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COMPARISON OF BODY SHAPE, BODY COMPOSITION AND SWEATING REACTION BETWEEN YOUNG MALE HIGHLANDERS OF PAPUA NEW GUINEA AND YOUNG MALE JAPANESE

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Abstract: Anthropometric measurements and measurements of sweating reaction during exercise were made on 11 young male highlanders in Beha Village at altitude between 1,500 meters and 1,800 meters above sea level in Papua New Guinea in August and 11 young male Japanese in Nishinomiya City in September. Measurements of local sweat rate and sodium concentration in local sweat during pedalling a bicycle ergometer at a constant work load of 450 Kg·m/min for 20 min. under a thermoneutral condition were made. New Guineans were significantly shorter in height, slightly lighter in body weight and had a lesser amount of body fat than Japanese. New Guineans showed significantly greater mean values of Rohrer's index and Brugsch's index than Japanese. Skinfold thickness for New Guineans was significantly thinner than that for Japanese. Physically New Guineans were more muscular and athletic when compared with Japanese. New Guineans showed considerably lower local sweat rate and significantly lower Na concentration in local sweat than Japanese. Differences in anthropometric characteristics and sweating reactions between New Guineans and Japanese might reflect more advanced acclimatization to hot environments in New Guineans when compared with Japanese.

Introduction

It is known that body fat content, body composition, and body shape change according to climate in which individuals live, nutritional conditions as well as physical activities (Coon, Garn and Birdsell, 1950; Brožek, 1952; Keys and Brožek, 1953; Lewis *et al.*, 1960). The subcutaneous layer of fat keeps heat content from heat dissipation due to low thermal conductance of fat (Burton and Bazett, 1936) when net heat is transferred from the body into the environment. The decrease in the subcutaneous fat layer produces such a change in body shape that the ratio of body surface area to body weight is increased. Since heat dissipation from the body is proportional to body weight, a greater body surface area to body weight is favorable for heat dissipation from body to the environment. Thus, it is assumed that body composition and body shape are important factors in temperature regulation (Hori *et al.*, 1977). Evaporation of water from the skin surface is the main mechanism of heat dissipation in a hot environment. It is well known that sweating reaction of humans changes adaptively under the influence of climate and the degree of physical

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activities (Dill *et al.*, 1938; Adolph, 1946; Hori, 1977). The Eastern Highlands of Papua New Guinea at about 1,600 m above sea level, located in a tropical zone, have hot middays and cool nights throughout the year, while Japan, located in a temperate zone, has not summers and cold winters. Highlanders in Papua New Guinea have a high carbohydrate diet (Hipsley and Clements, 1950) and perform hard muscular exercise such as ascending steep slopes in their daily lives when compared with Japanese (Hori *et al.*, 1980). Thus, it is of interest to compare body composition, body shape and sweating reaction of highlanders in Papua New Guinea with those of Japanese. In the present study, an attempt has been made to investigate differences in physical characteristics and sweating reaction between young male highlanders of Papua New Guinea and young male Japanese and to discuss the differences from the viewpoint of physiology of climatic acclimatization of humans.

Materials and Methods

Eleven young male highlanders of Papua New Guinea and 11 young male Japanese were selected as the subjects in the present study. The study on the New Guineans was made in August 1978 in Beha Village at altitudes between 1,500 meters and 1,800 meters above sea level in the Eastern Highlands of Papua New Guinea, tropical zone (Fig. 1) and that on the Japanese was made in September in Nishinomiya City at sea level in Japan, temperate zone. Anthropometric measurements and measurement of sweating reaction during bicycle ergometer exercise were

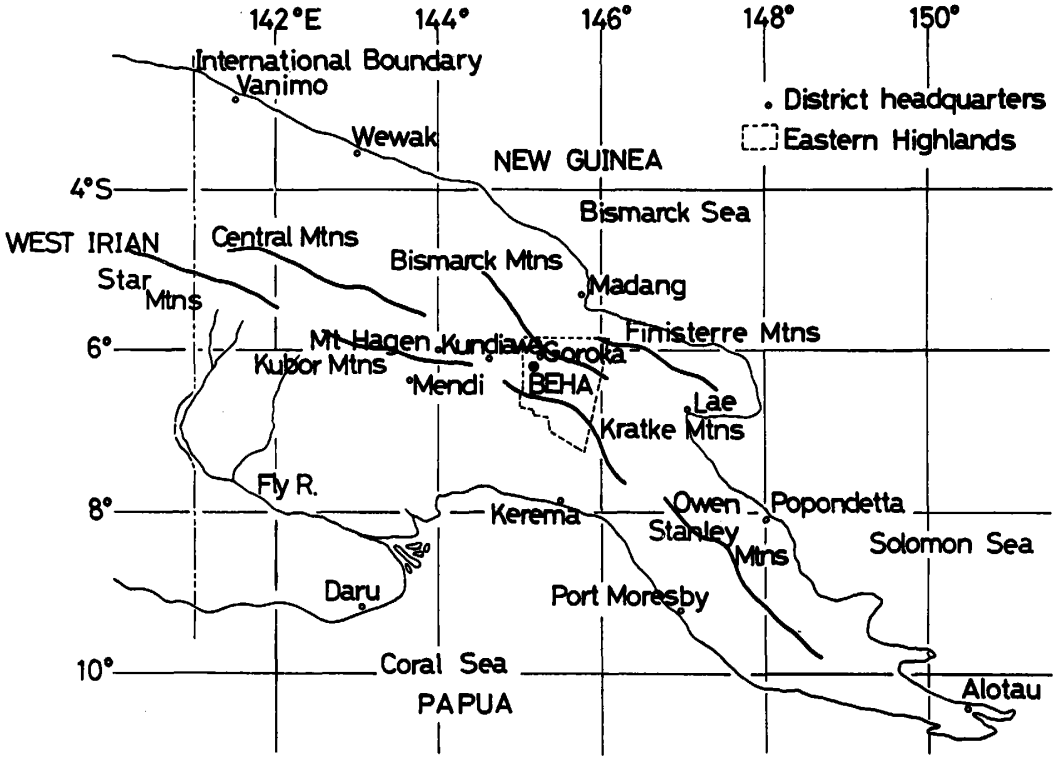

Fig. 1 Situation of Beha village.

made. The skinfold thickness was measured on the right side of the body with a caliper 2 sec. after a pressure of 10 g/mm^2 of the caliper jaw surface was applied to the skinfold. The skinfold sites measured and weighing factors used for calculating mean skinfold thickness are as follows (Hori *et al.*, 1974):

Chest	Abdomen	Waist	Subscapular	Upper arm	Thigh
0.143	0.139	0.139	0.143	0.141	0.295

The body fat (f %) was calculated from the mean skinfold thickness (X mm), body weight (W Kg) and body surface area (S m^2) using the following prediction equation (Hori *et al.*, 1974).

$$f=28.9\times\frac{S\times X}{W}+3.67$$

The bicycle ergometer test was performed at around 3 p.m. in a room of about 25°C. Subjects were instructed not to eat a meal and rest at least two hours prior to the experiment in order to minimize effects of specific dynamic action and physical activities. After sitting in a chair for 30 min. subjects clad in shorts rested on the saddle of a Monark bicycle ergometer for 10 min. and then pedalled at the constant work load of 450 Kg·m/min at the cycling rate of 50 rpm for 20 min. Samples of perspiration from the back were collected at 10 min. interval during exercise using the filter paper method (Ohara, 1966). The Na concentration in the sweat was determined by using eluates with distilled water from the filter papers by flame photometry.

Results

1. Anthropometric measurements

The physical characteristics of New Guineans and Japanese are shown in Table 1. The mean value of height for New Guineans (157.1 cm) was significantly smaller than that for Japanese (169.5 cm). The mean value of body weight for New Guineans (59.2 Kg) was slightly smaller than that for Japanese. New Guineans showed greater mean value of chest girth and smaller mean values of upper arm girth and

Table 1 Characteristics of subjects

	Number	Age (yr)	Height (cm)	Weight (Kg)	B.S.A. (m^2)	Circumference (cm)		
						Chest	Upper arm	Thigh
New Guineans	11	25.5 ±3.9	157.1 ±6.7**	59.2 ±5.8	1.63 ±0.09	88.0 ±3.3	26.4 ±1.9	49.6 ±2.8*
Japanese	11	23.6 ±1.4	169.5 ±5.2	62.4 ±7.0	1.74 ±0.14	86.5 ±4.1	27.1 ±1.2	52.1 ±2.1

B.S.A.: Body surface area.

Mean values are given with their standard deviations.

* Significant differences between two groups.

* at 5% level ** at 1% level.

thigh girth. Among these differences, the mean of thigh girth for New Guineans was significantly smaller than that for Japanese. The physical status of New Guineans was characterized by short stature and a stocky body shape.

2. Skinfold thickness

The mean values and their standard deviations of regional skinfold thickness were shown in Table 2. All the mean values of regional skinfold thickness measured for New Guineans were significantly smaller than those for Japanese. New Guineans showed much less individual variations in skinfold thickness.

Table 2 Skinfold thickness of subjects

	Chest	Abdomen	Waist	Subscapular	Upper arm	Thigh
New Guineans	5.1±0.7*	6.0±1.0**	6.0±1.5**	8.1±1.2*	3.9±0.7**	5.8±1.2**
Japanese	10.6±6.2	15.1±8.0	13.6±7.3	12.6±5.1	8.6±4.3	11.2±4.9

Mean values are given with their standard deviations.
* Significant differences between two groups.
* at 2% level ** at 1% level.

3. Physical and nutritional indices

The physical and nutritional indices for New Guineans and Japanese are shown in Table 3. New Guineans showed significantly greater mean values of Rohrer's index and Brugsch's index. Greater values of Rohrer's index and Brugsch's index for New Guineans reflect their more stocky body shape compared with Japanese. The mean values of body fat content (4.92 Kg) and body fat percentage (8.31%) for New Guineans were significantly smaller than those (8.24 Kg and 13.2% respectively) for Japanese. The mean value of mean skinfold thickness for New Guineans (5.8 mm) was significantly smaller than that for Japanese (11.8 mm). Thus it can be said that the body shape and body composition of New Guineans are an athletic type.

Table 3 Physical and nutritional indices

	Rohrer's index	Brugsch's index	Body fat (Kg)	Body fat (%)	M.S.T. (mm)
New Guineans	152.8±13.3**	56.1±2.3*	4.92±0.58*	8.31±0.48**	5.8±0.8*
Japanese	128.2±14.8	51.0±3.8	8.24±3.01	13.2±3.4	11.8±5.4

M.S.T.: Mean skinfold thickness.
Mean values are given with their standard deviations.
* Significant differences between two groups.
* at 1% level ** at 0.1% level.

4. Local sweat rate and sodium concentration in sweat

In Table 4, the peak local sweat rate, peak Na concentration in local sweat and

Table 4 Comparison of peak local sweat rate, peak Na concentration in local sweat and mean Na concentration in local sweat between young male highlanders of Papua Newguinea and young male Japanese

	Vp (mg/cm²/min)	Cp (mEq/L)	$\bar{C}$ (mEq/L)
New Guineans	0.644±0.292	26.9±14.0*	24.1±13.5*
Japanese	0.721±0.337	43.6±11.6	40.1±11.3

Vp: Peak sweat rate, Cp: Peak Na concentration,
C: Mean Na concentration.
Mean values are given with their standard deviations.
* Significant difference between two groups at 5% level.

mean Na concentration in local sweat for New Guineans are compared with those for Japanese. New Guineans showed considerably smaller mean value of peak local sweat rate (0.644 mg/cm²/min) than Japanese (0.721 mg/cm²/min) though this difference was not statistically significant. The mean values of peak Na concentration in local sweat (26.9 mEq/L) and mean Na concentration in local sweat (24.1 mEq/L) for New Guineans were significantly smaller than those for Japanese (43.6 mEq/L and 40.1 mEq/L respectively). Sweating reaction of New Guineans was characterized by lower sweat rate and lower Na concentration in sweat compared with that of Japanese.

Discussion

It has generally been believed that nutritional conditions, physical activities and climate affect physical status of men (Hipsley and Clements, 1950; Coon *et al.*, 1950; Lewis *et al.*, 1960). The growth of children is retarded by low caloric intake and low protein intake. Since most of the energy and protein in the diet for highlanders in Papua New Guinea comes from the sweet potato, the intake of energy always seems low and intake of protein, especially animal protein, is low when compared with Japanese (Hipsley and Clements, 1950; Norgan *et al.*, 1974; Hori *et al.*, 1980). As was pointed out by Coon *et al.*, (1950), tropical natives tend to have smaller stature as a result of long-term exposure to a hot climate than temperate natives. Thus, it can be said that the shorter stature of New Guineans is due in part to their smaller intake of energy and protein and due in part to their long residence in a tropical climate. It is known that a rise in ambient temperature results in a decrease in the energy intake, and a decrease of body fat content is caused by low energy intake and hard muscular exercise (Pascale *et al.*, 1955; Parizkova, 1959). Consequently, the thinner subcutaneous fat layer and less body fat content for New Guineans might be induced by the lower energy intake due to higher ambient temperature and walking long distances and ascending steep slopes often loaded with heavy baggage in their daily lives. Since a thicker deposit of subcutaneous fat prevents heat dissipation from body into the environment when ambient temperature is lower than skin temperature, a thinner layer of subcutaneous fat is favorable for temperature regulation of the body in hot climates. In an attempt to estimate

changes in body composition and body shape as induced by different climates, we plotted body weight and body fat content against height in Fig. 2. In this figure,

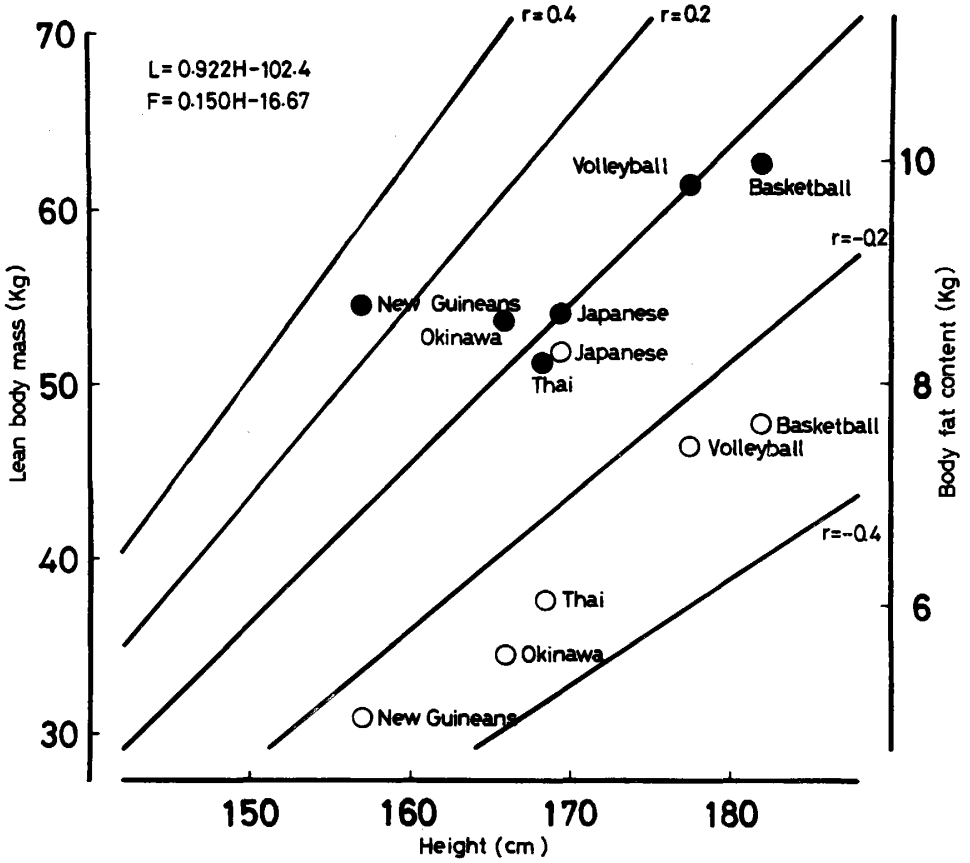

Fig. 2 Comparison of height, lean body mass and fat content among New Guineans. Thai and Japanese.

anthropometric data on 119 young male Japanese were used as a reference (Hori *et al.*, 1977) and those on residents in Okinawa (a subtropical zone in Japan) and Japanese athletes (volleyball players and basketball players) were used for the sake of comparison (Hori *et al.*, 1974; Tanaka *et al.*, 1977). Mean values of height, lean body mass and body fat content in the reference group were 171.0 cm, 55.3 Kg and 9.1 Kg respectively. Standard lean body mass (L Kg) and standard body fat content (F Kg) were obtained from height (H cm) by using the following prediction equations:

$$L=0.992\,H-102.4 \quad \text{and} \quad F=0.150\,H-16.67.$$

In this figure, the degree of overweight of lean body mass or underweight of lean body mass (fatness or leanness) is expressed as a percentage deviation of the actual lean body mass (body fat content) from the standard lean body mass (body fat content), "r". As shown in this figure, New Guineans were shorter in height and heavier in lean body mass than Japanese; natives in Thai and Okinawa had less body

fat content, while Japanese athletes were taller, heavier, and had less body fat content. When these groups were compared with a reference group with relative values as different from standard lean body mass and standard body fat content, New Guineans showed heavier lean body mass and smaller body fat content, while natives of Thai and Okinawa and Japanese athletes showed smaller body fat content. Thus, plotting of lean body mass and body fat content against height is a feasible method of estimating changes in physique, body composition and body shape as induced by different climates in which individuals live. It is known that sweating reaction changes with acclimatization to heat (Adolph, 1946; Dill *et al.*, 1938). The inhabitants acclimatized to hot environments from childhood sweat less in spite of the same rise in core temperature in heat, and Na concentration in their sweat is lower when compared with individuals unacclimatized to heat (Kuno, 1956; Hori *et al.*, 1976). The results presented in Table 4 that show New Guineans had less local sweat rate and lower Na concentration in sweat are consistent with the reports described above. The amount of evaporation of sweat is in proportion to sweat rate when sweat rate is low, but an increase in sweat rate represents wasted sweat which is not used for heat dissipation after the skin surface becomes a completely wetted area. The amount of evaporation of sweat from skin surface is in proportion to the vapor pressure difference between sweat and surrounding air when the wetted area is the same and this vapor pressure difference is increased by a decrease in Na concentration of sweat (Hori *et al.*, 1976). Thus, the evaporation of sweat for New Guineans appears to be more effective for cooling the body than that for Japanese. Since evaporation of sweat from the skin surface is the mechanism of heat dissipation for man in hot environments, better efficiency of sweating of New Guineans for heat dissipation can be considered as an important form of physiological adaptation to tropical climate.

