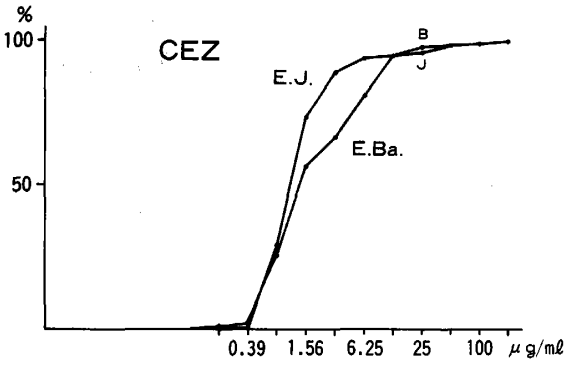

Figure 4

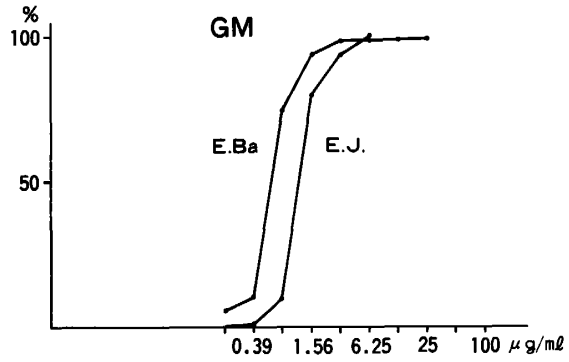

Figure 2

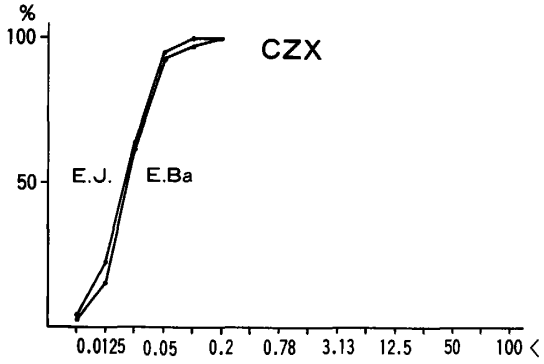

Figure 5

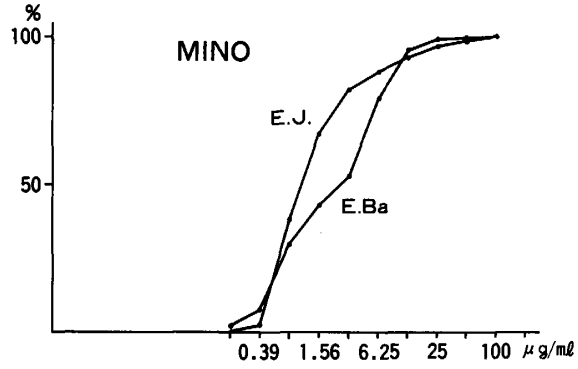

Figure 3

Figs. 1 to 5
Cumulative MIC distribution curves, expressed as percentage of isolates, based on the data in Table 3 and Table 4. ABPC: ampicillin, GM: gentamycin, MINO: minocycline, CEZ: cefazolin, CZX: ceftizoxime, E. Ba.: *E. coli* of Bangladesh, E. J.: *E. coli* of Japan.

of illness. Further studies on distribution and density of pathogens in non-diarrheal stools and in the environment, and of susceptible hosts, are required in order to fully understand the epidemiology of these diseases.

Isolation frequencies of EPEC have previously been reported (Edelman *et al.*, 1983; Komalarini *et al.*, 1977; Machii *et al.*, 1983; Sen *et al.*, 1983; Utsunomiya *et al.*, 1982), but the data varied according to the season and the technical effort applied to the detection. Therefore, comparative evaluation of results from different studies requires some scepticism.

In the detection of pathogens in the present study, 6 strains of ETEC were found by the Biken-test (modified Eleck's method), while tox gene-encoded DNA (ent plasmid) was detected in 7 strains by colony hybridization method. About 50% of *E. coli* isolates which were agglutinated on slide glass by anti-sera (mixture of monovalent anti-O and anti-K) to EPEC, following heat treatment were not agglutinated by monovalent anti-O sera in the test tube method. This means that test tube agglutination of heat treated organisms with monovalent anti-O sera is an essential part of isolate identification. EHEC is a newly recognized group of enteropathogenic *E. coli* (Levine and Edelman, 1984), which was characterized by its antigen O157: H7 (Levine and Edelman, 1984), by negative fermentation of sorbitol (Farmer III, and Davis, 1985), and further, by negative decarboxylation of ornithine and lysine (Haldane *et al.*, 1986). In the present study, strains with negative sorbitol-ornithine-lysine reactions, and bearing antigen O157 were regarded as EHEC, though termed probable EHEC, since anti-H7 anti-serum was not available.

The drug sensitivity patterns of bacteria in a given district may be greatly influenced by the pattern of consumption of antimicrobial agents; in some instances, this has been definitely proved (Glass *et al.*, 1980; Towner *et al.*, 1980). It is immediately recognizable that the consumption rate of antibiotics is far greater in Japan than in Bangladesh, especially of cephalosporines. Nevertheless, the sensitivity patterns of isolates from both countries were quite similar, suggesting that appearance of antibiotic-resistant strains is not necessarily accompanied by large scale use of antibiotics.

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バングラデシュおよび日本で分離した大腸菌の性状について

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バングラデシュおよび日本で分離した下痢便由来の大腸菌について，腸管病原菌の頻度および薬剤感受性を比較検討した。腸管病原菌の頻度はバングラデシュの192株中30株（15.6%），日本の157株中9株（5.7%）とその差は約3倍であった。薬剤感受性はアンピシリン（ABPC），ゲンタマイシン（GM），ミノマイシン（MINO），セファゾリン（CEZ），セフチゾキシム（CZX）の5薬剤について，それぞれの最小発育阻止濃度（MIC）をもって比較した。ABPCのMICでバングラデシュの株が日本の株よりもやや高値を示した他は，両国の株間に感受性の差は殆どみられなかった。抗生剤の使用頻度・使用量および耐性菌の出現状況の関係，また両国における社会的・自然的環境と病原菌の分布などについて考察を加えた。

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NOTES ON BLACKFLIES (DIPTERA: SIMULIIDAE) FROM SULAWESI, INDONESIA

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Abstract: A total of nine taxa of Simuliidae, including six new species, were collected from northern Sulawesi, Indonesia. All belong to the genus *Simulium* Latreille, and are assigned to the subgenera *Nevermannia* Enderlein {*S.* (*N.*) *aureohirtum* Brunetti and another unnamed species}, *Morops* Enderlein (1 new species), *Gomphostilbia* Enderlein (3 new species and another unnamed species) and *Simulium* Latreille s. str. (2 new species). Descriptions of all the new species are provided, with keys for their separation. Notes on their taxonomic relationships and the ecology of the immature stages are given.

INTRODUCTION

The simuliid fauna of Indonesia has not been studied since Edwards (1934) described 11 new species from Sumatra, Java and Bali, making a total of 18 species for this archipelago, although those of neighbouring areas in the Oriental Region have recently been studied (e. g. Sabah by Smart and Clifford, 1969; Philippines by Takaoka, 1983)

The present paper reports the results of blackfly collections made for the first time in Sulawesi during 1985 as part of the Royal Entomological Society's "Project Wallace Expedition". The majority of blackfly specimens examined were collected by the junior author; in addition some other samples which were collected by other investigators were loaned for study from British Museum (Natural History). In total, nine taxa of simuliids are treated, consisting of one known species, two unnamed species and six new species. The new species are described and keys are provided for all stages. Notes on their taxonomic affinities with related species are presented, as well as notes on their ecology.

MATERIALS AND METHODS

1. Study Area

Collections were made in two areas of northern Sulawesi during August and September 1985: —

a) The Toraut/Tumpah river system in the Dumoga-Bone Reserve (0°34′N; 123°54′E). The River Tumpah is a wide (20-30 m) but shallow river flowing on a rock bed with little trailing

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vegetation through primary forest. It descends steeply, and was sampled at different altitudes from 550 to 200 m, where it joined the R. Toraut, a similar sized river. The R. Toraut then flows through the farmland areas of the Dumoga Valley, where small tributaries flowing off the surrounding hills were sampled, as well as the irrigation channels on the valley floor.
b) Small streams flowing into Lake Mooat (0°45′N; 124°27′E) at an altitude of 1,100 m. Most of the streams sampled flowed through coffee plantations, but two were sampled in the forest. All were small (maximum of 2 m wide) and fairly slow flowing.

2. Taxonomic Study

The majority of the taxonomic specimens used were collected as larvae and pupae, from which adult males and females were reared. The specimens were preserved in 80% ethanol. The laboratory procedures used in this work were almost the same as those described by Takaoka (1983). The measurement of the hind basitarsus and tarsal claw tooth follows that of Davies and Györkös (1987). The morphological features and terms used follow those of Crosskey (1969)

3. Ecological Study

At each collecting site, the river width, depth and altitude were noted. In order to investigate the velocity preferences of each species, artificial substrates were suspended in six different velocity ranges for three sites in the R. Tumpah and one in the R. Toraut, using an experimental design described in Roberts and Okafor (1987). However, very few larvae colonized either polythene stripes or strings, so the experiment was abandoned. Instead, as far as possible, all the pupae within the sampling site were collected up. Where each pupa was found, the substrate type was noted and the velocity measured using a 2 cm wide ruler calibrated against a velocity meter (see Roberts and Okafor, 1987), which allows spot measurements of velocity. This method involves some error in underestimating high velocity preferences, particularly in small streams, but allows comparison of velocity preferences of different species in the same river.

Disposition of Type Specimens

All holotype, allotype and some of paratype specimens are in due course deposited in British Museum (Natural History), London, and some paratypes are also deposited in Bernice P. Bishop Museum, Honolulu, Hawaii.

Systematics

The present study follows the classification of Crosskey (1981), in which subgenus *Eusimulium* Roubaud within the genus *Simulium* Latreille s. l. is divided into two subgenera: *Eusimulium*, which is restricted for the *aureum* group, and *Nevermannia* Enderlein, which is used for the rest of *Eusimulium* in old sense. Accordingly, nine taxa collected from Sulawesi are classified as follows:

Genus *Simulium* Latreille s. l.

Subgenus *Nevermannia* Enderlein

ruficorne group

S. aureohirtum Brunetti, 1911

feuerborni group

S. sp. A

Subgenus *Morops* Enderlein

S. disneyi sp. nov.

Subgenus *Gomphostilbia* Enderlein

ceylonicum group

S. sulawesiense sp. nov.

S. torautense sp. nov.

S. rosemaryae sp. nov.

S. np. B

Subgenus *Simulium* Latreille s. str.

melanopus group

S. dumogaense sp. nov.

S. tumpaense sp. nov.

KEYS TO THE SPECIES OF SIMULIIDAE IN SULAWESI

Adult females

1. Basal section of R haired, claws with large basal tooth 2
   Basal section of R bare, claws simple or with small basal tooth 6
2. Katepisternum bare ..... *S. (N.) aureohirtum*
   Katepisternum haired 3
3. Pleural membrane haired ..... *S. (N.) disneyi*
   Pleural membrane bare 4
4. Mid basitarsus entirely dark brown; mandible without teeth on outer margin ..... *S. (G.) sulawesiense*
   Mid basitarsus whitish yellow on basal 1/3 or 1/2; mandible with teeth on outer margin 5
5. Mid and hind tibiae brown except basal extreme pale white ..... *S. (G.) torautense*
   Mid and hind tibiae white on basal 1/2 and brown on distal 1/2 ..... *S. (G.) rosemaryae*
6. Claws with small basal tooth ..... *S. (S.) dumogaense*
   Claws without tooth ..... *S. (S.) tumpaense*

Adult males

1. Basal section of R haired 2
   Basal section of R bare 4
2. Katepisternum bare ..... *S. (N.) aureohirtum*
   Katepisternum haired 3
3. Hind basitarsus slender, parallel-sided (Fig. 11b) ..... *S. (G.) torautense*
   Hind basitarsus enlarged, wedge-shaped (Fig. 12b) ..... *S. (G.) rosemaryae*
4. Hind basitarsus white on basal 1/4 and brownish black on the rest (Fig. 13b); style with non-serrated, pointed basal protuberance (Fig. 26d) ..... *S. (S.) dumogaense*

Hind basitarsus white on basal 1/2 or a little more and brownish black on the rest (Fig. 14b); style with serrated basal protuberance (Fig. 27d) ……*S.* (*S.*) *tumpaense*

Pupae

1. Gill with 6 filaments …… 2
   Gill with 8 filaments …… 5
2. Last abdominal segment with terminal hooks; cocoon wall pocket-shaped, without distinct high anteroventral neck …… 3
   Last abdominal segment without terminal hooks; cocoon shoe-shaped, with distinct high anteroventral neck …… 4
3. Tergum 6 without spine-combs; last abdominal segment lacks grapnel-like hooklets… ……*S.* (*N.*) *aureohirtum*
   Tergum 6 with spine-combs; last abdominal segment with a few grapnel-like hooklets ……*S.* (*G.*) *rosemaryae*
4. Trichomes on head and thoracic integument branched ……*S.* (*S.*) *dumogaense*
   Trichomes on head and thoracic integument simple ……*S.* (*S.*) *tumpaense*
5. Stalk of ventral pair of filaments short and dorsal and middle triplets not arising on the same vertical plane (Fig. 28b); cocoon with anterodorsal projection (Fig. 33a)…… ……*S.* (*G.*) *torautense*
   Stalk of ventral pair of filaments long and dorsal and middle triplets arising nearly on the same vertical plane (Fig. 30c); cocoon without anterodorsal projection …… ……*S.* (*G.*) sp. B

Larvae

1. Ventral papillae present …… 2
   Ventral papillae absent…… 5
2. Postgenal cleft shorter than postgenal bridge …… 3
   Postgenal cleft much longer than postgenal bridge …… 4
3. Postgenal cleft a little shorter than 1/2 of postgenal bridge; abdomen with distinct dorsal colored pattern ……*S.* (*N.*) sp. A (*feuerborni*-group)
   Postgenal cleft a little shorter than postgenal bridge; abdomen without any dorsal colored pattern ……*S.* (*N.*) *aureohirtum*
4. Cephalic apotome pale with faint positive head spots (Fig. 41a); posterior abdominal segments covered dorsally with branched minute spines……*S.* (*G.*) *rosemaryae*
   Cephalic apotome largely darkened centrally and posteriorly (Fig. 40a); abdominal segments covered dorsally with simple minute spines ……*S.* (*G.*) *torautense*
5. Postgenal cleft subtriangular, gradually narrowed anteriorly (Fig. 42b) …… ……*S.* (*S.*) *dumogaense*
   Postgenal cleft rounded, widest at basal 1/3 (Fig. 43b) ……*S.* (*S.*) *tumpaense*

Species Accounts

Subgenus *Nevermannia* Enderlein

Simuliun (*Nevermannia*) *aureohirtum* Brunetti

Simulium aureohirtum Brunetti 1911: 283-288; Edwards, 1934: 134-137.

Simulium (*Nevermannia*) *aureohirtum*: Ogata, 1956: 61-62; Ogata, 1966: 129.
Simulium (*Eusimulium*) *aureohirtum*: Puri, 1933: 1-7; Ogata and Sasa, 1954: 325; Ogata, Sasa and Suzuki, 1956: 73; Crosskey, 1973: 423; Takaoka, 1976: 170-171; Takaoka, 1979: 382-384; Takaoka and Suzuki, 1984: 11-12.
Eusimulium aureohirtum: Orii, Uemoto and Onishi, 1969: 1-13
Simulium (*Eusimulium*) *tuaranense* Smart and Clifford, 1969: 40-43. Syn. by Crosskey 1973.
Simulium (*Eusimulium*) *philippinense* Delfinado 1962: 47-62. Syn. by Takaoka 1983.

Simulium (*N.*) *aureohirtum* was originally described from Umling, Assam, India (Brunetti, 1911). The female, male, pupa and larva of this species were redescribed by Puri (1933) and Takaoka (1979). The morphological characters of the Sulawesi specimens conform at all stages to the redescription given by Takaoka (1979), which was based on Taiwanese specimens, except that upstanding hairs on the male scutellum are not brown but pale, and the upper eye of the male is composed of medium-sized facets in 22 or 23 horizontal rows in place of 18 rows. With regard to the latter character, a reexamination showed that the Taiwanese male specimens practically have the same rows of medium facets in the upper eye as do the Sulawesi specimens. In redescription, a few short rows of facets near the under margin unfortunately escaped the counting.

This species belongs to the *ruficorne* group and is widely distributed in the Oriental Region and parts of the Palaearctic Region. This is a first record for this species from Sulawesi.

Material examined: 4 females, 12 males, 24 pupae and 14 mature larvae, SULAWESI: Dumoga-Bone Reserve, tributaries of R. Toraut, D. M. Roberts, Sept. 1985; 1 female (BMNH), collected by light trap, Dumoga-Bone Reserve, Project Wallace Base Camp area, R. H. L. Disney, Sept. 1985.

Ecological notes: The Sulawesi population of this species may be autogenous, as already reported elsewhere by Takaoka and Noda (1979). It was found in some small streams 20 cm-2 m wide flowing into the R. Toraut in the Dumoga Valley at an altitude of 200 m, where it was associated with *S.* (*G.*) *torautense, S.* (*S.*) *tumpaense* and *S.* (*G*). *rosemaryae*. It was also found by itself in a stream flowing into Lake Mooat at an altitude of 1,100 m. Immature stages were normally attached to dead leaves or trailing grass.

Distribution: India, Sri Lanka, Thailand, China (Yunnan), Sumatra, Java, Borneo, Sulawesi (new record), Philippines, Taiwan, Japan.

Simulium (*Nevermannia*) sp. A

This species seems to be assignable to the *feuerborni* group, as defined by Datta (1973), by the gill histoblast with six filaments, the small postgenal cleft (a little less than 1/2 as long as postgenal bridge), and the presence of the characteristic dorsal colored pattern on the larval abdomen. The morphological characters of the larva mostly agree with those of *S.* (*N.*) *feuerborni* reported from Java and Bali (Edwards, 1934). The dorsal colored pattern on the larval abdomen, which seems to be species-characteristic in this species group, is very similar to each other. The adult and pupal specimens are needed for final identification.

This represents the first distribution record of the *feuerborni* group in Sulawesi.

Material examined: 2 early mature larvae, 4 immature larvae, SULAWESI: Dumoga-Bone Reserve, tributary of R. Tumpah, D. M. Roberts, Sept. 1985.

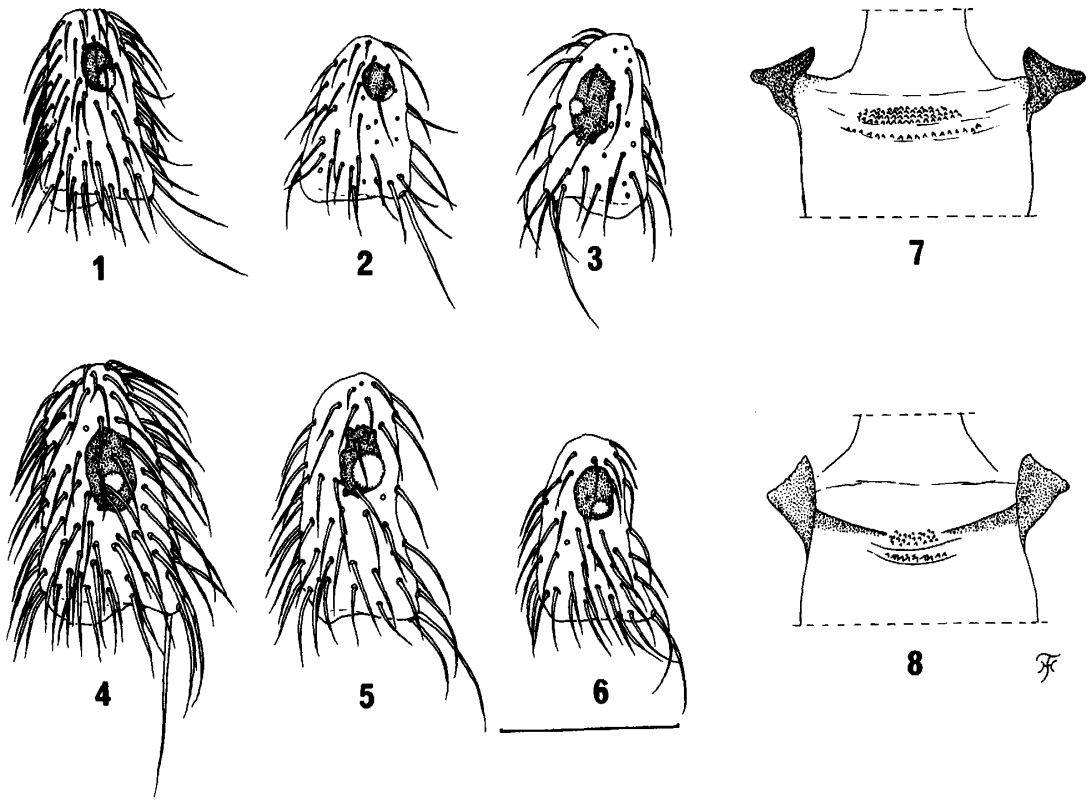
Ecological notes: Unknown.

Distribution: Sulawesi.

Subgenus *Morops* Enderlein

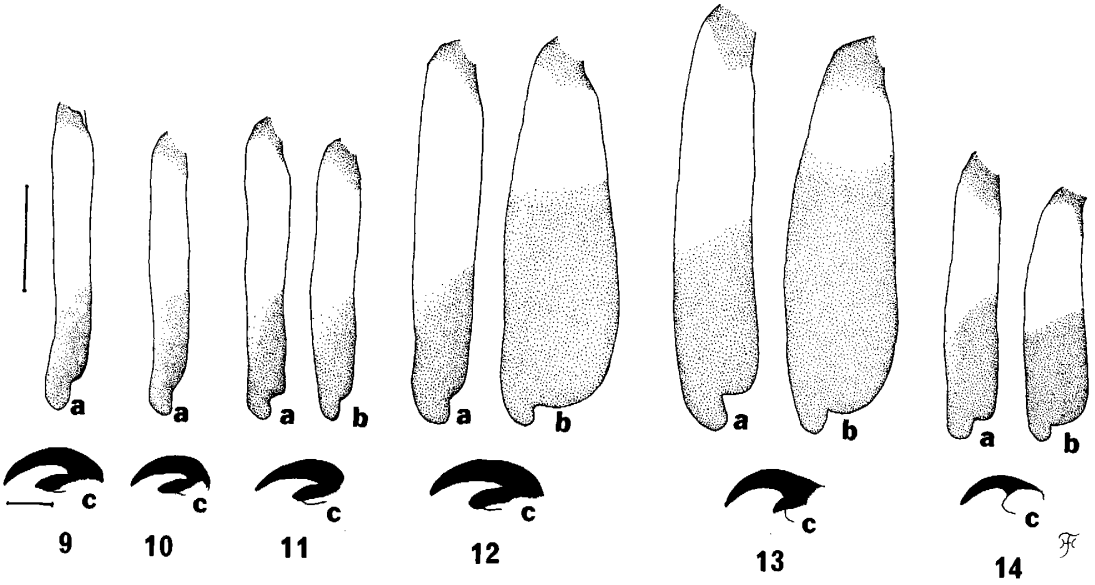
***Simulium* (*Morops*) *disneyi* sp. nov.**

Female. Body length 3.2 mm. Wing length 2.0 mm. *Head.* Nearly as wide as thorax. Frons brownish black, semishiny, whitish grey pruinose, and covered densely with whitish yellow appressed pubescence; frontal ratio 1.6:1.0:3.0; frons-head ratio 1.0:6.4. Clypeus brownish black, semishiny, whitish grey pruinose, and densely covered with whitish yellow appressed pubescence, and sparsely with dark hairs. Antenna composed of 2+9 segments, brown except scape, pedicel and base of 1st flagellar segment yellow. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.06:2.6; 3rd segment normal in shape and size, and with small sensory vesicle which is about 1.6×as long as wide, and about 1/4×as long as 3rd segment (Fig. 1). Maxilla with 12 inner teeth and 13 outer teeth. Mandible serrulated only on inner side, with 24 teeth, and devoid of any distinct teeth on outer side. Cibarium without denticles. *Thorax.* Scutum faintly whitish grey pruinose on brownish black background, with 3 faintly discernible longitudinal black vittae (1 medially and 2 submedially); scutum densely covered with whitish yellow appressed pubescence. Scutellum dark brown, faintly whitish grey pruinose, and with long and short hairs. Postscutellum brownish black, faintly-whitish grey pruinose, and bare. Pleural membrane with 4 pale hairs on upper

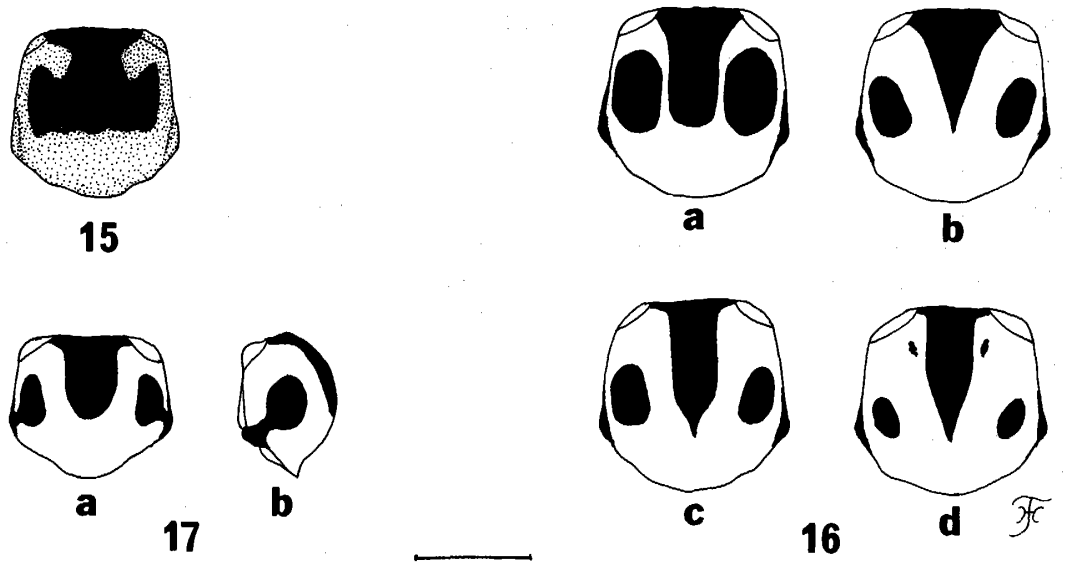


Figs. 1-8 Third segments of maxillary palps of female adults showing sensory vesicle (Figs. 1-6) and cibaria (Figs. 7 and 8): 1, *S. disneyi*; 2, *S. sulawasiense*; 3, *S. torautense*; 4, *S. rosemaryae*; 5 and 7, *S. dumogaense*; 6 and 8, *S. tumpaense*. Scale 0.1 mm.

region. Katepisternum longer than deep, and with numerous whitish yellow hairs as well as numerous dark hairs. *Legs.* Fore coxa whitish yellow, mid coxa dark brown and hind one brown. Fore and mid trochanters brown, and hind one whitish yellow. All femora brown with distal 1/3 of hind femur dark brown. Fore tibia brown with median portion largely pale brown on outer surface. Mid tibia brown except extreme base whitish yellow, and hind tibia whitish yellow on basal 1/5, and brown on the rest (somewhat darkened distally with distal 1/3 dark brown) and with dark trace of subbasal ring laterally; hind tibia covered with white appressed pubescence on posterior and lateral surfaces of basal 3/5 of their shaft. Fore tarsi brownish black; basitarsus cylindrical, 5.5×as long as its greatest width. Mid tarsi dark brown to brownish black. Hing tarsi brownish black except basal 2/3 of basitarsus (though base of basitarsus brown) and basal 1/2 of 2nd segment white; basitarsus (Fig. 9a) narrow, nearly parallel-sided. Calcipala (Fig. 9a) well developed, about 7/10×as wide as width of distal portion of basitarsus, and its tip reaching distal 1/3 of 2nd tarsal segment. Pedisulcus well developed. Claw (Fig. 9c) with basal tooth, which is 1/2×length of claw. *Wing.* C with spinules and hairs. Sc setate throughout its length on undersurface. Basal section of R haired. R_1 with spinules and hairs. R_2 with dark hairs. Basal cell absent. *Haltere* white, with petiole dark. *Abdomen.* Basal scale dark brown with white hair fringe. Dorsal surface of abdominal segments dark grey with tergites dark brown to brownish black, and sparsely covered with dark hairs; tergites of segments 6–8 shiny. *Genitalia.* Sternite 8 bare medially and with about 22 stout hairs laterally on each side. Anterior gonapophysis simple, membraneous, and with numerous microsetae; inner margin moderately sclerotized; posteromedial corner largely rounded (Fig. 18a). Genital fork (Fig. 18a) reversed-Y shaped, with well sclerotized stem; arms diverged laterally, and with well sclerotized distal ridge which is somewhat produced anteriorly. Paraproct (Figs. 18a, c) simple, with about 30 stout hairs on



Figs. 9-14 Hind basitarsi and tarsal claws: 9, *S. disneyi*; 10, *S. sulawesiense*; 11, *S. torautense*; 12, *S. rosemaryae*; 13, *S. dumogaense*; 14, *S. tumpaense*. a, hind basitarsus of female; b, hind basitarsus of male; c, female tarsal claw. Scales 0.2 mm for basitarsi, and 0.02 mm for tarsal claws.



Figs. 15-17 Scutal patterns of male adults: 15, *S. torautense*; 16, *S. dumogaense*. a-d, showing different patterns; 17, *S. tumpaense*. a, dorsal view; b, lateral view. Scale 0.5 mm.

lateral and ventral surface. Cercus (Fig. 18c) semilunar in lateral view, about 1/2×as long as wide, and moderately setose. Spermatheca (Fig. 18b) ellipsoidal, about 1.4×as long as wide, well sclerotized except small area of tubular base unsclerotized, and with minute internal setae.

Male, pupa and larva: Unknown.

Type specimen: Holotype female (BMNH), slide mounted, SULAWESI: Dumoga-Bone, Project Wallace Camp area, captured by light trap, R. H. L. Disney, Sept. 1985.

Ecological notes: Unknown except the fact that the gravid female was caught by light trap.

