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STUDIES ON THE SUSCEPTIBILITY OF *BIOMPHALARIA PFEIFFERI RUEPPELLII* (DUNKER) AND *B. SUDANICA* (MARTENS) TO *SCHISTOSOMA MANSONI* IN ETHIOPIA

YOUICHI ITO[1], HIROSHI ITAGAKI[2] AND TEFERRA WONDE[3]

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Abstract: *Biomphalaria pfeifferi rueppellii* and *B. sudanica* collected at various sites in Ethiopia, were exposed to Ethiopian strain of *Schistosoma mansoni*. Striking differences were observed in susceptibility of the two species of snail vectors to the Ethiopian strain. The infection rate of *B. pfeifferi rueppellii* with *S. mansoni* ranged from 67 to 100 %, while that of *B. sudanica* was only 9 %. It appeared that *B. pfeifferi rueppellii* would serve as the most important intermediate vector to *S. mansoni* in Ethiopia.

Two species of vector snails of *Schistosoma mansoni, Biomphalaria pfeifferi rueppellii* (Dunker) and *B. sudanica* (Martens), are found in Ethiopia (Brown, 1964). *B. pfeifferi rueppellii* is found throughout the plateaux in a wide variety of habitats, and also in the small streams and temporary pools. On the other hand, the distribution of *B. sudanica* is confined almost exclusively to the lake areas located in the southern part of the Rift Valley.

Ayad (1956), Lemma (1969) and Ito et al. (1973) surveyed the foci of schistosomiasis mansoni in Ethiopia and concluded that the endemic foci of schistosomiasis mansoni had a sporadic distribution.

It is contemplated that the distribution of vector snails and their susceptibility to *Schistosoma mansoni* play a significant role in the sporadic nature of the endemic foci.

In the present study, two species of *Biomphalaria* snails from Ethiopia were tested for their susceptibility to Ethiopian strain of *S. mansoni*.

MATERIALS AND METHODS

The present experiments were made in the Imperial Central Laboratory & Research Institute, Addis Ababa.

Biomphalaria pfeifferi rueppellii and *B. sudanica* from Ethiopia were utilized for the experiments. *B. pfeifferi rueppellii* was collected from several small streams mainly along Asmara Road, while *B. sudanica* from the Lakes, Ziwai and Awasa (Fig. 1).

1 Dept. of Parasitology, National Institute of Health, Kamiosaki, Shinagawa, Tokyo, Japan. 2 Dept. of Parasitology, Azabu Veterinary College, Fuchinobe, Sagamihara, Japan. 3 Dept. of Medical Zoology, Imperial Central Laboratory & Research Institute, P. O. Box 1242, Addis Ababa, Ethiopia.

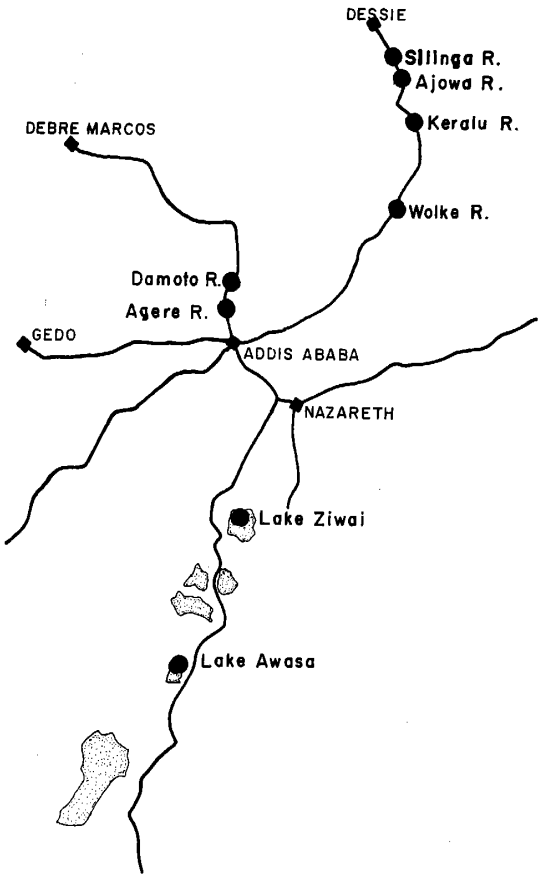

Fig. 1 The map showing the places of snail collection in Ethiopia.

After breeding the snails in the laboratory for two months or more, snails were removed to a beaker containing a small amount of water, and the beaker was placed under an electric light to check the natural infection with the trematode cercariae before the experiments could be undertaken. Only non-infected snails were used for the present study.

A strain of *Schistosoma mansoni* from Ethiopia which was obtained from the stool of an Ethiopian patient was used for the experimental infection. The Egyptian strain of *B. pfeifferi* was exposed to the miracidia and then the mature cercariae were inoculated intraperitoneally into white mice.

The mice infected with *S. mansoni* were killed 8 to 12 weeks after the infection. Then small portions of the liver were examined under microscope for the presence of ova and two or three infected liver fragments were used for the collection of eggs. Liver was homogenized in a motor with cold water, and the suspension transferred to a flask filled with cold water for the sedimentation. After sedimentation for 20 minutes the sediment was brought into a 500 m*l* conical flask with water warmed at 28–30 C for the hatching of miracidia. The flask was covered with strips of black vinyl-tape except its top portion. The flasks were placed under a 100-watt electric light bulb and within one hour the miracidia migrated to the surface so that they could be used to infect snails. The miracidia were counted under dissecting

microscope, 300 miracidia placed into each of the beakers filled with 200 m*l* tap water kept at room temperature for 2 or 3 days, to which 30 snails were placed in each one of them. The beakers were maintained at room temperature overnight. In the next morning, all snails were removed to an aerated aquarium operated by small pumps. The snails were maintained in the aquarium for three months and fed on cabbage leaves twice a week. At the end of a three month period, the snails were dissected and examined for sporocysts and cercariae of *Schistosoma mansoni*.