Acknowledgement

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パプアニューギニア高地人と日本人の体型，体構成および発汗反応の比較

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海抜 1500 m-1800 m に住んでいるパプアニューギニア高地人の成人男子11名と西宮市在住の日本人成人男子11名について，身体計測と運動中の発汗反応の測定を行った。パプアニューギニア人の測定は8月に，日本人の測定は9月に行った。発汗反応の測定は25°C の室内で午後3時より開始した。仕事量 450 kg•m/min の自転車労作計を用いたペダル踏み運動を20分間行わせ，背部の局所発汗速度と汗の Na 濃度を発汗カプセル沪紙法で測定した。パプアニューギニア高地人は日本人と比較して，身長が低く，体重は軽く，皮下脂肪厚が薄く，体脂肪含有量が少なかった。これらの差はいずれも統計学的に有意であった。パプアニューギニア高地人のローレル指数および比胸囲は日本人のそれらより有意に大きかった。パプアニューギニア高地人の局所発汗速度は日本人のそれより可なり低く，又前者の汗の Na 濃度は日本人のそれより有意に低かった。パプアニューギニア高地人の身体的特徴および彼等の発汗反応が日本人のそれと異なっているのは，彼等が日本人より高温環境によく馴化したことによると思われる。

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RESISTANCE TO CHALLENGES WITH HOMOLOGOUS *TRYPANOSOMA GAMBIENSE* IN MICE TRANSFERRED MOUSE IMMUNE SPLEEN CELLS OR IMMUNE SERA

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Abstract: To know the mechanism of protection from the homologous *Trypanosoma gambiense* infections in mice, the incidence of passive transfer of specific resistance to challenges with the parasites was examined in mice inoculated with immune spleen cells and immune sera separated on days 3 to 21 (day 0=immunization with microsomal fraction, 144,000×g sediment of *T. gambiense* homogenate, in complete Freund's adjuvant).

Passive transfer of specific resistance was successful only when immune spleen cells separated on days 3 and 5 were employed and the resistance was extinguished by treating the recipients with dextran sulfate 500 (2 mg/mouse), a macrophage-dysfunctional agent.

In case of immune sera, a powerful resistance to the infection was inducible by transferring immune serum separated on study day 7. About one-third of respective mice transferred immune sera separated on days 5, 14, and 21 were able to conquer the infection with 3×10^3 parasites but none of the mice transferred immune serum separated on day 3 overcame the challenge.

INTRODUCTION

Protective immunity has been successfully induced in animals immunized with homogenate antigen of trypanosomes (Weitz, 1960; Seed, 1963; Miller, 1965; Furuya, 1977; Osaki and Furuya, 1978) but the defensive mechanism has been discussed mainly on humoral immunity (Seed and Gam, 1966; Takayanagi and Enriquez, 1973; Seed, 1977).

In our serial immunological study in mice (Furuya, 1977; Osaki and Furuya, 1978), animals received homogenate or microsomal antigen of *Trypanosoma gambiense* together with adjuvant were able to overcome challenges with the homologous parasites given on and after day 3 of immunization irrespective of the agglutination antibody titer of their sera. This might suggest that factors other than humoral ones are also involved in the elimination of the parasites in these animals. This report aims to define the cellular and humoral factors controlling the protection from *T. gambiense* infections in mice by transferring immune spleen cells and immune sera in combination with dextran sulfate 500, a macrophage-dysfunctional agent (DS 500).

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Materials and Methods

Animal and antigen: Forty to sixty days old female ddY mice and *T. gambiense,* strain Wellcome, obtained from the Department of Protozoology and Parasitology, Research Institute for Microbial Diseases, Osaka University and maintained for years in our laboratory by serial passages in mice, were used for the experiment.

Microsomal fraction was prepared from the homogenate of the parasites isolated from the blood of heavily infected mice following Lanham and Godfrey's method (1970) by differential centrifugation (Furuya, 1977), and used as antigen.

Transfer of immune spleen cells and immune sera: Antigens were administered intraperitoneally at a dose level of 2 mg protein per mouse with an equal volume of Freund's complete adjuvant. Immune spleen cells and immune sera were taken from donor mice on study days 3, 5, 7, 14, and 21 (day 0=immunization).

Spleens removed from the donors were washed in cold RPMI 1640 medium, pH 7.2 containing 25 m*M* HEPES and 0.02% 2Na–EDTA, and the tissues were minced into small pieces and pressed gently between two pieces of glasses to give single cell suspensions. The spleen cells were filtered through cotton wool to remove large clots before they were washed twice with the above cold medium by centrifuging at $170 \times g$ for 10 min and adjusted to a final concentration of 1×10^8 cells/m*l* with the same medium. Immediately after transferring 0.5 or 1 m*l* of the suspension intraperitoneally, recipient mice were challenged with 50 parasites by the same route.

A part of the recipients transferred spleen cells taken from the donor mice on day 3 were treated with DS 500 (2 mg/head) intraperitoneally just before challenges.

Normal or immune serum (0.25 m*l*) was injected into the recipient mice intraperitoneally 5 hours after challenges with 3×10^3 parasites by the same route.

Results

All of the untreated and those mice transferred normal spleen cells died 4 to 6 days after challenges with 50 parasites. The recipients transferred immune spleen cells separated from the donors on days 3 and 5 offered a strong resistance to the challenge. Per cent survival and mean survival days of the dead mice in the recipients transferred immune spleen cells separated on day 3 were 72.2 and 8.8 ± 0.6, respectively. The specific resistance to the infection was lowered in mice transferred spleen cells separated on day 5. Neither protection nor longevity was induced into mice by transferring 5×10^7 or 1×10^8 immune spleen cells separated on days 7, 14, and 21 (Table 1).

All of the mice transferred normal mouse sera died 4 to 6 days after infections but those transferred immune sera separated on day 3 died 6 to 8 days after infections and mean survival days of the dead mice were extended to 7.2 ± 0.2. A mighty resistance to the infection was inducible in mice by transferring immune sera separated on day 7. About one-third of respective mice transferred immune sera separated on days 5, 14, and 21 conquered the infection and mean survival days of the dead mice were also extended to about 10 days (Table 1).

Table 1 Resistance to challenges with *Trypanosoma gambiense* in mice transferred immune spleen cells and immune sera

immunization term of donor mice in days	immune spleen cells				immune sera		
	5×10^7 cells/mouse		1×10^8 cells/mouse				
	per cent survival in recipient mice	MSDs of the dead mice	per cent survival in recipient mice	MSDs of the dead mice	antibody titer of donor mice	per cent survival in recipient mice	MSDs of the dead mice
3	72.2 (13/18)	8.8±0.6	—		original	0 (0/9)	7.2±0.2
5	11.8 (2/17)	6.2±0.6	80.0 (4/5)	5.0±0	1: 8	27.3 (3/11)	10.1±0.8
7	0 (0/ 7)	4.0±0	0 (0/5)	5.0±0.3	1: 16	90.0 (9/10)	9.0±0
14	0 (0/ 7)	4.6±0.3	0 (0/5)	4.6±0.4	original	30.0 (3/10)	7.0±1.8
21	0 (0/ 7)	4.2±0.2	0 (0/5)	5.0±0	1: 8	40.0 (4/10)	9.5±1.5

MSDs: mean survival days (mean±S. E.)

— : not examined

Agglutination antibody titer was measured with pooled serum.

Recipient mice transferred immune spleen cells and immune sera were challenged with 50 and 3×10^3 parasites, respectively.

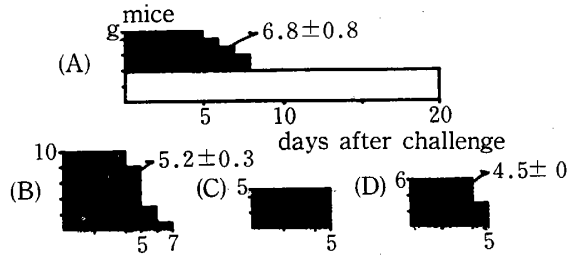

Fig. 1 Effect of treatment with dextran sulfate 500 on resistance to challenges with *Trypanosoma gambiense* in mice transferred immune spleen cells (A): transfer of immune spleen cells, (B): transfer of immune spleen cells and treatment with dextran sulfate 500, (C): treatment with dextran sulfate 500 alone, (D): nontreatment, □: survival, ■: death

To inflict injuries on phagocytes in the immune spleen cells and in the recipients, mice were injected DS 500 intraperitoneally immediately after transfer of immune spleen cells separated on day 3 and were challenged with the parasites uninterruptedly. The passive resistance induced in mice by immune spleen cells was cut off completely by treating the mice with DS 500 (Fig. 1).

Discussion

All of the mice immunized with microsomal antigen in adjuvant were able to overcome the challenges given on day 3 or 14 but agglutination antibody titers in their immune sera were strikingly low (Furuya, 1977; Osaki and Furuya, 1978).

To analyze the mechanism of defense against infections with the parasites, the resistance to infections was examined in mice transferred immune spleen cells and immune sera.

Passive transfer of specific resistance was accomplished by the use of immune spleen cells separated on days 3 and 5 of immunization. Campbell and Phillips (1976) reported that specific resistance to *T. rhodesiense* was transferable to the recipients by immune sera or B lymphocytes.

Moreover, Takayanagi and Nakatake (1975) reported that glass-adherent, antibody-forming cell population among immune mouse spleen cells was effective in preventing experimental infections with *T. gambiense* in recipient mice. In the present study, the spleen cell suspension contains different sorts of cells such as mononuclear and polymorphonuclear phagocytes, T- and B-lymphocytes, plasma cells and others. If any one of B lymphocytes, plasma cells and antibodies produced in recipients by these two kinds of cells is to be involved in the sweeping away of the parasites, mice transferred immune spleen cells taken from different study days, 7 to 21, should have overcome the challenges. But, neither protection nor longevity was observed in those mice.

Hahn (1974) and Hahn and Bierther (1974) reported the selective damage by DS 500 to mononuclear phagocytes. In the present experiment, recipient mice treated with DS 500 immediately after transfer of immune spleen cells were not able to evade the challenges with the parasites. From this, macrophages are likely to play a key role in the removal of parasites in the recipient mice. The reason why specific resistance was transferred by spleen cells within only 5 days after immunization might be that, in the early stage of immunization, the donor's spleen was filled with macrophages seemingly being participated in the transmission of antigenic informations to lymphocytes. These macrophages were probably activated by a certain stimulation because normal macrophages do not phagocytize trypanosomes in the absence of specific antibodies. But whether these macrophages have already been activated by phagocytizing the antigens in the donors and this status has been maintained in the recipient mice or these macrophages were activated by lymphokines produced by donor's T lymphocytes in the recipients is not known.

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Trypanosoma gambiense 免疫マウスの脾細胞あるいは抗血清を移入したマウスの同一原虫感染に対する抵抗性

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Trypanosoma gambiense に対するマウスの感染防御機構の解明を図る目的で，同原虫由来マイクロソーム画分を抗原として Freund's complete adjuvant と共に免疫した。免疫後3～21日にわたり経日的に得た免疫マウスの脾細胞あるいは抗血清を正常マウスに伝達し，同原虫感染に対する抵抗性を観察した。

免疫3あるいは5日目の脾細胞を移入したマウスのみ50個の原虫攻撃に対して特異抵抗性を示した。この抵抗性は脾細胞移入直後の硫酸デキストラン500による処理で消滅した。

抗血清の受身伝達では，免疫7日目の血清に非常に強い抵抗性付与能が認められた。また免疫5，14，及び21日目の血清を受身伝達したマウスの約1/3は 3×10^8 個の原虫攻撃に抵抗したが，免疫3日目の血清にはこれらの効果は認められなかった。

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DIC 症候群類似の症状を呈したクロロキン耐性熱帯熱マラリア症の一例

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昭和56年7月10日　受付

ま え が き

日本におけるマラリアは，古くは土着のマラリアも存在し，地域的に相当数の発生も見た[1]が大正期以降はその数も著しく減少し，第二次世界大戦後の一時期は復員兵による外来の輸入マラリアが猛威を振い二次感染も少数ながら発生したが[2]現在は土着マラリアは沖縄の一部[3]を除いてはほとんど消滅している。

しかし，近年我国が経済発展をとげ国民の多数が世界各地に観光，出張滞在をするようになり，輸入マラリアが今日大きなトピックスとなりつつある。このような情況のもとで，東南アジア・中南米・アフリカなどの熱帯各地を旅行または滞在した場合はマラリア感染の機会が多く，しかもマラリアに対する啓蒙教育が不完全であったため予防および治療に対する関心が乏しいことから，大友ら[4][5]の10年間にわたる連続調査によれば我国民で海外で感染し，帰国後発病したマラリア患者は年間わかっただけで平均50名を越え，推定では，100名に及ぶとみられ，そのうち数人の死亡例をみているのが現状である。

東南アジア地域のマラリア症のうち，ベトナム3国，フィリピンなどの熱帯マラリアはクロロキン耐性株であることが知られており[6]，この傾向は東南アジア全域に拡大されつつあり，更にこの熱帯マラリアはファンシダール耐性の傾向[7]も示して来ている。これらのことが熱帯マラリア患者の治療を困難ならしめている。

我々はフィリピンのパラワン島でクロロキン耐性熱帯熱マラリアに罹患し，重症化しDIC[8][9] (Disseminated intravascular coagulation) 症候群を呈した患者を経験したのでここに報告する。

症　例　(Table 1)

患者は21歳の無職の男性である。中肉中背で生来健康で特記すべき既往症はなく，家族歴にもアレルギー素質その他特記すべきことはない。

昭和55年3月5日より蝶類採集の目的でフィリピン (Philippines) のパラワン (Palawan) 島のプエルト・プリンセサ (Puerto Princesa) に渡航しガイドをやといバルサハン (Balsahan) のジャングル地帯に入り，13日間蝶の採集を行った。その行動を要約すると次のとおりである。

3月5日マニラ (Manila) に到着し翌日プエルト・プリンセサに渡った。翌日 (7日) より，同市を基点に，7日バルサハン，8日イラワン (Irawan)，9日サルコット山 (Mt. Salakot)，10日再びバルサハン，11日イラワンにて，それぞれ蝶類採集に従事した。さらに，1日おき13日から再び同島ジャングル地帯のラングアン (Languun) 及び周辺での蝶採集が開始された。発症日より，通常の平均的潜伏期を逆算すると，同日付近に感染があったことを推測させる。20日にプエルト・プリンセサに戻り，この翌日，既に過度の疲労感を自覚しているが，他の所見に欠け明らかな発症は認めていない。22日さらにマニラに戻り，2泊滞在の後24日帰国した。

その間，マラリア予防の目的でクロロキン製剤 Nivaquine (ニバキン) を週1回 200 mg 1錠を内服し，帰国後も発症までに2回内服していた。

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Table 1 Case History (Y.I. male, 21 age)

Snbjective symptoms: Remittent fever, Headache and muscle pain on lower limbs

Past history: none

Family history: none

Present history: Patient has experienced a trip collecting butterflies in Parawan lsland, Philippines, during 5th through 24th of March, 1980. On 28th, March, fatigue in the morning and high fever attack with chilling sensation up to 40.3°C of body temperature, on 29th, March, body temperature down with perspiration in the morning and up again to 40°C with chilling sensation. At night of 29th, 10 pm, patient was admitted in the Dokkyo University Hospital

Present status: Anemic face with slight jaundice, Puls rate 84/minute, normal blood pressure (115/75), body temperature 39.3°C, no lymphnode swelling, liver enlarging palpable as two fingers wide with pain, no spleen palpable and no neurological symptom.