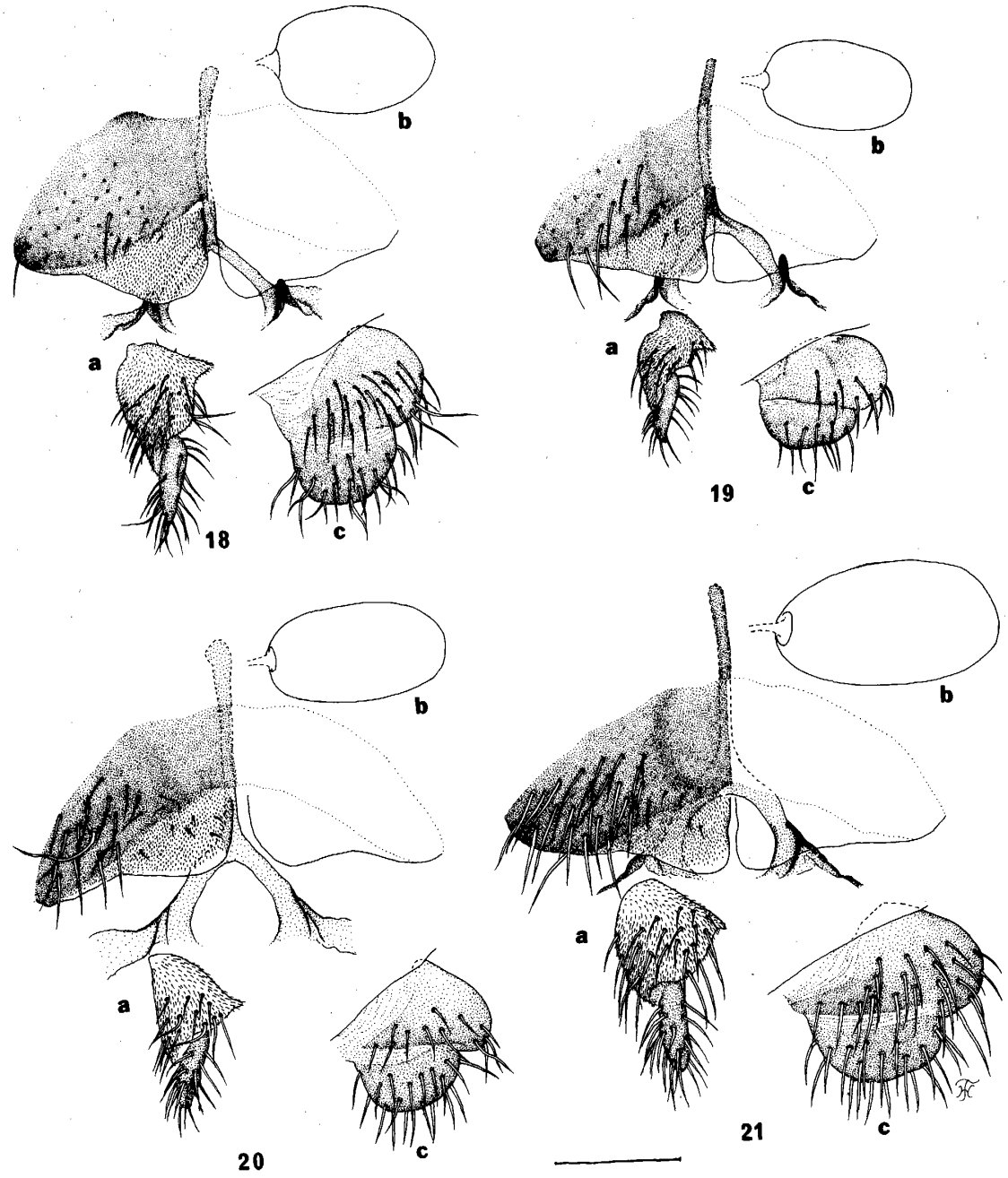
Distribution: Sulawesi.

Remarks: This new species is named after Dr. R. H. L. Disney who collected this species. This is assigned to the subgenus *Morops*, because hairs are present on both katepisternum and pleural membrane. This species seems close to *S.* (*M.*) *liliwense* Takaoka, 1983 and *S.* (*M.*) *salazarae* Takaoka, 1983 from the Philippines in having the small number of hairs on the pleural membrane, dark coloration of the hind tibia, and similar genitalia. However, it differs from the latter two species by the small sensory vesicle (its length against length of 3rd segment of maxillary palp: 1/4 versus 1/2.6 or 1/2.8), and by the small number of inner teeth on the mandible (24 versus 28 or 30).

Subgenus *Gomphostilbia* Enderlein

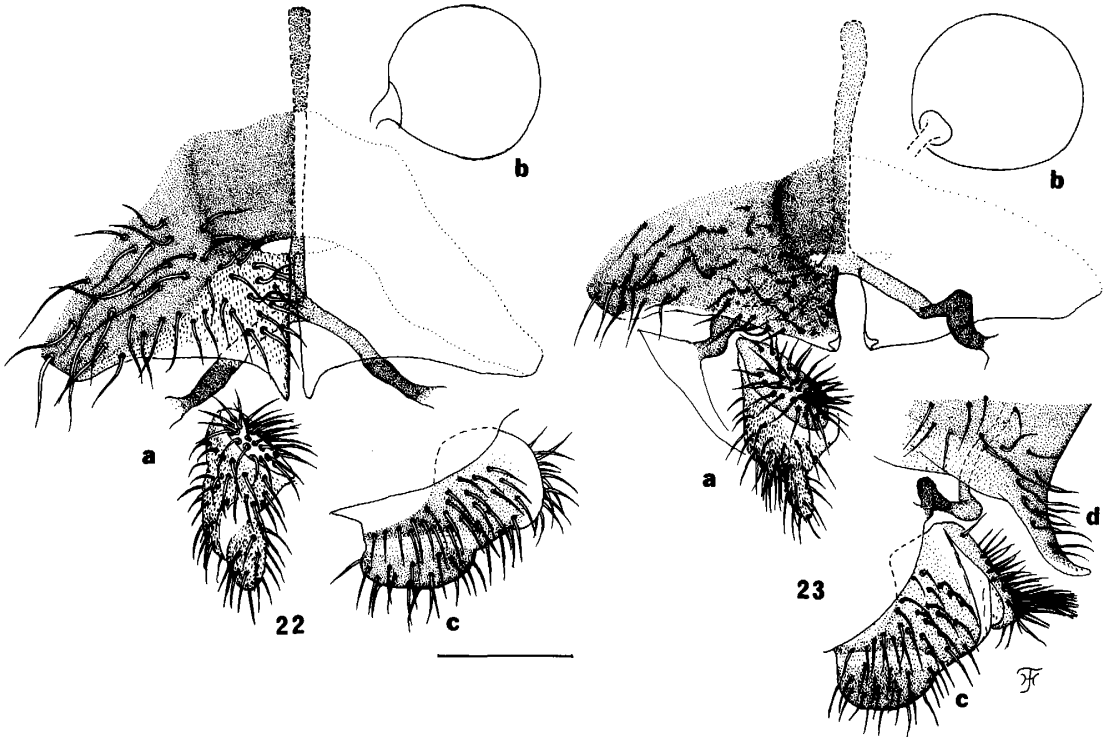
***Simulium* (*Gomphostilbia*) *sulawesiense* sp. nov.**

Female. Body length 2.5 mm. Wing length 1.8 mm. *Head.* Slightly narrower than width of thorax. Frons brownish black, semishiny, whitish grey pruinose, and covered densely with whitish yellow appressed pubescence; frontal ratio 1.9:1.0:2.0; frons-head ratio 1.0:3.6. Clypeus brownish black, semishiny, whitish grey pruinose, and densely covered with whitish yellow appressed pubescence. Antenna composed of 2+9 segments, brown except scape,

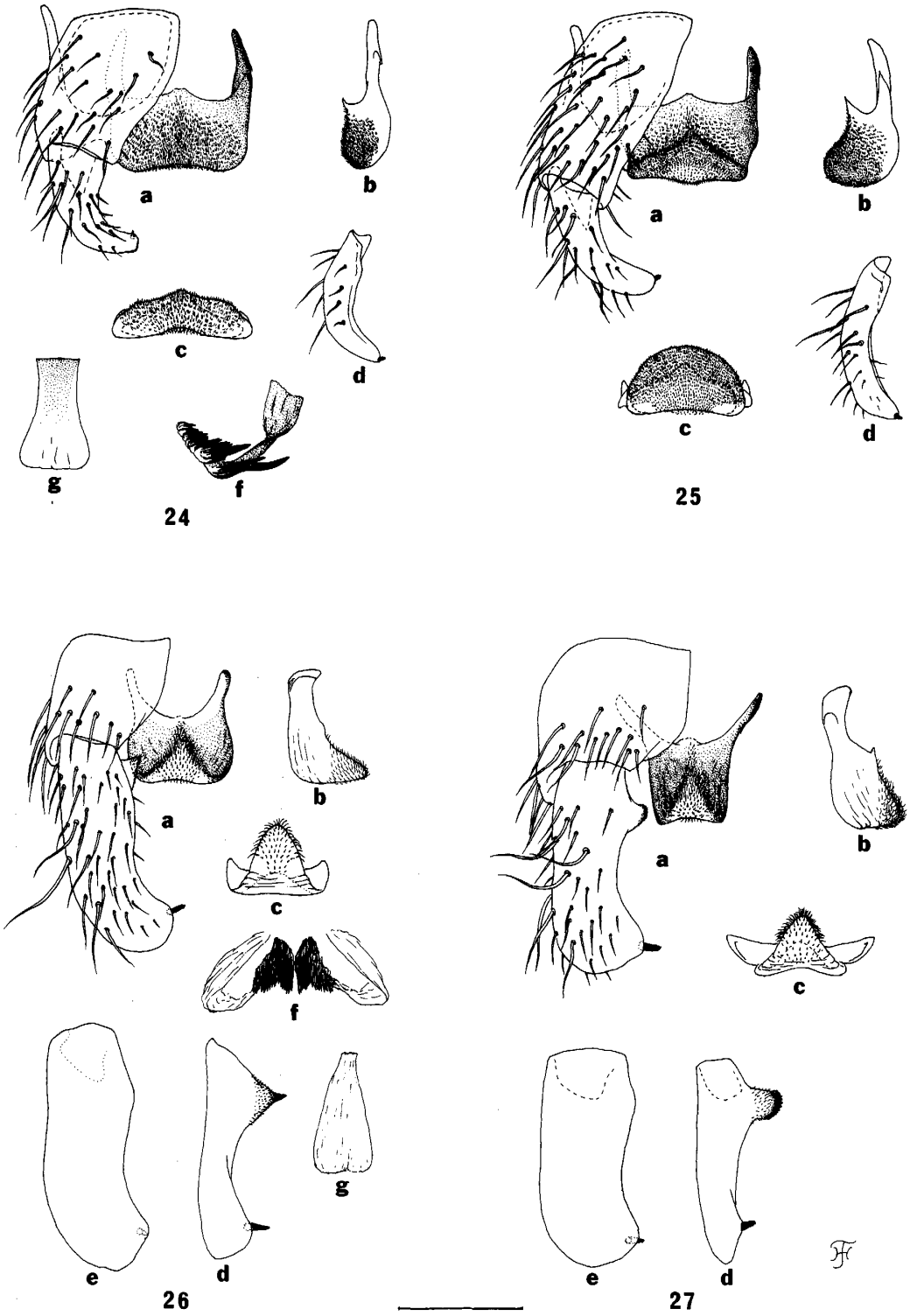


Figs. 18-21 Female genitalia: 18, *S. disneyi*; 19, *S. sulawesiense*; 20, *S. torautense*; 21, *S. rosemaryae*. a, 8th sternite, anterior gonapophyses, genital fork, paraproct and cercus in situ (ventral view); b, spermatheca; c, paraproct and cercus in side view. Scale 0.1 mm.

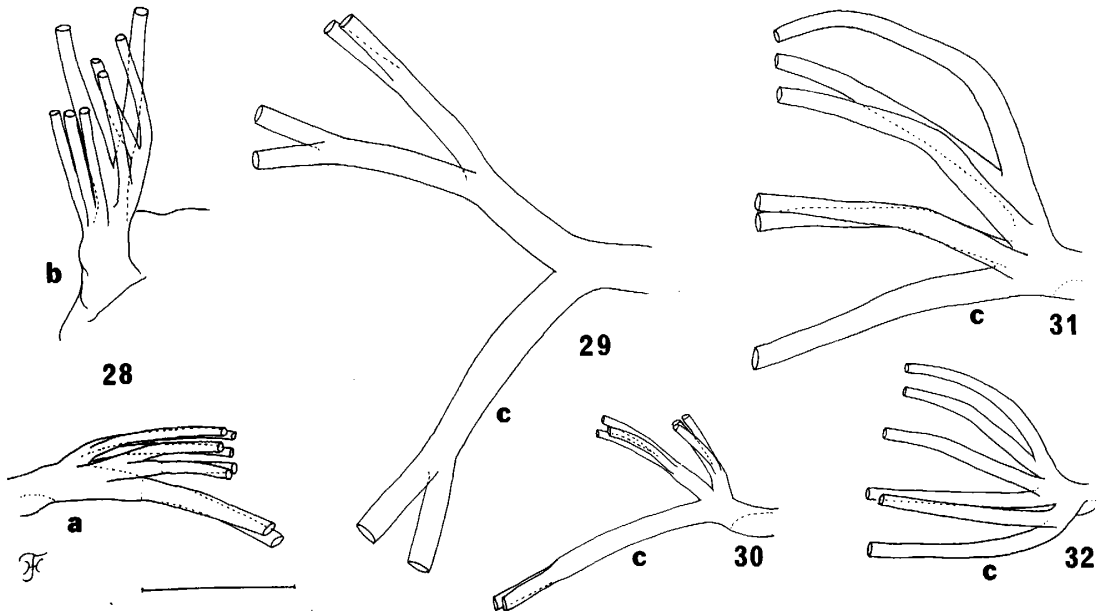
pedicel and base of 1st flagellar segment yellow. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.03:1.9; 3rd segment normal in shape and size, and with sensory vesicle which is about 1.5×as long as wide, and 1/5×as long as 3rd segment (Fig. 2). Maxilla serrated on both sides, with 6 inner teeth and 12 outer ones. Mandible serrulated only on inner side with 16 teeth and smooth on outer margin. Cibarium without denticles. *Thorax.* Scutum faintly whitish grey pruinose on brownish background, and densely covered with whitish yellow appressed pubescence. Scutellum dark brown, faintly whitish grey pruinose, and with hairs (but all lost, then their size and color unknown). Postscutellum brownish black, faintly whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and with several dark hairs. *Legs.* Fore coxa whitish yellow, mid coxa dark brown and hind one brown. All trochanters brown except hind one whitish yellow. All femora brown with distal 1/3 of hind femur dark brown. Fore tibia brown with median portion largely somewhat pale brown on outer surface. Mid tibia brown with basal 1/6 white. Hind tibia white on basal 1/3, then gradually darkened towards distal 1/3 and dark brown on distal 1/3, and densely covered with white appressed pubescence on posterior and lateral surfaces of basal 1/2 of its shaft. Fore tarsi dark brown, and basitarsus cylindrical, 6.0×as long as its greatest width. Mid tarsi dark brown. Hind tarsi dark brown to brownish black except basal 2/3 of basitarsus (though base of basitarsus brown) and basal 1/2 of 2nd segment white; basitarsus (Fig. 10a) narrow, nearly parallel-sided. Calcipala well developed, about 2/3×as wide as distal portion of basitarsus, and its tip reaching distal 1/3 of 2nd segment. Pedisulcus well developed. Claw with basal tooth,



Figs. 22 and 23 Female genitalia: 22, *S. dumogaense*; 23, *S. tumpaense*. a, 8th sternite, anterior gonapophyses, genital fork, paraproct and cercus in situ (ventral view); b, spermatheca; c, paraproct and cercus in side view; d, submedian lobe of 8th sternite with anterior gonapophysis in side view. Scale 0.1 mm.



Figs. 24–27 Male genitalia: 24, *S. torautense*; 25, *S. rosemaryae*; 26, *S. dumogaense*; 27, *S. tumpaense*. a, coxite, style and ventral plate in situ (ventral view); b, ventral plate in side view; c, ventral plate in end view; d, style in side view; e, style viewed from ventrolaterally; f, paramere; g, median sclerite. Scale 0.1 mm.



Figs. 28-32 Basal portion of pupal gills: 28, *S. torautense*; 29, *S. rosemaryae*; 30, S. sp B; 31, *S. dumogaense*; 32, *S. tumpaense*. a, dorsal view; b, inside view; c, outside view. Scale 0.3 mm.

which is 1/2×length of claw (Fig. 10c). *Wing.* C with spinules and hairs. Sc haired throughout its length on undersurface. Basal section of R haired. R_1 with spinules and hairs. R_2 with dark hairs. Basal cell absent. *Haltere* white, with petiole dark. *Abdomen.* Basal scale ocherous with white hair fringe. Dorsal surface of abdominal segments dark brown to brownish black except 2nd segment pale, and sparsely covered with dark hairs; tergites of segments 6-8 shiny. *Genitalia.* Sternite 8 bare medially and with about 18 stout hairs laterally on each side. Anterior gonapophysis simple, membraneous, and with a few short hairs as well as numerous microsetae; inner margin moderately sclerotized; posteromedial corner rounded and transparent (Fig. 19a). Genital fork (Fig. 19a) reversed-Y shaped, with well sclerotized stem; arms diverged laterally, with well sclerotized distal ridge which is produced anteriorly. Paraproct (Figs. 19a, c) simple, with about 10 stout hairs on lateral and ventral surface. Cercus (Fig. 19c) semilunar in lateral view, about 1/2×as long as wide, and moderately setose. Spermatheca (Fig. 19b) ellipsoidal, about 1.7×as long as wide, well sclerotized except small area of tubular base unsclerotized, and with minute internal setae.

Male, pupa and larva: Unknown.

Type specimen: Holotype female (BMNH), slide mounted, SULAWESI: Dumoga-Bone, Project Wallace Base Camp area, caught by light trap, R. H. L. Disney, Aug. 1985.

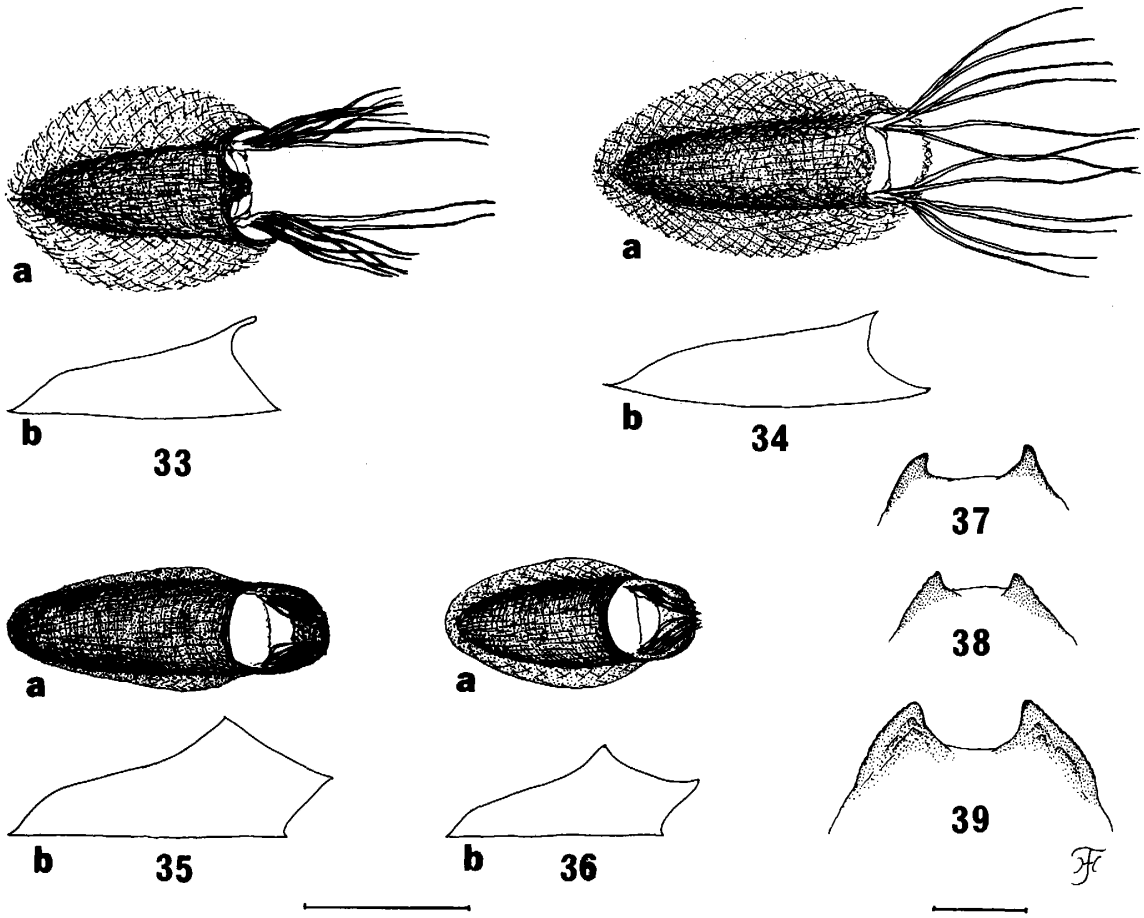
Ecological notes: The breeding habitats are unknown.

Remarks: This new species seems close to *S.* (*G.*) *miblosi* Takaoka, 1983 from Mindanao Island in the Philippines in having mandible devoid of teeth on the outer margin, small sensory vesicle and somewhat enlarged calcipala. However *S.* (*G.*) *sulawesiense* sp. nov. is different from the latter species by the fore and mid tibiae which are almost brown in this new species but are yellowish white on the basal 1/2 or more in the latter. In addition, the number of stout hairs on the sternite 8 greatly differs between the two species (ca. 40 versus 100).

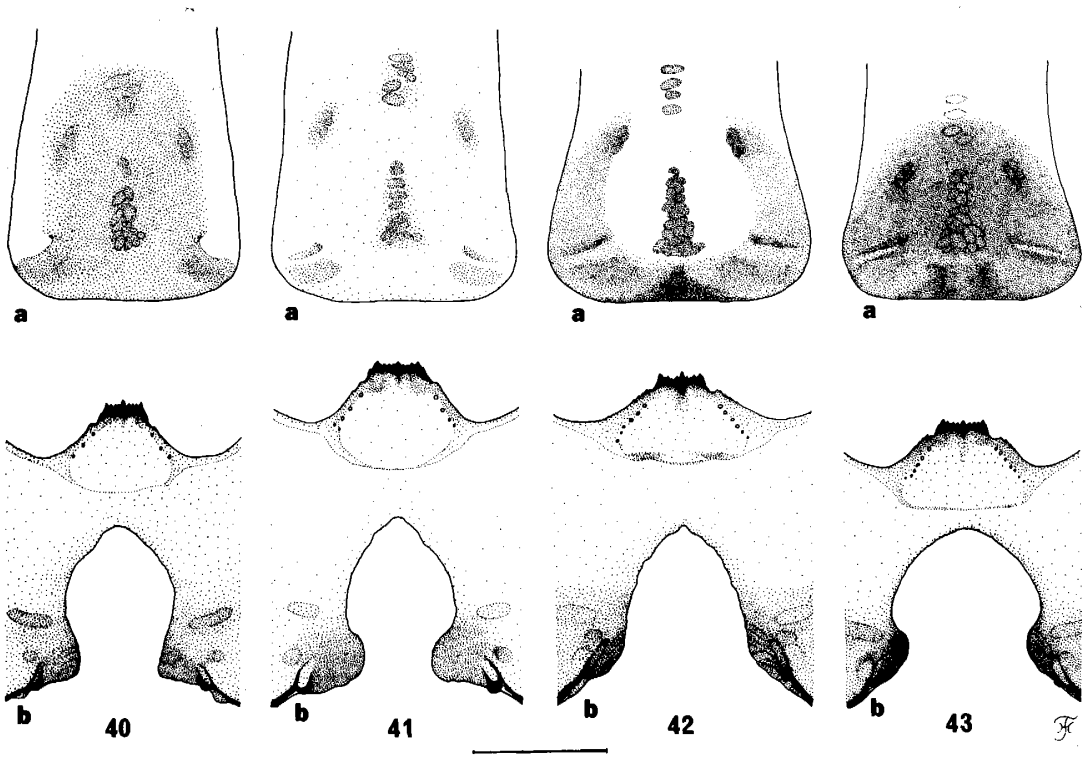
This species has also some similarities to *S.* (*G.*) *ceylonicum* (Enderlein), 1921 from Sri Lanka in the feature of the female mandible, and the shape and coloration of hind leg (Davies and Györkös, 1987). However, in the latter species, the greatest width of frons is much narrower than the height of frons (1.0:1.35) and the sensory vesicle is large (1/2×length of 3rd segment of palps). Further, this new species seems related to *S.* (*G.*) *dola* Davies and Györkös, 1987 from Sri Lanka in having the similar female mandible, small sensory vesicle and wide frons. However, there are differences in the shape of the hind femur, the coloration of the hind tibia and the tarsal claw ratio to its tooth which separate this species from *S.* (*G.*) *dola*.

***Simulium* (*Gomphostilbia*) *torautense* sp. nov.**

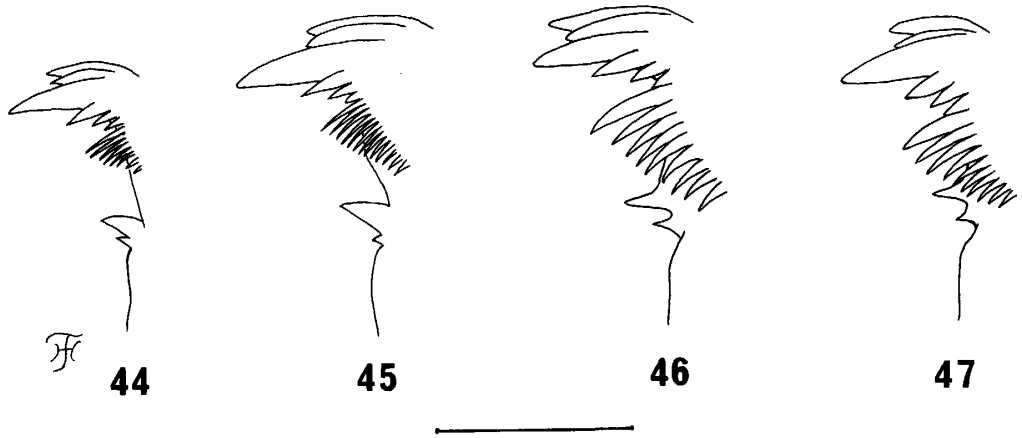
Female. Body length 2.3-2.7 mm. Wing length 1.8-2.0 mm. *Head.* Slightly narrower than width of thorax. Frons brownish black, semishiny, whitish grey pruinose, and covered densely with whitish yellow appressed pubescence; frontal ratio 1.3:1.0:2.5; frons-head ratio 1.0:5.8. Clypeus brownish black, semishiny, whitish grey pruinose, and densely covered with



Figs. 33-39 Pupae and cocoons (Figs. 33-36) and terminal hooks of pupal abdomen (Figs. 37-39): 33 and 37, *S. torautense*; 34 and 39, *S. rosemaryae*; 35, *S. dumogaense*; 36, *S. tumpaense*; 38, *S.* sp. B. a, dorsal view of pupa and cocoon; b, side view of cocoon. Scales 2.0 mm for pupae and cocoons, and 0.1 mm for terminal hooks.



Figs. 40-43 Larval head capsules: 40, *S. torautense*; 41, *S. rosemaryae*; 42, *S. dumogaense*; 43, *S. tumpaense*. a, cephalic apotome; b, hypostomium and postgenal cleft. Scale 0.2 mm.



Figs. 44-47 Tips of larval mandibles: 44, *S. torautense; 45, S. rosemaryae*; 46, *S. dumogaense*; 47, *S. tumpaense*. Scale 0.05 mm.

whitish yellow appressed pubescence. Antenna composed of 2+9 segments, brown except scape, pedical and base of 1st flagellar segment yellow. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.1:2.3; 3rd segment normal in shape and size, and with medium-sized ellipsoidal sensory vesicle which is about 2.3×as long as wide, and about 1/2.5×as long as 3rd segment (Fig. 3). Maxilla serrated on both sides, with 11 teeth on each side. Mandible serrulated on both sides, with 9 or 10 outer teeth and about 25 inner ones. Cibarium without denticles. *Thorax.* Scutum faintly whitish grey pruinose on brownish black to black background, with 3 faintly discernible longitudinal black vittae (1 medially and 2 submedially); scutum densely covered with whitish yellow appressed pubescence, intermingled with brown ones. Scutellum dark brown, faintly whitish grey pruinose, and with long and short upstanding dark hairs. Postscutellum brownish black, faintly whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and with numerous whitish yellow hairs intermixed with dark hairs. *Legs.* Fore coxa yellow, and mid and hind coxae brownish black. All trochanters dark brown to brownish black. All femora dark brown to brownish black. Fore tibia brownish black with median portion largely pale brown on outer surface, and covered densely with white appressed pubescence on outer surface of basal 3/5 of its shaft; mid and hind tibiae brownish black except extreme base whitish yellow (basal pale portion of hind tibia more distinct than that of mid one, occupying basal 2/5 on posterior surface, thus bearing its border with dark area oblique in lateral view), and densely covered with white appressed pubescence on posterior and lateral surfaces of basal 3/5 of their shaft; Fore tarsi black; basitarsus cylindrical, 5.9×as long as its greatest width, and with sparse dorsal hair crest. Mid tarsi brownish black to black except basal 1/2 of basitarsus whitish yellow. Hind tarsi brownish black to black except basal 5/8 of basitarsus (though base of basitarsus brownish black) and basal 1/2 of 2nd segment white; basitarsus (Fig. 11a) narrow, nearly parallel-sided. Calcipala and pedisulcus well developed. Claw with basal tooth, which is 1/2×length of claw (Fig. 11c). *Wing.* C with spinules and hairs. Sc with several hairs on basal 1/2 on undersurface. Basal section of R haired. R_1 with spinules and hairs. R_2 with dark hairs. Basal cell absent. Hair tuft of stem vein brownish black. *Haltere* white, with petiole dark.