現 病 歴

3月28日の朝に不快感を自覚し，同夜半より悪感，戦慄を発現し約1時間半の後 40.3°C まで発熱を生じた。29日午前9時頃発汗と共に下熱した。同日午後1時頃再び約1時間の悪感戦慄の後，40°C の発熱をみ，同午後10時獨協医科大学アレルギー内科へ，某医よりの紹介で緊急入院となった。

現症の概要

入院時体温 39°C で，脈拍数90/分，血圧 108/60 mmHg であった。頭色は蒼白かつ軽度黄疸様を呈した。頭痛を有し，眼瞼結膜・眼球結膜にはそれぞれ貧血・黄疸はなく，表在リンパ節の腫脹もなかった。胸部所見では聴診上で異常所見はなかった。腹部所見では，肝は軽度腫大し，弾性軟，圧痛あり，脾はこの時は触知しなかった。しかし，後にシンチグラムで腫大が確認された。その他，神経学的所見などには，異常は認めなかった。

検査所見 (Table 2)

入院時，貧血はなかったが，白血球数 2900/

Table 2 Clinical Findings at the begin of hospitalization

RBC	518 10^4/mm^3	GOT	92 K.U.
RBC	15.4 g/dl	GPT	53 K.U.
Ht	42 %	Al-P	21.1 K.A.U.
WBC	2900 /mm^3	OAP	220 G.B.U.
Meta.	7 %	LDH	618 W.U.
Stab.	52	γ-GTP	38 mU/m*l*
Seg.	11	M. G.	13 U
Eo.	1	Bil	2.0 mg/d*l*
Mo.	8	D. B.	1.3 mg/d*l*
Lym.	19	I. B.	0.7 mg/d*l*
At y-Lym.	2		
Pl.	3.5 10^4/mm^3	Urobilinogen (U.)	++
Ret.	6 ‰		
ESR (1 hr.)	2 mm	Bleeding Time	1 min.
CRP	+3	Coag. T.	10 min.
		PT.	10.8 sec.
		(cont.	9.9 sec.)
		PTT.	46 sec.
		(cont.	32 sec.)
		Fibrinogen	220 mg/d*l*
		FDP	<40 μg/m*l*

mm^3，血小板数 $3.5\times10^4/mm^3$ と共に著名な減少を示した。白血球分画像では桿状球52%，分葉球11%と左方偏位を呈し，単球は8%と軽度増加していた。血沈は1時間値2mm，2時間値8mmとむしろ遅延していたが，CRPは3+であった。GOT 92 K.U., GPT 53 K.U., Al-P 21.1 K.AU., LDH 618 W.U., M. G 13 U., 13 il, 2.0 mg/dl (D.B 1.3 mg/dl IB 0.7 mg/dl) と，軽度～中等度肝機能障害を示し，黄疸がみとめられた。しかし，血液凝固系には異常はなかった。

臨床経過並びに治療 (Fig. 1 及び Table 3)

熱型は Fig. 1 のごとく，周期不定の間歇熱が持続した。第2病日に末梢血液塗抹標本で ring form 及び trophozoite のマラリア原虫が確認された。原虫数が次第に増加したので，第4病日より治療を開始した。

通常の投与法に従って，2日間クロロキン塩基にして 1200 mg まで投与したが，原虫数の減少はきわめて軽度だったので，ピリメサミン・スルフォモノメトキシン（P剤・S剤）の合剤に切り換えた。

第5病日に P, S 剤各々 50 mg, 1 g を経口投与したところ翌日下熱したが末梢血中の原虫数に変化をみなかったため，さらに前日の半量を追加投与し，同日晩より塩酸キニーネ 1.5 g/日の投与を開始したが，第7病日再び発熱し難聴をも発現したため P・S 合剤を増量し，さらに同夜塩酸キニーネ 300 mg を点滴静注した。同時に下熱の目的で hydrocortisone 100 mg を静注した。そのためか原虫数はその後に減少をみた。

第8病日には，すでに末梢血中よりの ring 及び trophozoit はすでに消失していたが，尚も発熱は持続したので Predonisolone 15 mg/日の経口投与を開始した。

第9病日には下熱したが，さらに P.S 合剤，キニーネは継続投与し，各剤の合計がそれぞれ 275 mg, 5.5 g, 10.3 g となったときに投与を中止した。Predonisolone は漸減中止した。

第13，14病日に，再び発熱及び白血球数増多を見たが，末梢血中原虫は認められなかった。経過中，白血球数に関しては入院後，段階的改善を示した。

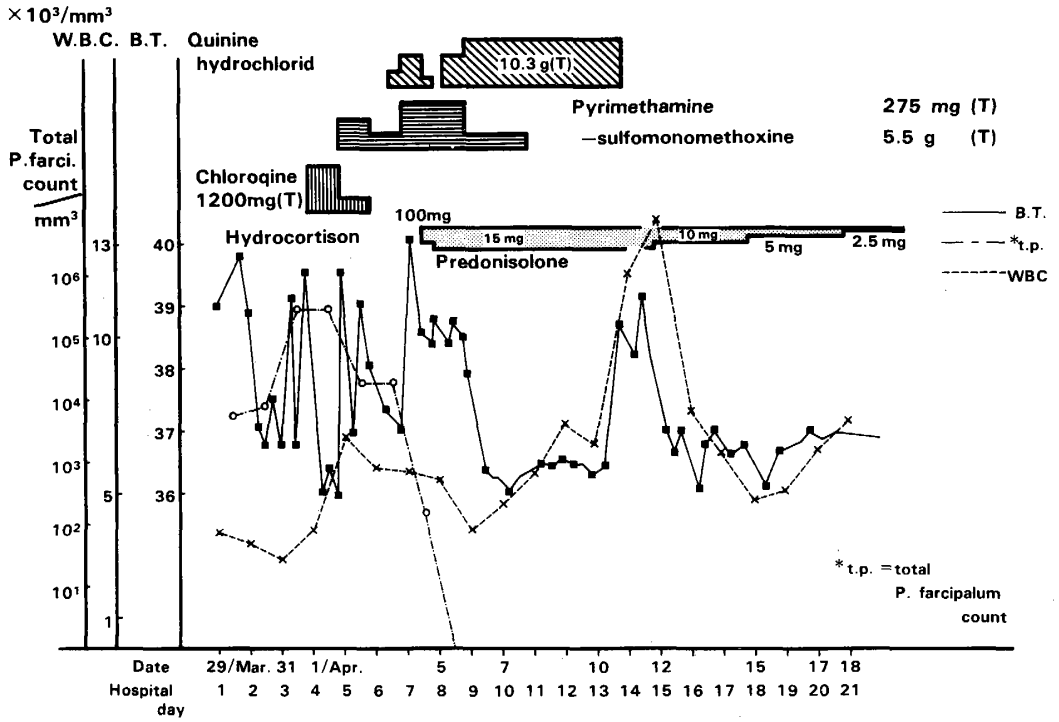
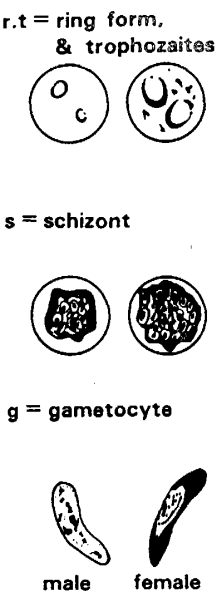


Fig. 1 Clinical Course I

Table 3 Changes of P. farciparum count in relation to Therapeutic Process

Date	29 mar.	30	31	1 Apr.	2	3	4	5	6
Hospital day	1	2	3	4	5	6	7	8	9
Parasite Stage	r.t	r.t	r.t	r.t	r.t s	r.t g	r.g	g	g
WBC	3700	3400	2900	3800	6800	5800	5700	5400	3800
P/W ratio	31/20	203/100	1495/15	1530/20	253/100	167+1*/50	4+1*/200	0+1*/400	0+1*/200
Total asexual parasites/mm³	5,735	6,902	289,033	290,700	17,204	19,488	142	0	0
log (asexual parasites/mm³)	3.75	3.83	5.46	5.46	4.23	4.28	2.15	0	0
sexual parasite (gametocyto)	0	0	0	0	0	16	29	14	19
log (sexual parasites/mm³)						1.2	1.4	1.1	1.3
Therapy (per os)									
Chloroquine (mg)				900	300				
Pyrimethamine (mg)					50	25	75	75	25
Sulfomonomethoxine (g)					1	0.5	1.5	1.5	0.5
Quinine HCl (g)						0.5	1.0	1.0	1.5
〃 (g) (i.v)							+0.3		



なお，第6病日より，末梢血中に Gametocyte が出現し，これにより本症例は熱帯熱マラリアであることが確定した。

凝固系異常 (Fig. 2 および Table 4)

次に我々が興味を持った，経過中に生じた凝固異常に関して述べる。

入院時すでに血小板数は減少し，血漿フィブリノーゲンは当初正常であったが経過と共に漸時減少した。FDP (fibrin degradation products) は漸時増加し，第6-7病日以降，すなわち末梢血中よりの ring 及び trophozoite の減少期あるいは消失の後にその最大値を示した。この時期の血小板数，血漿フィブリノーゲン量及び FDP の実測値は各々 $1.2\times10^4/mm^3$, 80 mg/dl 及び 10 μg/ml 未満であった。

同時に，この時期の出血，凝固時間は正常であったが，プロトロンビン時間は 12.3 秒でコントロールの 9.8 秒に比してあきらかに延長がみられた。

上記所見を総合すると臨床症状は出ていないが，DIC 症候群の検査成績に似ている。さらに，アンチトロンビン III (11.5 mg/dl) の減少，SK 活性プラスミン (30 min) の亢進もみられたので，この考え方は妥当のものであろう。

上記所見に対して患者は新鮮血 400 ml の輸血と1日 9000 U 約2日間のヘパリン療法（このヘパリンの投与量は，通常の投与法に比べ，はるかに少量であったが）の後，Fig. 2 のごとく改善を見た。前記した難聴も，発症後約1週間の経過の後消失した。

その他の検査所見では，Fig. 2 及び Table 4 の如く肝機能は入院後ほぼ10日目以降に正常化した。一方経過につれて貧血が出現し，低補体価は当初よりあったが，共にやや長期にわたって段階的改善をみた。尚，貧血に関して，網状赤血球の増加はあったが，直接・間接クームス試験はいずれも陰性であった。

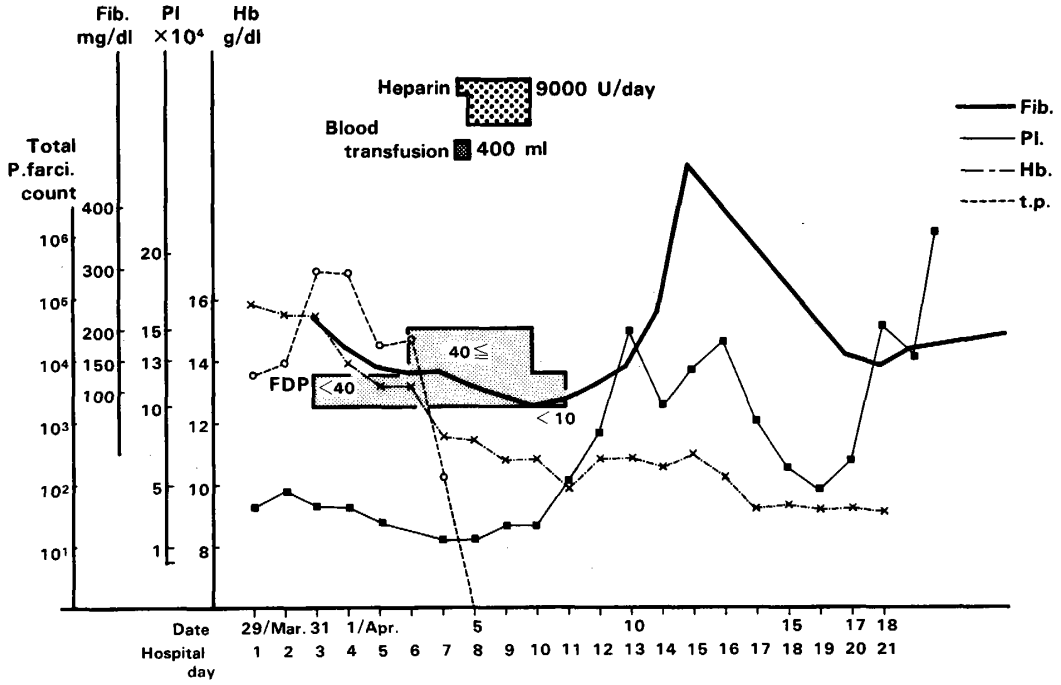


Fig. 2 Clinical course II (coagulation study, therapy and P. farciparum count)

Table 4 Hematological and immunological studies and P. farciparum count during hospitalization

Day		29 Mar.	31	1 Apr.			5				10						15				18
Hospital day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Platelet	×10⁴/mm³	3.4	4.8	3.5	3.5	2.5		1.4	1.2	2.2	2.1	5.0	8.2	14.9	9.9	12.2	14	8.8	5.9	4.4	6.4	15.0
Fibrinogen	×10²mg/dl			2.2	1.7	1.4	1.3	1.3	1.1		0.8	0.9	1.1	1.4	2.6	4.7					1.5	
FDP	μg/ml				<40	40≦	→		40≦		<40	<10	→									
Prothronbin Time	sec.	nl	→				↑↑	↑↑			↑↑											↑ (29/Apr. nl)
Coagulation T.	sec.	nl	→																			
Bleeding T.		nl	→																			
Anti thrombin III	mg/dl							11.5			17.5							26.8				
ESR	(1 hr.) mm			2			4									35					20	
WBC	×10³/mm³	3.7	3.4	2.9	3.8	6.8	5.8	5.7	5.4	3.8	4.6	5.6	7.1	6.5	12	13.8	7.6	6.3	4.6	5.0	6.3	7.3
RBC	×10⁶/mm³			5.2			4.4					3.2										(26/Apr. 3.2)
Hb	g/dl	15.8	15.5	15.4	13.9	13.1	13.1	11.5	11.4	10.8	10.8	9.9	10.8	10.8	10.5	10.9	10.2	9.1	9.2	9.0	9.1	9.0 (9/May 12.3)
CH_{50}						(3)*		(8)*			15		25									
C_3C						32		24			27		30		44			51			47	
Total Parasites	×10³	5.7	6.9	29	29	17	19	0.1	0	0	→											

* Kept in a refrigirator for 7 days.

考　　察

熱帯性マラリアは別名悪性マラリアと呼ばれ，24時間の周期で発熱し，血中の環状体が急激に増加し易いこと，脳症状をおこし易いことでよく知られている。この症状は脳症状は起さなかったが，短時日に体力の消耗が著しく，発病直後に入院したにもかかわらずクロロキン耐性株であった為に初期の治療が充分に効かず，キニーネの内服でも不充分で，キニーネの点滴およびＰ剤とＳ剤の内服でやっと危険期を脱出したものである。

疫学的にはパラワン島の熱帯マラリアはクロロキン耐性株が多いことは寄生虫学者間ではよく知られているが，海外渡航者の殆ど全員が熱帯病の知識なしに出かけている現在，クロロキン剤の内服で安心して流行地に滞在することは当然のことで，この点からみてこの症例は貴重な症例である。

熱帯熱マラリア原虫の診断については，標本虫がクロロキンの影響をうけて変化しているが，特有の熱型や環状体が一個の赤血球中に２個以上出現すること，それが短時日に増加すること，被変性赤血球の形が増大しないこと，schizont の形，gametocyte の出現などから容易である。一般にマラリアでは白血球の減少はよく知られているが，治療経過を追って虫体の出現消失および血液所見を追求した例は必ずしも多くはない。本報告では症状の経過と密接に関係して血小板の著減，血漿フィブリノーゲンの減少，FDP の増加をみとめ，肝障害（これはキニーネ内服が関係する可能性あり）があり，低補体価およびプロトロンビン時間の延長，その他の現象を観察しえた。DIC 症候群によるショック発現にいたらなかったとはいえ，上記所見は DIC 所見と関連を有するものである。

熱帯熱マラリア死亡例と DIC 症候群との関係はなお討議の余地があるが，本症例は討議の材料として充分に価値のあるものである。

結　　語

クロロキン耐性の熱帯熱マラリア患者（男21歳）１名を発病の初期から観察して，各種の臨床検査の結果とくに血漿の線溶系を中心に DIC 症候群にいたる段階の成績をみとめ治療により全治したので，その場合の諸検査の回復も含めてここに症例報告した。

稿を終るに臨み御指導と薬剤の供与をいただいた，岐阜大学寄生虫学教授大友弘士博士，独協医科大学医動物学教授山本久博士，独協医科大学アレルギー内科教授牧野荘平ならびに日本熱帯医学協会に深い感謝の意を表します。

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A CASE STUDY ON CHLOROQUINE RESISTED FALCIPARUM MALARIA COMBINED WITH DIC RESEMBLING SYMPTOMS

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A male patient of 21 of age was infected with *Plasmodium falciparum* resisted to chloroquine during his stay in Parawan Island, Philippines.