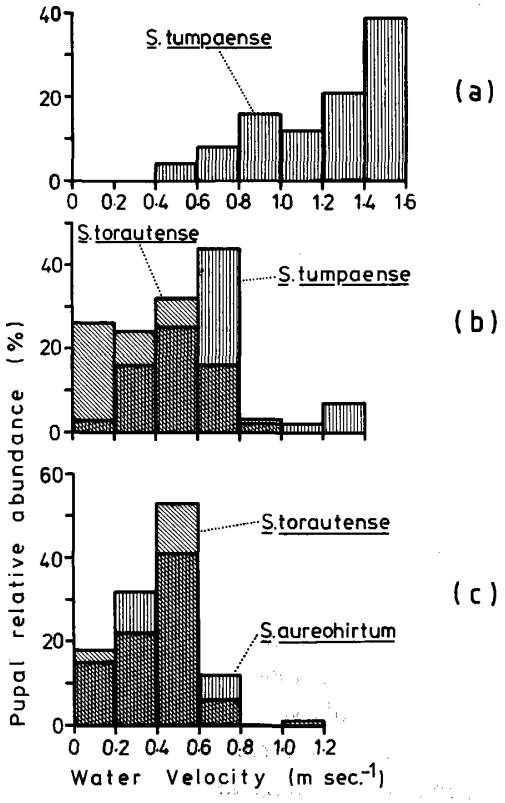


Fig. 48 Water velocity preferences for three species of blackflies: —a) *S. tumpaense* in the R. Tumpah; b) *S. torautense* and *S. tumpaense* in a stream flowing into the R. Toraut; c) *S. torautense* and *S. aureohirtum* in a second stream flowing into the R. Toraut.

Abdomen. Basal scale dark brown to brownish black with white hair fringe. Dorsal surface of abdominal segments dark brown to brownish black except basal 2/3 of 2nd segment pale brown, and sparsely covered with dark hairs; tergite of 2nd segment broadly white pruinose; tergites of segments 6-8 shiny. *Genitalia.* Sternite 8 bare medially and with about 18 stout hairs laterally on each side. Anterior gonapophysis simple, membraneous, and with several short hairs as well as numerous microsetae; inner margin moderately sclerotized; poster omedial corner largely rounded and transparent (Fig. 20a). Genital fork (Fig. 20a) reversed-Y shaped, with well sclerotized stem; arms diverged laterally, and with no projection. Paraproct (Figs. 20a, c) simple, with about 12 stout hairs on lateral surface. Cercus (Fig. 20c) semilunar in lateral view, 2/5×as long as wide, and moderately setose. Spermatheca (Fig. 20b) ellipsoidal, about 1.8×as long as wide, well sclerotized except small area of tubular base unsclerotized, and with minute internal setae.

Male. Body length 2.5-2.8 mm. Wing length 1.8-1.9 mm. *Head.* Width slightly wider than thorax. Holoptic; upper eye consisting of 15 horizontal rows and 15 vertical columns of large facets on each side. Face brownish black, and silvery white pruinose. Clypeus brownish black, silvery white pruinose and densely covered with whitish yellow pubescence interspersed with dark hairs. Antenna composed of 2+9 segments, yellowish brown to brown except scape, pedicel and base of 1st flagellar segment yellow; 1st flagellar segment elongated, about 1.6×as long as 2nd flagellar segment. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.3:3.1; 3rd segment not enlarged and with small sensory vesicle. *Thorax.* Scutum brownish black, with shiny, faintly-whitish grey pruinose pattern as shown in Fig. 15, and uniformly covered with bright brassy appressed pubescence (though relpaced by whitish yellow appressed pubescence on shoulders, and intermixed with whitish yellow pubescence on prescutellar area). Scutellum dark brown to brownish black, with long and short dark hairs. Postscutellum brownish black, faintly whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum with numerous dark hairs. *Legs.* Colourd as in female except tibial pale portions less distinct than female. Fore basitarsus almost cylindrical, 6.1×as long as its greatest width, and with sparse hair crest. Hind basitarsus (Fig. 11b) parallel-sided, about 1/2×as wide as greatest width of hind tibia which is equal to that of hind femur. Calcipala and pedisulcus well developed. *Wing.* C with spinules and dark hairs. Sc with a few hairs near base. Basal section of R fully haired. R_1 with a single row of spinules. R_2 with dark hairs. Basal cell absent. Hair tuft of stem vein dark brown. *Haltere* dark creamy, with petiole dark. *Abdomen.* Basal scale brownish black with dark hair fringe. Dorsal surface of abdomen brownish black, and sparsely covered with dark hairs; tergite of segment 2 broadly white pruinose, tergites of 5, 6 and 7 each with a pair of shiny, faintly-grey pruinose patches laterally. *Genitalia.* Coxite (Fig. 24a) nearly rectangular in ventral view, and about 1.4×as long as its greatest width. Style (Fig. 24d) small, about 4/5×as long as coxite, strongly curved dorsomedially at distal 1/3, and with a single apical spine. Body of ventral plate flat (Fig. 24a), rectangular in ventral view, about 1/2×as long as its width, slightly concave on posterior margin, slightly produced ventrally forming very low posteromedial process, and densely covered with microsetae ventrally, posteriorly and dorsomedially; basal arms of moderate size, directed forwardly and slightly inwardly. Paramere (Fig. 24f) of moderate size, and with 3 long hooks and 8-10 shorter ones. Median sclerite (Fig. 24g) broad, plate-like, somewhat divergent distally and ended roundly.

Pupa. Body length (excluding gill filaments) 2.5-2.9 mm. *Head* and *thorax.* Integument

yellowish brown, covered moderately with tubercles. Head with 1 facial pair and 3 frontal pairs of simple trichomes. Thorax with 5 pairs of simple, slender trichomes. Gill (Figs. 28a, b) with 8 filaments arranged in 3, 3, 2 groups from above downwards; dorsal triplet group very shortly stalked or even sessile, giving rise to 3 filaments, of which 2 filaments usually arranged in pair, having very short secondary stalk; middle triplet group arising inwardly from dorsolateral surface of main stalk a little distal to base of dorsal triplet, shortly stalked, and giving rise to 2 paired filaments (very shortly stalked) and 1 isolated filament; ventral pair of filaments shortly stalked, much longer and thicker than other 6 filaments in dorsal and middle groups which are almost the same in size (length 3.0-3.8 mm versus 1.8-2.6 mm); all filaments tapering toward tip, with numerous sharp transverse ridges, and covered densely with minute tubercles. *Abdomen.* Tergum 1 pale ocherous, with 2 simple setae (1 long and the other short and very slender) on each side, and covered with no tubercles. Tergum 2 pale ocherous, with 5 simple short setae and 1 simple long seta on each side. Terga 3 and 4 each with 4 hooked spines along posterior margin on each side. Tergum 5 lacking spine-combs. Terga 6-9 each with spine-combs on each side. Tergum 9 with a pair of simple terminal hooks (Fig. 37). Sternum 5 with 2 simple hooks submedially on each side. Sterna 6 and 7 each with 2 simple hooks widely spaced (1 submedially and the other laterally) on each side. Last segment with a few grapnel-like hooklets ventrolaterally. *Cocoon* (Figs. 33a, b) wall pocket-shaped, closely woven, extending ventrolaterally forming wide flange, bearing thick anterior margin and with small to medium anterodorsal projection.

Mature larva. Body length 4.7-5.0 mm. Body with transverse, sepia bands dorsally, each on thoracic segments 1-3 (though band on segment 2 indistinct in most specimens), and abdominal segments 1-4, and almost entirely sepia dorsally on abdominal segments 5-8 (though medially discolored to varying extents in most specimens). Cephalic apotome (Fig. 40a) more or less largely darkened except narrow lateral portions and anterior portion whitish yellow; head spots different in darkness against dark background, from much darker than, or as dark as, background (then positive), to lighter than background (then negative); further, in some larvae, some spots positive while others negative. Antenna composed of 4 segments, and longer than stem of cephalic fan; proportional length of segments 1-31.3:1.1:1.0. Cephalic fan with about 34 main rays. Mandible (Fig. 44) with normal arrangement of teeth, and without supernumerary mandibular serrations. Hypostomium (Fig. 40b) with a row of 9 apical teeth; medial tooth longest, followed by corner teeth, and 3 intermediate teeth (almost equal in size) on each side shortest; lateral margin smooth; hypostomial setae 3 or 4 in number, lying subparallel to side margins. Postgenal cleft (Fig. 40b) deep, about 4.5× as long as postgenal bridge, widest in the middle and somewhat constricted at base. Thoracic cuticle bare. Abdominal cuticle moderately covered with simple minute spines dorsally on segments 5-8. Rectal gill lobes compound, each lobe with 5-7 finger-like secondary lobules. Anal sclerite of usual X-form, with posterior arms about 1.2× as long as anterior ones, and broadly sclerotized at base between anterior and posterior arms. Ventral papillae well developed. Posterior circlet with about 68 rows of about 12 hooks.

Type specimens: Holotype female (BMNH), slide-mounted; allotype male; paratypes, 6 females, 8 males, 67 pupae and 10 mature larvae, SULAWESI: Dumoga-Bone Reserve, tributaries of R. Toraut, D. M. Roberts, Sept. 1985.

Ecological notes: This was the dominant species in the small tributaries (1-5 m wide) flowing into the R. Toraut in the Domoga Valley and was found in moderate velocities up to 0.6 m/

sec. (Fig. 40). Small numbers were also found in slow-flowing stretches of the R. Tumpah up to altitudes of 550 m. Most were found attached to trailing grass, only a few on rocks. It was associated with *S.* (*N.*) *aureohirtum, S.* (*S.*) *tumpaense* and *S.* (*G.*) *rosemaryae.* None was found around Lake Mooat (1,100 m altitude).

Distribution: Sulawesi.

Remarks: This new species seems closely related to *S.* (*G.*) *batoense* Edwards, 1934 from East Java, *S.* (*G.*) *siamense* Takaoka and Suzuki, 1984 from Thailand, and *S.* (*G.*) *krombeini* Davies and Györkös, 1987 from Sri Lanka, in having the dark coloration of the legs, parallel-sided male hind basitarsus, and deep larval postgenal cleft. However, this species is easily distinguished from these species by the presence of anterodorsal projection on the cocoon (Figs. 33a, b) and the branching of the pupal respiratory filaments (Figs. 28a, b).

Simulium (*Gomphostilbia*) *rosemaryae* sp. nov.

Female. Body length 2.5-2.8 mm. Wing length 2.4-2.5 mm. *Head.* Slightly narrower than width of thorax. Frons brownish black, faintly-whitish grey pruinose, and covered densely with whitish yellow appressed pubescence interspersed with dark hairs; frontal ratio 1.35: 1.0:2.0; frons-head ratio 1.0:5.2. Clypeus brownish black, semishiny, whitish grey pruinose, and densely covered with whitish yellow appressed pubescence, interspersed with several dark long hairs. Antenna composed of 2+9 segments, brown except scape, pedicel and base of 1st flagellar segment yellow. Maxillary palp with 5 segments proportion of 3rd, 4th and 5th segments in length 1.0:1.0:2.4; 3rd segment (Fig. 4) normal in shape and size, and with medium-sized, ellipsoidal sensory vesicle which is about 2.0×as long as wide, and 1/3×as long as 3rd segment. Maxilla serrated on both sides, with 16 outer teeth and 11 inner ones. Mandible serrulated on both sides, with 13 outer teeth and about 30 inner ones. Cibarium without denticles. *Thorax.* Scutum brownish black, with shiny, faintly whitish grey pruinosity, and densely covered with whitish yellow appressed pubescence, intermingled with brassy appressed pubescence. Scutellum dark brown, faintly-whitish grey pruinose, and with long and short upstanding dark hairs. Postscutellum brownish black, faintly-whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, and with numerous dark hairs, interspersed with whitish yellow pubescence. *Legs.* Fore coxa pale yellow, and mid and hind coxae brownish black. All trochanters dark brown except hind one pale yellow. All femora dark brown to brownish black. Fore tibia white except distal 1/4 brownish black; mid tibia white except distal 1/3 brownish black; hind tibia white on basal 1/2, gradually darkened towards distal 1/4, and brownish black on distal 1/4; all tibiae covered with white appressed pubescence on basal 2/3 or 3/4. Fore tarsi black; basitarsus nearly cylindrical, 5.0×as long as its greatest width, and with sparse dorsal hair crest. Mid tarsi black except basal 1/3 of basitarsus whitish yellow (its border not well defined). Hind tarsi black except basal 2/3 of basitarsus (though base of basitarsus brownish black) and base of 2nd segment white; basitarsus (Fig. 12a) narrow, nearly parallel-sided. Clacipala and pedisulcus well developed. Claw (Fig. 12c) with basal tooth, which is 1/2×length of claw. *Wing.* C with spinules and dark hairs. Sc almost fully haired. Basal section of R haired. R_1 with spinules and dark hairs. R_2 with dark hairs. Basal cell absent. Hair tuft of stem vein brownish black. *Haltere* white. *Abdomen.* Basal scale dark brown to brownish black with white hair fringe. Dorsal surface of abdominal segments dark brown to brownish black except basal 1/2 of 2nd segment whitish yellow to pale yellowish brown, and sparsely covered

with dark hairs and pale pubescence; tergite of 2nd segment broadly white pruinose; tergites of segments 6-8 shiny. *Genitalia.* Sternite 8 bare medially and with about 26 stout hairs laterally on each side. Anterior gonapophysis simple, membraneous, and with a few short hairs as well as numerous microsetae; inner margin slightly concave medially, and narrowly sclerotized; posteromedial corner rounded and transparent (Fig. 21a). Genital fork (Fig. 21a) reversed-Y shaped, with well sclerotized stem; arms broad, diverged laterally, and with no marked projection. Paraproct (Figs. 21a, c) simple, with about 20 stout hairs on lateral surface. Cercus (Fig. 21c) semilunar in lateral view, 2/5×as long as wide, and moderately setose. Spermatheca (Fig. 21b) ellipsoidal, about 1.8×as long as wide, unpatterned, and well sclerotized except small area of tubular base unsclerotized.

Male. Boby length 2.8-3.0 mm. Wing length 2.4-2.5 mm. *Head.* Width slightly wider than thorax. Holoptic; upper eye consisting of 13 horizontal rows and 10 vertical columns of large facets on each side. Face brownish black, and silvery white pruinose. Clypeus brownish black, silvery white pruinose and densely covered with whitish yellow pubescence interspersed with dark hairs. Antenna composed of 2+9 segments, yellowish brown to brown except scape, pedicel and base of 1st flagellar segment yellow; 1st flagellar segment elongated, about 1.8×as long as 2nd flagellar segment. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.2:3.2; 3rd segment not enlarged and with small sensory vesicle. *Thorax.* Scutum brownish black, with semishiny, faint, whitish grey pruinose pattern similar to that of *S.* (*G.*) *torautense* (although less distinct) and uniformly covered with whitish yellow to yellow appressed pubescence intermixed with brassy one. Scutellum dark brown to brownish black, with long and short dark hairs. Postscutellum brownish black, faintly whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum with numerous dark hairs interspersed with whitish yellow ones. *Legs.* Fore coxa yellow, mid and hind coxae brownish black. All trochanters brownish black except hind one yellowish brown. Fore tibia white except basal small area pale brown and distal 1/3 brownish black, and covered densely with white appressed pubescence on basal 4/5; mid tibia brownish black except basal 2/5 white; hind tibia brownish black except basal 1/3 white; white areas of mid and hind tibiae covered with white appressed pubescence. Fore tarsi black; basitarsus almost cylindrical, 6.1×as long as its greatest width, and with sparse hair crest. Mid tarsi black except basal 1/4 whitish yellow (though its border not well defined). Hind tarsi black except basal 2/5 and a little more of basitarsus and base of 2nd segment white (though base of basitarsus dark brown); hind basitarsun (Fig. 12b) expanded, wedge-shaped in lateral view, 3×as long as its greatest width, and equal in width to hind tibia which is about 1.4×as wide as hind femur. Calcipala and pedisulcus well developed. *Wing.* C with spinules and dark hairs. Sc bare or with a few hairs near base. Basal section of R fully haired. R_1 with a single row of spinules. Basal cell absent. Hair tuft of stem vein dark brown. *Haltere* dark creamy. *Abdomen.* Basal scale brownish black with dark hair fringe. Dorsal surface of abdomen brownish black except basal 1/2 of segment 2 dark yellow to pale brown, and sparsely covered with dark hairs; tergite of segment 2 broadly white pruinose; tergites of 5, 6 and 7 each with a pair of shiny, faint, grey pruinose patches laterally. *Genitalia.* Coxite (Fig. 25a) nearly rectangular in ventral view, and about 2.0×as long as its greatest width. Style (Fig. 25d) small about 4/5×as long as coxite, gently curved dorsomedially, and with a single apical spine. Body of ventral plate (Fig. 25a) flat, rectangular in ventral view, a little longer than 1/2 its width, nearly parallel-sided (although posterior 1/2 somewhat narrowed), slightly

convex medially on posterior margin, produced ventrally forming round posteromedian process, and densely covered with microsetae ventrally, posteriorly and dorsomedially; basal arms of moderate size, directed forwardly. Paramere of moderate size, and with 3 long hooks and 8-10 shorter ones. Median sclerite broad, plate-like, somewhat divergent distally and ended roundly, as in *S.* (*G.*) *torautense*.

Pupa. Body length (excluding gill filaments) 2.6-3.3 mm. *Head* and *thorax.* Integument yellowish brown, covered densely with tubercles. Head with 1 facial pair and 3 frontal pairs of simple trichomes. Thorax with 5 pairs of simple, slender trichomes. Gill (Fig. 29c) with 6 filaments arranged in 3 pairs; ventral pair of filaments with long stalk, dorsal and middle pairs having primary stalk in common which is about 1/2× as long as that of ventral pair, and is divergent from the latter stalk making a right angle; secondary stalk of middle pair subequal to or a little longer than that of dorsal pair, and also subequal to their common primary stalk; all filaments subequal in length and thickness to each other, almost as long as pupal body, tapering toward tip, with numerous sharp transverse ridges, and covered densely with minute tubercles. *Abdomen.* All terga pale ocherous. Tergum 1 moderately tuberculate, with 1 simple long seta on each side. Tergum 2 moderately tuberculate, with 5 simple short setae and 1 simple long seta on each side. Terga 3 and 4 each with 4 hooked spines along posterior margin on each side. Tergum 5 lacking spine-combs. Terga 6-9 each with spine-combs on each side. Tergum 9 with a pair of terminal hooks, outer margin of which is serrulated (Fig. 39). Sternum 4 with 2 simple or bifid hooks submedially on each side. Sternum 5 with 2 bifid hooks submedially on each side. Sterna 6 and 7 each with 2 hooks widely spaced (1 submedially-situated hook bifid, and the other laterally-situated simple or bifid) on each side. Last segment with a few grapnel-like hooklets ventrolaterally. *Cocoon* (Figs. 34a, b) wall pocket-shaped, closely woven, extending ventrolaterally forming wide flange, and bearing narrow anteroventral neck (though this neck absent in some cocoons); anterodorsal margin of opening not thickened, though slightly raised medially, but with no distinct projection.

Mature larva. Boby length 5.2-5.8 mm. Body grey, with transverse, sepia bands dorsally, each on abdominal segments 3 and 4 (though usually discontinued medially), and almost entirely sepia dorsally on abdominal segments 5-8 (though discolored medially to varying extents in most specimens). Cephalic apotome (Fig. 41a) pale, with faint positive head spots. Antenna composed of 4 segments, and longer than stem of cephalic fan; proportional length of segments 1-3 1.2:0.9:1.0. Cephalic fan with about 28 main rays. Mandible (Fig. 45) with normal arrangement of teeth, and without supernumerary mandibular serrations. Hypostomium (Fig. 41b) with a row of 9 apical teeth; median tooth as long as corner teeth, and longer than 3 intermediate teeth on each side, which are subequal in size to each other; lateral margin smooth; hypostomial setae 4 or 5 in number, lying subparallel to side margins. Postgenal cleft (Fig. 41b) deep, arrow-head shaped, about 3.6× as long as postgenal bridge, widest in the middle and markedly constricted at base. Thoracic cuticle bare. Abdominal cuticle moderately covered with branched (into 3-5) minute spines dorsally on segments 5-8. Rectal gill lobes compound, with 0-3 finger-like secondary lobules. Anal sclerite of usual X-form, with posterior arms about 1.2× as long as anterior ones, and broadly sclerotized at base between anterior and posterior arms. Ventral papillae well developed. Posterior circlet with about 86 rows of about 13 hooks.

Type specimens: Holotype female (BMNH), slide-mounted; allotype male, slide-mounted;

paratypes, 10 females, 7 males, 10 pupae and 4 mature larvae, SULAWESI: tributaries of Lake Mooat (near Kotinomobagu), D. M. Roberts, Sept. 1985.

Ecological notes: This species was found in streams (20 cm-2 m wide) flowing through both forest and coffee plantations into Lake Mooat (altitude of 1,100 m). The streams had low velocities (0.1-0.4 m/sec.) and the larvae were attached to dead leaves and trailing vegetation. They were found in association with *S.* (*S.*) *dumogaense*. Six pupae were also found in a small stream (2 m wide) flowing through farmland into the R. Toraut (at an altitude of 200 m).

Distribution: Sulawesi.

Remarks: This new species seems related to *S.* (*G.*) *metatarsale* Brunetti, 1911, *S.* (*G.*) *tenuistylum* Datta, 1973, from India, *S.* (*G.*) *tokarense* Takaoka, 1973, *S.* (*G.*) *okinawense* Takaoka, 1976 from Japan, *S.* (*G.*) *inthanonense* Takaoka and Suzuki, 1984 from Thailand, and *S.* (*G.*) *ela* Davies and Györkös, 1987 from Sri Lanka by having the enlarged, wedge-shaped male hind basitarsus (Fig. 12b). However, *S. rosemaryae* is readily separated from these known species and also from all the other members of the *ceylonicum* group by having the pupal gill with six filaments in place of eight filaments. The two Philippine species, *S.* (*G.*) *baisasae* Delfinado, 1962 and *S.* (*G.*) *ambigens* Delfinado, 1969, both belonging to different groups, have the 6-filamented pupal gill (Takaoka, 1983). However, the shape of genitalia in both sexes, and the female claws (simple or with small tooth) are quite different from those of the present new species.

Simulium (*Gomphostilbia*) sp. B

Female, male, larva : Unknown.

Pupa. Body length (excluding gill filaments) 2.3 mm. *Head, thorax* and *abdomen* similar to those of *S.* (*G.*) *torautense* except following features: Stalk of dorsal triplet of gill filaments arising upwards making a right or a little greater angle against stalk of ventral paired filaments, and stalk of middle triplet arising nearly on the same vertical plane (Fig. 30); ventral paired filaments with elongated stalk (Fig. 30), much longer and thicker than other 6 filaments in dorsal and middle groups which are almost the same in size (length about 2.8 mm versus 1.6 mm); terminal hooks weakly serrulated near apex (Fig. 38). *Cocoon* similar to that of *S.* (*G.*) *torautense* but without anterodorsal projection.

Specimens examined: 3 pupae, SULAWESI: Dumoga-Bone Reserve, tributary of R. Toraut, D. M. Roberts, Sept. 1985.

Ecological notes: This species was collected together with *S.* (*G.*) *torautense* in a small tributary flowing into the R. Toraut.

Distribution: Sulawesi.

Remarks: There is a possibility that this is the pupa of *S.* (*G.*) *sulawesiense* which was herein described based on the only one female adult specimen. However, the identity of this species remains unsolved because of the insufficient material.

This species seems very close to *S.* (*G.*) *sundaicum* Edwards, 1934 from East Java in having the similar branching method of pupal gill filaments and simple slipper-shaped cocoon, although the female of the latter species differs from that of *S.* (*G.*) *sulawesiense* by having the dark subbasal ring on the hind tibiae.

Subgenus *Simulium* Latreille s. str.

***Simulium* (*Simulium*) *dumogaense* sp. nov.**

Female. Body length 2.7-3.3 mm. Wing length 2.7 mm. *Head.* Narrower than width of thorax. Frons black, shiny, and covered with several dark hairs along lateral margins and near lower margin; frontal ratio 1.2:1.0:1.5; frons-head ratio 1.0:4.9. Clypeus black, shiny, whitish pruinose, and moderately covered with dark hairs. Antenna composed of 2+9 segments, brown except scape, pedicel and base of 1st flagellar segment yellow. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.2:2.5; 3rd segment (Fig. 5) normal in shape and size, and with medium ellipsoidal sensory vesicle which is about 1.5×as long as wide; sensory vesicle with wide opening (Fig. 5). Maxilla serrated on both side, with about 15 teeth on each side. Mandible serrulated on both sides, with 14-16 outer teeth and about 30 inner ones. Cibarium with a transverse row of about 15 small denticles and a cluster of numerous small denticles on median space, as shown in Fig. 7. *Thorax.* Scutum brownish black to black, shiny, faintly-whitish grey pruinose and with a broad band of iridescence on each side along lateral borders; the 2 bands bent inwards near anterolateral corners; scutum uniformly covered with dark hairs. Scutellum brownish black to black, faintly-whitish grey pruinose, and with long and short upstanding dark hairs. Postscutellum brownish black to black, faintly-whitish grey pruinose, and bare. Pleural membrane and katepisternum bare. *Legs.* Fore coxa whitish yellow, and mid and hind coxae brownish black. All trochanters, femora and tibiae brownish black; fore tibia with whitish sheen widely on outer surface, and mid and hind tibiae also with whitish sheen on posterior surface along basal 1/2 of shaft. Fore tarsi black; basitarsus dilated, about 4.1×as long as its greatest width, and with conspicuous, thick dorsal hair crest. Mid tarsi brownish black to black except basal 4/5 of basitarsus and basal 1/2 of 2nd segment whitish yellow. Hind tarsi brownish black to black except basal 1/2 of basitarsus (though base of basitarsus brownish black) and 2nd segment whitish yellow; basitarsus (Fig. 13a) narrow, nearly parallel-sided. Calcipala and pedisulcus well developed. Claw (Fig. 13c) with small basal tooth. *Wing.* C with spinules and hairs. Sc fully haired on undersurface. Basal section of R bare. R_1 with spinules and hairs. R_2 with hairs. Basal cell absent. Hair tuft on stem vein dark brown. *Haltere* pale white with petiole dark brown. *Adbomen.* Basal scale brownish black with dark hair fringe. Dorsal surface of abdominal segments brownish black to black except segment 2 brownish, and sparsely covered with dark hairs; tergite of 2nd segment with broad transverse, iridescent, silvery white pruinosity; tergites of segments 5-8 shiny. *Genitalia.* Sternite 8 (Fig. 22a) bare medially, and with about 24 dark long hairs on each side; posterior border of sternite 8 widely concave, and well demarcated from anterioir gonapophyses. Anterior gonapophysis (Fig. 22a) simple, membraneous, nearly triangular in shape, with short projection extending backwards on posteromedial corners, and covered sparsely with about 20 dark long hairs; inner margin nearly straight and weakly sclerotized; posterior margin thin, transparent and bare. Genital fork (Fig. 22a) reversed-Y shaped, with slender, well sclerotized stem; arms diverged laterally, and with heavily sclerotized ridge distally but having no marked projection. Paraproct (Figs. 22a, c) produced downwards, with nearly transparent rounded plate facing ventromedially, and moderately setose on lateral surface; ventromedial plate with about 25 pale slender hairs on its surface. Cercus (Fig. 22c) very short, rounded posteriorly, and moderately setose. Spermatheca (Fig. 22b) globose, and well sclerotized except small area of tubular base unsclerotized.

Male. Body length 3.0-3.4 mm. Wing length 2.5 mm. *Head.* Width slightly narrower than thorax. Holoptic; upper eye consisting of 17 horizontal rows and 13 or 14 vertical columns of large facets on each side. Face black, and white pruinose. Clypeus black, white pruinose and sparsely covered whth dark hairs. Antenna composed of 2+9 segments, brown except scape, pedicel and base of 1st flagellar segment yellow; 1st flagellar segment elongated about 1.7×as long as 2nd flagellar segment. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.2:2.5; 3rd segment not enlarged and with small ellipsoidal sensory vesicle. *Thorax.* Scutum black, with brilliant iridescent, silvery white pruinosity laterally and posteriorly and in 2 narrow submedian bands extending from anterolateral corners so as to enclose a black non-iridescent spot on each side and to leave middle of scutum black widely—this iridecent pattern is somewhat variable with individuals: in 6 of 10 males examined, middle non-iridescent band is nearly paralled-sided on posterior 2/3 and with rounded posterior end, and black spot on each side is large as shown in Fig. 16a; in 2 males, middle band is cuneiform and black spot on each side is of medium size as shown in Fig. 16b; in 1 male, though lateral black spots are subequal in size to those in the preceding 2 males, middle band is parallel-sided on anteior 2/3 and narrowed posteriorly (Fig. 16c); in 1 male, lateral black spots are small, and additional anterior pair of small black spots are present, though somewhat faint, as shown in Fig. 16d; scutum uniformly covered with brown appressed hairs which appear bright in certain angles of light. Scutellum black, white pruinose, somewhat iridescent and with dark long hairs. Postscutellum black, white pruinose, sowewhat iridescent and bare. Pleural membrane and katepisternum bare. *Legs.* Coloured as in female except hind basitarsus (Fig. 13b) with more dark portion than in female. Fore basitarsus dilated, about 4.6×as long as its greatest width, and with conspicuous thick hair crest. Hind basitarsus (Fig. 13b) enlarged, 4.0×as long as its greatest width, and subequal in width to hind tibia. Calcipala and pedisulcus well developed. *Wing.* C with spinules and dark hairs. Sc bare. Basal section of R bare. R_1 with a single row of spinules. Basal cell absent. Hair tuft on stem vein with dark hairs. *Haltere* white with petiole dark brown. *Abdomen.* Basal scale black with dark hair fringe. Dorsal surface of abdomen brownish black to black, and sparsely covered with dark hairs; tergites of segments 2, 4, 5, 6 and 7 each with a dorsolateral pair of iridescent, silvery white pruinose spots, which are broadly connected in the middle to each other in segment 2 but are narrowly connected in the remaining segments along anterior margin. *Genitalia.* Coxite (Fig. 26a) quadrate in ventral view, and a little longer than width. Style (Fig. 26e) elongate, much longer than coxite, nearly parallel-sided, about 3×as long as wide, somewhat flattened dorsoventrally, and with a single apical spine; style with basal protuberance produced dorsomedially, which is pointed apically (Fig. 26d). Body of ventral plate (Fig. 26a) nearly quadrate in ventral view, slightly shorter than width, and with hairy posteromedian process produced ventrally; posterolateral margins of posteromedian process not serrated, though slightly uneven (Fig. 26c); basal arms of moderate size, and somewhat divergent. Paramere (Fig. 26f) of moderate size, and with numerous small hooks. Median sclerite (Fig. 26g) broad, plate-like, and rounded distally. *Pupa.* Body length (excluding gill filaments) 3.2-3.4 mm. *Head* and *thorax.* Integument brown, covered densely and elaborately with tubercles. Head with 1 facial pair and 2 frontal pairs of bifid or trifid trichomes. Thorax with 5 pairs of 3-6 branched trichomes. Gill (Fig. 31c) with 6 filaments arranged in pairs, upper pair of filaments shortly stalked but middle and lower pairs almost sessile; all filaments subequal in length and thickness, tapering toward tip,

about 1/3×length of pupal body, with numerous sharp transverse ridges, and covered densely with minute tubercles. *Abdomen.* Tergum 1 pale ocherous, with 2 simple setae (1 long and the other short) on each side, and covered with no tubercles. Tergum 2 with 6 simple short setae in a row, of which 4 are stout and spinous on each side. Terga 3 and 4 each with 4 hooked spines along posterior margin on each side. Terga 5-7 lacking spine-combs. Tergum 8 with spine-combs composed of about 8 spines on each side. Terminal hook absent. Sternum 5 with 2 simple hooks submedially on each side. Sterna 6 and 7 each with 2 simple hooks, 1 submedially and 1 laterally on each side. Grapnel-like hooklets absent. *Cocoon* (Figs. 35a, b) shoe-shaped, tightly woven, and with narrow flange along ventrolateral margins; opening with thick lim.