Typical fever attacks occurred just after coming back to Japan and he was hospitalized. The patient turned to worse within a few days due to high fever attacks and acute increase of parasitemia even by oral administration of chloroquine, and then he was treated with quinine intravenously as well as orally, in combination with pyrimethamine and sulfamono-methoxine, which resulted to establish complete cure of this patient.

Among his clinical course, such findings were positive in high grades as leucopenia, thrombocytopenia, prolongation of prothrombin time, decrease of fibrinogen, antithrombin III and fibrin degradation products (FDP) in blood. Highly activation of SK plasmin was also recognized.

Those findings were resembled with DIC syndrom even though without actualization in this patient. Blood transfusion, medication of corticosteroid and heparin were very useful for recovery from those situation.

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FUNDAMENTAL STUDIES ON THE LEUKOCYTE CHEMOTACTIC FACTOR IN LYMPHOKINES OF DOGS INFECTED WITH *BABESIA GIBSONI*

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Abstract: The agarose plate method using dog neutrophils and human monocytes as migrating indicator cells were used to demonstrate neutrophil chemotactic factor and monocyte chemotactic factor, respectively, in dogs infected with *Babesia gibsoni*. The experiments were done in the supernatant from *in vitro* cultures of spleen cells, peripheral blood lymphocytes as well as extracts of spleen cells.

In particular, it was suitable to use canine neutrophils as migrating indicator cells for demonstrating neutrophil chemotactic factor in 2.5×10^5 cells per well of normal canine leukocytes incubated for 2 hours in agarose plate with 10% inactivated normal canine serum. Supernatants from *in vitro* cultures of canine spleen cells (3 weeks postinfection) and peripheral blood lymphocytes (3 days postinfection) showed an increased chemotactic activity of neutrophils as compared with that of normal dogs. Similarly, supernatants from *in vitro* cultures of canine spleen cells (2, 3 and 25 weeks postinfection) and peripheral blood lymphocytes (7 days postinfection) showed a tendency to contain monocyte chemotactic activity.

INTRODUCTION

Recently, infection with *Babesia* (*B*) *gibsoni* in dogs has been reported from various regions of Japan (Akashi *et al.*, 1969; Kusunoki, 1971; Noda, 1977, 1978). In a series of previous studies by the authors on experimental protozoan diseases using animals based on cell-mediated immunity, it was reported that macrophage activation was associated with increased cell size, spreading on glass, adhensiveness to a charged surface, phagocytic activity and digestive capacity, and with increase in contents of some lysosomal enzymes (Ishimine *et al.*, 1978, 1979; Makimura & Suzuki, 1977; Noda & Suzuki, 1977; Sethi *et al.*, 1975; Shirahata *et et.*, 1975, 1976, 1977; Nakabayashi *et al.*, 1978). It was suggested that the cell-mediated immunity by the macrophages and lymphocytes towards *B. gibsoni* infection may be involved in the protective immunity (Ishimine *et al.*, 1978, 1979; Suzuki, 1976). On the other hand, it has been suggested that the initial reaction of antigen with a few specifically sensitized lymphocytes results in the production of soluble mediators, also called lymphokines (LKs), which through their biologic activity, are capable of recruiting host inflammatory cells, activating them, and keeping them at the site (Boyden, 1962; Fudenberg *at al.*, 1976; Weissmann, 1978). However, there is a

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surprising paucity of research regarding the LKs and monocyte function in normal dogs and also concerning the basic and clinical cell-mediated immunity of canine diseases. In consequence, the authors tried to examine the effect of LKs on the peripheral blood monocyte chemotaxis, as well as on the neutrophils in *B. gibsoni* infected dogs by detecting the chemotactic factor in the LKs.

Materials and Methods

Babesia species: The strain of *B. gibsoni* used in this study was maintained by canine passages in our department (Ishimine *et al.*, 1978, 1979). This was originally obtained from a hunting dog that contracted the parasite in Hyogo Prefecture in Japan.

Experimental animals: Six adult mongrel dogs, weighing approximately 10 kg, were used in the experiments. They were inoculated intravenously (i.v.) with approximately 8.5×10^9 parasitized erythrocytes which had been harvested from a splenectomized dog wherein 30% of the erythrocytes contained *B. gibsoni*. Peripheral leukocytes were examined at the hours of 3, 6 and 12, and on days 1, 3, 7, 10, 14, 21 and above 175 postinfection (p.i.). For the 2nd experiment, dogs were also inoculated with the parasitized erythrocytes, and the materials were taken from 2 of the dogs on the 2nd and 3rd weeks representing the acute stage, and above the 25th week p.i. as the chronic stage of the infection. For the chronic cases in the canine experimental babesiosis the dogs were challenged with the same parasitized erythrocytes i.v. 25 weeks after the 1st inoculation to obtain hyperimmunized dogs as donors of the immune lymphocytes. The hyperimmunized spleen cells were taken on the 4th week after the challenge.

Preparation of Babesia lysate antigen: Three volumes of PBS were added to one packed volume of the parasitized erythrocytes. The suspension was homogenized with a glass homogenizer and ultrasonic vibrator (100 W, Kubota Insonator, Model 200, Tokyo) for 5 minutes, and stored for at least 48 hours at −80 C. The antigen was thawed, rehomogenized, washed 5 times with PBS and recentrifuged at 17,300 g for 20 minutes. The final sediment was homogenized with 1.5 volume of PBS and lyophilized in 0.5 m*l* quantities. When needed, 50 mg of lyophilized antigen was mixed with 2 ml of PBS in a 10 m*l* conical centrifuge tube and centrifuged at 365 g for 30 minutes. The supernatant fluid was collected and stored at −80 C to serve as the "*Babesia* lysate antigen, or BLA".

Preparation of lymphokines (LKs): Spleen cells and lymphocytes from peripheral blood were collected from normal and *Babesia* immune dogs by means of a slight modification of the Conray-Ficoll method (Tsuji, 1971). The cells were washed twice with HBSS. Normal and *Babesia* immune spleen lymphoid cell suspensions were incubated with 50 μg BLA in a humidified 5% CO_2 incubator at 37 C for 24 hours. These lymphoid cell cultures were centrifuged at 1000 g for 30 minutes. The supernatants thus obtained were filtered through a millipore membrane filter (0.3 μ, type PH, Millipore Co., USA) and were used as the normal cultured supernatant and *Babesia* immune LKs. They were diluted 1 : 2 with medium TC–199.

Preparation of spleen cell extract: Suspension of 1×10^8 spleen cells per m*l* of

medium TC–199 was repeated by dry-melting (—80 C and 37 C) and sonicated by the ultrasonic vibrator for 5 minutes. The suspension was filtered through a millipore membrane filter.

Preparation of agarose plate: 0.12 g of agarose was dissolved in 45 m*l* distilled water and 4.5 m*l* double concentrated medium TC–199 containing 1% heat-inactivated canine or human serum was added and kept at 48 C. After suspension, 5 m*l* of this mixture was poured into a petri dish for tissue culture. Three m*l* of this mixture was placed on a slide glass at 4 C and allowed to solidify. The agarose plate used for normal canine neutrophils was prepared to contain 10% heat-inactivated calf serum (CS) or canine serum. As shown in Fig. 1, these wells, 3.0 mm in diameter, were punched into the agarose medium with a steel punch. Ten μl of indicator cells as previously described were poured into the outside well and 10 μl of medium TC–199 into the inside well. The plate was incubated for 2 hours to evaluate chemotaxis of neutrophils. Incubation was for 18 hours at 37 C in CO_2 incubator. After that, the petri dish or slide glass was fixed in methanol overnight at 4 C. The agarose was discarded and the cells were stained with Giemsa or Wright stain.

Measurement of chemotaxis: Measurement of chemotaxis was made according to the method of Nelson (1975). The measurement of chemotaxis was calculated as the ratio A: B; that is, A, the chemotactic distance between a central well containing indicator cells and outside well containing the sample for measurement of chemotaxis or positive chemotaxis, and B, the natural chemotactic distance from central well to the inside well containing medium TC–199 as control or spontaneous migration. This was determined by the use of microscope.

Results

Spontaneous migration of normal canine neutrophils: Patterns and measurements of spontaneous migration in normal canine neutrophils of the peripheral blood are shown in Chart 1. The migration distance of normal canine neutrophils increased parallel with the incubation period and also with the increase in the number of the cells. Addition of serum into the agarose plate is necessary for the migration of the cells. Migration increased by adding small amounts of serum to the agarose plate. The migration distance and cell number were observed to decrease remarkably when heterologous serum was used instead of autologous serum. The difference in the migration distance between the slide glass and petri dish used as glass plates was investigated to simplify this method. No remarkable difference was demonstrated in both materials as shown in Chart 1. To incubate, it was necessary to keep the slide glass on a flat surface. In this experiment, the authors found it suitable to use canine neutrophils as migration indicator cells for demonstrating neutrophil chemotactic factor (NCF) in 2.5×10^5 cells per well of normal neutrophils. They should be incubated for 2 hours in agarose plate with 10% inactivated normal canine serum.

Neutrophil chemotactic assay: Neutrophil chemotactic assay was carried out using supernatant from spleen cell cultures of three normal canine samples. The chemotactic index ranged from 0.6 to 1.3 and the average was 1.0 ± 0.2, as shown in Chart 2.

a. Arrangement of wells in agarose plate

b. Pattern of differential migration in response to chemotactic factor

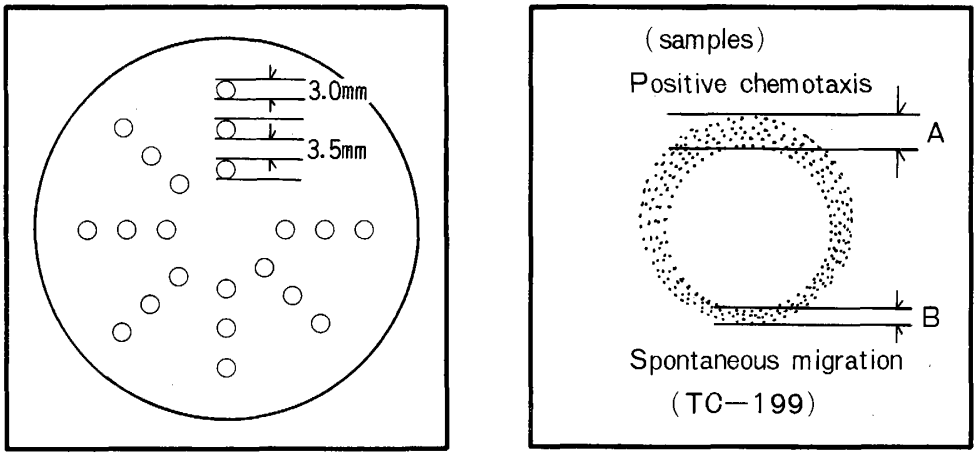


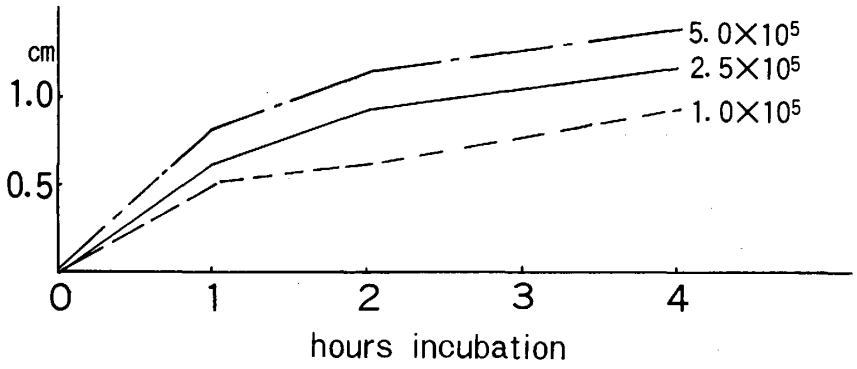
Fig. 1 Differential migration of canine neutrophils in the leukocytes from a well in the supernatant from the spleen cells of dogs in the acute infection of *Babesia gibsoni*.

The chemotactic index of the supernatant from spleen cell cultures from acute cases of canine babesiosis showed 1.1 ± 0.2. A slight increased chemotactic activity of neutrophils was also detected in some cases. The neutrophil chemotactic index of chronically infected babesiosis was 1.0 ± 0.2. As shown in Chart 3, supernatant from peripheral blood lymphocyte cultures showed a slight increase in chemotactic activity at 3 and 7 days postinfection (p.i.) of *B. gibsoni* compared with that of normal dogs. There was a tendency for this activity to gradually decrease up to the 21st day p.i.

Monocyte chemotactic assay: When canine peripheral blood monocytes were used as migration indicator cells for demonstrating NCF, migration of neutrophils present in small numbers was also observed at the same time. Thus, it was impossible to calculate the chemotactic index accurately. For this reason, human peripheral blood monocytes were used as migration indicator cells for demonstrating NCF in these experiments. The monocyte chemotactic index of spleen cell extracts from normal dogs was shown to be 1.0 ± 0.1 (0.8–1.0). The monocyte chemotactic indices in the acute stage of 3 weeks p.i. and chronic stage of 29 weeks p.i. were 1.1 ± 0.1 (1.0–1.1) and 1.1 ± 0.2 (0.9–1.2), respectively. As shown in Chart 4, the difference in chemotactic response to monocytes was demonstrated in some cases of *B. gibsoni* infection compared with that of the control. The monocyte chemotactic index of supernatant from normal canine spleen cells was 0.9 ± 0.2 (0.9–1.2) and that of the supernatant from acute cases of *B. gibsoni* infected dogs, 2 and 3 weeks p.i., was 1.2 ± 0.1 (1.0–1.3) and 1.1 ± 0.2 (0.9–1.5), respectively. Increased monocyte activity was demonstrated compared with that of normal dogs. The monocyte chemotactic index, shown in Chart 5, of supernatant from *Babesia* chronically infected monocyte chemotactic activity was also observed. Monocyte chemotactic activity was observed to be present in the supernatant from peripheral lymphocyte cultures 7 days p.i. as shown in Chart 6.

Chart 1 Measurement of spontaneous migration in normal dog peripheral blood neutrophils.

a. Effects of the number of leukocytes added per well and incubation time



cells/well	spontaneous migration (cm) (hours incubation)		
	1	2	4
5.0×10^5	0.8 ± 0.1	1.1 ± 0.2	1.3 ± 0.6
2.5×10^5	0.6 ± 0.1	0.9 ± 0.1	1.1 ± 0.4
1.0×10^5	0.5 ± 0.03	0.6 ± 0.4	0.9 ±0.3

b. Effect of varying dog serum concentration in agarose medium

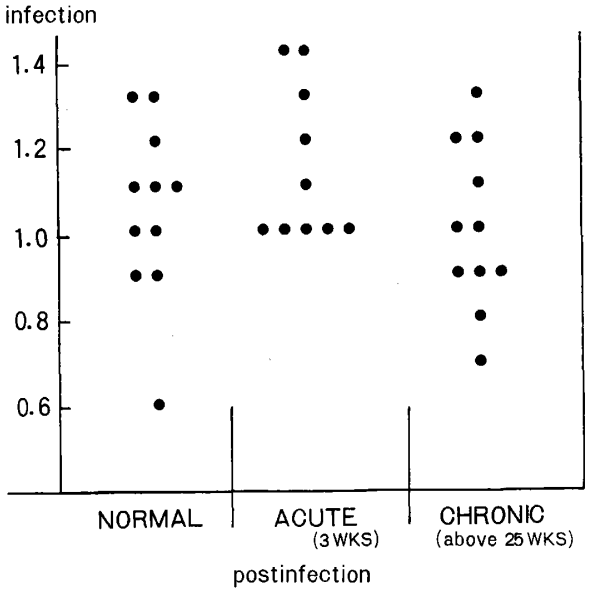
Dog—serum	Spontaneous migration (cm)
10 %	1.2 ± 0.2
2.5 %	0.9 ± 0.1
0 %	0.3 ± 0.2

c. Differential migration on petri dish and slide glass

Materials	Spontaneous migration (cm)
Petri dish	0.8 ± 0.1
Slide glass	0.7 ± 0.1

Notes: The values shown are mean±standard error within representative experiments done independently at least five times

Chart 2
Chemotactic response of dog neutrophils to supernatants from spleen cells of normal dogs with acute infection and chronic infection.



Migration ratio	No. of examined		
	11	10	11
Mean±S.D.	1.0±0.2	1.1±0.2	1.0±0.2
Range	0.6—1.3	0.9—1.4	0.8—1.4

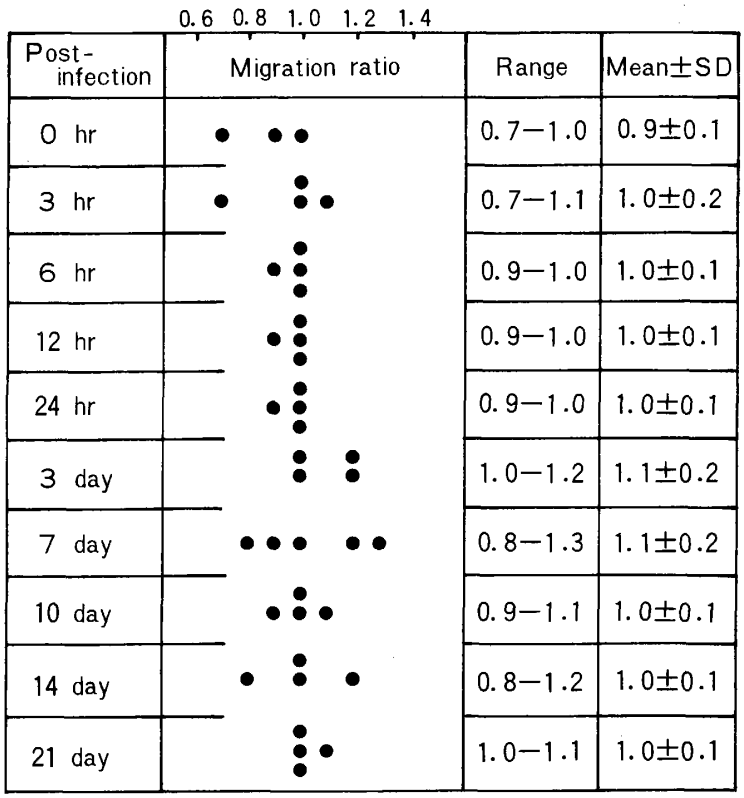
Notes:

$$\text{Migration ratio} = \frac{\text{Distance of positive chemotaxis}}{\text{Distance of spontaneous migration}}$$

Discussion

The major defense mechanism towards *Babesia* infection in dogs is generally thought to be the cell-mediated immunity (Ishimine et al., 1978, 1979; Makimura & Suzuki, 1977; Noda, 1978; Suzuki, 1976). Based on various findings by the authors in the past experiments, it has been suggested that the cell-mediated immunity by the macrophages, lymphocytes and the LKs may be involved in the protection immunity (Ishimine *et al.*, 1979; Suzuki, 1976). These findings included the increase of the monocytes in the peripheral blood, the phagocytosis of the parasitized erythrocytes by the reticuloendothelial cells, especially the splenic macrophages, the increased parasitemia caused by splenectomy in the chronic cases, and the accelerated phagocytosis of the parasitized erythrocytes by the immune monocytes from the chronic cases in comparison with those from the normal dogs in *in vitro* cultures. On the other hand, it is known that polynuclear leukocytes and monocytes in infectious diseases migrate to the site of infection (Fudenberg et al., 1976; Weissmann,

Chart 3
Chemotactic response of dog neutrophils to supernatants from peripheral blood lymphocytes in dogs intravenously infected with *B. gibsoni*.



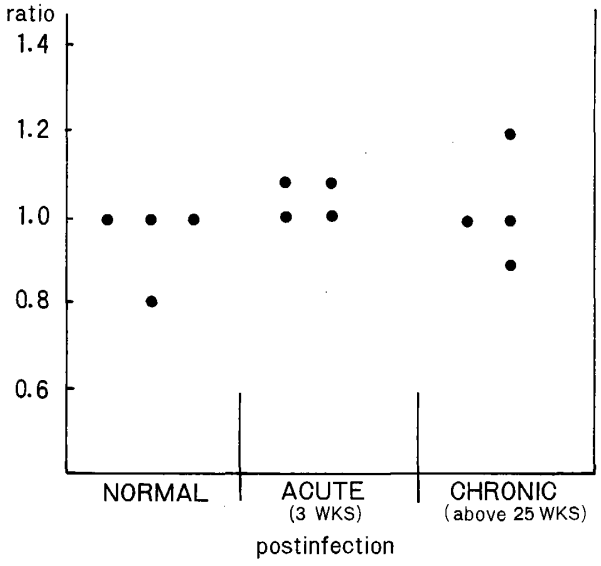
Post-infection	Migration ratio	Range	Mean±SD
0 hr		0.7—1.0	0.9±0.1
3 hr		0.7—1.1	1.0±0.2
6 hr		0.9—1.0	1.0±0.1
12 hr		0.9—1.0	1.0±0.1
24 hr		0.9—1.0	1.0±0.1
3 day		1.0—1.2	1.1±0.2
7 day		0.8—1.3	1.1±0.2
10 day		0.9—1.1	1.0±0.1
14 day		0.8—1.2	1.0±0.1
21 day		1.0—1.1	1.0±0.1

Notes: See Chart 2

1978). The movement of leukocytes has been divided roughly in two ways, the original or spontaneous mobility or negative chemotaxis, and movement towards a factor or positive chemotaxis. Several methods have been used for measuring chemotaxis such as the membrane filter method (Boyden, 1962), and the recently devised agarose plate method (Tautz, 1974; Tsuji & Que, 1976).

In this experiment, the authors thought of using canine monocyte for the monocyte chemotactic measurement, as indicator cells. The purification method of canine peripheral blood monocyte is not yet well established up to now. For this reason, canine monocyte chemotaxis was measured by using human peripheral blood monocytes as migration indicator, instead. This is because there are some problems encountered in the use of human monocytes. One is the property or function of monocytes which may differ in different animal species, and the other is the nonspecific effect of the materials produced by *Babesia* sensitized-lymphocytes on these monocytes. The former statement is yet to be considered, but for the latter there have been some reports on positive chemotaxis nonspecifically induced by human monocytes in mouse spleen cell extracts in cases of malaria infection (Wyler & Gollin, 1977).

Chart 4
Chemotactic response of human monocytes to extracts of spleen cells from normal dogs with acute infection and chronic infection.



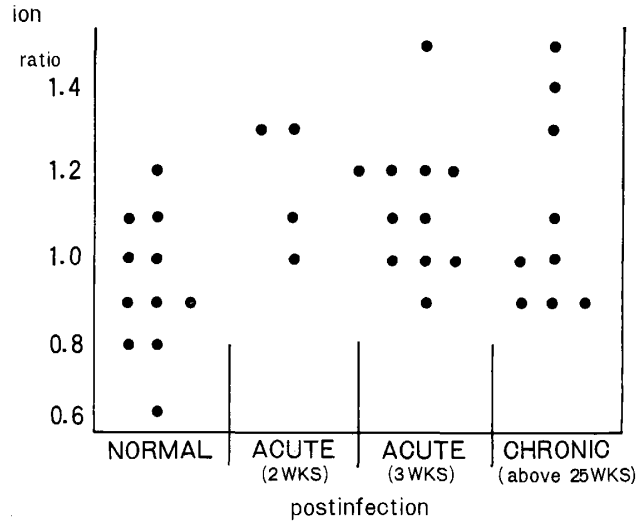
Migration ratio	No. of examined		
	4	~4	4
Range	0.8—1.0	1.0—1.1	0.9—1.2
Mean±S.D.	1.0±0.1	1.1±0.1	1.1±0.1

Notes: See Chart 2

In the early stage of *Babesia* infection in animals, an increase in the number of neutrophils and monocytes is observed in the peripheral blood (Holbrook et al., 1968; Simpson, 1974; Trubowitz, 1968). Recently, the presence of neutrophil chemotactic factor in the LKs, produced by sensitized lymphocytes with specific antigen, has been reported (Ishimine *et al.*, 1979; Suzuki, 1976; Fudenberg et al., 1976). However, no report has been made yet concerning the presence of neutrophil chemotactic factor in canine babesiosis. In the present study the authors believe that there is small quantity of chemotactic factor present in the spleen cell culture, especially in the peripheral blood lymphocyte supernatant. This suggests that there is a response of the sensitized lymphocytes to *Babesia* infection in dogs as a defense mechanisms against the parasitic infection. In the present study it was observed that a small quantity of monocyte chemotactic factor is present in the spleen cell extracts and the culture supernatant from chronically infected dogs.

It has been reported that immunity against *Babesia* infection is brought about by the state of nonsterile immunity or premunition, and that the protozoa is hidden in the host for a fixed period after recuperation from the infection (Noda, 1977). It is also supposed that *Babesia* latent infection enables the lymphocytes to keep it

Chart 5 Chemotactic response of human monocytes to supernatants from spleen cells of normal dogs with acute infection and chronic infection.



Migration ratio	No. of examined 11	4	11	9
Mean±S.D.	0.9±0.2	1.2±0.1	1.1±0.2	1.1±0.2
Range	0.6—1.2	1.0—1.3	0.9—1.5	0.9—1.5

Note: See Chart 2

Chart 6 Chemotactic response of human monocytes to supernatants from peripheral blood lymphocytes in dogs intravenously infected with *Babesia gibsoni*.

Postinfection (Days)	0.8 1.0 1.2 1.4 1.6 Migration ratio	Range	Mean ±S.D.
0		0.9—1.1	1.0±0.1
1		0.9—1.2	1.0±0.1
3		0.9—1.2	1.1±0.1
7		0.9—1.5	1.2±0.2
10		0.9—1.4	1.1±0.2
14		0.9—1.5	1.1±0.2

Notes: See Chart 2

sensitized from the specific antigen *in vivo*, hence, the long duration of monocyte chemotactic factor emission.

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実験的バベシア感染犬におけるリンホカイン中の白血球走化性因子に関する基礎的検討

伊藤　守・牧村　進・鈴木　直義

実験的 *Babesia gibsoni* 感染犬における脾臓細胞培養上清，リンホカインの好中球および単球走化性因子について基礎的検討をおこない，以下の所見を得た。

1) 犬好中球走化性の測定にはアガロース・プレート法を用いて犬末梢血液白血球数，約 2.5×10^5/well, 10%非動化犬血清含有アガロース・プレートにて2時間の培養条件が最適であった。

2) 犬好中球走化性は，バベシア感染後3週のリンホカインおよび感染後3日の末梢血液リンパ球培養上清中に認められた。

3) 単球走化性はバベシア感染後3週および慢性期のリンホカイン，ならびに感染後7日以降の末梢血液リンパ球培養上清中に認められた。

以上のことから，犬ピロプラズマ感染に伴って，白血球走化性因子は感染犬の脾臓細胞および末梢リンパ球培養上清中に出現することが明らかになった。

帯広畜産大学家畜生理学教室

Errata of the publication by Takaoka, H. *et al.* appeared in the Vol. 9, No. 3/4, 1981.

page	line	error	correct
187	seventh line from the bottom	1981	1982
188	first line from the top	monitered	monitored
〃	eighth line from the bottom	tract	thorax
189	second line from the top	$\sum_{m=1}^{12}[(\bar{T}_i-T_0)/12]$	$\sum_{n=1}^{12}[(T_i-T_0)/12]$
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〃	third line from the bottom	1981	1982

Japan. J. Trop. Med. Hyg., Vol. 9, No. 3, 4, 1981, pp. 187–197

DEVELOPMENT OF *ONCHOCERCA VOLVULUS* LARVAE IN *SIMULIUM OCHRACEUM* AT VARIOUS ALTITUDES IN GUATEMALA WITH SPECIAL REFERENCE TO THE AMBIENT TEMPERATURE

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Abstract: Females of *Simulium ochraceum* which were fed on a carrier of the Guatemalan strain of *Onchocerca volvulus* microfilariae were maintained under natural conditions at five different altitudes (350–1,500 m) within and outside of the onchocerciasis endemic zone. The results suggested the probability that these insects' chance of surviving increases with altitude. The infective stage of *O. volvulus* larvae was reached on the eighth day at Mária Santísima (alt. 650 m). At higher altitudes (1,200–1,500 m), however, no larval development beyond the late first stage occurred, except in one *S. ochraceum* female (at Guatemala City, 1,500 m) which yielded late second-stage worms on day 16. The length of the gonotrophic cycle was three or four days irrespective of altitude. Heat accumulation, calculated by means of a self-registered thermometer, suggested that the predicted period required for larvae to reach the infective stage in the vector varies considerably with altitude (4.4–28 days). The relationship between the rate of development of *O. volvulus* larvae at varying altitudes, and ambient heat accumulation, is also discussed.

INTRODUCTION

Many factors influence the transmission dynamics of onchocerciasis. Ambient temperature is one of the most important of them, affecting as it does the parasite's development in the blackfly vector as well as the latter's survival. From previous laboratory experiments, we reported the development of *Onchocerca volvulus* larvae to the third stage in *Simulium ochraceum* Walker (the principal vector of onchocerciasis in Guatemala) under simulated day and night temperatures (Takaoka *et al.*, 1981). This occurred even when night temperature dropped below 16.9 C (the predicted development zero point), although larval development-time was extended. These results suggested that the speed of *O. volvulus* larval development in field population of *S. ochraceum* might vary with heat accumulation at differing altitudes.

The present study was thus undertaken to determine the probability of survival of *S. ochraceum*, and the development of *O. volvulus* larvae within it, at selected altitudes

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where ambient heat accumulation was being monitered.

Materials and Methods

Duplicate experiments were conducted in November and December, 1979, (*i.e.* during the early dry season — the period when workers harvesting coffee are most frequently exposed to blackfly bites). Females of *S. ochraceum* used in the project were captured between 09.00 and 12.00 hr at a coffee plantation, (Finca Mónica Ivoné) located on the southwestern slopes of Atitlán Volcano (Department of Suchitepequez).

Field procedures for the capture and maintenance of simuliids were the same as in previous work (Takaoka *et al.*, 1981). The *O. volvulus* microfilaria-carrier used in these studies was a 40 year-old man, with density of 45 mf/mg (based upon one shoulder biopsy). After collection, tubes holding individual blood-fed female blackflies were randomly divided into four (in November) or five (in December) groups, each consisting of 80–100 insects. Transportation from the collecting site to Escuintla (the lowest experimental altitude) took about two hours. Further one-and-a-half hours were required for the journey to the other locations. During this period the simuliids were held in an insulated portable cooler in which ice held the temperature to 15–20 C. Immediately upon arriving at the selected locations where the adults of *S. ochraceum* were to be maintained, all dead or moribund flies were removed. As a result, 13.1% (in November) and 10.7% (in December) of the total females were omitted. The tubes containing the remaining blackflies were packed horizontally in coverless plastic boxes, each with a capacity for 60 individuals. The boxes wrapped in damp towels were placed in a plastic bag to secure high humidity. These boxes were then aligned in wooden weather-instrument shelters (40 cm × 40 cm × 50 cm), standing one metre above the ground. Each shelter also held a hydrothermograph to record air temperature and humidity during the course of the experiment. The environmental shelters for housing the simuliids were stationed near the national highway from Escuintla to Guatemala City — at the Health Centre in Escuintla (alt. 350 m), at finca Mária Santísima (650 m), at finca Barretál (1,250 m), at finca Rincón (1,200 m) and at the laboratory, Servicio Nacionál de Erradicación de la Malaria, Guatemala City (1,500 m). Human onchocerciasis and *S. ochraceum* adults were known to be present only at Mária Santísima, Barretál and Rincón. All sites were visited each day thereafter. The number of live blackflies was recorded, and dead ones, if present, were removed and preserved in the laboratory freezer (at −19 C) for later dissection.

All dead examples were dissected into head, tract and abdomen, and teased out in 0.9% saline solution using a binocular stereomicroscope. The number of larvae present was counted and the developmental stages were identified by size and morphology, following Duke (1968) and Bain (1969). Also, follicular development of the ovaries was examined to estimate the duration of the gonotrophic cycle at ambient temperatures.

Heat accumulation (Day·C) reveals the sum of daily heat accumulation (supposedly allowing the larvae of *O. volvulus* to develop in the vector). Daily heat

accumulation was directly calculated from the air temperature graphs according to the following formula: $\sum_{m=1}^{12}[(\overline{T}_i - T_0)/12]$, always providing that T_i (the mean temperature of every two hours) exceeds T_0 (the predicted developmental zero point for *O. volvulus* larvae in *S. ochraceum*). The predicted developmental zero point and the thermal constant used in this study are 16.9 C and 45 Day·C respectively according to our previous study (Takaoka *et al.*, 1981).