Mature larva. Body length 5.0-6.0 mm. Body dark grey. Cephalic apotome (Fig. 42a) pale except portions along both sides and along posterior margin darkened; head spots markedly positive. Antenna composed of 4 segments, and longer than stem of cephalic fan; proportional length of segments 1-3 1.6:1.6:1.0. Cephalic fan with about 38 main rays. Mandible (Fig. 46) with normal arrangement of teeth, and without supernumerary mandibular serrations. Hypostomium (Fig. 42b) with a row of 9 apical teeth; median tooth slightly longer than corner teeth; corner teeth slightly longer than 3 intermediate teeth on each side, of which middle tooth smaller than its side teeth; lateral margin serrated; hypostomial setae 6-8 in number, lying divergent posteriorly from lateral margins. Postgenal cleft (Fig. 42b) deep, about 2.4×as long as postgenal bridge; both sides converging from base anteriorly. Thoracic and abdominal cuticle bare. Rectal gill lobes compound, each lobe with 6-9 finger-like secondary lobules. Anal sclerite of usual X-form, with posterior arms about 1.4×as long as anterior ones; anterior arms broadly sclerotized. Ventral papillae absent. Posterior circlet with about 102 rows of about 16 hooks.

Type specimens: Holotype female (BMNH), slide-mounted; allotype male, slide-mounted; paratypes, 4 females, 9 males, 15 pupae, and 3 mature larvae, SULAWESI: Dumoga-Bone Reserve, tributary of Lake Mooat (near Kotinomobagu), D. M. Roberts, Sept. 1985.

Ecological notes: This species was only found in one small (1.5 m wide) slow-flowing stream near Lake Mooat. It was attached to trailing grass in association with *S.* (*G.*) *rosemaryae.*

Distribution: Sulawesi.

Remarks: This new species may be assigned to the *melanopus* group, defined by Takaoka (1983) by having the unpatterned scutum, claws with small basal tooth, and paraproct with ventrointernal plate in the female, the 6-filamented pupal gill and shoe-shaped cocoon. The female of this species is separated from all the known members of this group by the shape of the anterior gonapophyses and paraprocts (Fig. 22a). The characteristic, iridescent, pruinose scutal pattern of the male is very similar to that of *S.* (*S.*) *laterale* Edwards, 1933 from Sabah (Smart and Clifford, 1969), but the genitalia is quite different from each other. Particularly, the style of this species, which has the pointed basal protuberance (Fig. 26d), is distinct within the *melanopus* group. It is noteworthy that the similar basal protuberance in the male style is also possessed by the *novolineatum* group in the Oriental Region {e. g. *S.* (*S.*) *fenestratum* Edwards, 1934 from Sumatra}

The larva of this species also differs from all the other related species by the shape of postgenal cleft (not widely rounded but gradually narrowed anteriorly) (Fig. 42b).

***Simulium* (*Simulium*) *tumpaense* sp. nov.**

Female. Body length 2.0-2.6 mm. Wing length 1.7-2.2 mm. *Head.* Narrower than width of thorax. Frons black, shiny, and covered with several dark hairs along lateral margins and near lower margin; frontal ratio 1.2:1.0:1.1; frons-head ratio 1.0:3.5. Clypeus black, shiny, whitish grey pruinose, and moderately covered with dark hairs. Antenna composed of 2+9 segments, brown except scape, pedicel and base of 1st flagellar segment yellow. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.2:2.5; 3rd segment (Fig. 6) normal in shape and size, and with small ellipsoidal sensory vesicle which is about 1.4×as long as wide. Maxilla serrated on both sides, with 12 outer teeth and 10 inner ones. Mandible serrulated on both sides, with 12 or 13 outer teeth and about 24 inner ones. Cibarium with a transverse row of about 10 small denticles and a cluster of numerous small denticles on median space, as shown in Fig. 8. *Thorax.* Scutum brownish black to black, shiny, faintly-whitish grey pruinose and somewhat iridescent, specially along lateral borders; scutum uniformly covered with dark appressed hairs. Scutellum brownish black, faintly-whitish grey pruinose, and with long and short upstanding dark hairs. Postscutellum brownish black to black, faintly-whitish grey pruinose, and bare. Pleural membrane and katepisternum bare. *Legs.* Fore coxa whitish yellow, and mid and hind coxae brownish black. All trochanters whitish yellow except mid one brown. Fore femur brown to dark brown, becoming darker towards distal end; mid and hind femora brownish black except extreme base of hind femur yellow. Fore tibia brownish black with median portion whitish yellow largely on outer surface; mid tibia brownish black except extreme base whitish yellow; hind tibia brownish black with more distinct whitish yellow portion at base than mid tibia, pale portion more extended on posterior surface than on anterior surface, thus its border with dark area oblique in lateral view; fore tibia with whitish sheen widely on outer surface, and mid and hind tibiae also with whitish sheen widely on posterior surface along basal 1/2 of shaft. Fore tarsi black; basitarsus dilated, about 4.7×as long as its greatest width, and with medium dorsal hair crest. Mid tarsi brownish black to black except basal 2/3 of basitarsus whitish yellow. Hind tarsi brownish black to black except basal 1/2 of basitarsus (though base of basitarsus brownish black) and 2nd segment whitish yellow; basitarsus (Fig. 14a) narrow, nearly parallel-sided. Calcipala and pedisulcus well developed. Claw (Fig. 14c) simple, without tooth. *Wing.* C with spinules and hairs. Sc fully haired on undersurface. Basal section of R bare. R_1 with spinules and hairs. R_2 with hairs. Basal cell absent. Hair tuft on stem vein dark brown. *Haltere* white. *Abdomen.* Basal scale brownish black with pale hair fringe. Dorsal surface of abdominal segments brownish black to black, and sparsely covered with dark hairs; tergite of 2nd segment with broad transverse whitish pruinosity; tergites of segments 5-8 shiny. *Genitalia.* Sternite 8 (Fig. 23a) of moderate size, much produced posteriorly, forming submedian triangular lobes, and with numerous stout hairs laterally and on these submedian lobes. Anterior gonapophysis (Figs. 23a, d) reduced, membraneous, and not well demarcated from posterior margin of produced submedian lobes of 8th sternite; gonapophysis-fused triangular lobes (Figs. 23a, d) transparent narrowly along posterior border and with small transparent tip bent ventrally on posteromedial corners. Genital fork (Fig. 23a) reversed-Y shaped, with stout, well-sclerotized stem; arms diverged laterally, and with heavily sclerotized distal ridge but having no marked projection. Paraproct (Figs. 23a, c) produced downwards, with brownish, elliptical, chitinized plate facing anteroventrally and somewhat medially, and moderately setose on lateral surface; this

anteroventral plate furnished with about 40 hairs on its surface, most of which are pale and slender, but several hairs near posterior margin of plate are dark, somewhat stouter, and situated very close together, appearing, in lateral view, as dark bundled hairs; deep groove present laterally and posteriorly between chitinized plate and paraproct proper. Cercus (Fig. 23c) very short, rounded posteriorly, and moderately setose. Spermatheca (Fig. 23b) globose, and well sclerotized except small area of tubular base unsclerotized.

Male. Body length 2.3-3.0 mm. Wing length 1.6-1.9 mm. *Head.* Width slightly wider than thorax. Holoptic; upper eye consisting of 14 or 15 horizontal rows and 13 or 14 vertical columns of large facets on each side. Face black, and silvery white pruinose. Clypeus black, silvery white pruinose and sparsely covered with dark hairs. Antenna composed of 2+9 segments, yellowish brown to brown except scape, pedicel and base 1st flagellar segment yellow; 1st flagellar segment elongated, about 1.7×as long as 2nd flagellar segment. Maxillary palp with 5 segments, proportion of 3rd, 4th and 5th segments in length 1.0:1.4:2.7; 3rd segment not enlarged and with small sensory vesicle. *Thorax.* Scutum brownish black, with brilliant iridescent silvery pruinosity laterally and posteriorly and in 2 broad submedian bands extending from anterolateral corners so as to enclose a black non-iridescent spot on each side and to leave middle of scutum black widely, though lateral iridescent band usually disconnected to posterior iridescent pruinosity in front of wing base, as shown in Figs. 17a, b; scutum uniformly covered with brown appressed hairs which appear bright in certain angles of light. Scutellum brownish black, silvery white pruinose, somewhat iridescent and with long hairs. Postscutellum black, white pruinose, somewhat iridescent and bare. Pleural membrane and katepisternum bare. *Legs.* Coloured as in female. Fore basitarsus dilated, about 5.0×as long as its greatest width, and with medium thick hair crest. Hind basitarsus (Fig. 14b) only slightly enlarged, gradually widened to distal end (or widened towards basal 1/3, then nearly parallel-sided up to distal end), about 3.8×as long as its greatest width, and about 3/4×as wide as greatest width of hind tibia. Calcipala and pedisulcus well developed. *Wing.* C with spinules and dark hairs. Sc bare. Basal section of R bare. R_1 with a single row of spinules. Basal cell absent. Hair tuft on stem vein dark brown. *Haltere* white. *Abdomen.* Basal scale black with yellowish hair fringe. Dorsal surface of abdomen brownish black to black, and sparsely covered with dark hairs; tergites of segments 2, 4, 5, 6 and 7 each with a dorsolateral pair of silvery pruinose spots connected dorsally to each other along anterior margin; these pruinose spots somewhat iridescent in certain angles of light. *Genitalia.* Coxite (Fig. 27a) quadrate in ventral view, and a little longer than width. Style (Fig. 27e) elongate, much longer than coxite, nearly parallel-sided, about 2.6×as long as wide, somewhat flattened dorsoventrally, and with a single apical spine; style (Fig. 27d) with basal protuberance produced dorsomedially, which is serrated distally. Body of ventral plate quadrate in ventral view (Fig. 27a), slightly concave on posterior margin, and with low setose posteromedian process produced ventrally; posterolateral margins of posteromedian process (Figs. 27b, c) uneven, stair-stepped but not serrated; basal arms of moderate size, and widely divergent. Paramere of moderate size, and with numerous short hooks. Median sclerite broad, plate-like, and rounded distally.

Pupa. Body length (excluding gill filaments) 2.4-2.6 mm. *Head* and *thorax.* Integument yellowish brown, covered moderately with tubercles. Head with 1 facial pair and 2 frontal pairs of simple trichomes. Thorax with 6 pairs of simple trichomes. Gill (Fig. 32c) with 6 filaments arranged in pairs, very shortly stalked; all filaments subequal in length and thick-

ness, tapering toward tip, about 2/5×length of pupal body, with numerous sharp transverse ridges, and covered densely with minute tubercles. *Abdomen*. Tergum 1 ocherous, with 2 simple setae (1 long and the other short) on each side, and covered sparsely with minute tubercles. Tergum 2 ocherous on basal 1/3 to 1/2, and with 6 simple short setae in a row, of which 3 are stout and spinous, on each side. Terga 3 and 4 each with 4 hooked spines along posterior margin on each side; tergum 3 ocherous on basal 1/3. Terga 5-7 lacking spine-combs. Tergum 8 with spine-combs in transverse row on each side. Terminal hook absent. Sternum 5 with 2 simple hooks submedially on each side. Sterna 6 and 7 each with 1 simple hook submedially on each side and lacking outer hook. Grapnel-like hooklets absent. *Cocoon* (Figs. 36a, b) shoe-shaped, closely and tightly woven, and with narrow flange along ventrolateral margins which is distinctly wider than that of *S.* (*S.*) *dumogaense*; opening with thick rim.

Mature larva. Body length 4.5-5.0 mm. Body pale grey to dark greenish grey. Cephalic apotome (Fig. 43a) generally pale on anterior 1/2 and dark on posterior 1/2; head spots positive (though usually submerged in dark ground color) or negative. Antenna composed of 4 segments, and longer than stem of cephalic fan; proportional length of segments 1-3 1.6: 1.6:1.0. Cephalic fan with about 38 main rays. Mandible (Fig. 47) with normal arrangement of teeth, and without supernumerary mandibular serrations. Hypostomium (Fig. 43b) with a row of 9 apical teeth; median tooth as long as corner teeth, and longer than 3 intermediate teeth on each side, which are subequal in size to each other; lateral margin serrated; hypostomial setae 6 or 7 in number, lying divergent posteriorly from lateral margins. Postgenal cleft (Fig. 43b) deep, rounded, constricted near base, and about 7.5×as long as postgenal bridge. Thoracic and abdominal cuticle bare. Rectal gill lobes compound, with 6-9 finger-like secondary lobules. Anal sclerite of usual X-from, with posterior arms about 1.8×as long as anterior ones; anterior arms broadly sclerotized. Ventral papillae absent. Posterior circlet with about 88 rows of about 13 hooks.

Type specimens: Holotype female (BMNH), slide-mounted; allotype male, slide-mounted; paratypes, 25 females, 24 males, 20 pupae, and 44 mature larvae, in alcohol, SULAWESI: Dumoga-Bone Reserve, R. Tumpah, D. M. Roberts, Sept. 1985; 2 females, 2 males, pinned, 7 pupae, 4 pupal skins, 1 cocoon, 4 mature larvae and 11 immature larvae in alcohol (BMNH), Dumoga-Bone, Dumoga Valley, irrigation channel to R. Toraut, near Project Wallace Camp area, G. B. White, Dec. 1985.

Ecological notes: This was the dominant species in the large rivers Toraut and Tumpah (20-30 m wide) and was most abundant in the highest velocities (Fig. 48a). Large numbers were also present in small feeder canals (1 m wide) of the Toraut irrigation system in the Dumoga Valley, where velocities over 2 m/sec. were recorded. A few were found in rapids in small tributaries of the R. Toraut (Fig. 48b). In the R. Toraut and R. Tumpah, larvae were usually attached to dead leaves, with a few on rock; in the irrigation canals, they were attached to trailing vegetation. They were found at altitudes of 200-500 m in the R. Tumpah, and were also present in streams flowing into Lake Mooat (1,100 m).

Distribution: Sulawesi.

Remarks: This new species also belongs to the *melanopus* group, and the pupa and larva have a close similarity to those of the other members of the group. The female of this species lacks a claw tooth, as does the female of *S.* (*S.*) *discrepans* Delfinado, 1969 from the Philippines (Takaoka, 1983). However, there is a difference in the shape of the anterior gonapophyses

and paraprocts between the two species. This species possesses a characteristic, iridescent, pruinose pattern on the male scutum (Fig. 17a), which is, however, different from that of *S.* (*S.*) *dumogaense* and *S.* (*S.*) *laterale*. The male genitalia resembles that of *S.* (*S.*) *dumogaense* except the style with a serrated basal protuberance (Fig. 27d). By having this form of the style, *S.* (*S.*) *tumpaense* is easily separated from all the other members of the *melanopus* group.

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インドネシア，スラウェシで採集されたブユについて

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インドネシアのスラウェシ島北部の，Dumoga-Bone国立公園内で採集されたブユの標本を分類学的に検討した。その結果，既知種1種，新種6種を含む9種が分布していることが分かった。これらはブユ属（*Simulium*）の4亜属（*Nevermannia, Morops, Gomphostilbia, Simulium* s. str.）に分類された。これらブユ種の成虫，蛹，幼虫期の検索表を作り，新種の記載を行った。また幼虫の生息状況に関しても簡単に触れた。

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NOTES ON THE INABILITY OF AUTOGENOUS DEVELOPMENT OF OVARIAN FOLLICLES IN *SIMULIUM RUFICORNE* MACQUART AND *S. NIGRITARSE* COQUILLETT FROM PLATEAU STATE IN NIGERIA

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Abstract: The expression of autogeny in *Simulium ruficorne* and *S. nigritarse*, both belonging to the *ruficorne* species group, from Plateau State in Nigeria was examined by maintaining the newly emerged females with a 30% sucrose solution for five days. The result showed no evidence of autogeny. The interspecific difference in the reproductive strategies was briefly discussed in comparison with the autogenous species belonging to the same species group.

INTRODUCTION

Autogeny is known in several species of Simuliidae, most of which are arctic or subarctic species (for review see Anderson, 1987). Autogenous species is generally very rare in warmer ecosystems. In this respect, it is of great interest that autogeny was reported in two subtropical or tropical blackfly species i. e. *Simulium ornatipes* Skuse and *S. aureohirtum* Brunetti, both of which have a wide distribution in the Australasian and the Oriental region, respectively (Hunter, 1977; Takaoka and Noda, 1979; Takaoka, 1988). These two species belong to the *ruficorne* species group of *Simulium* (*Nevermannia*), which is one of the most distinctive species groups within the subgenus *Nevermannia* and even within the genus *Simulium* in that it has extended its distribution in four zoogeographical regions (i. e. Ethiopian, Palaearctic, Oriental and Australasian regions). The successful colonization of remote islands by *S. aureohirtum* was briefly discussed considering autogeny for the first gonotrophic cycle as a favorable reproductive strategy, coupled with the probable preference of female adults to feed on birds for subsequent gonotrophic cycles and larval adaptabilities to slow-flowing waters like trickles and irrigation channels or even almost stagnant waters like in paddy fields (Takaoka and Noda, 1979). The same speculation may be applied to some of other members of this group, which have such a wide distribution as does *S. aureohirtum*. However, little information is available on autogeny or anautogeny, although blood feeding habits of adults and larval habitats of some widespread species are known (Crosskey and Büttiker, 1982).

In this study, *S. ruficorne*, the type of the species group, was examined, since this species is widely distributed throughout the African continent, its neighbouring islands, parts of

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Europe, Arabian Peninsula and Middle East (Crosskey, 1967). *Simulium nigritarse,* one of its West African allies, was also studied.

Materials and Methods

Pupae of these two species were collected from various streams in and around the Plateau State in Nigeria during June-August in 1985. Emergent females were individually maintained with a 30% sucrose solution at a temperature of 22°C. Six days after emergence, the ovaries of all the flies were dissected, and developmental state of the primary follicles was examined according to the criteria defined by Christophers (1911) and later modified by Mer (1936).

Results and Discussion

The material was scarce but the result clearly showed no evidence of autogeny in females of *S. ruficorne* and *S. nigritarse* at least in some populations of the northern Nigeria. The primary follicles were all small and spherical (size, ca. 40-45 μm in diameter in *S. ruficorne* and ca. 35-40 μm in *S. nigritarse*) (Table 1) and had no yolk granules. It is considered that these two species need a blood meal to complete ovarian development. However, the present result does not preclude the possible existence of autogenous strains of *S. ruficorne* in other different areas, as Rivosecchi *et al.* (1969) suggested *S. ruficorne* in Yemen to be autogenous.

Simulium ruficorne is morphologically very similar to *S. aureohirtum* and *S. ornatipes*, and is able to utilize similar types of waters as immature habitats (Crosskey and Buttiker, 1982). However, it is intriguing, as the result shows, that there are interspecific differences in the reproductive methods (i. e. autogeny vs. anautogeny) in this *ruficorne* group. The lack of autogeny in *S. ruficorne*, if proved also in other populations, may indicate that the strategies by which this species had attained its wide distribution are different from those employed by *S. aureohirtum* or *S. ornatipes.* The biological features other than autogeny may be the principal factors that enabled *S. ruficorne* to widely extend its distribution. Crosskey

Table 1 Developmental stage of ovarian follicles in two species of the *ruficorne*-group in the Plateau State, Nigeria, maintained with a 30% sucrose solution for five days after emergence

Localities	*S. ruficorne*		*S. nigritarse*	
	No. flies examined	Follicular stage	No. flies examined	Follicular stage
Ibangi	2	Ia	2	Ia
Bukururu	—	—	1	Ia
Kuru	1	Ia	2	Ia
Hoss	—	—	1	Ia
Meijuju	2	Ia	—	—
Meigem	1	Ia	27	Ia
Kudaru*	4	Ia	1	Ia

*Kaduna State

and Büttiker (1982) considered that since it has successfully colonized even unstable habitats such as those in the desert and semi-desert wadis, the differing oviposition method enabling the eggs to survive long dry periods in damp stream beds might be employed by *S. ruficorne,* in addition to its larval capacity to withstand unusually polluted waters and high temperatures.

On the other hand, about 3/5 of the total species in the *ruficorne* species groups (ca. 50 spp.) were reported to live in the African continent and its islands whereas only one (*S. aureohirtum*), and three species (*S. ornatipes* and two related taxa) are known in the Oriental and the Australasian region, respectively (Crosskey, 1987). The fact seems to suggest that this species group must have evolved in Africa and may have diverged from there into the surrounding regions. If anautogeny is a primitive feature and autogeny is a derived one, as viewed by Downes (1971), it is further inferred that one of the phylogenetic lines, which had gained the ability to produce eggs autogenously, could have advanced farther eastwards from Africa through Middle East and Asia into Australia.

Further studies are needed to determine how wide the trait "autogeny" is spreading in this species group. Such studies of other members in and outside Africa may provide further information as to whether or how autogeny played a role as one of the reproductive strategies in the process of divergence of this species group.

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The author thanks Dr. H. Takahasi, the leader of the JICA based Japan-Nigeria Joint Project to University of Jos, under which this work was carried out.

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ナイジェリアのプラトー州における2種のブユ，*Simulium ruficorne* と *S. nigritarse* の未吸血性卵巣発育の欠如について

高岡　宏行

ブユ属のなかで *ruficorne* 種群は旧大陸に広く分布するが，東洋区とオーストラリア区にそれぞれ分布する2種では，未吸血性卵巣発育が知られている。そこでこの種群の大半が分布するエチオピア区の種についても，同様の性質が見られるかを検討するため，ナイジェリアのプラトー州の数箇所でこの種群に属する2種のブユ，*S. ruficorne* と *S. nigritarse* について羽化後の卵巣発育を調べた。その結果，いずれのブユ種でも蔗糖を与えただけでは卵巣は Ia 期のままで全く発育しないことが分かった。

大分医科大学医動物学教室　　(〒879—56) 大分県大分郡挾間町医大ケ丘1—1506

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41　パキスタン・Sind州住民の寄生虫に対する免疫・疫学的調査成績

近藤力王至　（金沢大・医・寄生虫）
佐野　基人　（浜松医大・寄生虫）
中島　康雄　（山梨医大・寄生虫）
宮本　健司　（旭川医大・寄生虫）
茅根　士郎　（麻布大・獣医・寄生虫）
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（埼玉医大・寄生虫）
赤羽　啓栄　（福岡大・医・寄生虫）
S. S. Ahmed　（Sind大・動物）

我々はパキスタン・Sind州において1986年12月から翌年1月までの2カ月間に，住民149名について，寄生虫症に関する免疫・疫学的調査を行った。*Toxoplasma* 抗体陽性者：Latex凝集反応では34.2%と，石川県住民のそれ（11.7%）よりもかなり高い値であった。蠕虫類に対する抗体保有状況は次の如くであった。Ouchterlony法：犬回虫幼虫ES抗原（TcnLES）では1.8%，旋毛虫幼虫抽出抗原（TspLEX）では10.9%，広東住血線虫成虫抗原（AnqAEX）では7.4%，犬糸状虫成虫同抗原（DiAEX）では1.6%，肝蛭成虫同抗原では1.6%の抗体保有者がみられたが，剛棘顎口虫幼虫抽出抗原に対する抗体保有者はみられなかった。カウンター電気泳動法：TcnLES，TspLEXに対する抗体保有率はそれぞれ1.6，4.9%を示した。ELISA：TcnLES，TspLEX，AngAEX，DiAEXに対する平均抗体価はそれぞれ1.0±0.3，1.0±0.2，1.2±0.3，1.1±0.3，抗体陽性者率ではそれぞれ0.0，0.0，4.2，3.9で，いずれも石川県住民のそれとは大きな差はみられなかった。この地方は乾燥地帯であり，住民の蠕虫類感染者およびその抗体保有者は少ないものの，沈降抗体保有状況からみて，旋毛虫，広東住血線虫については潜在的感染者がいるものと思われた。

42　ネパール王国の寄生虫症

伊藤　洋一　（北里大・医・寄生虫）
村田　良介　（予研）
曾田　研二（横浜市大・医・公衆衛生）
堀内　　勁（聖マリアンナ医大・小児科）

1986年度の感染症基礎調査はネパール王国において実施された。調査は感染症全般にわたって行われたが，ここでは内部寄生虫症に限ってその状況を報告する。(1) マラリア：タライ平野を主体に900万人の人々が感染の危険に曝されている。1984年より急激な増加が生じ，1985年には国内で42,321人の年間患者発生数をみるに至っている。*A. annularis*，*A. fluviatilis*，*A. maculatus*，A. minimusが媒介種として知られており，地区により優占種が異なる。(2) カラ・アザール：ネパール政府は1980—85年の間Dhanusa地区の住民および地区病院における患者の発生状況を調査し，35名の患者を検出した。付近から3種類のPhlebotomus属が採集されたが，*Ph. papatasi* が総捕獲数の75%を占め，媒介種の可能性が示唆された。また，Joshiらは各地の病院14施設において1980—84年の間の患者数を調べ，604名の患者の内47名が死亡していることを報告している。(3) フィラリア症：各地に広く流行しているものと推定される。Jung (1973) は中部地区の住民5,302名を検査し，479名 (9.0%) のmf陽性者を検出している。Rajbhandari (1986) は西部の3地区で652名を検査し，45名（6.9%）のmf陽性者を検出している。(4) 包虫症：Joshiは1979—83年の間にカトマンズ市付近の3病院で外科手術を受けた27,288名の患者の記録から76例の包虫症を見出している。

また，近くの屠殺場で処理される家畜を調査し，水牛601頭（18.6%），羊・山羊645頭（6.0%），豚65頭（15.5%）に寄生を認めた。(5) 腸管内寄生虫症：蠕虫種として回虫，鉤虫（アメリカ・ズビニ），鞭虫，蟯虫の感染が蔓延し，原虫種として赤

痢アメーバ，ランブル鞭虫などの寄生が認められる。

43 ガーナの一地方村における腸管寄生虫感染状況の調査

伊藤　誠，高柳　坦，佐藤　重房
（名古屋市大・医・医動物）
鳥越　貞義　（三重大・医・小児科）
R. K. アンテソン（ガーナ大・野口研）

国際協力事業団（JICA）の野口研プロジェクトの一環として，1983—1984年にかけてガーナの一地方村において腸管内寄生虫感染状況の調査を行った。この村（Fetteh）は首都アクラの西方70 kmの海岸沿いに位置する人口約2,000人の半農半漁の村で，野口研の Field Station となっている。

全年齢を対象に集めた糞便は，直接塗抹法ホルマリン・エーテル法で検査した。1983年の9—11月（A期間）1984年1—4月（B期間）の調査の後 pyrantel pamoate による集団駆虫を試み，同年4—8月に再度調査した。

その結果，蠕虫類では，A・B両期間とも回虫卵陽性率が最も高く（各63, 61%），鉤虫卵（各30, 25%），鞭虫卵（各23, 27%）がこれに続き糞線虫（各3，11%）も検出された。原虫類では大腸アメーバシスト（各18, 27%）が最も多く検出された。

年齢別では0.5—1歳でほとんどの寄生虫に対して感染が起こり始め，陽性率は年齢と共に高くなり，4—6歳ですでに80%以上が何らかの寄生虫に感染していた。回虫卵陽性率は，4—6歳でピークに達した後，横ばいであったが，鉤虫，鞭虫卵陽性率は徐々に上昇し，10—19歳でピークに達した後，減少する傾向を示した。

駆虫の結果，回虫卵および鞭虫卵陽性率はそれぞれ7.4%, 11.6%と有意に減少したが，鉤虫卵陽性率は20%と，減少は有意ではなかった。駆虫後の調査期間が4カ月と長かったために，この間に再感染が起こり，この駆虫薬の効力を過小評価していることも考えられた。

44 ナイジェリア・ジョスにおける学童の腸管内寄生虫調査

金子　清俊　（愛知医大・寄生虫）
高橋　基久，高橋　利幸，高橋　弘
（国際協力事業団）
Shonekan, R.A.O
（ジョス大学・微生物）

ナイジェリア連邦共和国に対する医療協力活動の一部門として，“小児下痢症”の調査が進められた。その一環として学童の寄生虫調査が行われた。調査地ジョスは首都ラゴスの北東約950 km，標高1,200 mほどの高原に位置し，人口はおよそ35万の小都市である。調査は市内の小学校4校を選定し，朝登校した学童に糞便容器を渡して，直ちに採便できた者，合計620名（年齢4—18歳）を対象とした。検査はホルマリン・エーテル法で行い，鉤虫卵陽性者のみ一部濾紙培養法を実施した。

成績：糞便検査の結果，蠕虫6種類と原虫6種類を含む計12種類の腸管内寄生虫が検出された。蠕虫寄生者は全体の22.6%で，そのうち回虫は9.5%，アメリカ鉤虫が5.3%，鞭虫が7.4%，糞線虫が0.5%，小形条虫が0.3%，テニア sp.が0.2%であった。一方，原虫寄生は全体の44.2%に認められ，大腸アメーバが36.3%，赤痢アメーバが13.4%，小形アメーバが8.7%，ヨードアメーバが6.6%，ランブル鞭毛虫が8.9%，メニール鞭毛虫が5.2%であった。