Results

Heat accumulation at five locations of differing altitude

Table 1 presents the temperature conditions at the five experimental stations. The temperature-fluctuation patterns together with the daily heat accumulations during four days in December are shown in Figure 1. The December-thermal pattern for Barretál was lacking due to a faulty thermograph. As was to be expected, the mean daily temperature decreased as altitudes rose, and temperatures fluctuated remarkably by day and night at all altitudes observed.

Actual heat accumulation during the November experiment is illustrated in Figure 2. At Barretál and Guatemala data were insufficient for prediction of the time required for larvae to develop into the infective stage. Heat accumulation increased linearly at Escuintla and Mária Santísima, but irregularly in Guatemala

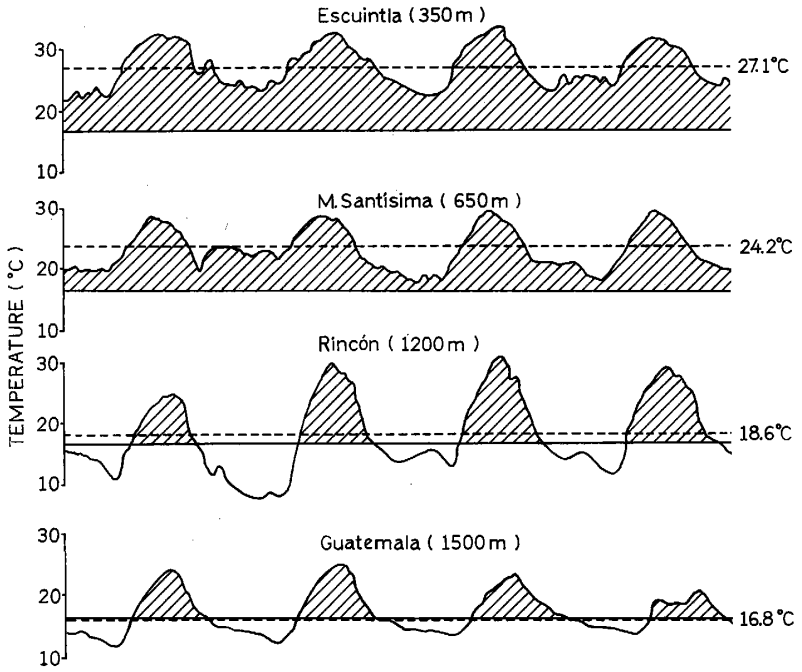

Figure 1 The fluctuations in air temperature during four days in December at four distinct altitudes, and effective temperatures above 16.9 C for *O. volvulus* larval development in *S. ochraceum*. (Areas indicated by oblique lines represent heat accumulation; solid horizontal line represents 16.9 C (developmental zero point); dashed line represents mean air temperature).

City, due to the fact that the temperatures did not reach 16.9 C on the seventh, eighth and ninth days.

Table 2 shows the predicted periods (in days) required for larval development to the infective stage, based on the mean daily heat accumulation. Predicted

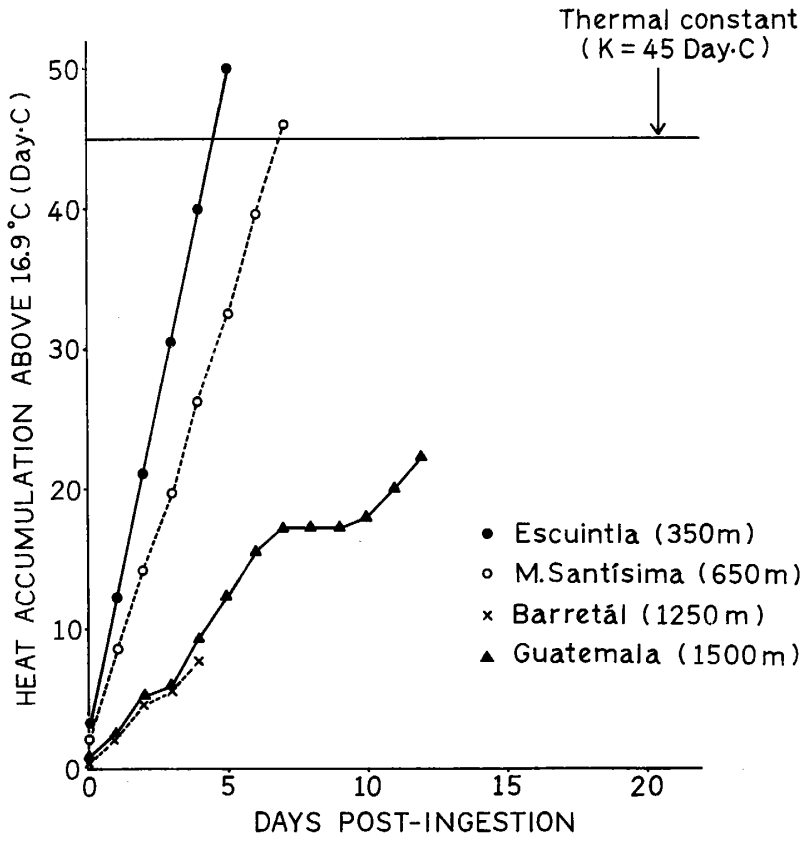

Figure 2 The heat accumulation during the course of the November experiment at four different altitudes.

Table 1 Daily temperature (C) at five localities of differing altitude in Guatemala (1979)

Place (Altitude)	Date	Mean	(Range)	Maximum		Minimum	
				Mean	(Range)	Mean	(Range)
Escuintla	Nov. 4–10	27.2	(25.7–29.5)	31.4	(30.8–32.2)	23.0	(21.8–27.0)
(350 m)	Dec. 8–20	27.1	(26.1–27.9)	33.1	(32.3–35.0)	22.6	(21.0–24.0)
Mária Santísima	Nov. 4–14	22.8	(21.8–23.3)	28.9	(26.0–30.8)	18.5	(17.0–19.6)
(650 m)	Dec. 8–21	24.2	(22.2–26.5)	30.1	(29.0–31.6)	19.6	(17.0–21.6)
Rincón (1200 m)	Dec. 8–21	18.6	(17.0–20.2)	26.7	(24.8–31.0)	13.1	(8.5–16.2)
Barretál (1250 m)	Nov. 4–7	17.9	(17.4–18.5)	22.1	(21.4–23.0)	13.6	(13.0–14.0)
Guatemala	Nov. 4–16	17.2	(13.1–19.3)	23.0	(15.8–27.6)	12.8	(11.2–15.5)
(1500 m)	Dec. 8–21	16.8	(14.7–18.7)	23.0	(19.9–26.2)	12.7	(8.6–14.5)

Table 2 Daily heat accumulation and predicted period required for development of *O. volvulus* larvae ot the third stage in *S. ochraceum*

Place and Month	Daily heat accumulation		Period for larval development in days	
	Mean	(Range)	Mean	(Range)
Escuintla				
November	9.8	(8.8–11.7)	4.6	(3.9– 5.1)
December	10.2	(9.2–11.0)	4.4	(4.1– 4.9)
Mária Santísima				
November	6.1	(4.9– 8.5)	7.4	(5.2– 9.1)
December	7.5	(5.3– 9.6)	6.0	(4.7– 8.5)
Rincón				
December	2.9	(2.0– 4.0)	15.5	(11.3–22.5)
Barretál				
November	1.8	(1.5– 2.0)	25.0	(22.5–30.0)
Guatemala				
November	1.8	(0.0– 3.7)	25.0	(12.8–)
December	1.6	(0.6– 2.6)	28.0	(17.3–75.0)

developmental periods varied considerably, with a tendency for prolongation as altitude rose.

Survival of the blackfly host

Figure 3 illustrates the survival curves for batches of female *S. ochraceum* fed on a carrier of *O. volvulus* and maintained at five different altitudes. The data suggest the probability that survival decreases with loss of altitude. In both experiments, very small number of simuliids survived beyond the predicted day when third-stage larvae should have appeared in the head. At Escuintla, 1.1% (1/90 blackflies in November) and 1.6% (1/62 blackflies in December) of the initial host population survived through the fifth day, while at Mária Santísima, 4.4% (4/90 blackflies in November) and 6.3% (4/64 blackflies in December) lived beyond the eighth and sixth day respectively. At Rincón, Barretál and Guatemala City, the only *S. ochraceum* to survive to the predicted day was one female (out of 90 experimented with at Barretál in November) which lived for 26 days.

Development of O. volvulus larvae in S. ochracrum

Figures 4 and 5 show the larval development of *O. volvulus* in *S. ochraceum* in relation to ambient heat accumulations at five differing altitudes. The mean daily heat accumulation obtained from the first four day's observation in November was used for Barretál instead of the actual one. Among 152 blackflies examined at Escuintla (350 m) no third-stage larvae were observed although two blackflies which died on the seventh day (in November) and sixth day (in December) yielded late second-stage larvae (Duke's stage K). However, at Mária Santísima (650 m), where the mean temperature was 22.8 C in November and 24.2 C in December,

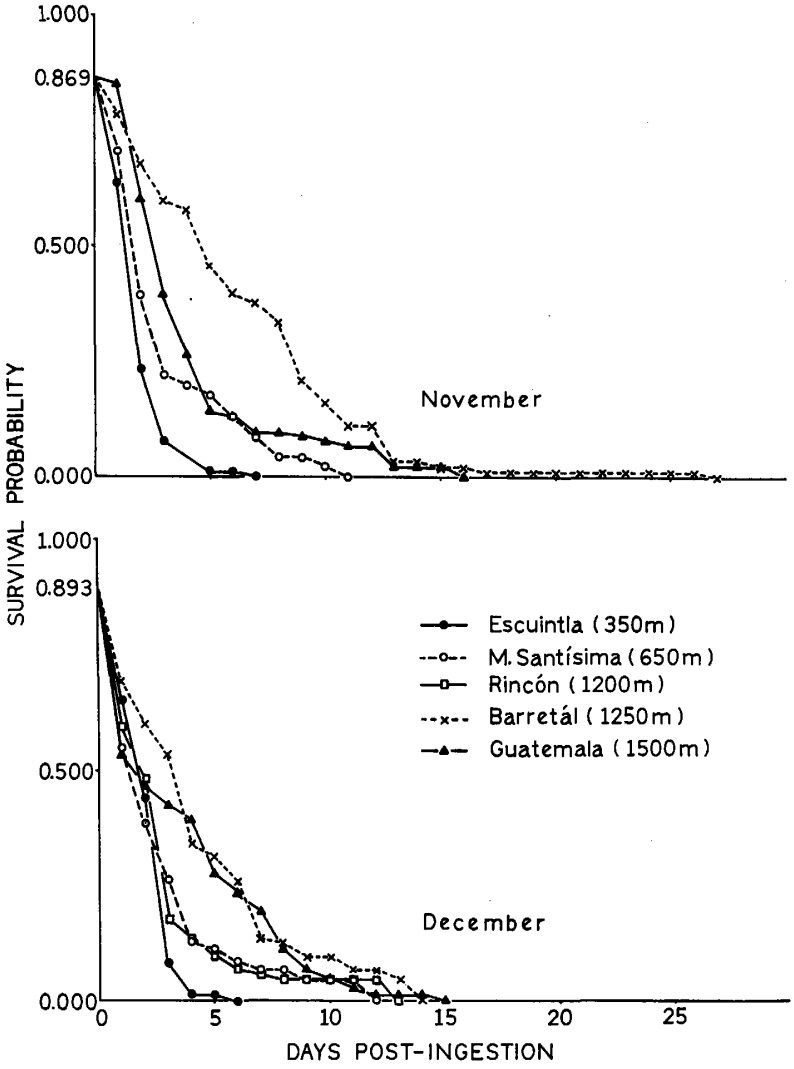

Figure 3 The survival curves for *S. ochraceum* females which were fed on a carrier of *O. volvulus* microfilariae and maintained at five different altitudes.

larval development to the infective stage occurred on the eighth day (in November) and ninth day (in December), although it was somewhat asynchronous as evidenced by several stages within individual blackflies (Table 3). On the other hand, no development beyond late first stage (H) was observed in simuliids held at Barretál (1,250 m) and Rincón (1,200 m). Unfortunately, the single example that survived 26 days at Barretál did not harbour parasites. Similarly, larval development beyond stage F was not seen in Guatemala City (1,500 m), except in the case of one female which died on the 16th day in November, harbouring five late second-stage larvae (K and J).

Length of gonotrophic cycle

At all selected locations, except in Rincón, fully-grown eggs were first found in

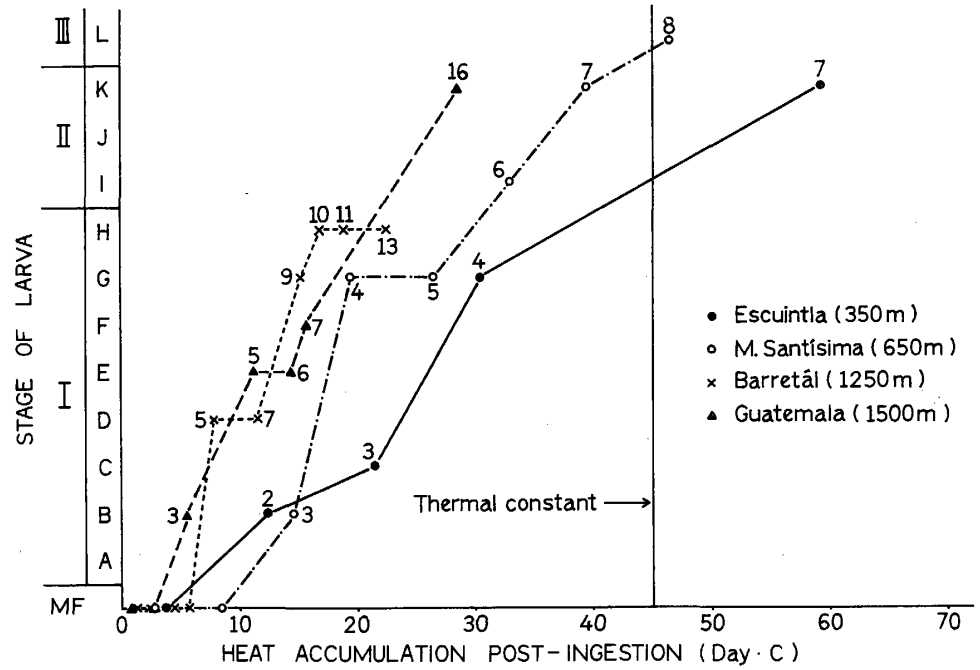

Figure 4 The relationship between the development of *O. volvulus* larvae in *S. ochraceum* and the ambient heat accumulation at four altitudes in November. (Numbers in the figure show days after ingestion of microfilariae).

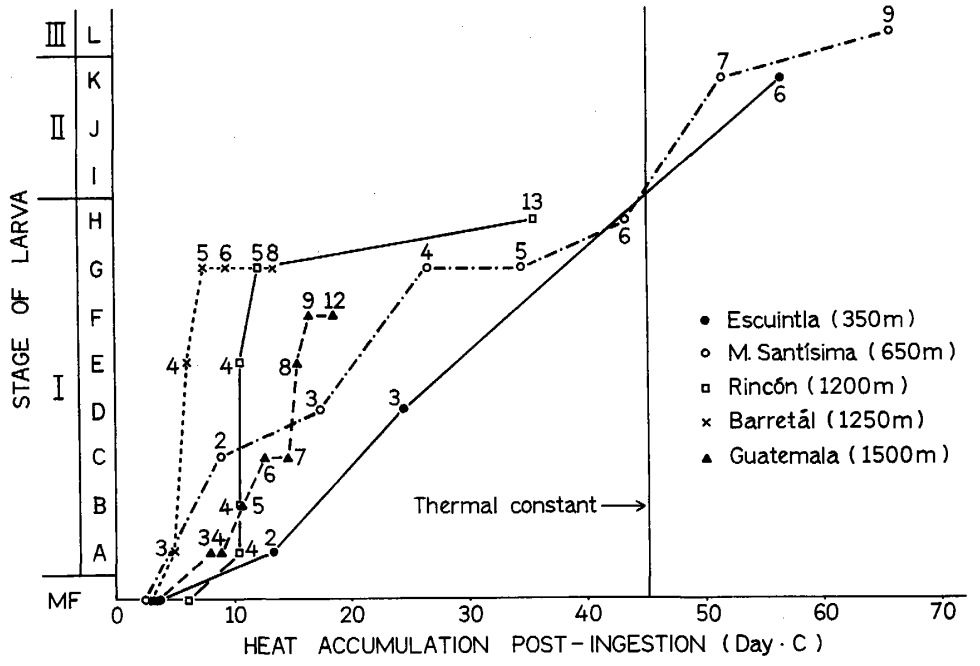

Figure 5 The relationship between the development of *O. volvulus* larvae in *S. ochraceum* and the ambient heat accumulation at five altitudes in December. (Numbers in the figure show days after ingestion of microfilariae).