45 フィリピン・ボホール島における住血吸虫症，特に中間宿主貝 *Oncomelania quadrasi* の生息地の特徴について

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ボホール島は北緯10度・東経124度に位置し，その東方がレイテ島南部に，西方がセブ島に面している。その形はほぼ楕円で，4,117 km²の広さをもつ。島内の日本住血吸虫症流行地は Ipil 川の左岸に，しかも省内46自治体中の2町，Talibon と Trinidad に限られて存在する。我々は1981年以降，この地域の中間宿主貝生息状況の調査ならびに疫学的調査を行ってきた。その結果，1984年と1985年に新しい中間宿主貝生息地が6カ所で見つかった。これらの生息地は，住血吸虫症患者の居住地に近接していた。この島における中間宿主貝生息地は現在では11カ所となり，それらはいずれも Ipil 川に流入する3水系に沿って局在している。中間宿主貝の生息には好適であるように見えるが，実際には生息していない地域の地形を調べたが，中間宿主貝生息地はすべて砂壌土で pH 6.5以下の地域であった。この地域は，3－5年毎に3－4カ月間も続く深刻な旱魃に見舞われる。中間宿主貝 *O. quadrasi* は湧水を水源にもつ山襞の湿地帯と，それにつながる細い水路にのみ見出される。そこには *Melania* 属，*Segmentina* 属，*Lymnaea* 属など普通の淡水産巻貝も生息しており，このことはこれらの生息地が年間を通じ決して，もしくは滅多に干上がらない事を示している。我々の得たこれらの知見は，ボホール島における日本住血吸虫流行地の，著しい局在性の解明にいくつかの手掛りを与えてくれた。

46 日本住血吸虫感染マウスにおける変異原物質 Trp-p-2 の体内代謝について

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（岡山大・医・寄生虫）
有元佐賀恵，綿矢　有佑，早津　彦哉
（岡山大・薬・薬品化学）

日本住血吸虫感染における発癌の問題に関連して，肝の薬物代謝酵素のチトクローム P 450の代謝活性を調べた。*in vitro* では肝の P-450の活性は，感染により低下することがわかった。*in vivo* で確認するため，トリプトファン熱分解物由来で強い変異原性を持つ Trp-p-2 を静注し，体内からの消失速度を調べた。又，日住感染時に肝に蓄積する，Schistosome pigment と同一と言われているヘマチンと Trp-p-2 との吸着を調べた。

日住セルカリア30隻感染後12週の ddY マウスと対照非感染マウスに，2 mM Trp-p-2 を0.3 m*l* 静注し，0.5，1，3，6，18時間に経時的に血液を採血し，血清中の Trp-p-2 とその代謝活性である N-OH-Trp-p-2 を，Ames test により測定した。Trp-p-2 濃度は静注後両群共に急速に減少した。1時間では両群に差はみられなかったが，感染群では3時間で約1.8倍，6時間では約2.7倍と有意に高く代謝の遅れが認められた。一方，代謝活性体である N-OH-Trp-p-2 は，非感染対照群では静注1時間後に鋭いピークがみられた。感染後は3時間後にピークがみられたが，その高さは1/3にとどまった。*in vivo* においても *in vitro* でみられたと同様，代謝活性の低下が確認された。

Trp-p-2 と Schistosome pigment の本質であるヘマチンとの吸着について，0.02mM Trp-p-2 2 m*l* とヘマチン1 mg，5 mg，10 mg を37℃，1時間 incubation し，その上清中の残存 Trp-p-2 を分光学的に測定した。ヘマチン1 mg で上清中の Trp-p-2 は35%，5 mg で0.5%に減少し強い吸着が認められた。このことは，Trp-p-2 は Schistosome pigment に，生体力，特に肝に特異的に吸着され，局所的に肝細胞は高い変異原物質に暴露される可能性を示唆する。

47 当教室を訪れた在留外国人の寄生虫感染，特に赤痢アメーバと日本住血吸虫について（1986—1987年の成績）

山田　稔，吉川　尚男，松田　信治，
松本　芳嗣，手越　達也，陳　錫慰，
塩田　恒三，吉田　幸雄
（京都府医大・医動物）
金　竜起，小林　祥男
（京都市立病院・伝染病）
藤本荘太郎，中島　正継
（京都第2日赤病院・消化器科）

〈症例1〉男，37歳。粘血便中に多数の赤痢アメーバ（Eh）栄養型と少数の嚢子を認む。血清診断抗体陽性（慶応大・医・寄生虫実施，以下同じ）。大腸ファイバーにて多数のびらんを認め，生検切片中に栄養型を認める。チニダゾール（2 g/日，5日）にて治癒するもその後嚢子排出者となる。その後チニダゾール（2 g/日，5日），メトロニダゾール（2 g/日，10日）で治癒せず，カルバルソン（0.75 g/日，分3，10日）でやっと治癒した例である。〈症例2〉男，34歳。大腸アメーバ（Ec）の嚢子，ブラストシスチス・ホミニス（Bh）陽性。〈症例3〉女，31歳。EhとEcの嚢子，Bh，小形アメーバ嚢子陽性。メトロニダゾール（2.25 g/日，5日）にて症例3は治癒するも症例2は未だBh陽性。〈症例4と5〉夫37歳，妻32歳。夫Eh嚢子とBh陽性。妻Bh陽性。妻はチニダゾール（2 g/日，5日）で治癒。症例3，4共抗Eh抗体陰性。〈症例6〉女，24歳。フィリピン出身。急性虫垂炎にて虫垂摘出。虫垂組織中に多数の日本住血吸虫卵（Sj），糞便検査で回虫，鞭虫，Sj卵陽性。肝機能正常。肝脾腫殆どなし。肝生検，直腸生検にてもSj陽性。Ouchterlony，IEP，COP共強陽性（広島大・医・寄生虫実施）。メベンダゾール（200 mg/日分2，3日），プラジカンテル（50 mg/km/日，1日）で治癒。治癒後血清反応は陰転した。結論として，熱帯諸国との国際交流や熱帯諸国を旅行した外国人の訪日する機会の増加する昨今，輸入寄生虫病が増加する恐れが示唆された。

48 ケニア国住民における尿検査紙法によるビルハルツ住血吸虫症患者の発見

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木村　英作，勝又　達哉
（長崎大・熱帯医研・寄生虫）

ケニア国コースト州クワレ地区の一部落住民，約1,400名を対象に，プラジカンテル治療前（検査数915名）および3カ月後（検査数635名）の2回，尿検査紙を用いて血尿・蛋白尿を半定量的に検出し，ビルハルツ住血吸虫（ビ吸虫）の虫卵排泄との関係を調べた。治療の前後において，血尿・蛋白尿のレベル（−，±，1＋，2＋，3＋）とそのおのおののレベルに於ける虫卵陽性者の割合には強い関連性が認められた。また，血尿および蛋白尿と，ビ吸虫卵の排泄数にも強い相関があった。尿検査紙法でどの程度正確に虫卵陽性者を選び出せるかを調べるため，いくつかの判定基準についてsensitivity（虫卵陽性者のうち検査紙が陽性と判定される者の割合）とspecificity（虫卵陰性者のうち検査紙が陰性の者の割合）を比較した。血尿或は蛋白尿だけを基準とするとsensitivity或はspecificityのいずれかが低すぎた。血尿と蛋白尿を組み合わせ“血尿±以上，或は蛋白尿1＋以上”という基準を用いると十分に高いsensitivity（74.6%）とspecificity（85.4%）が得られた。プラジカンテル治療後，住民の虫卵陽性率は54.2%より17.8%に（減少率67.2%），重症度（虫卵数の幾何平均/10 m*l* 尿）は66.7より18.4に（減少率72.4%）下がったが，この基準によるsensitivityは69.0%，specificityは82.0%と治療前に比し殆ど差がなかった。この結果は，尿検査紙法が治療後で住民の虫卵陽性率や重症度が，急激に低下した場合にも応用できることを示している。本法は検鏡による虫卵確認の過程を省くことが出来るので，大規模なビ吸虫症コントロールにおいて薬物治療を容易にするであろう。

49 ケニア国のビルハルツ住血吸虫症流行地における水中セルカリア濃度

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（長崎大・熱帯医研・寄生虫）
D. Migwi　（KEMRI，ケニア）
宇賀　昭二　（神戸大・医・医動物）
行天　淳一　（愛媛大・医・寄生虫）

ケニア国のビルハルツ住血虫症流行地クワレ地区の小部落で住民がよく接触する水場において，水中のセルカリア濃度の測定と感染貝の調査を行った。この部落の住民の感染率は60%で，感染の強さは（排泄虫卵数で表す）は10.5 eggs/10 m*l* 尿であった。部落を流れる4つの川より，22個所を調査地に選んだ。セルカリアメトリーと貝の調査は，月2回行った。1985年11月より1987年5月までの成績を報告する。

調査地のセルカリアの分布：

Mwele川では，1年7カ月の観察期間中に調査した10個所中，8個所で多くのセルカリアが発見された。ある所では50 *l* 中28隻ものセルカリアが検出された。Mtsangatam川では8個所中3個所に，Mbadgi川では2個所中2個所でセルカリアが検出された。Tswele川では，セルカリアは検出されなかった。

感染貝の分布：

Mwele川，Mtsangatam川，Mbadgi川には感染貝は検出されたが，Tswele川には感染貝は検出されなかった。

セルカリアメトリーと感染貝調査結果の比較：

1年7カ月間に22地点で，749回調査が行われた。そのうちセルカリアも感染貝も検出された回数は31回，セルカリアのみ検出49回，感染貝のみ検出は45回であった。

水中セルカリア濃度と感染貝数の季節的変動：

水中セルカリア濃度も感染貝数も3－5月にピークとなる。以後数は減少するが，11—12月に再び小さなピークがみられた。

50 糞線虫感染犬および猿における糞便検査法の比較

塩飽　邦憲，千種　雄一，角坂　照貴，
金子　清俊　（愛知医大・寄生虫）

慢性感染時の糞便中R型幼虫の減少の原因と考えられる要因のうち，腸管内でのR型幼虫数の変動，糞便中でのR型幼虫数の時間的変化を *S. stercoralis* 感染犬で調べると共に，感染犬および感染猿において4種の糞便検査法（ベールマン法，MGL法，濾紙培養法，薄層塗抹法）について比較検討を行った。

感染犬では腸管を下るにつれて内容物1 g当たりのR型幼虫数の減少が認められ，排出された糞便では更に減少していた。排出直後の糞便について，希釈法による幼虫数を100%として4種の糞便検査法を比較すると，ベールマン法とMGL法（ガーゼ無）が82%，MGL法（ガーゼ有）で62%，濾紙培養法で56%の回収率を示した。糞便中での動きのあるR型幼虫数は排出直後を100%とすると，2時間後で85%，6時間後で72%，12時間後で33%，24時間後では8.6%と著しく減少した。犬の糞便検査については，ベールマン法とMGL法が最も優れており，次いで濾紙培養法，薄層塗抹法であった。猿では，糞便が粘着性に富むために，MGL法が最も優れており，次いでベールマン法であった。

慢性感染時の糞便中R型幼虫数の減少の原因については，雌成虫生殖器の萎縮および産卵数の減少，雌成虫数の減少が大きく影響しているが，それ以外に，腸管内でのR型幼虫数の減少も関係していた。糞便検査法については，排便後遅くとも6時間以内に検査すべきであり，MGL法とベールマン法が幼虫の回収率では優れていたが，実用的には佐藤ら（1984），安里ら（1983）の報告のように，回収率が高く，手抜も簡単なMGL法と同定の安易な濾紙培養法の併用が優れていると考えられる。

51 ウェステルマン肺吸虫成虫主要抗原のELISA反応性について

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（宮崎医大・寄生虫）

ウェステルマン肺吸虫成虫（PW）抽出液をSephacryl S-300ゲル濾過で分画すると，分子量の異なる2つの主要抗原（PW-1，PW-2）が得られた。Ouchterlony反応で，PW-1は虫卵抗原と，PW-2はES抗原との間に一致する沈降線を形成した（第39回日本寄生虫学会南日本支部大会）。今回は，肺吸虫症患者血清，実験的肺吸虫感染ネコ血清，および他の寄生虫症患者血清を対象に，PW-1とPW-2のELISAによる反応性を比較した。2週毎に経時採血した感染猫プール血清について両抗原を用いてELISAを行ったところ，急性期（4－8週）ではPW-1よりもPW-2に対して高い値が得られた。しかし，慢性期（20－24週）では両抗原の間で同等の反応性がみられた。更に個別血清について両抗原に対する抗体価の相関を調べてみると，やはり，急性期の血清ではPW-2に対して高い値が得られた。次に肺吸虫症患者血清について反応性をみると，両抗原の間で同等の反応性がみられ，高い相関を示すことから，今回検討した患者血清のほとんどは慢性期のものと思われる。次に肺吸虫症以外の寄生虫症患者血清を用いて，PW-1およびPW-2の交差反応性を調べてみると，両抗原共に各種住血吸虫症の血清に対して陽性反応を示した。そのほかの線虫感染症や横川吸虫症の血清に対しては，交差反応は殆ど見られなかった。住血吸虫症患者血清に対する交差反応は，PW-1の場合には抗原を90℃，30分処理することにより大幅に低下させることができた。また，PW-2の場合には，被検査血清を200倍に希釈することによって除くことができた。

52 旋毛虫抗原遺伝子の構造と発現

菅根　一男　　（信州大・医・寄生虫）

組換えDNA技術を応用し，蠕虫由来の抗原タンパクの性質およびその遺伝子の構造を明らかにし，採取した抗原タンパクの免疫診断への実用化を検討するために，先にplasmid vector, pBR322を用いて旋毛虫抗原遺伝子のcloningを行い報告した。今回は次のstepとしてλ-phage系のexpression vectorであるλgt11を用いて抗原遺伝子を発現させ，そのcDNA sequenceを明らかにしたので報告する。実験方法は，旋毛虫感染幼虫より抽出したpoly A^+ mRNAよりGublerの方法で合成したcDNAをメチル化した後，linkerをligateしEcoRI処理後λ gt 11に組みこんだ。そしてpackagingしてcDNA libraryを得た。次に抗原タンパクを産生するcloneを選択するために，これを*E. coli*に感染させ培地中に形成されたplaqueをIPTGを含んだnitrocellulose filterに移し，目的のcloneを一次血清として旋毛虫感染マウス血清，二次血清としてHRP conjugatedヤギ抗マウスIgG血清を用いた酵素抗体法でscreeningした。その結果，primary screeningで3×10^4個のplaque中4個のpositive cloneが認められた。この4個のcloneにつきsecondary screeningを行い，insertの長さを調べたところ3個は900 b，1個は150 bであった。900 bの3個のcloneにつき制限酵素のcutting siteを調べたところ，同じ部位に位置し，この3個のcloneは同一のものと推定された。この900 bのcDNAの構造を明らかにするために，plasmid vector, pTZ 18Rに入れてdideoxy chainterminination法でDNA sequenceを調べ，computerで解析を行った。その結果得られた900 bのcDNAの3'末端にはpoly A additional signalおよびpoly Aが認められ，塩基配列をアミノ酸配列にして調べたところopen reading frameが存在しややAT richであった。

53 Control of *Aedes* vectors of dengue haemorrhagic fever in Singapore

Chan Kai Lok (Department of Zoology, National University of Singapore)

航空機事情により，来日不可能であった。講演内容は，日本熱帯医学会雑誌，第16巻，第2号，113—120頁に掲載した。和文抄録なし。

54 「北スマトラ地域保健対策プロジェクト」のマラリア媒介蚊対策（'86・87）

安野　正之　（公害研・生物環境）
高木　正洋　（予研・衛生昆虫）
菊池　哲志，徳久　秀二，鈴木　猛
（JICA プロジェクトチーム）
池本　孝哉　（帝京大・医・寄生虫）

インドネシア北スマトラ州のマラッカ海峡に面した漁村で，マラリア・ベクター・コントロール・オペレーションを試行中である。

媒介種 *An. sundaicus* が，屋外吸血，屋外休息傾向のため DDT 残留噴霧は無効である。幼虫の多数発生を見るのは，日当たりの良い汽水域のみである。そこで総合防除による幼虫対策が選択された。

環境改変による防除としては，ラグーンの水路化，一部の溝の埋め立てを行った。結果は良好である。シェイディングも有効である。グッピーの大量放逐による，生物（学）的防除の評価はまだ下せない。高密度に定着した発生源での抑制効果は実証された。失効要因である捕食魚，水質変化，干上がり，散逸，藻類対策の確立が急がれる。Temephos による薬剤防除は，対象面積が散布能力を越えない限り，概ね所期の成果を期待できることがわかった。

86年9月開始の予備試行では，成虫密度の上昇を抑えることは出来なかった。汽水域氾濫原の排水対策が未了のため，降雨で発生源が対処可能面積以上に拡大したためである。87年4月以降の試行では，当初少降雨であったのと氾濫原対策が一段落した結果，密度は低く推移している。環境管理が地域防除成否の鍵であることの証左であろう。防除活動が本格化するにつれ，住民との摩擦も顕在化する。住民の社会経済的基盤や，固有の価値観を十分考慮した衛生教育を志向すべきであろう。

55 A small-scale field trial of S-31183 for the control of *Anopheles minimus* in Thailand

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(Mahidol University, Thailand)
神田　錬蔵
(聖マリアンナ医大・病害動物)
(和文抄録なし)

56 ヌマカ属の羽音捕獲野外実験

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ヌマカ属雌雄捕獲の効率を上げる為の野外実験を，森林内の隔離された発生源ペラク州ポンドク・タンジュン・ヒリーの油ヤシ栽培地内と，セランゴール州バタンダ・ベルジュンタイの広大な開けた沼地の2カ所を選んで行った。

捕獲には灯提とプラスチック円柱を用い，ドライアイスとハムスターをくっつけ，最も効率よい羽音の振動数を発する方法をとった。ヌマカは500 Hz を発信するスピーカーとハムスター1匹とドライアイスを同一灯提内に入れ，一方雄は350 Hz のスピーカーをプラスチックシリンダー内に入れ，ハムスターとドライアイスをくっつける方法が効果的であった。それら両者を毎日発信すると，多数の蚊を捕獲出来たなかでも，広大な土地では経産率が下がるが，新しく生産される蚊を減らすには至らなかった。

森の中の沼では蚊の数は極端に下がるが，森から出てくる蚊が残っていて出てくるため経産率は下がらなかった。

57 オンコセルカ症における媒介者防除

水谷　澄　（日環センター）

オンコセルカ症媒介者としてブユ防除は，もっぱら，幼虫対策としての河川への薬剤投入という形で進められている。しかし本症の流行地である西アフリカと中米においては，発生河川の形態が

著しく異なり，西アフリカの大河で行われている *S. damnosum* に対する航空機などからの薬剤投入方式は，中米の山岳地帯を流れる渓流では適用出来ない。

グアテマラの主媒介種は *S. ochraceum* である。この種は渓床勾配20～40°，流水量18～180 *l*/min（Yamagata, 1984），流速41～66 cm/sec，水深0.2～2.0 cm（Yamagata and Kanayama）の条件を持つ，コーヒー園内または周辺の山間渓流で高い発生がみられている。

山間における問題点は発生源の発見にある。山歩きによる探索活動での調査による，発生源地図の作成が重要である。

一方殺虫剤の投与に関して Temephos を用いた実験によると，処理する薬量にかかわらず，水量の少ない流れでは到達距離も短く，0.18 *l*/秒の流れでは，10分間流水量当たり 2 ppm でも200 ppm でも到達距離は25 m と短かかった。また 1 *l*/秒前後の流れでも有効距離は100 m程しか得られない。この理由として，流水量の少ない所では特に土壌への吸着が著しいことが，室内試験，食塩を流して行った実験や多くの実地試験で確められた。（Umino and Suzuki, 1984; Kamimura ら，1985; Tabaru ら，1982）

この結果，流れの大小にかかわらず Temephos 24 g（a. i. 1. 2 g）を袋に入れ，50～100 m ごとに 2 週間隔で投入すれば，いずれの発生源においても満足すべき結果が得られるという，小渓流型のブユ幼虫駆除方式が確立された。

パイロット地区での防除作業は1979年に Lavaderos で開始され，その後1983までに 6 地区で開始された。

その結果，Lavaderos では 4 年後に ABR は1/100近くまで減少し，他の地区でも1983年には処理前の 1 /10以下となった。またいずれも媒介種の伝播可能密度以下となり（Yamagata ら，1985），上記方式がグアテマラにおけるオンコセルカ症媒介種駆除に十分な効果を発揮したことが証明された。

今後に残る問題点として，1）現状の投薬量の低減化，すなわち投入量の減少と投入間隔の延長が可能かどうか。2）さらに有効距離の長い殺虫原体または製剤の開始の心要性。3）媒介種の薬剤感受性の定期的なチェック。4）抵抗性発現時の代替殺虫剤の準備。といったことがあげられている。

58 カダヤシとメダカにおける蚊幼虫天敵魚としての能力の比較

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神田　錬蔵
（聖マリアンナ医大・病害動物）

メダカと蚊幼虫とが共存していた水域にカダヤシを放魚し，それが定着した後に蚊幼虫の消失した多数の例が，佐藤ら（1972）によって報告されている。

ここでは，カダヤシとメダカとでは，蚊幼虫の天敵魚としての能力に，どのような差異があるかを観察したので報告する。

1．カダヤシないしメダカが高密度に繁殖する水域の，1 ㎡当たりの生息密度は両種とも300～500尾程度であった。両種の混生する水域でも同じ程度であった。

2．自生する個体の胃内容物は，両種とも広食性で，その水域の食物事情に左右され，種間差は認められなかった。

3．室内試験で，カダヤシは人工飼料の有無にかかわらず蚊幼虫をよく捕食したが，メダカでは捕食数が大きく左右され，蚊幼虫の捕食数は著しく減少した。

4．異種に対する攻撃性を調べたところ，グピー稚魚に対しては，カダヤシの場合人工飼料の有無にかかわらずこれを攻撃して捕食したが，メダカでは顕著な差が認められなかった。

5．カダヤシとメダカを 1 番ずつ段階的に10番まで混生させたところ，10日の内には全ての水槽でメダカが死亡するに至り，その捕食回帰直線は $Y=-0.08X+9.4444$ であった。

以上のことから，カダヤシはメダカに比べて，他の生物に対する攻撃性が強く，蚊幼虫捕食数も多かった。すなわち，蚊幼虫天敵魚としてはカダヤシの方がメダカよりもはるかに優れていることが示された。

59 1986年マレーシア・サラワク州におけるデング熱の流行と媒介蚊の生態

宮城　一郎　　(琉球大・医・保健)
Chang Moh Seng　(クチン国立病院)

1986年マレーシア・サラワク州のクチン国立病院媒介蚊防除圧班と共同で，文部省科学研究助成金により蚊科の系統分類学的研究を実施した。その際，サラワク州における最近のデング熱の流行の疫学とその媒介蚊に関する資料についても検討し，現地調査をする機会を得た。

サラワク州におけるデング熱（出血性デング熱を含む）の流行は1975年より徐々に目立ちはじめ，1982年届出患者120名（内血清学的確定者41名)，1983年516名(内243確定)，1984年491名，1985年45名（内14確定)，1981年210名（内65確定）であった。1986年の場合210名中96名 (54%) がクチンなど主要都市を含む第一地区から患者が発生しており，発生のピークは9月から10月にかけてみられた。7月から10月にかけ各地で166個の産卵用トラップを人家内外，庭，森林に放置し，次の5種のシマカ亜属の蚊を得た。*Ae. albopictus, Ae. albolineatus, Ae. malayensis* および *Ae. boharti*，後2者はサラワクから新記録であった。*Ae. albopictus* の発生は572個体で著しく，*Ae. aegypti* は16個体と少なかった。又，65地区で合計3,262軒を調査した結果686軒，1,126容器にシマカ種の幼虫が発生しており house index は21%，Breseau index は35であった。いずれの地区でも *Ae. aegypti* の発生が *Ae. albopictus* より少なかった。この様にサラワク州のデング熱流行地帯では *Ae. albopictus* の発生が著しく，本種のデング熱媒介蚊としての重要性は *Ae. aegypti* と同等と考えられている。

60 タイ国北部の山地および平地における日本脳炎・デング熱媒介蚊の調査

森　　章夫　　(長崎大・医・医動物)
五十嵐　章
(長崎大・熱帯医研・ウイルス)

タイ国北部の海抜1,000mを越える地点に居住するカレン，モン等の山地民の日本脳炎およびデング熱に対する抗体保有率は，いずれも平地に住むタイ族に比べ低いことが明らかにされている。この原因を明らかにするため，標高の異なる地点で日本脳炎とデング熱の媒介蚊の調査を行った。方法はタイ国北部のチェンマイ県で1,000mを越える地点，平地およびその中間の畜舎にライトトラップを設置し，飛来する蚊を同じ時間採集し，その中から日本脳炎媒介蚊を取りだした。またデング熱媒介蚊については，山地と平地の集落で住居の内外の発生源の調査を行った。その結果，日本脳炎の媒介蚊である *Culex tritaeniorhyrchus, Cx. gelidus, Cx. fscocephala* は1,000mを越える地点では，集落が山の尾根にあって最も近い水田から35km隔っているモン族の村では，まったく採集されなかった。カレン族の村でも1,000m以上の地点では水田に隣接するにもかかわらずこれらの種類の蚊はたいへん少なかった。しかし標高が低くなるに従って，これら3種類の蚊の数は増加した。デング熱の主要伝播蚊である *Aedes aegypti* は平地の村では，いずれの集落でも幼虫のみられる発生源が多数存在するのに対し，1,000mを越える地点で調査を行ったカレン，モンの集落ではどちらもこの蚊の主要な発生源となる水がめ，小型の人工容器がたいへん少なく，*Ae. aegypti* の幼虫は採集できなかった。わずかに少数の *Ae. albopictus* が集落内の竹の切株から採集されたのみである。このことから1,000m以上の山地の住民は日本脳炎，デング熱に感染する機会が平地民に比べ大変少ないと思われる。

61 蛇毒の免疫

沢井　芳男　　(日本蛇族学術研)

蛇毒の治療血清は，1890年に北里・ベーリングの破傷風およびジフテリアの血清療法が蛇毒の領域に導入され，1895年にカルメットによりインドコブラ血清が開発されて以来，世界各国でそれぞれの蛇毒で免疫された単価，あるいは多価血清が実際の毒蛇咬症の治療に用いられた。わが国でもハブおよびマムシ血清が作られるようになった。

しかしその後毒蛇咬症の病因が追及され，その過程で，蛇毒による致死，あるいは局所の病変を起す因子等が分離され，それぞれの因子に対する

免疫が要求されるようになった。例えばハブ毒にみられる致死あるいは局所の出血，壊死に対する中和抗体がそれである。従って単価血清であっても，蛇毒の中のいくつかの重要な病害因子に対応する血清の中和価を検討する必要が生じた。トキソイドについても同様であって，蛇毒の毒性因子の免疫原性をそこなわないような不活化方法が問題となる。

また最近は蛇毒の鑑別方法としてELISAが開発されているが，ここでは各蛇毒の中で最も特異性の高い因子に対する免疫抗体の研究が必要になる。

62 ヤマカガシ抗毒素の試作およびその臨床効果について

川村　善治，沢井　芳男
（日本蛇族学術研）

ヤマカガシはわが国で北海道を除く本州，四国，九州に生息している。最近，この蛇による全身性の出血および血液凝固阻止等を呈した重症例が多い。これまでに10数例が報告されている。一般に毒蛇咬症は急性疾患であるが，ヤマカガシ咬症は異例で3～4時間，又はそれ以上の潜伏期間を有する為，重篤になるかの診断が咬症時に大変難しい。昭和59年には急性中毒による死亡例が発生したのを契機に，我々は兎および山羊を用いてヤマカガシ抗毒素の試作を行った。ついで2例の重症患者の治療に応用して効果を確める事が出来たので，報告する。免疫原としてはヤマカガシのトウベルノイ腺から抽出した毒液を凍結乾燥後，1/60 M PBS（pH 7.0）で4 mg/mlに溶解し，ホルマリン（2％）まで加えて不活化し抗原とした。免疫にはウサギ(Lot. 1，2)および山羊2頭(Lot 3）を用い，抗毒素を試作した。免疫動物の粗血清の精製は，塩析法でウサギでは硫酸ナトリウム（Na_2SO_4)を使用し，山羊では硫酸アンモニウム（$(NH_4)_2SO_4$およびペプシン消化法を用いた。治療には精製抗毒素を10 mlバイアルとし凍結乾燥した。それぞれの抗毒素の力価0.1 mlが，ヤマカガシ毒の11, 27, 28 mldを中和した。これらの抗毒素の治療効果については，Lot.1および2をヤマカガシ咬症患者の重症例に投与し，いずれも出血傾向および血液の凝固異常を抑制し患者を回復させる事ができた。

症例1．20歳男性。1987年6月5日11時豊田市逢妻町自宅附近の小川で右手背第4.5指根部を受傷，手背に軽度の腫脹，14時間後に局所の出血の為，医療センターで創部を縫合したが止らず，菊池病院に入院，歯齦および注射局所より持続性出血と潜血尿を呈する。30時間後に抗血清10 mlを静注した。3時間後に出血が止まり，血清病もなく，6月15日退院した。

症例2．9歳男子。1987年9月9日16時30分，岡崎市中之郷町の用水路附近で右手背第4.5指根部を受傷，その後，虚脱状態にあり，1時間後に岡崎病院に入院，ステロイドを注射する。局所は軽度の腫脹と潜血尿あり，次第に歯齦出血が亢進し，四肢等まで出血斑を呈す。9月11日41.5時間後に抗血清10 mlを静注し，24時間後に，血尿，歯齦出血，皮下出血がとまり，治癒した。

63 ヤマカガシ毒の免疫学的研究：特に血液凝固活性化因子を中心に

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本間　　学　　（群馬大・医・病理）

多くの蛇毒が血液凝固活性化を含むが，その作用は第X因子活性化作用を持つラッセルクサリヘビ毒をはじめ，Bothrops属のトロンビン様作用，エキスクサリヘビやヤマカガシ毒のプロトンビン活性化作用など，多種多様である。このような血液凝固活性化因子によって引き起こされるDICや急性腎不全が，毒蛇咬症における重要な問題となっている。