Table 3 Development of *Onchocerca volvulus* in *Simulium ochraceum* which were fed on a carrier of the Guatemalan strain of *O. volvulus* microfilariae and kept under natural conditions at Mária Santísima (650 m above sea level)

Days post-infection	No. flies examined	No. flies harboring larvae	Total no. larvae	Mean no. larvae/fly (Range)	Stage of larvae (%)			
					Mf.	L_1	L_2	L_3
November, 1979								
1	24	24	8,291	345.5 (20–893)	100.0	0.0	0.0	0.0
2	22	22	3,064	139.3 (7–512)	100.0	0.0	0.0	0.0
3	15	13	1,244	82.9 (0–268)	94.7	5.3	0.0	0.0
4	2	2	4	2.0 (1– 3)	50.0	50.0	0.0	0.0
5	2	2	3	1.5 (1– 2)	0.0	100.0	0.0	0.0
6	4	3	16	4.0 (0– 7)	0.0	93.7	6.0	0.0
7	5	4	13	2.6 (0– 6)	0.0	30.8	69.2	0.0
8	3	2	13	4.3 (0– 11)	0.0	0.0	23.1	76.9
9	0	—	—	— (–)	—	—	—	—
10	2	1	1	0.5 (0– 1)	0.0	0.0	0.0	100.0
11	2	2	25	12.5 (10– 15)	0.0	0.0	0.0	100.0
December, 1979								
1	25	25	8,382	335.3 (54–825)	100.0	0.0	0.0	0.0
2	14	14	1,441	102.9 (0–357)	99.9	0.1	0.0	0.0
3	9	9	1,639	182.1 (10–398)	98.8	1.2	0.0	0.0
4	9	8	40	4.4 (0– 22)	52.5	47.5	0.0	0.0
5	1	1	8	8.0 (8.0)	0.0	100.0	0.0	0.0
6	2	1	3	1.5 (0– 3)	0 0	100 0	0.0	0.0
7	1	1	4	4.0 (4.0)	0.0	0.0	100.0	0.0
8	0	—	—	— (–)	—	—	—	—
9	2	1	7	3.5 (0– 7)	0.0	0.0	42.9	57.1
10	0	—	—	— (–)	—	—	—	—
11	1	1	12	12 (12)	0.0	8.3	25.0	66.7

experimental hosts three days after ingestion of the blood-meal. Essentially, the length of gonotrophic cycle proved to be three days irrespective of altitude. The single exception was Rincón, where four days were required.

Discussion

In the laboratory study (Takaoka *et al.*, 1981), it was shown that *O. volvulus* larval development in *S. ochraceum* occurred at diurnal/nocturnal temperatures where the latter were below 16.9 C (the predicted developmental zero point). In the present study, larval development in the vector species was investigated in relation to ambient heat accumulation at different altitudes (350–1,500 m). Unfortunately, the results demonstrated significantly-reduced probabilities of host-survival in the field when compared with corresponding laboratory temperatures

(Takaoka *et al.*, 1981). Increased mortality might well have been caused by high midday temperatures, since temperatures above 28 C are debilitating for *S. ochraceum* (Takaoka *et al.*, 1981 and Monroy, 1979). Under these circumstances, only a few blackflies survived to the day when larvae might have reached the infective stage. The completion of *O. volvulus* larval development in *S. ochraceum* was observed only at Mária Santísima (where mean temperatures were moderate, ranging from 21.8–26.5 C) with the developmental period being 8 or 9 days, nearly equal to that predicted by heat accumulation at this medium altitude. It is notable that the mean temperature range there coincides with that optimal for the vector's ability to transmit *O. volvulus* in the laboratory experiment (Takaoka *et al.*, 1981). On the other hand, at lower altitude (350 m) with high mean temperature (25.7–29.5 C) larvae reached only the late second stage even in females (which died on day 6 and 7) surviving beyond day 5 (*i.e.* the predicted day when larvae might be expected to develop to the infective stage at this altitude). This somewhat retarded development despite the sufficient heat accumulations might be associated with the initial cooling of females to 15–20 C during the transportation soon after the ingestion of blood meal. At higher altitude (1,200–1,500 m) no blackflies lived to the predicted critical day (15th–28th day) except one female at Barretál, and no development occurred beyond the late first stage in blackflies dying during the seventh through 13th day after ingestion of microfilariae. Therefore, it was not ascertained whether *O. volvulus* larvae could develop to the infective form in accordance with ambient heat accumulation in nature; although as shown in Figures 4 and 5, larval development (though limited to the first stage) appeared to require less heat accumulation at higher altitudes. This suggested that the first-stage larval development could occur even at temperatures below 16.9 C. The partial development to the late second stage seen in one female dying on day 16 at Guatemala City (1,500 m) seems to indicate that the extended larval development to the infective stage would take place in low percentages even at higher altitudes where night temperature drops far below the developmental zero point (about 17 C). In such cases the developmental period would probably require more than two weeks as suggested in Table 2. From the epidemiological point of view, it appears of great importance that the duration of extrinsic development of *O. volvulus* larvae in *S. ochraceum* varies considerably by altitudes (*i.e.* with temperature conditions) within endemic areas. As already reported by previous workers (Garms, 1975, Collins, 1979, and Garms and Ochoa, 1980) natural infection rates of *S. ochraceum* for third-stage larvae of *O. volvulus* in Guatemalan endemic zones were variable ranging from 0.09–3.2%. This would be probably explained partially by the different speeds of larval development in the vector simuliids and by the different longevities of the host insects within endemic areas. In this connection it should be also pointed out that the increased probability of simuliid's survival at higher altitudes may be to some extent reduced by the increased risk of exposure to natural hazards when the female oviposits and takes the subsequent blood-meals. This is because more frequent gonotrophic cycles might be repeated in the host blackfly before the larvae therein becomes infective at higher altitudes than at lower or medium ones, since the duration of one gonotrophic cycle is nearly the same — three days — even at higher altitude.

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中米グァテマラの温度条件の異なる種々の標高における *Onchocerca volvulus* 幼虫の媒介ブユ *Simulium ochraceum* 体内での発育

高岡宏行[1,2], K. M. Hansen[2], 高橋　弘[2], J. O. Ochoa[2], E. L. Juarez[2]

中米グァテマラの標高の異なる5地点（350-1,500 m）に設置した百葉箱内で *Onchocerca volvulus* ミクロフィラリア保有者から吸血させた媒介ブユ *Simulium ochraceum* を飼育し，その後の *o. v.* 幼虫の発育と同時に設置した自記温度計より算出された有効温量との関係を調べた。標高 350 m の Escuintla では有効温量から4日目に第3期幼虫の出現が予測されたが第6日，7日目に死亡したブユ雌でもまだ第2期後期幼虫しか見られなかった。一方，標高 650 m の Mária Santísima ではほぼ有効温量から推測されるとおりに第8日目に第3期幼虫への発育が観察された。ここより標高の高い3地点（1,200 m, 1,250 m, 1,500 m）では，各々15日，25日，25-28日目に第3期までの幼虫発育が予測されたが飼育したブユの大半がこれらの期間内に死亡したことにより十分な観察を行いえなかった。これら3地点では，飼育開始後 7-13 日の間に死亡したブユ体内で見出された幼虫はまだ第1期後期までしか発育していなかった。しかしながら標高 1,500 m の Guatemala 市において飼育開始後16日目に死亡した1個体のブユから第2期後期まで発育した幼虫が見出されたことから，標高の高い場所でも非常にゆっくりとではあるが *o. v.* 幼虫の第3期までの発育は可能であることが示唆された。

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THE FIRST RECORD OF HUMAN INFECTION WITH *DIPHYLLOBOTHRIUM CAMERONI* RAUSCH, 1969[1]

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Abstract: A diphyllobothriid cestode, which has been spontaneously expelled from a 57-year-old seaman at Fukuoka City, on December 1969, was sent to us for identification. The appearance of yellowish-brown, thickset, complete strobila (about 600 mm in length) was evidently different from usual forms of the known diphyllobothriid cestodes from humans. It was tentatively reported as "another marine species of the genus *Diphyllobothrium* from a man in Japan" (Kamo *et al.*, 1978). After careful morphological examinations it is now identified as *Diphyllobothrium cameroni* Rausch, 1969, which has been found in a Hawaiian monk seal (*Monachus schauinslandi*) from Midway Atoll, Leeward Island in the Pacific Ocean. In the relative position of the uterine pore, within the genital atrium, and other morphological details, it agrees closely with Rausch's description. The discrepancies in dimensions of the seminal vesicle and egg size are probably attributable to the considerably greater size of our specimen, which had grown well in larger space of human intestine. Its eggshell surface observed by SEM exhibited the typical characteristics (deep, numerous pits, with rough intervening surface) as the eggs of cestodes from the marine habitat according to Hilliard (1972). It seems to be the first and unusual case of human infection with *Diphyllobothrium cameroni* Rausch, 1969 (new Japanese name: cameron retto jochu). The source of human infection is quite obscure.

INTRODUCTION

In such a country as Japan, where raw or undercooked marine fishes are an important and traditional part of diet, it seems to be fairly strange that a diplogonadic tapeworm, *Diplogonoporus grandis* has been the only known species from human cases among various diphyllobothriid cestodes from marine habitat.

In order to explore such paradoxical situation, a review study of the so-called broad tapeworm in Japan was initiated by Kamo *et al.* in 1972, suspecting the occurrence of human infection with some kind of marine diphyllobothriids mistaken for the common species, *Diphyllobothrium latum*.

Recently the evidence of human infection with a certain marine species of the genus *Diphyllobothrium* was demonstrated by Kamo *et al.*, 1977, and subsequently a new marine species from a man was described as *Diphyllobothrium yonagoensis* n. sp.

1 This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture. 2 Department of Medical Zoology, Tottori University School of Medicine, Yonago City, Japan. 3 Department of Environmental Medicine, Shimane Medical University, Izumo City, Japan. 4 Department of Parasitology, School of Medical Technology, Kyushu University, Fukuoka, Japan.

by Yamane *et al.*, 1981.

In addition the authors, in 1978, briefly reported another *Diphyllobothrium* species of marine origin which had been spontaneously expelled from a 57-year-old seaman at Fukuoka City in 1969. The species was identified as *Diphyllobothrium cameroni* Rausch, 1969, and its description is given here.

Materials and Methods

The worm was preserved in 10% formalin solution, and then it was in a contracted condition, which had been spontaneously expelled from a 57-year-old seaman, H. U., who lives in Fukuoka City, Kyushu, Japan, on a day in December, 1969. A whole mount preparation of some mature proglottids was prepared, staining in Semichon's acetic carmine. Serial sections of mature proglottids were also prepared in horizontal, sagittal and transversal planes, staining in modified trichrome stain solution. The eggshell surface was observed by the scanning electron microscopy.

Observation

Strobila muscular, yellowish-brown in colour; margins slightly serrate. Strobila 650 mm long with maximum width 9.5 mm, attained about 200 mm behind from the scolex. Segments wider than long, with length increasing posteriad. Length/width ratio of premature segments about 1 : 12, of mature segments with maximum width about 1 : 8, and of terminal segments as much as 1 : 2.5.

Maximum thickness of strobila 2.0 mm (Figs. 1, 2). Excretory system including longitudinal and transverse ducts in parenchyma and numerous interconnected

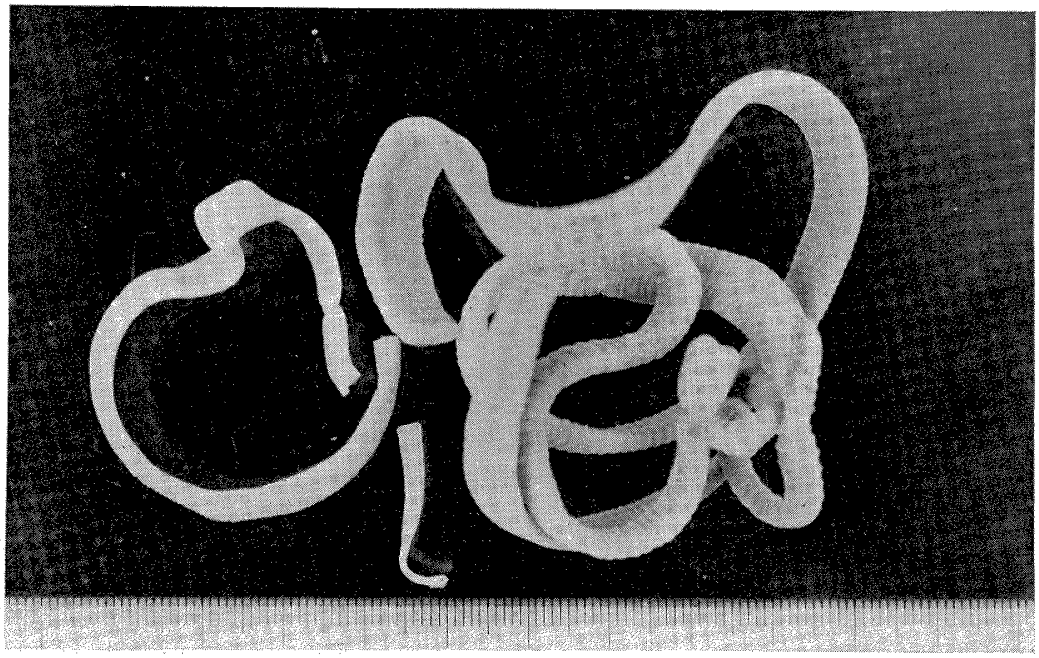

Fig. 1 Whole body.

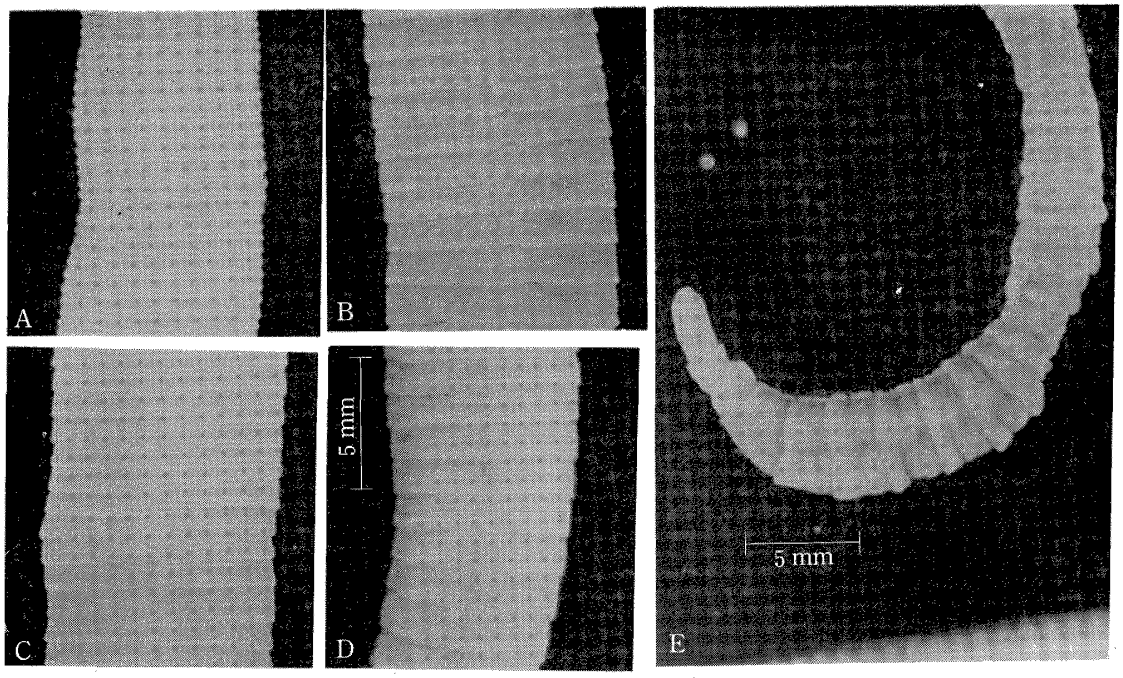

Fig. 2 Proglottids (no staining).
A: 100 mm behind from the scolex, B: 200 mm behid, C: 300 mm behind, D: 400 mm behind, E: terminal

ducts in cortical region. Innermost layer of longitudinal muscle fibres strongly developed, as much as 0.5 mm thick. Scolex lanceolate, pointed at apex, 2.2 mm long $\times$ 1.3 mm wide (lateral view) $\times$ 0.96 mm thick. Bothria deep, extending full length of scolex and diversing at apex; lips of bothria slightly rolled. Neck absent (Fig. 3). Genital pore situated ventrally on midline at anterior margin of segment, covered by velum of preceeding segment. Cirrus sac elongate, 0.515 mm long $\times$ 0.288 mm in diameter. Apex of cirrus sac attached dorsally by fibres confluent with those of longitudinal muscle layer. Cirrus sac opening anteriorly into genital atrium. Seminal vesicle elongate, 0.340 mm dorsoventral dimension by 0.247 mm in diameter, situated posteriorly near dorsal end of cirrus sac, and connected with latter by short duct. Subspherical testes numerous, arranged in one layer in lateral fields (in irregular layer two to three deep in near central field). Vagina running anteriad, then turning ventrad along posterior surface of cirrus sac, opening in genital atrium immediately posterior to opening of cirrus sac. Bilobed ovary situated transversely at posterior margin of segment dorsal to uterus; ovarian lobes extending laterad beyond uterine loops. Vitellaria abundant, forming two lateral fields; medial margins of vitelline (and testine) fields diversing posteriad, only slightly overlapping ends of uterine loops and ovarian lobes. Uterine loops extending laterad and anterolaterad, reaching level of anterior margin of genital atrium (Fig. 4).

When much distended with eggs, gravid uterus may extend posteriad into adjacent segment. Uterus opening into posterior wall of genital atrium (Fig. 5). Terminal portion of uterus thick walled, with valvelike structure near orfice. Eggs

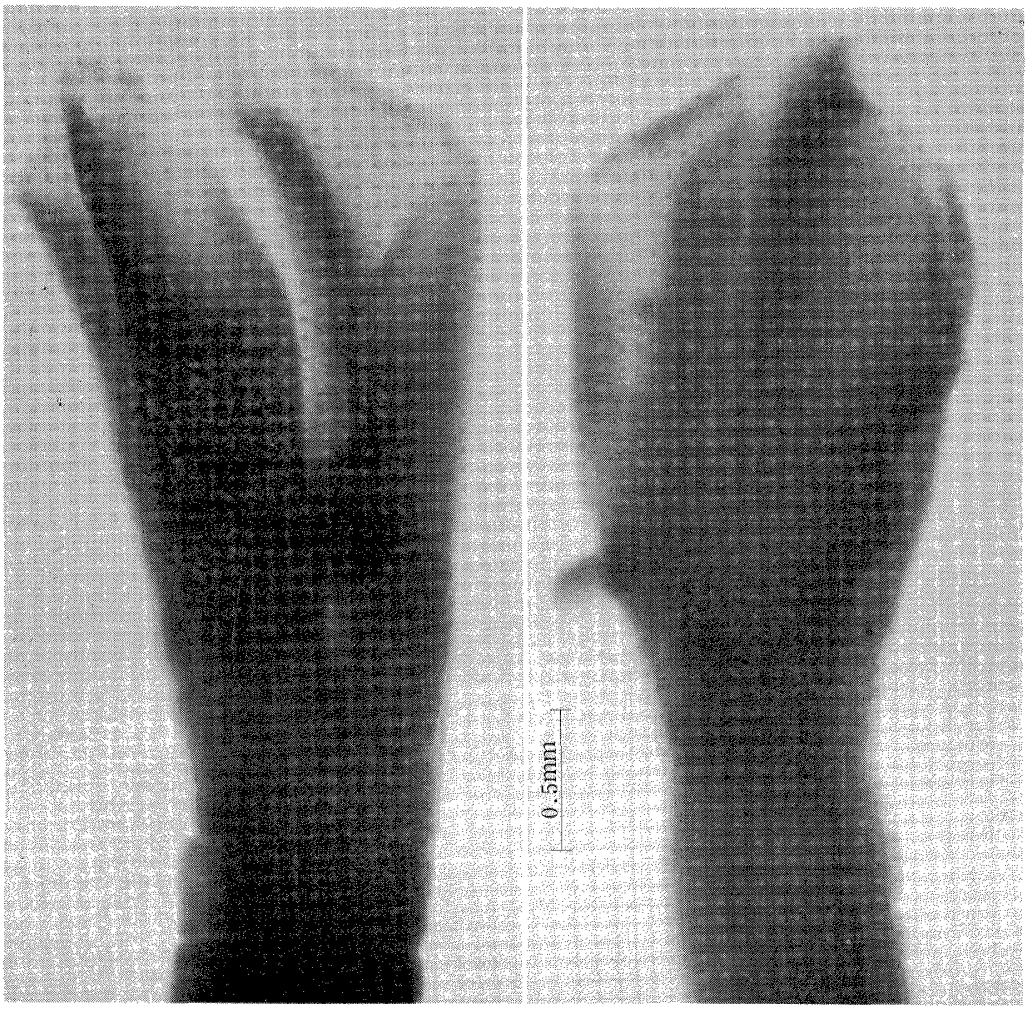

Fig. 3

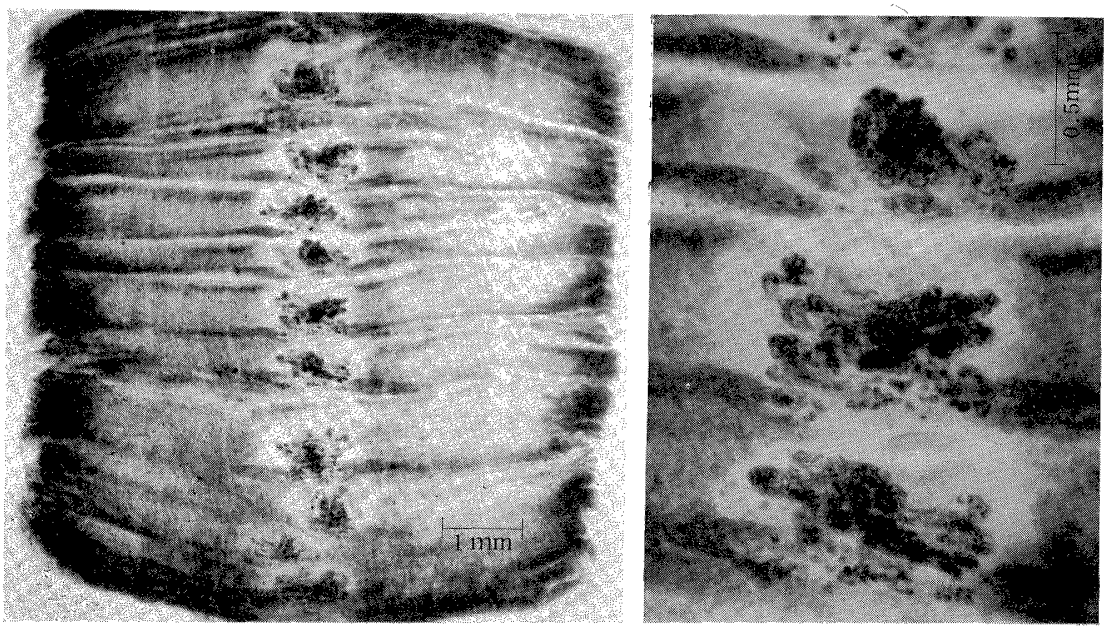

Fig. 4

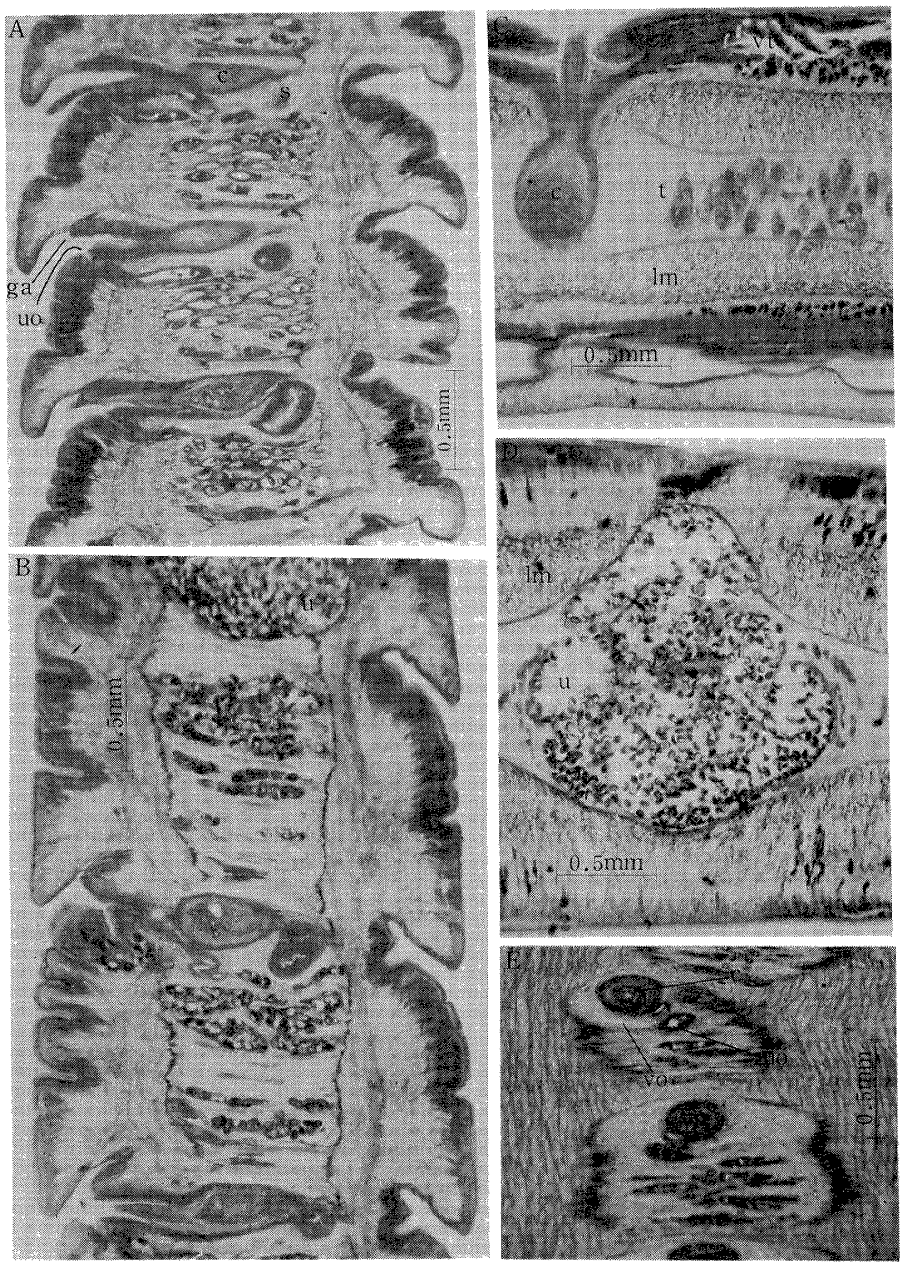

Fig. 5 Sections of mature proglottids.

A, B: sagittal sections, showing arrangement of genital organs, C, D: cross sections, E:horizontal section

c: cirrus sac, ga: genital atrium, lm: longitudinal muscle layer, s: seminal vesicle, t: testis, u: uterus, uo: uterine opening, vo: vaginal opening, vt: vitelline follicle

ellipsoidal, without apical knob, 45.9–56.7 μ (avg 50.9$\pm$2.5) $\times$ 35.1–42.4 μ (avg 39.5$\pm$1.5). Large (irregular sized), deep pits distributed densely on the surface of

Fig. 3 Scolex, dorsoventral (left) and lateral (right) view.

Fig. 4 Whole mount preparation. right: uterine field (larger magnification)

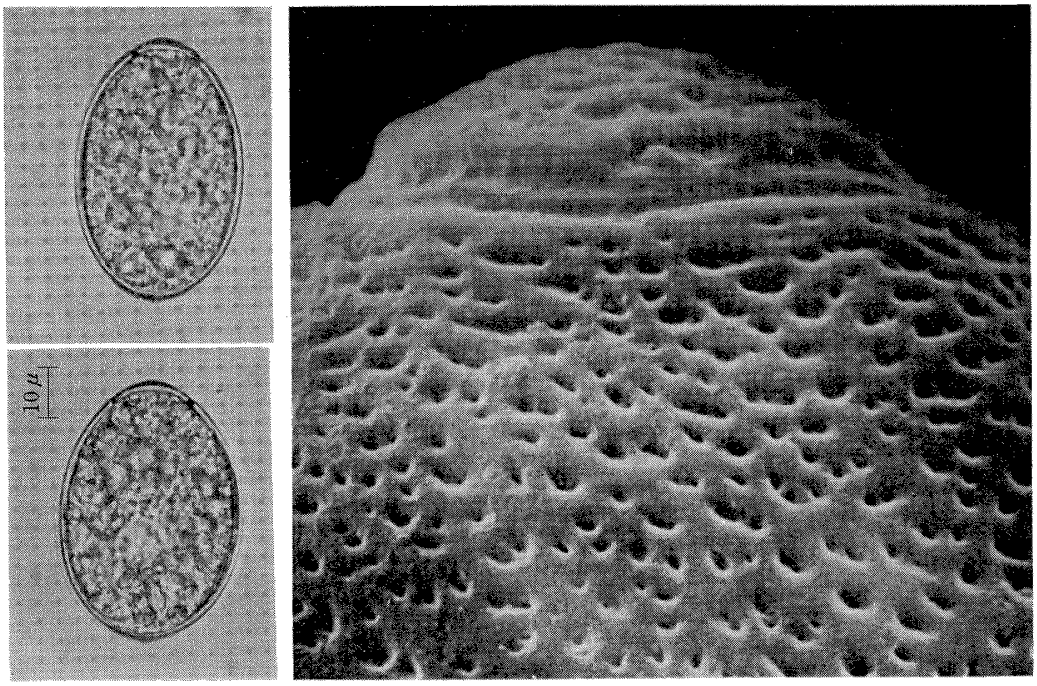

Fig. 6 Eggs and egg-shell surface by SEM(× 5000).

eggshell (0.8–1.4 μ thick in lateral) (Fig. 6).

Discussion

As described above our specimen from a seaman seems clearly to be identical in almost all morphological details with the description of *Diphyllobothrium cameroni* Rausch, 1969, which had been found from the Hawaiian monk seal (*Monachus schauinslandi*) collected at Midway Atoll, Leeward Islands. The discrepancies in some values of measurements such as diameters of the seminal vesicle and egg sizes are probably attributable to the considerably greater size of our specimen, which had grown in larger space of human intestine.

The Hawaiian monck seal is distributed in lat. 20–30 N around the Hawaii Islands, while its food habits still obscure according to Nishiwaki (1965). The source of human infection with this worm is quite unknown, although it should be found from some marine fishes as the common food between seal and human.

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The authors wish to express our heartfelt thanks to Dr. R. L. Rausch, Division of Animal Medicine, School of Medicine, University of Washington, Seattle, Washington, U. S. A., for his kindness and encouragements through providing with many specimens or papers of various diphyllobothriid cestodes. To Mrs. S. Wakahara, Miss M. Hasegawa, and Mr. Y. Sugihara, Dept. of Medical Zoology, Tottori Univ. School of Med., appreciation is expressed for their technical assistances.

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Diphyllobothrium cameroni Rausch, 1969
—カメロン裂頭条虫（新称）— の人体寄生第一例

加茂　甫・山根　洋右・川島健治郎

福岡市在住の男子船員（当時57歳）から，1969年12月に自然排出された，裂頭条虫と思われる虫体が同定のため送られてきた。虫体は頭節を有する完全な標本であったが，小柄（約 600 mm）で，肉厚の，ずんぐりした黄褐色の外観を呈し，一見してこれまで人体寄生種として知られている裂頭条虫とは異なるもののように思われた。著者らはこれを，人体寄生裂頭条虫のさらに新しい海洋種として，特徴の概要をとりあえず学会に報告しておいたが（加茂ら，1978），このたび *Diphyllobothrium cameroni* Rausch, 1969 として同定を確定し，和名としてカメロン裂頭条虫を提唱した。本種はミッドウェー諸島近海で捕獲されたタイヘイヨウモンクアザラシ（*Monachus schauinslandi*）から得られた標本に基づいて、Rausch (1969) が命名記載した種類である。人体から得られた標本は，形態的特徴が極めてよく Rausch (1969) の記述に一致し，とくに子宮孔が生殖孔の後壁に開口する点が他種に見られない特徴である。貯精嚢や虫卵などの計測値が人体排出標本で全般的に大きいのは，アザラシの腸管にくらべて広大な人の腸管内で，大きく成長できたことによる差異であろうと思われる。走査電顕による卵殻表面像は，深い点刻が密に分布し，点刻間の面がきめ粗い性状を示し，Hilliard (1972) のいう海洋性の特徴を認めた。これはカメロン裂頭条虫の人体から見出された最初の例であるが，恐らく偶発的なものであろう。感染源は人間とタイヘイヨウモンクアザラシとの共通の食物となっている海産の魚類であろうと思われるが，まったく不明である。

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