また，ヤマカガシ毒ではマウスへのi.v.投与では非常に強い致死作用（LD_{50} 5.3 μg/20 g mouse）を示すが，皮下や筋肉内投与ではその毒性は1/20～1/30に低下する。このことは致死作用が，血液凝固活性と密接に関連していることを示唆している。

また，日本のヤマカガシと同じナミヘビ科に属する蛇（タイヤマカガシ，オオミズヘビ，カラミズヘビ，ヒロクチミズヘビ，マングローブヘビ）の毒の血液凝固活性化作用を調べ，これらの毒と

の免疫交差について検討した。

ナミヘビ科では，近縁のタイヤマカガシ毒のみ同じく *in vitro* でプロトロンビン活性化作用を示し，他の4種は第X因子活性化作用を示した。また，抗ヤマカガシ血清によりタイヤマカガシ毒の血液凝固活性化致死活性を抑えることができ，これらの2種の毒が類似した抗原性を持つことが示された。

さらに，クサリヘビ科のエキスクサリヘビ（II, X activator)，ラッセルクサリヘビ（X, V activator)，マライマムシ(I, X, XIII activator)の毒との免疫交差を調べた。その結果，Ouchterlony法による沈降線の形成は認められなかったが，抗エキスクサリヘビ血清によって，ヤマカガシ毒の血液凝固活性と致死活性が抑えられた。このことは，全く異なった科に属するヤマカガシとエキスクサリヘビの毒に含まれるプロトロンビン活性化因子が，分子量（それぞれ約170,000と56,000）が異なっているにもかかわらず，類似した抗原性を持つことを示しており，非常に興味深いことである。

64 ELISAによるハブ毒および抗体価の測定

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血中または組織中のハブ毒や抗体価は，ウサギ皮内注射法で測定しているが，この方法は感度がそれ程高くなく，微量の毒量や低レベルの抗体価は測定できないため，もっと感度の高い測定法の開発を目的にELISAのハブ毒への応用を試みた。

ハブ咬症の際の注入毒素量や，トキソイド接種後または咬症経験者の免疫獲得状況などを調査するためには，ウサギ皮内注射法よりもっと低いレベルまで測定できる毒素，および抗体価測定法の開発が必要だからである。

結果

1．ELISAによるハブ粗毒の測定可能域は10 μg～200 μgだった。

2．ハブ粗毒500 μgを注射したウサギの大腿筋の残留毒素量は，30分後：445 μg，1時間後：380 μg，3時間後：220 μg，5時間後：105 μg，24時間後：25 μgとかなり長時間高濃度を持続したが，あらかじめ抗毒素1.0 m*l*(644単位)を静注したウサギでは，15分後：161.39 μg，30分後：88.25 μg，1時間後：33.59 μg，3時間後：1.41 μgと30分以内で大部分は中和され，3時間以内でほぼ完全に中和された。

3．ELISAによる抗HR-1価，抗HR-2価の測定可能域は，いずれも0.02～0.10 u/m*l*だった。

4．抗毒素1.0 m*l*抗HR-1価＝644単位)を静注したウサギでは，1時間後：4.4 u/m*l*，3時間後：2.8 u/m*l*，8時間後：2.5 u/m*l*，24時間後：1.5 u/m*l*，48時間後：1.0 u/m*l*，72時間後：0.6 u/m*l*とかなり長時間高い単位を維持したが，筋注では48時間後の0.9 u/m*l*が最も高く，筋注より静注の方がはるかに効率がよかった。

5．ハブ研究部職員の抗体保有状況を調査した結果，咬症経験者および乾燥毒取扱い者に抗体の存在を認めた。

65 コブラ神経毒の免疫化学について

楊　振忠　（精華大・生命科学研）

台湾コブラ毒から分離，結晶化されたCobrotoxinは分子量7,000の塩基性ポリペプチドで，Postsynaptic トキシンに属する。S-S結合と分子内に埋れたTyr-25はCobrotoxinの生物活性の発現に必須な構造を保つのに必要であり，Lys-47とArg-33の陽イオン群が，運動神経終板のAchRの陰イオンの部位と特異的に作用することによって，神経筋伝導をブロックする。

Cobrotoxinの免疫によって得られた家兎抗血清に，当量のCobrotoxinを混合してできた抗原抗体複合物を，0.53 Mギ酸（pH 2.05）に溶解，Sephadex G-100のカラムでゲル濾過を行って，抗体と抗原を分離した。精製抗体は100% Cobotoxinと反応して沈殿物を作る。そのFabフラグメントとCobotoxinが反応してできた可溶性抗原抗体複合物の分子量（約157,000）から，Cobotoxinは1分子について，3つの抗体結合部位を持っていると推定される。

家兎抗血清の中には，上述の沈降性抗体のほかに，非沈降性抗体が含まれている。非沈降性抗体

はCobotoxinと反応して複合体を形成するが沈殿しない。それは本抗体がCobotoxin分子中3つの抗原決定基のうち2つ，または1つとしか反応しないためである。

Cobotoxinで免疫したマウスの脾臓細胞と骨髄ガン細胞の融合によって，モノクロナール抗体を生産するHybridoma cell lineが確立され，その大量培養およびマウス腹水ガンの引発によって，モノクロナール抗体が大量に生産された。Cobrotoxin Sepharoseカラムによるアフィニティ精製抗体は，IgG_{2a}に属し，2分子のCobotoxinと反応することが証明され，毒ヘビ咬症の治療効果が抗血清に比して46倍高い。

最近，逆相HPLCによるペチプドmappingで抗原ペプチドが分離，モノクロナール抗体の併用による，更に進んだ研究が，抗原抗体作用の解明に寄与することを期待している。

66 マングースの血清中の3種の抗出血因子の精製

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香村　昂男　（沖縄県公害衛研）
与那覇和雄，当山　清善
（琉球大・農化）
川村　善治　（日本蛇族学術研）

マングース血清中の3種の抗出血因子をSephadex G-200およびTSK gel DEAE-5PWを用いて高速液体クロマトグラフティーで約70倍に精製した。収率は約7％～10％であった。

各種精製標品は，ポリアクリルアミドディスク電気泳動的に均一であった。各標品の分子量は，ゲル濾過法により65,000と求められた。また，SDS-ディスク電気泳動により求められた分子量は69,000であることからマングース血清中の3種の抗出血因子は，単一のサブユニットから構成されていることがわかった。各精製抗出血因子は，ハブ毒の出血因子であるHR 1およびHR 2以外に，他の出血毒（サキシマハブ，ヒメハブ，トカラハブ，ニホンマムシ，タイワンハブ）作用も阻害した。

精製抗出血因子は60℃まで安定であり，また，pH 2—11で安定であった。ハブ毒との間に沈降反応が認められないことから，それらの因子は免疫グロブリンではなく，その動物が本来保有している物質であることがわかった。

これらの3種の抗出血因子のアミノ酸組成は，ほとんど同一であった。

67 蜂刺症および蚊刺症の免疫学的研究

加納　六郎　（東京医歯大）

近年蜂刺症が非常に増加し，特に社会生活をしている攻撃性の強いスズメバチ科とミツバチ科の蜂による被害が多い。その中でも最も多いのが，本州，四国，九州では，キイロスズメバチ，北海道では，ケブカスズメバチであり，これに次いでアシナガバチ類である。これらは人家の軒下や庭などに，営巣するためである。また長野県では，地中の巣の幼虫を採るために，クロスズメバチの被害が多い。一方ミツバチは，養蜂家の被害が多い。日本では年間30—40人が蜂刺症により死亡しており，多い年は70人を越えている。これら死亡者の大部分は，蜂毒の直接作用によるものではなく，反復刺されてのアナフィラキシー・ショックによるものである。しかも1時間以内に死亡する者が多い。

一方蚊刺傷でも体質により，症状の激しい者があり，稀に重症例や死亡例がみられる。特に特異体質の子供に多く，アカイエカやヒトスジシマカなど普通種によるものが多い。これもアレルギー，およびアナフィラキシーによるものである。

これから牧野荘平博士らによる蜂刺症のアレルギー症状と，大滝倫子博士らによる蚊刺症のアレルギー症状の症例報告がある。

68 野性蜂刺傷によるアレルギー症状

牧野　荘平，生井聖一郎，池森　亨介
（獨協医大・アレルギー内科）

蜂刺傷は通常軽度の局所反応を示すのみであるが，強い局所反応，全身反応はIgE抗体を介するI型アレルギー反応によっている。我々は栃木県下の農山村地域を対象として，蜂アレルギーの発生頻度を調査し，一部の有症者について免疫学的検討を行った。

1．地域調査：人口約8万の農業林業地域を対

象として，地区衛生役員を介してアンケート調査を行った。101名の有症者の回答を得たが，その症状は，局所反応のみ57%，じんま疹などの軽度全身反応32%，意識障害を伴う重症全身反応５%，遷延型局所反応５%であった。原因蜂はアシナガバチが大部分であったが，スズメバチによる刺傷では重症全身反応が多かった。

地区の一部の保育園から中高校生についても施設を通じて調査し，0.8%に何らかの症状があった。よって，地区全体としては0.4%の有症率となったが，実際は１%弱と推定される。

２．免疫学的検討：発見された種々の重症度を示す有症者29名についてスズメバチ，クロスズメバチ，アシナガバチの蜂毒による掻皮反応を行い，１例を除き全例が１つ以上の毒抗原に陽性反応を示し，14名につき RAST を行い同様に全例陽性反応を示した。この結果は，本調査における症状が IgE 抗体を介していたことを示している。

外国市販蜂毒抗原（Pharmacia）についても皮内反応と RAST で検討し，同種蜂では本邦蜂と共通抗原性が高いことが示され，また，スズメバチ毒，アシナガバチ毒間では共通抗原性が高いが，ミツバチ毒とは共通抗原性が低いことを認めた。

69　蚊刺症

大滝　倫子，岡　　恵子
（東京医歯大・皮膚科）

蚊刺による皮膚反応は刺された頻度，年齢の経過により変遷していくことが従来より欧米諸国で報告されている。わが国でも同様の傾向が見られるか否かを調べるため *Aedes albopictus* を用い，百数十人の被験者の前腕を刺させ，時間経過に伴う皮膚反応の変化を観察した。被験者は１歳より68歳まで各年齢層に亙っている。蚊刺による皮膚反応は二峰性を持つ。つまり蚊刺直後に生ずる即時反応：紅斑膨疹を特徴とし30分を頂点として１～２時間で次第に減弱あるいは消退する。５～６時間後より再燃し始め，瘙痒を伴う浸潤性紅斑を特徴とし，24時間を頂点とする遅延反応の２者である。年齢とともに，この２つの反応は変わる。幼児期には即時反応は生ぜず，強い遅延反応を示す例が多く（Type I），加齢に従い Type II：即時反応陽性，遅延反応陽性となる。さらに年をとると Type III：即時反応は出るが，遅延反応は出なくなる。老齢となると Type IV：即時反応も遅延反応も陰性率が高くなる。即時反応即ちアレルギーI型か，また遅延反応即ちIV型なのかは証明されていない。これら被験者に対し，*Aedes albopictus* 唾液抗原に対する特異 IgE を ELISA 法で測定したところ，実際の蚊刺による即時反応の程度と IgE score とは相関した。

一方，一般的蚊刺反応の他に，重症型蚊刺症がある。第１例は12歳女児，５－６歳より発症。蚊に刺される度に，局所は発赤腫脹し血疱を形成後潰瘍化し，蚊刺後数時間で40℃の発熱を伴う。原因種は *Aedes* および *Armigeres*。現在19歳で同様の状態が続く。第２例は10歳より発症。原因種は *Culex* と推定。13歳で，malignant histiocytosis で死亡。これら重症蚊刺症の発症機序は，アレルギーIII型の Arthus 反応によるものと推定されている。このような重症蚊刺症の報告は，１例を除きわが国に限られており，その死因の多くは malignant histiocytosis である。これらの点も解明されるべき，今後の研究課題である。

70　ナイジェリア国，ジョス大学病院における乳児健診の実態に関する研究

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はじめに：西アフリカに位置するナイジェリア国において，我々は国際協力事業団（JICA）の医療協力プロジェクトの一貫として，国立ジョス大学医学部と共同で小児下痢症に関する研究を行って来た。この研究において乳児健診の分析は村落部，および大学病院における下痢症患児の実態調査の基礎データとなるもので，重要な意味を有している。乳児健診ではユニセフの護助による予防接種の徹底が主な目的であり，同時に体重測定ならびに健康・栄養指導が無料で実施されていた。

対象および方法：1984年ならびに85年の２年間に受診した1,742名を対象とし，その健診記録をもとに母親および乳児の実態について調査分析を

行った。

結果：受診した母親の年齢は12歳から50歳までに分布し，最も多い年代は20代前半であった。児は第1子から第13子までに分布し，第1子が最も多く全体の約20%を占めていた。第1子を出産する母親の年齢は，10代が最も多く64.0%を占めており，特に15歳から20歳が多かった。10代の間に最高7人の出産をしており，一方わずかではあるが10代前半の低年齢出産，および30歳以上の高齢初産が存在していることが明らかとなった。またひとりの母親の平均出産数は約6人であることがわかった。初回受診時の月齢は，1カ月以内の児が最も多く49.0%を占め，再診では6カ月以上の児が多く見られた。児の月齢別平均体重曲線を見ると1960年頃のわが国の状況に近いことが明らかとなった。

結語：10代前半の低年齢出産，および10子以上の多産も存在はするが，症例数は比較的少なかった。体重曲線から，戦後数年間のわが国の乳児の発育状況よりは上回っていることが示された。また本曲線からは，極端な栄養障害などの問題点を見出すことはできず，その原因としてこの健診に訪れる集団が都市部の比較的社会階層の高い集団に属していること，および少なくとも乳児期の体重増加に必要な栄養が，母乳により確保されているためと考えられた。

71 フィリピン・ネグロス島民に対する医療援助の概要
—過去5年間のまとめと，今後の医療援助についての考え方—

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1983年より1987年の5年間，フィリピン・ネグロス島北部，Bacolod市周辺の貧民に対する医療援助を行った結果と，守口市ロータリークラブの寄贈したKanlaon山麓医療相談所における日常医事相談業務の概要について報告する。

方法：毎年12月28日より1月3日のうちの5～7日間，Barangay(村落組織)の長，および当地ロータリークラブの後援にて各村落を移動訪問し，一日診療を行った。構成メンバーは医師10数名，看護婦および調整員，現地在員OISCA隊員ら約30名で，診療科目は内科，外科，小児科，耳鼻科，歯科などである。

結果：過去5年間の受診者総数は10,970名を数え，年齢層は0—89歳で，乳幼児と30—49歳代が多い。傷病大分類別にみた村民の罹患疾病は，呼吸器感染症が最も多く第1位を占め，次いで消化器疾患，神経感覚器の疾患，筋骨格系・結合織の疾患，感染症・寄生虫症，内分泌・栄養および代謝性疾患，皮膚科疾患，精神障害，循環器系疾患，血液疾患，泌尿生殖系疾患と続く。このうち乳幼児は低栄養と腸管感染症，成人では呼吸器感染症ならびに結核が比較的多くみられたのが特異的である。

喀痰排泄患者のZiehl-Neelsen法による結核菌検査では12.8%，345名中44名が陽性を示した。医療相談所における医事相談には，常駐看護婦がその任に当たっているが，感冒，頭痛，下痢症状および外傷に対する処置，高血圧指導，低栄養指導，妊娠や家族計画指導，腰痛対策などの相談が多い。

まとめ：2国間における政治的社会的問題のため，施行しうる医療に限界がある。従って短期間診療にならざるを得ないが，かような方法では疾病罹患の状況把握は可能であるが，臨床の経過観察は困難である。とりわけ肝結核の診断，ならびに排菌者の公衆衛生学的対処がむつかしい。

医学の領域のみならず，人文，社会科学的立場に沿った，よきコミュニケーションの確立を第一義課題とし，今後の医療援助活動をすすめていきたい。

72 開発途上国における栄養障害

鈴江緑衣郎　　(栄養研)

発展途上国の定義を，一応東南アジアとその周辺部に限ってみることにする。この地方は人口密度も高く，米を常用とし，タロ芋やキャッサバ，甘薯などの芋類，一部にはとうもろこしが常用されている。しかし野菜類の使用は，栽培野菜より自然に生えている木々の新芽や果物などが用いられ，むしろ野菜の消費は低い。動物性の食品とし

ては，海岸や河川の側では魚介を食用しているが，その量は多くはない。内陸では鶏や鶏卵，豚が中心であるが，宗教的なタブーが多い。大豆および大豆製品は中国系の住民，インドネシアで多く，椰子油，綿実油などの植物油の消費は比較的高い。このようにこの地域に住む一般の人々の日常食品は，概して低カロリー，低蛋白で，殊に動物性蛋白が少ない。東南アジアに住む人々の体格は一般に見て欧米人に比較して小さく，貧弱でそのうえ寿命も短く，耐久性や根気に乏しい。そのうえ結核やトラホームという，慢性伝染性疾患が広く存在している。これらは長い間に培われた低栄養からの影響と思われる。低栄養の影響として第一に注目すべきことは，低体重出生児の増加である。低体重出生児は母体の栄養状態と健康状態を反映するものであるが，パプア・ニューギニア25%や，ラオス，フィリピンの18%など，相当高率を示している。蛋白エネルギー低栄養症も多く，標準体重80%以下の5歳以下の子供はベトナムで43.6%，ラオス42%，パプア・ニューギニアで36%に達する。特別な栄養素の欠乏症としては，鉄欠乏による栄養性貧血も多い。発展途上国においては妊婦の2/3が，また非妊婦の1/2が栄養性貧血と言われ，母性の健康に重大な支障を来たしている。最近は食物に鉄を強化しているが，寄生虫による貧血も見られる。ヨード欠乏も地方性甲状腺腫としてみられ，聾や精神発達障害の原因となっている。中国に多く，ベトナム，フィリピンにも少し存在する。ビタミンA欠乏症も多く，角膜乾燥症やその他のA欠乏による眼疾患が多く見られる。その国はフィージー，カンボジア，ラオス，パプア・ニューギニア，フィリピン，ベトナムである。定期的に大量のAを投与したり，食品中へのA強化は行われている。以上述べた低栄養症のうち最も重大なのは良質蛋白質の欠乏症で，免疫力の低下をきたし伝染病にかかりやすくなり，また脳の発達も押えられる。将来大切なことは，良質蛋白質を大量に含む食品の開発と，栄養教育である。

73 アジアのらい

森　龍男　（国療・多摩全生園）

世界のねらいの分布を見ると最も密度の高いところはアフリカであるが，総人口の上からみれば，インド，東南アジアがより多くなっている。インドの南端のマドラス地方の1.9%から海岸に沿って東側を北上するに従って1.1%と減少し，その他の地区は1%以下である。WHOの推定では，インドのらい患者数は400万としているが，実際には1,000万の患者がいると推定される。インドにはイギリス統治時代に英国やベルギーのミッションが作った病院があり，らい患者を収容しているが，主として各州の衛生部が中心となって巡回診療を行い，ミッション，WHOおよび笹川記念保健財団などから寄附された治らい薬が無料で投薬されている。また昭和37年に日本の民間の援助によってJALMAが発足し，宮崎先生が初代所長となって行かれたことは周知のことと思う。JALMAではらい患者の外来，および入院の施設を持つと同時に診療班を組んで巡回診療も行ったわけであるが，これが現在もインド政府によって運営されている。タイ国には国立のらい療養所が2カ所あり，患者数は人口1,000人につき1人位で，新患の発生も年間3,000人位で横ばいの状態である。全国を16区域に分けて各区らい対策センターをおき，各県の衛生部と協力し配下の病院と協力してらいの診療にあたっている。治療法は無料，フィリピンにも国立らい療養所が2カ所あり，島々にいくつかの小さな療養所がある。巡回診療で，無料で治らい薬が配布されている。中国ではらい患者数は15万位，過去においてはらいの病院を建てて隔離する方法をとっていたが，現在では在宅治療の方針に切りかえ，各所の病院がらいの外来治療を行っている。治らい薬は国産で，勿論無料で投薬されているが，リファンピシンやランプレンなどに不足しているので，外国のミッションや笹川記念保健財団から寄贈を受けている。日本では昭和22年頃は，年間700名程の新患があったが年々減少し現在では35名で，病型もB群が多くなっている。タイ国ではL型とT型が半々位である。日本においても大学の外来患者の統計を見る

と，明治，大正，昭和の初期ではT型が多く，T型とL型が半々になったのは昭和30年頃である。リファンピシンを2日間内服するだけで，L型患者の全身らい菌が死菌となるので，感染源の減少は患者の減少につながると思う。

74 開発途上国における結核

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感染危険率：現在，結核蔓延状況を測定する最も信頼できる疫学指標は，結核感染危険率であるといわれ，未感染者が1年間に結核感染を受ける確率である。先進国の感染危険率は何れの国も0.1%以下となり，年間約10%の速さで改善をみている。これに対し，韓国，台湾などでは0.7%程度，年間減少率も5%を越えているが，他の多くの開発途上国では感染危険率は今でも1%を越えており，その減少の速度は極めて遅い。このため，世界の結核蔓延状況は，先進国，中進国，開発途上国と3群に分化しており，これらグループの格差，つまり，結核問題の南北格差はますます大きくなっているというのが現状である。

結核罹患率：全世界では塗抹陽性結核患者は，年々400万程度発生しており，菌陰性の肺結核(粟粒結核を含む)，肺外結核（結核性髄炎を含む）を加えれば年々1,000万人にものぼる結核患者が発生していると推定される。このうち80%以上は開発途上国の患者であり，そのうち70%以上はアジアの国々の患者である。

結核有病率：アジア，アフリカのいくつかの国では，大規模な結核実態調査が繰り返えされ，結核の実情が著しく明らかにされている。これらの成績をみると，単に結核有病率が高いだけでなく，結核治療に失敗して結核菌を排菌し続ける慢性排菌者や再発例など，治療の困難な患者が多く，結核問題をより複雑，困難にしている。

結核対策：これに対し，結核対策に用いることの出来る予算は，国民1人当たり0.1ドルにもならない国が大部分である。わが国の結核対策費，国民1人当たり7.44ドル（健康保険による医療費も含む）と比較すると開発途上国の困難さは明らかである。このような実情の中で結核対策をすすめるため，①国の大部分を占める農村でも実施できる，②永続的な対策を，③住民の要求に適合した形で，④地域の一般保健医療システムの中に統合して行うことを原則として，結核対策がすすめられている。

わが国の協力：わが国は結核対策には優れた経験を持っているので，開発途上国の結核対策には多くの面で協力が可能である。事実，結核研究所で行っている国際研修や，2国間の国際協力など活発にすすめられており，諸外国からも高く評価されている。

75 最近経験した熱帯病の症例

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鳴戸　弘，関　育子，表　光代
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松田　肇　（東大・医科研・寄生虫）

マラリア，肝炎，住血吸虫症等の熱帯病は，熱帯地方長期滞在日本人にとって健康管理上重要な疫患である。最近滞在中に罹患したこれらの熱帯病の例をいくつか経験し，さらに罹患状況を調査し集計したので報告する。

症例1．チュニジア滞在30歳女性。全身倦怠，39℃台の発熱で発症，黄疸が出現し肝炎が疑われて入院。精査の結果A型肝炎と診断された。発症1カ月前に生がきを食べており，これによる感染が強く疑われた。

症例2．ザンビア滞在27歳男性。地方の村で稲作技術の指導に従事していた。腰痛と血尿が出現したため現地病院を受診，尿沈渣よりビルハルツ住血吸虫卵が認められAmbilharにて治療を行った。

症例3．マラウイ滞在24歳男性。悪寒と共に39℃台の発熱があり，現在病院にて熱帯熱マラリアと診断された。クロロキン内服および静注により治療を受けたが下熱せず，キニーネ，ファンシダールを併用し発症15日目に下熱した。クロロキン耐性熱帯熱マラリアと考えられた。

症例4．エチオピア滞在33歳男性。湖の周辺で生活し，水辺に繁える草を食べることもあった。現地健康診断時に便より肝蛭卵が検出され，帰国後Bithionolにて治療を行った。

症例5．ケニア滞在27歳男性。61年2月頃より不眠を訴え，家に籠りがちになり，しだいに被害関係妄想が増強してきた。3月に入ると幻聴が出現，医師を受診し精神分裂病と診断された。

76 国連「水と衛生の10カ年計画」について

真柄　安基
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1．はじめに

開発途上国の60%の人々は安全な飲料水が得られず，また75%の人々はトイレ等の衛生施設の恩恵にも浴していない。さらに飲料水が得られても汚染されていたり，相当離れたところに存在するため，何千人の婦人や子供が水を運ぶために1日何時間を費している。その上，水道などの環境衛生施設があっても，維持管理が不適切なために，開発途上国では1日約25,000人以上の人々が死亡し，何百万人もの人が水に起因する病気で衰弱した状態にある。国連では，「水と衛生の10カ年計画（IWSSD)」を立て国連に加盟している世界各国が協力しあって，1981年から1990年までの間に安全な飲料水の供給と汚物の衛生処理に対策を構じ，1990年には全ての人類がそれらの恩恵を受けるよう世界的な事業が展開されている。その中で，この事業の展開状況を概要すると共に，そのさらなる展開に必要と思われる事項について概説したい。

2．IWSSDの状況

1981年以来，当初IWSSDの達成に必要と想定された年300億ドルの投資額の約1/3である，年100億ドル程度が投資されてきた。その結果都市部における水道の整備は，比較的順調に進行してきている。しかし，都市部の衛生設備や，農村部における水道，衛生設備については1981年以降顕著な進捗はない。その理由としては，開発途上国における人口増加が大きいこと，都市への人口移動が都市周辺のいわゆるスラムの形成につながっていること，農村部の貧窮状況が改善されないことなど各種の要因が指摘されている。また，整備されている施設の単価も，わずか約100ドル/人程度に過ぎないのである。

わが国も，IWSSDに積極的に協力しており，昭和56年から昭和59年迄の4年間での協力実績は437.5百万ドルでありODA総額の約3.1%を占めている。その後もIWSSD関連の協力額は増加しているものの，欧米の先進国に比べれば少ないといわざるを得ない。

水道や衛生設備は，地域の特性にあったものが要求されるものである。そのため，わが国の限られた資金，人材で効率的な協力事業を展開して行くためには，特にこの分野の国際協力を中心となって実施する機関・機構の強化が要望されている。また，開発途上国の技術水準や運営体制を踏まえた，地域の条件に合致した技術（適正技術）の開発も積極的に行う必要性が強く指摘されている。

3．おわりに

第2次世界対戦のわが国の状況から，今日までの過程を振り返ってみるとき，開発途上国の状況は解決できるものと信じている。「水道や汚物の衛生処理」は，まさに人間の尊厳を保つに必要な最低の要件である。開発途上国の深刻な衛生状況は，テレビや新聞からある程度は知ってはいても，日本の日常生活の中では実感として捉えがたく，単なる同情心に留まってしまうことが多い。

21世紀に向けて，わが国より豊かな社会を構築し，国際社会の一員として調和していくために求められている行動の1つが，IWSSDへのより積極的な関与だと考えている。

77 インドネシア各地の飲料水から検出された *Pseudomonas aeruginosa* の薬剤感受性について

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1985年と1986年に行った，インドネシア各地の飲料水の細菌学的調査の際検出された *Pseudomonas aeruginosa* の25菌株について，化学療法剤に対する感受性，およびpenicillin Gとcefazolinに対する β-lactamase活性を検討したので，その成績を報告する。

感受性テストの成績では，MIC値でcarbenicillin, cephaloridineおよびcefoxitinにお

いて1,000 μg/ml 以上, cefmetazole で500 μg/ml 以上, cephalothin で25 μg/ml 以上というような高い抵抗性を示す値が得られた。一方, tobramycin と amikacin では良好な感受性が示された。

同菌種について, 東邦大・医・微生物学教室の五島らが開発した方法を用いて, penicillin G と cefazolin に対する β-lactamase 活性を測定した。その結果は, すべての菌株で, 両薬剤に対する β-lactamase 活性は低いものであった。

現時点までの化学療法剤感受性テストの成績および β-lactamase 活性値と, 検出菌株の由来, 地域, 血清型別との間に, 特別な関係はみられていない。今後, 検討を継続していきたい。

78 海外発展途上国における最近2カ年の飲料水検査成績 (1985, 1986年)

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我々はこれまで, 熱帯発展途上国で上位罹患率を占めている経口感染症の感染源の1つとして, 飲料水の重要性を強調してきた。今回は, 第27回本学会総会での報告に引き続き, 熱帯各地すなわち, 東南アジア, 中近東, 東アフリカ, 中南米の多くの国々の首都に在留する邦人たちが実際に使用している飲料水について, 化学的および細菌学的分析を行ったのでその成績について報告する。調査は, 1985年3月から1986年10月までの2年間に行われた。飲料水は, あらかじめガス滅菌したポリ容器に直接採取し, 直ちに細菌学的検査 (一般細菌, 大腸菌群) を実施した。化学的分析は, 日本に持ち帰り行った。

1985年度の成績は, 次の様であった。水道水末端より遊離残留塩素が検出されたのはザンビア (22.2%), マレーシア (8.3%), インドネシア (3.9%) であった。しかし, これらは多分に個人的に殺菌剤を投入しているものと思われ, 行政上行われているか否かは疑問である。またベネズエラのミネラルウォーター (7検体) より, すべてに遊離残留塩素が検出された。糞便系汚染の直接的指標である大腸菌群は, ほとんどの国々の水道水, 井戸水より検出された。その中でもインドネシア (50.0%), タイ (44.2%), フィリピン (31.6%) と, 東南アジアの国々で検出率が高かった。1986年度の調査結果では, ビルマ (33.3%), メキシコ (14.3%), アラブ首長国連邦 (8.3%) の国々の首都飲料水より遊離残留塩素の検出がみられた。大腸菌群の検出も前年度と同様に, 東南アジアの国々に多くみられた。前回の報告とも合わせ, 最近4—5年は公共の給水源・施設の改善はほとんど見られないことがわかった。

79 インドネシア在留邦人の飲料水

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熱帯各地の発展途上国の在留邦人にとっては, いかにして安全な飲料水を確保するかがその国で健康な生活を送るための鍵を握っていると言っても過言ではない。そのために, 在留邦人の多くはミネラルウォーター, 煮沸水, 浄水器を利用し, 出来るだけ安全な飲料水を得るように努めている。今回, インドネシア在留邦人が実際に飲んでいる各種類の飲料水について, その細菌学的検査に焦点をしぼって検査を行ったので, その成績について報告する。

飲料水は, あらかじめガス滅菌したポリ容器に直接採取し, 直ちに細菌学的検査を行った。また, 調査は, 1985年および1986年の6—7月に行った。

その結果, 大腸菌群陽性または一般細菌陽性の飲料水を使用している人の寄生虫陽性率は, それぞれ75.0%, 81.3%とかなり高く, 経口感染症の感染源の1つとして重要であることが再確認された。煮沸水, 浄水, ミネラルウォーターからもかなりの割合で一般細菌, 大腸菌群が検出された。煮沸処理前後の細菌陽性率を比較してみると, 1985年, 1986年共に原水よりも煮沸処理水からの大腸菌群検出率が高いことがわかった。そして,

原水中細菌類が陰性であったものが，煮沸処理後には細菌類が陽性になっている検水の割合も50%前後に観察された。浄水器使用前後の細菌数陽性率は，浄水後には減少しており，ある程度効果があることが判明した。しかし，使用期間が6カ月以上になると，原水の細菌類陰性だったものが浄水後には陽性に転じている検水の割合が増え，浄水器の保守管理に問題があることが示唆された。

80 ナキウサギ（pika）の体温調節能—特に発熱特性について—

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松本　孝朗，大渡　　伸，范　　育仁
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ナキウサギ（pika）の体温が家ウサギより高い理由は，その高い熱産生能と低い熱放散能に起因するが，運動量・耳介の含む体表面積や行動性体温調節能などを統合して考えるべきである。ナキウサギは麻酔剤に高感受性を示す反面，腹腔内投与の外因性発熱物質（LPS-pyrogen）に反応しないと報告されている。

今回，私共は細い耳介静脈注入の困難を克服して，22℃室内に飼育のナキウサギ(n＝9，平均体重：262.2 g）を人工気象室（28℃，60% rh）に移し3.8 μg/kgのLPS-pyrogenを耳介静脈注入して発熱反応の有無を検索し，次の結果を得た。

(1)無麻酔，無拘束のナキウサギの平均直腸温（39.34℃）はLPS-pyrogen静注後，20分の潜時を経て上昇開始，最高直腸温40.23℃（△ Tre：0.73℃）を経て発熱持続時間70分の一峰性発熱曲線を示した。

(2)呼吸頻度（RR）は，LPS静注前値108 c/min，発熱極期100 c/min，発熱終了時92 c/minと変化し，これは家ウサギのRR変化率より小さく，熱放散反応の不備を示唆している。

(3)発熱上昇期にはふるえ（shivering）熱産生，立毛反射や体動減少など，熱産生反応や熱保存反応には首尾一貫性がみられ，この結果からナキウサギが家ウサギに劣らない体温上昇能のみならずLPS-pyrogenによる発熱特性を有することが明らかになった。

81 Dry Saunaによる暑熱負荷時の熱出納

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熱帯地非スポーツマンと，日本人スポーツマンを環境制御実験装置（28℃，60% rh）に安静にさせた後，低温サウナ（60—70℃）で30分間暑熱負荷を加え（n＝4　period），体重，皮膚温，心拍数，血圧，呼吸数，代謝量の変化を連続記録解析した。暑熱負荷中の熱帯地非スポーツマンの心拍数，代謝量の増加率は日本人スポーツマンのそれより低かった。また，熱帯地非スポーツマンでは，かなりの呼吸数増加が観察され，パンティング様の浅速呼吸がみられた。これらの結果は，(1)暑熱負荷中の心拍数と代謝量の変化は両被験者で平行推移すること，(2)暑熱負荷中の代謝量の変化率は熱帯地被験者において小さく，これは温熱感受性の閾値上昇による慣れの現象と考えられること，(3)暑熱負荷によって誘発された呼吸数の増加とパンティング様の浅速呼吸は，熱帯地住民の熱放散反応を特徴づけるものか否か今後の検討を要すること，などを示唆している。

82 暑熱順化に関する研究（第4報）—容量式湿度計・発汗カプセル法による解析

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気温28℃，湿度60%に調節された環境制御実験装置で暑熱地住民や日本人スポーツマンの両下肢に，温熱刺激（43℃温水，30分）を負荷して体温調節の熱放散反応(特に温熱発汗)を誘発し，サーモグラフィ装置や容量式湿度計—発汗カプセル法による発汗開始時間，発汗閾値口腔温，刺激中(20分間)，刺激後（5分間）の発汗量などを各種熱放散指標を同時記録，解析して，暑熱地住民・日本人スポーツマン・日本人非スポーツマンの3群についてデータを比較し暑熱順化の中枢性・末梢性機序解明に資した。

本研究では，特に(1)容量式湿度計—発汗カプセル法による発汗定量の校正曲線（calibration）の描記に新考案を試み，被験者に装着した発汗カプセルの頂部にもうけた小孔から，カプセルの下の皮膚の上に汗や30℃，0.45%NaClを0.01，0.02，0.03，0.04 mlの容量ずつマイクロピペットを用いて段階注入，高感度容量湿度計を介して相対湿度（% rh）曲線を連続記録し，これを絶対湿度に変換，最終的には発汗量をmg/cm²•minの単位で測定し，実記録中の発汗量の計測を可能とした。

(2)上記，発汗カプセル法（Fan-Kosaka method)による発汗潜時，発汗開始閾値口腔温はサーモグラフィ装置による胸部・腹部平均皮膚温の変化時点のそれらの一致，さらに容量式湿度計—発汗カプセル法は発汗量の正確な測定が可能である点，発汗解析に極めて有効な手段であることが明らかになった。

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41 IMMUNO-EPIDEMIOLOGICAL SURVEY OF HUMAN PARASITOSES IN SIND, PAKISTAN

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The immuno-epidemiological survey of parasitoses was carried out in the period from December, 1986 to January, 1987 on 149 inhabitants in Sind, Pakistan.

The positive rate of *Toxoplasma* antibody by latex agglutination test was 34.2%, the result was apparently higher than 11.7% of healthy person in Japan. The test antigens of helminths used were *Toxocara canis* larval ES (TcnLES), *Trichinella spiralis* larval extracts (TspLEX), *Angiostrongylus cantonensis* adult extracts (AngAEX), *Dirofilaria immitis* adult extracts (DiAEX), *Gnathostoma hispidum* larval extracts and *Fasciola hepatica* adult extracts. The positive rates of antibodies by Ouchterlony's method were 10.9% to TspLEX and 7.4% to AngAEX, those with countercurrent electrophoresis to TcnLES and TspLEX were 1.6% and 5.7% respectively. The positive rates of antibody titers by ELISA to AngAEX and DiAEX were 4.2% and 3.9% respectively, however, antibodies to TcnLES and TspLEX were not detected.

In contrast, those of Japanese were 0.8% to AngAEX, 2.6% to DiAEX, 1.3% to LES and 1.7% to TspLEX by means of ELISA. There were no significant differences in positive rates between Sind and Japan. Considering our present results, latent infections with *Trichinella* and *Angiostrongylus* in Sind were suggested.

42 RECENT STATUS OF PARASITIC DISEASES IN NEPAL

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Japan International Cooperation Agency decided to send the observation team composed of 6 members who cover public health, epidemiology, microbiology, parasitology and pediatrics in Nepal.

This pesentation is restricted only to the observations of parasitic diseases. (1) Malaria

is widely prevalent among inhabitants in the southern territory of Nepal along the boundary between India. It is estimated that about 60% of the population (nine millions) is exposed to the risk of the infection and it is one of the most important diseases in this country. The malaria situation became suddenly serious showing the number of patients of more than fourty thousands in 1985. It is evident that the prevavence is high in the southern parts of the country. (2) Kala-azar has been reported after 1980; the cases were 604 with the case fatality rate of 8.0 per cent.

(3) Filariasis seems to be prevalent not only among rural but also urban population. A survey indicated a high positive rate of microfilaria in blood as 7.1-9.2% among people in the central and western region. (4) Echinococcosis has been reported and a number of patients received the operation at the hospitals in Kathomandu.

(5) Rate of infestation of the inhabitants by intestinal parasites is very high all-over the country.

43 A SURVEY ON INTESTINAL PARASITES IN A RURAL VILLAGE IN GHANA

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A survey on intestinal parasites was carried in a rural village in Ghana. Stool specimens were collected from 198 persons with various ages in September-November 1983 (period A) and from 155 persons in January-April 1984 (period B). After masstreatment with pyrantel pamoate (Combantrin^{R}), 98 persons who took the brug examined in April-August 1984. Stool examinations were done by means of the direct and the Formalin-ether concentration methods.

In both period A and B, *Ascaris lumbricoides* was the most common helminth (63%, 61% respectively), followed by hookworm (30%, 25%), *Trichuris trichiura* (23%, 27%) and *Strongyloides stercoralis* (3%, 11%). The most common protozoan parasite was *Entamoeba coli* (18%, 27%). Both single and multiple infections with these species were found in more than 80% of residents in the age group ranging from 4 to 6 years old.

The treatment with the anti-helminth drug resulted in significant reduction in the positive rate of *A. lumbricoides* (7.4%) and *T. trichiura* (12%) but changes of the rates of hookworm (20%) and *S. stercoralis* (3%) were not significant. Re-infections which might take place during the period of the survey after the treatment may cause the underestimation of the effectiveness of the drug.

This study was supported in part by JICA and by Ghana Government.

44 SURVEY ON INTESTINAL PARASITES AMONG SCHOOL CHILDREN IN JOS, NIGERIA

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Survey on intestinal helminths and protozoa was made on the school children in Jos, Plateau State of Nigeria during the rainy season in June to September, 1985. A total of 620 stool samples were collected from 4 different primary schools, and they were examined by formol-ether concentration technics. In total 12 species of parasites were detected. They were composed of 6 species helminths and 6 protozoa. In 4 different school, helminthic parasites occurred in 22.6% (140/620). The overall positive rares of eggs in stool specimens were 9.5% for *Ascaris lumbricoides,* 5.3% for *Necator americanus* and 7.4% for *Trichuris trichiura.* The rate of infection the with other helminths were 0.5% for *Strongyloides stercoralis,* 0.3% for *Hymenolepis nana* and 0.2% for *Taenia* sp. On the other hand, intestinal protozoan cysts occurred in 44, 2% (274/620), including specific protozoa, such as *Entamoeba coli* (36.3%), *Entamoeba histolytica* (13.4%), *Endolimax nana* (8.7%), *Iodamoeba buetschlii* (6. 6%), *Giardia lamblia* (8.9%) and *Chilomastix mesnili* (5.2%). In Tudun-wada primary school the intestinal protozoan infections were found higher than in the other schools.

45 SCHISTOSOMIASIS IN BOHOL ISLAND, PHILIPPINES, WITH SPECIAL REFERENCE TO THE HABITAT CHARACTERISTICS OF THE VECTOR SNAIL, *ONCOMELANIA QUADRASI*

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Bohol Island lies between Southern Leyte in the east and Cebu in the west in latitude 10°N and longitude 124°E. Oval in shape, Bohol has an area of 4,117 square kilometers. The endemic focus of schistosomiasis in the island is confined only to the left bank of Ipil River, or two of 46 municipalities of the province, namely Talibon and Trinidad. We have conducted malacological and epidemiological surveys in the island in 1981. As a result six new snail colonies were found by us in 1984 and 1985. We were led by the proximity of the snail habitat to the residence of the infected individuals. There are now 11 snail colonies in the island, which are apparently confined to three water courses flowing into Ipil River. We studied the

topography of places that look suitable for snails but to not harbor them in the island. It was found that all snail positive areas are located the regions with sandy loam and with a pH of not more than 6.5. The area has seriously been visited by a drought for the period of 3 to 4 months every 3 to 5 years. *O. quadrasi* is found only in spring-fed swamps and its adjoining small creeks located in the folds of the hills, in which common fresh water snails such as *Melasia* sp., *Segmentina* sp. and *Lymnaea* sp. are observed, indicating that the habitats have never or seldom dried up all the year round. Our findings shed some light on the apparent restriction of the endemic focus of the disease in Bohol.

46 IN VIVO METABOLISM OF TRP-P-2 IN MICE INFECTED WITH *SCHISTOSOMA JAPONICUM* AND ITS ADSORPTION TO SCHISTOSOME PIGMENT

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Mutagen metabolizing activity of cytochrome P-450 in the liver was investigated in connection with the liver cancer and schistosome infections. The metabolizing rate was examined in vivo after intravenous injection of a strong mutages, Trp-p-2 derived from tryptophan pyrolysate. *Schistosoma japonicum* infected and age-matched control mice were injected with 0.3 m*l* of 2 mM Trp-p-2. The concentrations of Trp-p-2 and its metabolite (N-OH-Trp-p-2) in the serum were measured periodically by Ames test. Trp-p-2 concentration decreased immediately in both groups at one hour after the injection, but the difference was not notied. However, Trp-p-2 in infected mice remained significantly high in the serum at 3 and 6 hours as compared with that in control mice. N-OH-Trp-p-2 in infected mice increased gradually and showed a lower peak at 3 hours than that in control mice. The decrease of metabolizing rate in *S. japonicum* infected mice was confirmed *in vivo* as well as *in vitro*.

Adsorption of Trp-p-2 to hematin which is thought to be the same substance as schistosome pigment accumulated in the mouse liver when infected with *S. japonicum* was examined. Two milliliter of 0.01 mM Trp-p-2 were incubated with 1 mg, 5 mg, or 10 mg of hematin for one hour at 37°C. Remaining Trp-p-2 in the supernatant decreased to 35%, 0.5% and 1.3% of the original concentration, respectively. Storong affinity of Trp-p-2 to hematin was confirmed *in vitro*. It suggested that hepatic cells in schistosomiasis were exposed to highly concentrated Trp-p-2 or other mutagens which possess a high affinity to hematin.

47 PARASITIC INFECTIONS OF FOREIGN RESIDENTS DIAGNOSED IN OUR LABORATORY, DURING THE PERIOD 1986-1987, ESPECIALLY ON *ENTAMOEBEA HISTOLYTICA* AND *SCHISTOSOMA JAPONICUM*

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We examined intestinal protozoas and helminths among foreign residents during the period 1986-1987, and six cases of these are reported. Case 1: The patient (37-year old male) was suffered from *Entamoeba histolytica* (Eh). Many trophozoites and some cysts of Eh were found in the bloody stool. Serum antibodies to Eh were positive. He was treated with tinidazole (2 g/day, 5 days) and cured. He, however, excreted many cysts and so again was treated with tinidazole (2 g/5 days), but the cysts were still appeared. Additional treatment with metronidazole (2 g/day, 10 days) was conducted but it was not successful. He finally cured by carbarsone administration (0.25×3 g/day, 10 days). The results from Case 2 to Case 5 were as follows: Case 2 (34-year old male) was positive for cyst of *Entamoeba coli* (Ec) and *Blastocystis hominis* (Bh), and Case 3 (31-year old female) was positive for cyst of Eh, Ec, *Endolimax nana*, and Bh. Metronidazole treatment (2.25 g/day, 5 days) cured Case 3 but not Case 2 who had still Bh. Case 4 (37-year old male) and Case 5 (32-year old female), a couple, were positive for Bh and the former had cyst of Eh additionally. Only Case 5 was treated with tinidazole (2 g/day, 5 days) and cured. Serum antibodies to Eh in Case 3 and 4 were negative. Case 6: The patient (24-year old female), who is Phillipino and married with Japanese man, was suffered from acute appendicitis. After surgical operation, the ova of *Schistonoma japonicum* (*Sj*) were detected in the sections of the appendix. In her stool 3 kinds of helminth ova of *Ascaris lumbricoides, Trichuris trichiura*, and Sj were found. The former two parasites were successfully treated with mebendazole (100 mg×2/day, 3 days). Ova of Sj were detected not only in appendix and in the feces but also in the rectal mucosa and in the liver. Serum antibodies to Sj were strongly positive. One day dose of praziquantel (50 mg/kg) successfully cured the patient, and the Sj ova continued negative in stool and antibody test became negative thereafter.

In conclusion, we should pay attention to the importance of increasing source of parasitic infections with increase of foreigh residents with parasites.

48 SCREENING OF URINARY BILHARZIASIS USING REAGENT STRIPS IN KENYA

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The effectiveness of urinalysis reagent strips, which detect haematuria and proteinuria semi-quantitatively, in selecting *Schistosoma haematobium* egg-positive persons was studied in a community (population 1,465) in Kwale, Coast Province, Kenya. The study put particular emphasis on comparing the efficacy of reagent strips before and after chemotherapy.

A total of 915 subjects were examined in June/July, 1986 for *S. haematobium* eggs, haematuria and protenuria, which was followed by treatment with a single dose of praziquantel at 40 mg/kg in July/August. Three months after treatment, another round of urime examination was carried out including 635 subjects.

The levels of haematuria and proteinuria (negative, trace, 1+, 2+, 3+) were found to be strongly associated with prevalence of the egg positives. The degrees of urinary blood and proteis were also correlated positively with the number of eggs excreted in urine. The association and correlation were strong even after the treatment with praziquantel prevalence from 54.2 to 17.8% and intensity of infection from 66.7 to 18.4 eggs/10 m*l* of urine.

For the purpose of establishing a criterion of reagent strips, several different criteria were compared in terms of sensitivity (% of egg-positive cases who were also reagent strip positive) and specificity (% of egg-negative cases who were also reagent strip negative). Neither haematuria nor proteinuria alone was sufficient as a criterion due to low sensitivity or specificity. When a combined criterion "haematuria trace up or proteinuria 1+ up" was applied for the selection, reasonably high sensitivity (74.6%) and specificity (84.4%) were obtained before treatment. The reduced prevalence and intensity of infection after praziquantel treatment did not affect sensitivity (69.0%) and specificity (82.0%).

In controlling urinary schistosomiasis, chemotherapy may have to be repeated. Our present result indicated that reagent strips could be applicable even after substantial reduction of prevalence and intensity of infection by chemotherapy. The use of reagent strips would greatly simplify the selection of persons to be treated, and facilitate large-scale control activities.

49 CERCARIAL DENSITIES AT WATER CONTACT SITES IN AN ENDEMIC AREA OF URINARY SCHISTOSOMIASIS IN KENYA

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Studies on cercarial densities and vector snails (*B. globosus*) were carried out at selected water contact sites in a village in Kwale District, where *S. haematobium* was endemic. Prevalence rate and intensity of infection (geometric mean) in the village were 60% and 10.5 eggs/10 m*l* of urine respectively. Along four rivers (stream) running through the village, 22 study sites were selected, where cercariometry and snail surveys were carried out twice a mouth per site. Results obtained between November, 1985 and May, 1987 will be reported this time.

(Distribution of cercarial in the study area)

Along Mwele River, many cercariae were detected at 8 of 10 study sites in 19-months study period. At one site as many as 28 cercariae/50 m*l* of water was recorded. Cercariae were found at 3 of 8 study sites along Mtsangatamu River, and all 2 sites along Mbadzi River. However, no cercariae was recorded from Tswele River.

(Distribution of infected snails)

Infected snails were found in the Rivers of Mwele, Mbadzi and Mtsangatamu, but not in Tswele River.

(Comparison of results of cercariometry and snail survey)

A total of 749 times of survey was made at 22 study sites in 19 months. In 31 surveys, both cercariae and infected snails were detected. Whereas, only cercariae were detected in 49 surveys, and only infected snails in 45 surveys.

(Seasonal changes in cercarial densities and the number of infected snails)

Cercarial changes in cercarial densities and the number of infected snails reached the maximum in March-May. Another small peak was in November-December.

50 COMPARISON WITH METHODS OF FECAL EXAMINATION IN DOGS AND A MONKEY INFECTED WITH *S. STERCORALIS*

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The diagnosis based on fecal examinations of chronic strongyloidiasis is difficult because of low larval outputs in feces. Dogs infected with *S. stercoralis* also expelled no rhabditiform larvae in feces from about 10 weeks after infection. We repported that the decrease in larval outputs of feces related to a decrease in a number of parasitic females and to an atrophy of reproductive organs in parasitic females (1987). In the present study, fluctuations in a number of rhabditiform larvae in the intestine and feces were counted with various methods of fecal examination.

Numbers of rhabditiform larvae in contents of the intestine decreased with nearing the anus, then those in expelled feces decreased remarkably. Four methods of fecal examination were compared with a simple dilution method on feces just after defecation. Recovery rates of larvae were 82% for Baermann technique, 82% for formalin-ether centrifugation technique without a gauze, 62% formalin-ether centrifugation technique with a gauze and 56% for filter-paper cultures at 25°C for 35 hours, against the number of larvae counted with the simple

dilution method. Motile rhabditiform larvae in expelled feces decreased in number with time, 85% at 2 hours, 72% at 6 hours, 33% at 12 hours and 8.6% at 24 hours after defecation, at 25°C with the simple dilution method. It considers that feces with rhabditiform larvae should be examined within 6 hours after defecation. Although high recovery rates of larvae in feces of dogs and a monkey infected with *S. stercoralis* were obtained with Baermann technique or formalin-ether centrifugation technique, a combination of formalin-ether centrifugation technique and filter-paper cultures seems to be useful for its simplicity and identification of the species in practice.

51 ELISA REACTIVITY OF MAJOR ANTIGENS ISOLATED FROM *PARAGONIMUS WESTERMANI* ADULT WORMS

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Two antigenic fractions, termed PW-1 and PW-2, having different molecular weight, were isolated by Sephacryl S-300 gel chromatography of *Paragonimus westermani* adult worm extract. By Ouchterlony test, PW-1 was identified as egg-derived antigen, while PW-2 was identified as ES antigen. In the present study, ELISA-reactivity of PW-1 and PW-2 was tested against sera obtained from experimentally infeced cats, paragonimiasis patients, or from other helminthiasis patients. Pooled cat sera obtained at an acute stage of infection (4-8 weeks) showed higher ELISA value against PW-2 than against PW-1. On the other hand, pooled cat sera obtained at a chronic stage of infection (20-24 weeks) showed comparable ELISA value against two antigens. When correlation coefficiency of the ELISA values of the individual cat serum obtained at various time after infection was examined, PW-2 showed higher value against acute stage-serum than PW-1 did. Since sera from paragonimiasis patients showed comparable ELISA value against PW-1 and PW-2, these patients were assumed as chronic stage. Sera from schistosomiasis japonicum, mansoni, or haematobium showed significant cross-reactivity against PW-1 and PW-2. Sera from other helminthiasis, however, did not show cross-reactivity. The cross-reactivity of PW-1 observed against schistosomiasis sara was significantly reduced by pre-treatment by heating (90°C, 30 min) of PW-1, whereas the cross-reactivity of PW-2 against schistosomiasis sera was reduced by diluting test serum up to 200 fold.

52 STRUCTURE AND EXPRESSION OF A GENE ENCODING AN ANTIGEN OF *TRICHINELLA SPIRALIS*

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In order to collect a large amount of *T. spiralis* antigen which is useful for immunodiagnosis, recombinant DNA technique was used. Total RNA was extraced from infective larvae of *T. spiralis* by centrifugation through a cesium chloride cushion. Poly (A)-rich mRNA was isolated from total RNA with oligo (dT)-cellulose gel column. Double stranded cDNA was systhesized and ligated into the EcoRI site of β-galactosidase gene present in λgt 11 DNA and packaged. Phages containing inserts were infected to *E. coli* and screening of positive plaques was done by enzyme immunoassay. An antigenic fusion protein extracted from *E. coli* was demonstrated by Western blotting analysis. cDNA sequencing was done by dideoxy chain-termination method.

53 CONTROL OF *AEDES* VECTORS OF DENGUE HAEMORRHAGIC FEVER IN SINGAPORE

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(The author could not attend the meeting because of transportation trouble. The paper of the subject was submitted and appeared in Japan. J. Trop. Med. Hyg., Vol. 16, No. 2, pp. 113-120, 1988)

54 MALARIA VECTOR CONTROL OPERATION IN THE "NORTH SUMATRA HEALTH PROMOTION PROJECT"

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An integrated larval control operation against *Anopheles sundaicus* has been undergoing in an coastal area of North Sumatra, Indonesia to establish an alternative control measure to the DDT residual spraying, as the target species was found to be exophily and exophagous in the area.

Channeling of lagoons is promising. Both of the filling up roadside ditches and the shading on pouds were also effective, although they were only applicable for some habitats. The effectiveness of releasing guppies, as an biological control agent, is on the course of evaluation. The agent can successfully control the larvae, if the density of it is continuously high. Diminishing or vanishing of the agent was also observed, however, due to predation by other predacious fishes, intolerable changes of the water quality and/or quantity, and over dipersion. Larviciding by temephos was satisfactory.

On the preliminary operation conducted from September in 1986 our effort failed in

reducing the adult density due to the precipitation which expanded the habitat to an uncontrollable extent, before the scheduled environmental management was carried out. On the next one started from April in 1987 little precipitation and the effective drainage resulted in low adult density. This suggests the importance of the environmental management for the malaria vector control.

Some disagreement with the operation has arisen among villagers with progress of the activity. More serious health education taking account both of the socio-economic merit for villagers and their way of thinking in strongly needed.

55 A SMALL-SCALE FIELD TRIAL OF S-31183 FOR THE CONTROL OF *ANOPHELES MINIMUS* IN THAILAND

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The insect growth regulator, S-31183 (0.5 G formulation), was applied into two slow running streams (A and B) which were the breeding habitats of *Anopheles* vectors at the dosage of 5 ppb in February 1987. The flowing rates of the stream A and B were 2.5 *l*/sec and 5.2 *l*/sec, respectively. The evaluation of the efficacy was based on bioassay test of the water from the streams as well as on the age determination of the collected mosquitoes.

It revealed that this insect growth regulator diffused readily from the application sites along the streams. Twenty-four hours after appication the bioassay test of the water collected at the check points, 150 m (stream A) and 50 m (stream B) produced 100% and 96% emergence inhibition of *Anopheles maculatus,* respectively. Based on weekly observations it showed that S-31183 (0.5 G formulation) exhibited more than 70% emergence inhibition for *An. maculatus* and *An. minimus* for approximately one month. Likewise, the nulliparous population of *An. minimus* was also decreased. It might be suggested that this compound inhibited the emergence of the new generations of mosquitoes.

56 FIELD STUDY ON SOUND TRAPPING OF *MANSONIA* IN MALAYSIA

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Two systems for the effective trapping of *Mansonia* of both sexes were designed and

tested near an isolated forest swamp ecotype near an oil palm plantation at Kampong Pondok Tanjung Hilir, Perak and an open vast swamp at Batang Berjuntai, Selangor. The responce of *Mansonia* to the various wingbeat frequencies were monitored and confirmed. The effective frequencies together with dry ice and hamster as attractants were used with the lantern and cylinder traps. The two attractants enhanced the trapping efficiency significantly. The trapping system consisting of a speaker emitting 500 Hz of wingbeat frequency, a lantern, a hamster and dry ice was most suitable and effective for trapping female *Mansonia*. However, for the collection of males, the system with a speaker emitting 350 Hz, a cylinder, a hamster and dry ice proved to be good. Daily trapping at the 2 ecotypes with 2 system of traps have collected large numbers of both sexes of *Mansonia*. The trapping method reduced the palous rate extreamely in the vast swamp, but less effective in the isolated forest swamp, though the trapping should be continued and monitored due to hiden mosquitoes in the forest.

57 CONTROL OF THE VECTORS OF ONCHOCERCIASIS IN GUATEMALA

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Tactics for controlling blackflies, as vectors of onchocerciasis, have done mainly by means of larviciding to the breeding place, but the feature of the practical method between West Africa and Central Americas are greatly different because of the diferences of the figure of the rivers, and the method such as an airial spraying being conducted in the West Africa can not be adopted to Central Americas where the breeding place are small and deeply covered by canopies.

In the Republic of Guatemala, *Simulium ochraceum* is reported to be the main vector of the disease, where the species can be found breeding in the streams flown in the coffee plantation or steep mountainous area.

The typical feature of the breeding streams in Guatemala has 18-180 *l*/min in water amount, 41-66 cm/sec in velocity, 0.2-2.0 cm in depth and 20-40° of the grade of beds. The important factor to obtain a successful control is to find out breeding sites. So, the great effort must be devoted to the searching activity and the making maps of breeing sites in this country.

On the other hand, the laboratory and field experiment using temephos revealed that the insecticide carry did not depend on the amount or concentration of insecticide applied but on the amount of water discharge mainly by the absorption of the insecticide to the stream bed.

According to the experimental results, the larviciding method for the blackfly in such a streamlet as in Guatemala was established that a packed 24 g temephos 5% WP should apply at every 50-100 m of the breeding stream in every two weeks despite of the stream size.

The pilot control study in Guatemala iniciated at Livaderos in 1979 revealed that the ABR decreased by 1/100 in the pilot area and also 1/10 in other areas by 1983, showing the method valuable for control the vector of onchocerciasis.

Yet, the following items remain for further studies.

1 . Amount of dosing and period of application.

2. Developement of insecticides with long cally.
3. Periodical check of insecticide susceptibility of the target species.
4. Alternative insecticide against the development of insecticide resistance.

58 COMPARATIVE STUDIES OF POTENTIALITY AS TO NATURAL ENEMY OF MOSQUITOES ON *GAMBUSIA AFFINIS* AND *ORYZIAS LATIPES*

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Mosquito fishes (*Gambusia affinis*) and medakaes (*Oryzias latipes*) showed the different potentiallity as to natural enemy of mosquito larvae in the present study.

1) Population density of both species were 300-500/m^2 in the high density waters.
2) It was showed similar density in the mixed habitat.
3) They ate a similar food in the field, because there were same food in those stomach.
4) Mosquito fishes was more excelled than medaka, about a character of attack to mosquito larvae.
5) Mosquito fishes was inhabited in the filthy waters, but medaka did not live in the same waters.

59 OUTBREAK OF DENGUE AND BIOLOGIES OF THE VECTOR MOSQUITOES IN SARAWAK, MALAYSIA, 1986

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Dengue fever (DH) including Dengue Haemorrhagic (DHF) fever became of public health importance in Sarawak, since 1982 when the first epidemic occurred with 120 notified reported cases. Since then epidemics have been recorded in 1983 with 513, 1984 with 491, 1985 with 45 and 1986 with 210 cases.

Analysis of the serological results from the Institute for Medical Research in Kuala Lumpur revealed a dengue positivity rate averaging 52.5% between 1982-1985. The cases indicated significant increases in all the 7 divisions of Sarawak and 54% (96 cases) of the total 210 cases in 1986 were reported from the 1st division, near Kuching. In an effort to better understand the bionomics and association of the important vector, *Aedes aegypti* and the suspected vector, *Aedes albopictus* and other *Aedes* (*Stegomyia*) species in the transmission and propagation of DF and DHF, a collaborative entomological study was carried out

of by the Japanese Research team and the State VBDCP Headquaters, 1986. A total of 166 ovitraps were set indoor and outdoor in the garden and forest of Kampong Tebakang, Murara Tuang, Sir Aman, Mulu National Park and Bako National Park from July to October. The following 5 species of *Aedes* (*stegomyia*) were collected: *Ae. albopictus, Ae. aegypti, Ae. albolineatus, Ae. malayensis* and *Ae. boharti,* of which last two were new records in Sarawak. *Ae. albopictus* with 572 larvae was most common in indoor and outdoor and *Ae. aegypti* with 16 larvae was not as common as *Ae. albopictus* in both in and outdoors. A total of 686 positive houses and 1,126 positive breeding habitats in 65 localities covering 3,262 premises were detected by the VBCDP team. The mean *Aedes* house index in 21% and breteau index is 35. *Ae. aegypti* was not detected in 42 of the 65 localites surveyed as compared to only 3 localites for *Ae. albopictus.*

60 STUDIES ON THE VECTOR MOSQUITOES OF JAPANESE ENCEPHALITIS AND DENGUE FEVER AT HIGHILANDS AND LOWLANDS IN NORTHERN THAILAND

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Japanese encephalitis (JE) and dengue fever have been prevailing in Northern Thailand. Antibody positive rates against JE and dengue viruses among humans in lowlands were high, while they were very low among Hill Tribes living over 1,000 m above the sea level. In order to know the reason which makes this difference of positive rates of antibody against these viruses, the population of vector mosquitoes were compared among different altitude in Chiang Mai province from July to August, 1986.

The vector mosquitoes of JE were collected by light traps in animal pens for 2 hours after dark. Only a few number of *Culex tritaeniorhynchus* and *Cx. gelidus* were collected at the villages over 1,000 m above the sea level, even though there were large rice field near the villages. The numbers of mosquitoes increased of lower area. The survey of population density of the vector mosquitoes of dengue fever were carried out at the villages in lowlands and Hill Tribes. All receptacles inside and outside of house were observed, and mosquito larvae in receptacles were carried back to the laboratory to identify the species. Many *Aedes aegypti* were collected from the containers in the house and some *Ae. albopictus* were in many kinds of receptacle outside of house with *Ae. aegypti.* In the villages of Hill Tribes, any water containers with *Ae. aegypti* larvae was not found inside and around house. Only a few *Ae. albopictus* larvae were found in bamboo stumps.

From the results mentioned above, it makes clear that Hill Tribes living over 1,000 m above the sea level in Northern Thailand are bitten by very small numbers of the mosquitoes. This means that they have little chance to be infected with JE and dengue viruses.

61 IMMUNOLOGICAL ASPECT OF SNAKE VENOM

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Following the development of analytical studies on various kind of snake venom, certain principles which are responsible for lethal or local pathological changes induced by the envenomation have been separated from the venom. Thus, it is suggested that immunity against various venom factors is important to increase the effectiveness of antivenom. For example, not only anti-lethal activity but also anti-hemorrhagic or anti-necrotic immunity are requested in habu antivenom. In such a situation, immunity in snake venom is important for the improvement of potency of anti-venom, analysis of pathogenesis and application to identification of snake venom by ELISA.

62 STUDIES ON THE PREPARATION OF ANTI-YAMAKAGASHI (*RHABDOPHIS TIGRINUS*) ANTI-VENOM AND ITS CLINICAL APPLICATION

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Severe bites by Yamakagashi have been reported by several workers. Since 1985, study on preparation of Yamakagashi antivenom have carried out and applied clinically to the treatment of the patient.

Rabbits and goats were immunized the venom extracted from Duvernoy's glands of Yamakagashi. Toxoided venom by formalin and crude venom were used as immunizing antigen. Anti-venom separated from the blood of immunized animals was fractionated by sodium sulphate or ammonium sulphate and digested by pepsin. 0.1m*l* of the purified anti-venom neutralized 11, 27 and 80 mlds of the venom, respectively.

Three cases of severe bite by Yamakagashi were treated by intravenous drip of one vial (10 m*l*) of the anti-venom. The hemorrhagic diathesis and coagulation abnormality of blood of the patients recovered dramatically within few hours after the administration of the anti-venom.

63 IMMUNOLOGICAL STUDY ON YAMAKAGASHI SNAKE VENOM (*RHABDOPHIS T. TIGRINUS*) CROSS REACTION OF BLOOD COAGULATION ACTIVATOR WITH VENOMS OF SOME VENOMOUS SNAKES

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Many snake venoms contain blood coagulation activators; *Vipera russelli* venom containing factor X activator, *Bothrops* venoms containing thrombin-like enzyme, *Echis carinatus* and *Rhabdophis t. tigrinus* venom containing prothrombin activator. Coagulant factors contained in snake venoms cause DIC (Disseminated Intravascular Coagulation) and acute renal failure, and these are very important problems in snake-bite.

R. t. tigrinus venom shows a strong lethal activity i.v. injection (LD_{50} 5.3 μg/20 g mouse), although lethal activity becomes weaker by i.m. or s.c. injection (LD_{50} 147 μg and 184 μg/20 g mouse, respectively). These results suggest that lethal activity is closely connected with coagulant activity. *Vipera russelli* venom also shows similar lethal activity like *R. t. tigrinus* venom.

We investigated coagulant activities of some colubrid snake venoms (*Rhabdophis s. subminiatus, Enhydris bocourti, Enhydris chinensis, Homalopsis buccata and Boiga dendrophila*) and examined immunological cross reaction of coagulant factors against *Yamakagashi* snake venom. *R. s. subminiatus* venom activated prothrombin like *R. t. tigrinus* venom and the other venoms activated factor X. *R.t.t.* antiserum could repress the lethal and coagulant activities of only *R.s.s.* venom.

And we examined immunological cross reaction of *R.t.t.* venom against the venoms of *Echis carinatus* (factor II and X activator), *Vipera russelli siamensis* (factor X and V activator) and *Calloselasma rhodostoma* (factor I, Xand XIII activator). Although a precipitation line was not observed in a reaction by a micro-Ouchterlony method, only *Echis carinatus* antiserum could repress the lethal and coagulant activities of *R. t. tigrinus* venom. *R. t. tigrinus* belongs to a different family from *E. carinatus* and each prothrombin activator has a difference in molecular weight (170,000 and 75,000 respectively). It is interesting that these prothrombin activators showed a similar antigenesis.

64 APPLICATION OF ELISA FOR DETERMINATION OF VENOM AND ANTI-HEMORRHAGIC POTENCY OF HABU

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Experiments were performed to establish the quantitative methods for determination of venom and anti-hemorrhagic potency of Habu by ELISA.

In order to study the slight amount of venom remaining in the mascular tissue or blood circulation caused by envenomation, and the circulating anti-hemorrhagic potency lower than 1.0 u/m*l* caused by toxoid injections or envenomation, more sensitive methods for the determination of venom and anti-hemorrhagic potency than rabbits skin test was required.

The results obtained as follows.

1. The detection limit of Habu crude venom was 10 ng~200 ng.
2. The amount of venom remained in local mascular tissue of the rabbits which were challenged with 500 μg Habu venom were decreased to 445 μg, 380 μg, 220 μg, 105 μg and 25 μg after 1/2, 1, 3, 5 and 24 hours respectively. But rabbits which were previously injected Habu anti-venom 1.0 m*l* (644 units) were decreased to 161.39 μg, 88.25 μg, 33.59 μg and 1.41 μg after 1/4, 1/2, 1 and 3 hours respectively.
   The 500 μg of Habu venom were partially neutralized by 1 m*l* (644 untis) of Habu anti-venom within 1/2 hours and almost perfectly neutralized within 3 hours.
3. The detection limit of anti-hemorrhagic potency was 0.02~0.10 u/m*l* both anti-HR-1 and anti-HR-2.
4. Anti-hemorrahgic potency of rabbits injected Habu anti-venom 1.0 m*l* (anti-HR-1=644 units) intravenously were 4.4 u/m*l*, 2.8 u/m*l*, 2.5 u/m*l*, 1.5 u/m*l*, 1.0 u/m*l* and 0.6 u/m*l* after 1, 3, 8, 24, 48 and 72 hours respectively, but the hightest potency of rabbits injected intramuscularly were 0.9 u/m*l* 48 hours after injection. It suggests that intravenous injection of anti-venom is much more effective than intramuscular injection.
5. The staffs who had been bitten by Habu or using freeze-dried venom were detected anti-hemorrhagic potency, but another staffs could not detected.

65 IMMUNOCHEMICAL STUDIES ON COBRA NEUROTOXIN

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Cobrotoxin, a neurotoxic protein, was isolated in a crystalline state from venom of Taiwan cobra and was proved to be the main toxic protein in cobra venom. It is a small, basic protein consisting of a sigle peptide chain of 62 amino acid residues, cross-linked by 4 disulfide bonds.

Antisera have been prepared by injecting increasing doses of cobrotoxin with Freund's adjuvant into rabbits. 100% precipioable antibody (Ab) was isolated immunospecifically by gel filtration on sephadex G-100 after the Ag-Ab complex had been dissociated with 0.53 M formic acid-0.15 M NaCl, pH 2.05. The molecular weight of the soluble complex formed from its papain fragments and cobrotoxin providence that cobrotoxin has three Ab combining sites per molecule. When Ab preparations were applied to cobrotoxin-Sepharose collumn, more than twice the amount of Ab determined by precipitin reaction was always recovered from antisera and IgG. This indicates that rabbits hyperimmunized with cobrotoxin produce non-precipitating (58%) as well as precipiting Ab. Non-precipitating Ab can recognize only two Ag determinants out of the three and the precipitating Ab binds with the three. The neutralizing capacity of the precipitating and non-precipitating Ab increased 18-fold and 23

-fold, respectively, over that of the antisera.

Stable hybridoma cell lines producing monoclolal Ab to cobrotoxin were produced through fusion of NS-1 cells with BALB/c mouse spleen cells hyperimmunized with cobrotoxin. The hybrid cells were cloned and the Ab was produced in large amount both by cell culture and by inducing Ab in ascites to BALB/c mice. The monoclonal Ab purified by affinity chromatography was identified to be IgG_{2a} and proved to combine with two molecules of cobrotoxin. Monoclonal Ab are not only a valuable tool in studying the mechanism of interaction between Ag and Ab but also its therapeutic potency is 2-fold greater than that of non-precipitating Ab. Therefore, from conventional antisera to monoclonal Ab the therapeutic potency has increased 46-fold.

In view of the emerging value of reversed-phase HPLC in peptide mapping analysis, we used this technic for the separation of antigenic peptides and determined their structure and location in the sequence of cobrotoxin. From our preliminary results of sequence assignment on peptide fragments with antigenic activity, three candidates of antigenic determinants were found. In further study, well characterized monoclonal Ab will be used to select its specific antigenic determinant, and the mechanism of interaction between antigenic determinant and monoclonal Ab will be studied.

66 PURIFICATION OF THREE ANTI-HEMORRHAGIC FACTORS FROM THE SERUM OF *HERPESTES EDWARDSII*

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Three anti-hemorrhagic factors (AHF-1, AHF-2 and AHF-3) were purified from the serum of *Herpestes edwardsii,* a mongoose, by a combination of gel filtration on a Sephadex G-200 column and a high performance liquid chromatography with a TSK gel DEAE-5PW column. Each of the purified anti-hemorrhagic factors showed a single band on a polyacrylamide disc electrophoresis. The three anti-hemorrhagic factors neutralized the toxicity of the hemorrhagic venoms of 6 species of *Trimeresurus* and *Agkistrondon blomhoffi*. The factors inhibited also the hemorrhagic activity of HR 1 and 2, the hemorrhagic principles of *T. flavoviridis* Okinawa. AHF-1, AHF-2 and AHF-3 were stable at the temperature from 0°C to 60°C and at the pH between 2.0 and 11.0. The molecular weight of the three anti-hemorrhagic factors were estimated to be 65,000 and 69,000 by a gel filtration and a SDS-polyacrylamide gel electrophoresis, respectively. None of precipitin line was found for the purified anti-hemorrhagic factors with the venom of *T. flavoviridis* Okinawa or its hemorrhagic principles, HR 1 and HR 2. These results suggest that three anti-hemorrhagic factors were not immunoglobulins but natural immunity possessed by the animals. There were little difference in amino composition of three anti-hemorrhagic factors.

67 IMMUNOLOGICAL ASPECT OF HYMENOPTERA STINGS AND MOSQUITOES

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Cases of Hymenoptera stings are increasing recently, especially cases of stings by social wasps (Vespidae) and social bees (Apidae), which are very aggressive. In Japan *Vespa simillima* is the most dangerous species and *Polistes* spp. are in the next, because they build their nests under the eaves of houses or on the bushes in the gardens. In Nagano Prefecture, there are many cases of hornet (*Vespula lewisi*) stings, because the larvae and pupae in the underground nests are edible and tasty. Many beekeepers have been stung honeybee (*Apis mellifera*). In Japan usually 30-40, sometimes 70 persons die by Hymenoptera stings during a year. The greater part of the dead persons died not by the direct effects of the venoms, but by anaphylaxis of repeated stings. Many persons died within 1 hour by the Hymenoptera stings.

In the mosquito bites some persons show severe symptoms and rarely die. These cases are also caused by allergy or anaphylaxis.

Case report of Hymenoptera stings by Dr. Sohei Makino and case report of mosquito bites by Dr. Noriko Ohtaki will be given from now.

68 ALLERGIC SYMPTOMS DUE TO HYMENOPTERA STINGS

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Prevalece of Hymenoptera stinging hypersensitivity among approximately 80 thousand population in Yamizo district in Tochigi was studied through questionaires send to 1,000 public health workers in that area. One hundred and 1 cases with the hypersensitivity were reported. Fifty-seven per cent, 32%, 5%, and 5% of the 101 hypersensitive cases showed local reaction only, mild systemic reactions, severe reactions with loss of conciousness, and longlasting local reactions, respectively. Additional survey including school students suggested that about 1% of the population at large had experiences of Hymenoptera stinging hypersensitivity in their lives. Most of the causative insects were wasps.

Serum IgE antibodies to either one of hornet, wasp and bee venoms were found in all 29 hypersensitive cases examined except one case. All of 14 hypersensitive cases examined showed positive skin tests to either one of these 3 venoms. These observations showed that the hypersensitivity symptoms were induced by the significant coincidence of positive tests on RAST or intracutaneous tests was found between wasp and hornet venoms, but not between

waps/hornet and bee venoms. These results showed the antigenic cross-reactivity of the venoms of wasps and hornets.

69 MOSQUITO BITES

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To study the mechanism of the skin reaction to mosquito bites, 120 volunteers, aged 1 to 68 years old, were experimentally exposed to female mosquito, *Aedes albopictus*, and observed the skin reactions to the bites.

Mosquito bites caused two types of skin reaction; immediate and delayed. In immediate reaction wheal appearred immediately after the bite, and disappearred within 1 to 2 hours. Whereas, in the reaction of delayed type, the erythema appearred again 5 to 6 hours after the bites and enlarged gradually until 24 to 48 hours, then lasted for several days. The volunteers were divided into 4 groups according to the skin reactions, In the first group, any immediate reaction was not observed, but delayed one was. The second group had both reactions. The third group showed only immediate type of reaction. In the last group, no reaction was observed. The most of infants belonged to the first or the second group, while the aged volunteers belonged to the fourth group. Serum IgE, specific to mosquito saliva antigen, were measured with ELISA. The titer of IgE was highly correlated with the skin reaction of immediate type. The volunteers of the 2nd and the 3rd groups had a high IgE titer. On the contrary, in the first and the 4th groups IgE titer remained at a low level. It is suggested, therefore, that the immediate skin reaction is caused by a high titer of IgE, specific to mosquito saliva antigen.

We had experienced severe cases caused by mosquito bites. The first case was a 12 years old girl, when she visited first our clinic. She had suffered from edematous erythema with hemorrhagic central vesiculation and high fever after the mosquito bites since 5 or 6 years of age. The biting tests were done to make clear the causative species of mosquito. She had high fever, temporal lymphocytopenia and severe eruptions 12 hours after the bite of *Aedes aegypti* and 20 hours after the bite of *Armigeres subalbatus*, while only slight erythematous lesions were observed after the bite of *Culex tritaeniorhynchus* and *Anopheles sinensis*. The biopsy specimen from the bitten site were compatible with an Arthus reaction. The second case was a boy. He had high fever and hepatosplenomegary. The causative species were supposed to be *Culex* sp.. He died of malignant hystiocytosis (MH) at 13 years of age.

Eight severe cases of mosquito bites died of MH in Japan so far as reported. The reason of the relation between severe cases of mosquito bites and MH remains to be clarified.

70 THE RESEARCH FOR THE GROWTH OF INFANT IN JOS UNIVERSITY TEACHING HOSPITAL, NIGERIA

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The infantile diarrhea's research was carried out as one of JICA medical research project. This research for the growth of infant was investigated between 1984 and 1985 in Jos University. Teaching Hospital Nigeria. The vaccination, the measurement of body weight, and the guidance of nutrition were undertaken in the antenatal clinic service at Jos University Teaching Hospital. The age of mothers was between 12 and 50. The most at the first seen was the first half in twenties. The infants were between 1st and 13th. The most of number was the first baby by 20%. The most of age of mothers who delivered the first was teens by 64%. The maximum of mother in teenagers had 7 babies. There were some mothers over 30 years old at the first birth. One of the mother had 6 children meanly. The mean body weights in every month were very close to that of 1960 in Japan. Malnutrition in this study could not find out. This was thought that these people belonged to a high class society and took enough calories to gain the body weight.

71 MEDICAL SUPPORT FOR THE INHABITANT IN NEGROS ISLAND, PHILIPPINE

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Since 1983, medical examination of the poor and needy in Negros Island has been performed with the aid of Rotary Club. Total number of patients examined during five years (1983—1987) were enumerated 10,970 most of which were infant and thirty to forty-nine years old female (their mother). Results of the physical findings were ranked as following; first, the infectious respiratory diseases, then infectious intestinal disease, infectious eye disease, parasites disease, metabolic disorder or malnutrition, dermatological disease, psyphiatric disease, circulation system and anemia. It seems specific that so many infants had the malnutrition or intestinal infection, while many adults had infectious respiratory disease and lung tuberculosis. The Ziehl-Neelsen test of sputum which were examined by the probability sampling proved positive of 12.8% (44/345).

Any sufficient medical treatment and observation of the inhabitants could not have been done only because of our short staying (five or seven days per year), but of any inadequate communication between our medical team and the inhabitants.

72 MALNUTRITION IN DELELOPING COUNTRIES

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Undernutrition and specific deficiency states are the main problems in the developing countries. In many developing countries, growth failure in the pre-school group is widespread and no doubt a shortfall in both energy and proteins is involved. In those developing is involved. In those developing countries, chronic undernutrition as shown by a deficit in height for age continues to be the main problem, though severe forms of acute undernutrition (deficit of weight for height) also still exists in many areas with root crop staples. Problems of specific deficiency states like nutritional anemia, vitamin A deficiency and endemic goitre continue to pose a problem in several of the developing countries of Asia. Unlike protein-energy malnutrition, the prevention and control of which requires long-term deficiencies are amenable to direct interventions, e. g. nutrient distribution, fortification of foods, etc. Nutritional anemia is widespread problem and occurs even in some of the developed countries. Efforts are needed to control what appears to be predominantly an iron deficiency anemia, together with measures to eradicate worm infestations and infections which interfere with the biological utilization of foods. Goitre is a problem mainly in the mountainous areas of the Asian countries, though countries in the South Pacific are also affected. The patients of cretinism and deaf-mutism are found frequently. Some of the countries have a national programme for goitre control. In these countries that are making serious attempts to reduce the problem of protein-calorie malnutrition, the main strategies appear to be directed at the pre-school child, as well as at the pregnant and lactating women. Nutrition education has been the mainstay of most nutrition-based activities. Education in the use of locally available foods as weaning supplements is an important area in which greater efforts are needed at present.

73 LEPROSY IN ASIA

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The most density area of leprosy patient is Africa but the most population of leprosy patient is India and Southeast Asia. The numbers of leprosy patient in India estimate for 4 millions from presumption of WHO, but indeed there may be 10 millions of leprosy patient in India. These patients are partialy controled with the sanitary department of each province by a traveling clinic. All anti-leprosy drugs which are donated from Mission, WHO or Sasagawa Health Memorial Foundation, are supplied with cost-free. JALMA was established in Agra at 1962 by the support of Japanese citizens. JALMA have a out-patient clinic

of leprosy and facility of hospital treatment at the same time some teams go out the traveling clinic. Now this JALMA is managed by Indian Government.

There are two national leprosarium in Thailand. The density of leprosy patient is one per thousand, appearance of new leprosy patient is horizontal about 3 thousand in year. Leprosy control center which is set in each 16 block divided the Thailand to 16 provinces, examines leprosy patients cooperating with department of hygine in each prefecture and their adherent hospitals. All anti-leprosy drugs are supplied with cost-free of course.

There are two national leprosarium in Philippine and some islands have small leprosarium. Anti-leprosy drugs are supplied with cost-free by the traveling clinic.

People's Republic of China has about one hundred and fifty thousand leprosy patients, they took a policy to isolate the leprosy patient to hospital in the past, but now they changed to medical treatment at home and many hospitals have out patient of leprosy. DDS is made in their country and is supplied with cost-free, but rifampicin and B663 are donated from foreign Mission or Sasagawa Memorial Health Foundation.

In Japan, there are 7 hundred new leprosy patient in year at 1947, but now we have only 35 new leprosy patients in year. Almost all patients are borderline group patient. There was many tuberculoid leprosy patients in outpatient of Osaka University of Japan at about from 1900 to 1940, but the tuberculoid leprosy patient decreased to half and half of T and L type of leprosy in 1956. Now in Thailand, T and L type of leprosy patient is half and half. All of the leprosy bacilli are killed with 2 days administration of rifampicin, then the decrease of infection source may be influence to appearance of new leprosy patient.

74 TUBERCULOSIS IN DEVELOPING COUNTRIES

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(Abstract not received in time)

75 CASES OF TROPICAL DISEASES WHICH WERE EXPERIENCED RECENTLY

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Tropical diseases such as malaria, hepatitis, schistosomiasis are important diseases for long-term Japanese sojourners in tropical countries in processing their health control. Recently we experienced some of these diseases which ocurred during their stay, and further investigations were made on the diseases.

Case 1. 30 year old female staying in Tunisia. The initial symptoms were general

malaise and fever followed by icterus. She was admitted to a hospital under a suspicion of hepatitis, and a diagnosis of hepatitis A was made. Infection by raw oysters were strongly suspected because she had eaten them one month before the onset.

Case 2. 27 year old male staying in Zambia. He had been engaged in technical cooperation of agriculture. He visited a local hospital complaining of lumbago and hematuria. And eggs of *Schistosoma hematobium* was detected in his urine.

Case 3. 24 year old male staying in Malawi. He had a high fever accompanied by chill, and was diagnosed as falciparum malaria. Fever did not decrease in spite of the administration of chloroquine until 15th day of illness when quinine and fancidar were added for treatment. This case was regarded as a chloroquine resistant malaria.

Case 4. 33 year old male staying in Ethiopia. He was living in the neighborhood of a lake and had chances to eat grasses which were growing near the lake. Eggs of *Fasciola hepatica* was detected on health examination, and treatment was done with Bithionol.

Case 5. 27 year old male staying in Kenya. He began to complain of insomnia and tend to confine himself in his house. Delusion of persecution and reference increased followed by auditory hallucination. He consulted a doctor, and treatment was started under the diagnosis of schizophrenia.

76 IWSSD

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(Abstract not received in time)

77 DRUG SENSITIVITY OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM DRINKING WATER IN INDONESIA

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The present report the results of drug sensitivity and β-lactamase activities of *Pseudomonas aeruginosa* isolated from drinking water in Indonesia in 1985 and 1986.

Twenty-five strains were examined. Sensitivity testing to chemotherapeutic agents such as carbenicillin (CBPC), cephaloridine (CER), cephalothin (CET), cefoxitin (CFX), cefmetazole (CMZ), cefazolin (CEZ), cefusulodin (CFS), tobramycin (TOB) and amikacin (AMK) were examined. The minimum inhibitory concentrations (MIC) were measured in those strains which seemed to be resistant by disk agar diffusion method.

Resistance of all strains to CBPC, CER, CET, CFX, CMZ and CEZ were found at very

high concentrations exceeding 250 μg/ml or 1,000 μg/ml. However, almost all strains were sensitive to TOG and AMK.

The β-lactamase activities of benzylpenicillin (PCG) and CEZ in those strains were investigated by use of disk-agar substrate profile method. Results of β-lactamase activities in those strains were a low level, respectively.

78 BACTERIOLOGICAL AND CHEMICAL STUDY OF THE DRINKING WATER IN THE TROPICAL COUNTERIES. RECENT TWO YEARS OBSERVATION (1985, 1986)

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Diseases such as dysentry, endemic hepatitis and various parasitosis are still prevalent in the tropical under-development countries. We focus our attention to the drinking water, as it plays an important role in the outbreak and transmission of these diseases. We report here the recent 2 years results of the bacteriological and chemical study of the drinking water in these tropical countries. The results showed that the drinking water in these countries contained a large amount of microorganisms, especially in these of Indonesia, Thailand, Philippine, and chlorinization of drinking water was hardly carried out. The results suggest that drinking water, especially in the South-East countries, is polluted by fecal contamination with relatively high probability.

79 DRINKING WATER OF THE JAPANESE INHABITANTS IN INDONESIA

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In order to know the influence of the degree of the pollution of the drinking water upon the orally infected diseases, we carried out surveys of the drinking water at Jakarta in Indonesia. Water samples examined were tap and well water, boiled water, filtrated water and commercial mineral water. More than half of the tap water, boiled water as well as mineral water were contaminated with coliform bacilli. In the drinking water after boiling and kept in continers, the coliform bacilli were increased as compared with those of tap

waters. Same results observed in the filtrated water. The reason for this is supposed to poor managing of the water purifiers.

80 THERMOREGULATORY AND PYROGENIC RESPONSES IN PIKA (WHISTLE RABBIT)

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Pikas's better heat conservation ability thought to be due to its higher metabolic rate, poor heat dissipation through weak panting and smaller ear surface area. Although it's very sensitive to narcotics, it responds poorly to intraperitoneal lipopolysaccharide (LPS) pyrogen.

In this experiment 9 pikas (mean body weight: 262.2 g) reared at 22°C were subjected to intravenous LPS pyrogen (3.8 μg/kg) in the environmental control chamber (temp. 28°C and 60% r. h.). Throughout the experiment the animals were unrestrained in cages with thermister probe in situ deep in rectum and fixed to the tail by adhesive plaster. Fifteen minutes prior to i. v. pyrogen injection, rectal temperature (The) and respiratory rate (RR) were recorded every 5 minutes, and thereafter every 10 minutes. The LPS pyrogen evoked a monophasic fever (mean peak Tre: 40.23°C, mean ΔTre: 0.73°C$\pm$0.3°C) which persisted for 70 minutes after a mean latent period of 20 minutes. RR decreased from precontrol rate of 108 c/min to 100 c/min at the peak of fever to a low 92 c/min at the end of the pyrexia. It was also observed that the pika developed piloerection and shivering during the rising phase of the fever. Occasionally they licked their body but mostly they had minimal movement during the pyrexic period. These observation show that pika rabbits can elicit fever with i. v. LPS pyrogen but the heat loss mechanisms differ from those of albino rabbits.

81 HEAT BALANCE DURING THERMAL STIMULATION IN DRY SAUNA

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The subjects were a tropical resident (Tanzania, 37 years old, male) and a Japanese sportsman (sprinter, 33 years old, male) in this study. The changes in metabolic rate, respiratory rate, pulse rate, blood pressure, skin temperature and body weight during 30

minutes sauna heat load (60—70°C) were measured after 30 minutes resting in an environmental control chamber (room temperature 28°C, humidity 60%). The pulse rate in the tropical subject was higher than that of the Japanese sportsman (in both subjects, n=4 period of experiments), while the metabolic rate in the tropical subject was conversely lower. But the increase rate in pulse rate and metabolic rate during heat load in the tropical subject was lower compared to that of the Japanese subject. And considerable increase of respiratory rate and in a few case, the rapid and shallow breathing similar to thermal panting were observed in the tropical subject. From these results the following comments are drawn: (1) a pulse rate and metabolic rate during heat load changed in parallel in both subjects, (2) a stable and smaller change rate of metabolism during heat load observed in the tropical subject suggests the habituation due to a raised threshold of heat sensitivity, (3) a considerable increase of respiratory rate and the rapid and shallow breathing similar to thermal panting observed in the tropical subject suggests the survival of thermal panting still in human being.

82 DETERMINATION OF HEAT ACCLIMATIZATION BY CAPACITANCE HYGROMETER-SWEAT CAPTURE CAPSULE METHOD

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Measurements of sweat rate in tropical and Japanese subjects were performed in an environmental control chamber (room temperature 28°C, humidity 60%). Local heat load (43°C water bath) was applied on lower legs (30 min, and 20 min) to induce thermal sweating responses. The indicators of thermoregulatory heat loss responses such as sweat onset time, threshold oral temperature for sweating and sweat volumes (for 20 min during heat load, for 5 min after heat load) etc. were simultaneously measured by using thermography and capacitance hygrometer-sweat capture capsule method. By analyzing the data of tropical inhabitants and Japanese (sportsmen and non-sportsmen), the central and peripheral mechanisms of heat acclimatization were investigated. In this study, a new quantitative calibration by using capacitance hygrometer-sweat capture capsule method was devised, i. e., on the top of the capnule fixed to the skin of subject, a small hole was made, through which, subject's sweat or 30°C 0.45% NaCl solutions (0.01, 0.02, 0.03 and 0.04 ml) were precisely dropped into the capsule with a micropipette and hole sealed. Relative humidity changes (% r.h.) of the capsule were continuously recorded by capacitance hygrometer. By calculating the absolute humidity from relative humidity, sweat rate (mg/cm^2•min) could be obtained and sweat volume was quantitatively decided by the sweat rate. (Fan-Kosaka method). This new method made the measurement of sweat volume simple and accurate, and that these experimental modalities may be utilized in further determination of physiological mechanisms of hear acclimatization.

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