Results and Discussions

A total of 410 *Biomphalaria pfeifferi rueppellii* and 490 *B. sudanica* were exposed to Ethiopian strain of *S. mansoni*. The results which are summarized in Table 1,

Table 1 Susceptibility of *Biomphalaria pfeifferi rueppellii* and *B. sudanica* from Ethiopia to Ethiopian strain of *Schistosoma mansoni*

Collecting sites of snails	Species of snails	No. of snails exposed	No. of snails examined	No. of snails infected (%)	Mortality (%)
Silinga River	*B. p. rueppellii*	60	9	9(100)	85.0
Wolke River	〃 〃	60	4	4(100)	93.3
Keralu River	〃 〃	60	6	4(66.7)	90.0
Ajowa River	〃 〃	30	10	9(90.0)	66.7
Aegere River	〃 〃	70	4	4(100)	94.3
Damoto River	〃 〃	130	34	32(94.1)	73.8
Total		410	67	62(92.5)	83.7
Lake Awasa	*B. sudanica*	430	175	19(10.9)	59.3
Lake Ziwai	*B. sudanica*	60	40	0	33.3
Total		490	215	19(8.8)	56.1

reveal that almost all *B. pfeifferi rueppellii* collected from several areas encountered were infected with *S. mansoni*; the infection rate of the snails collected from the Silinga River, Wolke River and Aegere River was 100%, 90% of those from Ajowa River, 94% of those from Damoto River, and 67% of those from Keralu River were found infected with *S. mansoni*. A large number of *B. sudanica* collected from the Lakes, Awasa and Ziwai, however, remained non-infected with the Ethiopian strain of *S. mansoni*, namely, 19 out of 215 (9%) of the examined snails were infected with *S. mansoni*.

Meanwhile, a lot of snails exposed to the miracidia died, and the mortality of *B. pfeifferi rueppellii* ranged from 68 to 97% at the end of a three month maintenance period. On the other hand, the mortality of *B. sudanica* ranged from 20 to 93% and seemed to be slightly lower than that of *B. pfeifferi rueppellii*. The high mortality might be caused by unfavorable conditions due to maintenance and infection of the snails with *S. mansoni*.

Files and Cram (1949) reported that *B. pfeifferi* from Liberia was readily infected with four strains of *S. mansoni* from Puerto Rico and Venezuela and the infection rate ranged from 38 to 65%. Malek (1962) described in his guide book that both *B. pfeifferi* and *B. sudanica* served as the vector snails of *S. mansoni* in Africa. It is apparent from this experiment that *B. pfeifferi rueppellii* and *B. sudanica* from Ethiopia differ in their susceptibility to Ethiopian strain of *S. mansoni*; *B. pfeifferi rueppellii* could acquire infection, while only less than 10% of *B. sudanica* were infected with that of *S. mansoni* from Ethiopia.

Wright and Brown (1962), Brown (1964) and Suzuki et al. (personal communication) assumed that *B. pfeifferi rueppellii* had ubiquitous distribution on the Ethiopian plateaux, while *B. sudanica* seemed to be confined to the lakes near and around the Rift Valley. The authors also found that the distribution of *B. sudanica* was almost limited to the Lakes, Ziwai and Awasa situated in the southern part of the Rift Valley, Ethiopia.

These data indicate that *B. pfeifferi rueppellii* serves as the most important vector snail to the Ethiopian strain of *S. mansoni*, while *B. sudanica* seems to be fairly resistant to the latter.

It is hoped that comparative studies on the susceptibility of *Biomphalaria* snails from the neighbouring countries to the Ethiopian strain of *S. mansoni* would shed some light on this problem in the near future.

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The stimulating discussions with the Drs. C.T. Lo, a visiting research worker at the Institute of Pathobiology, Faculty of Science, Haile Sellassie I University, Addis Ababa and N. Suzuki, National Institute of Health, Tokyo are highly appreciated by the authors.

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Biomphalaria pfeifferi rueppellii (Dunker) 及び *B. sudanica* (Martens) のマンソン住血吸虫に対する感受性

伊藤洋一[1]・板垣博[2]・テフェラ・ウオンデ[3]

エチオピア産 *Biomphalaria* 属の2種の貝にエチオピア人の患者から分離したマンソン住血吸虫ミラシジウムを実験的に感染させ，その感受性を比較した。その結果，各地から採取した *B. pfeifferi rueppellii* では67～100% の感染率が得られたのに比し，*B. sudanica* ではわずかに9% の感染率しか得られなかった。また両種のエチオピアにおける分布状態を調査したところ，*B. pfeifferi rueppellii* はエチオピア全土に亘り分布しているのに比し，*B. sudanica* は南部湖水地区の一部の湖水にしかその棲息が認められなかった。

これらの結果より，エチオピアにおけるマンソン住血吸虫の主要な中間宿主は *B. pfeifferi rueppellii* であると考えられる。

1 国立予防衛生研究所　寄生虫部　2 麻布獣医科大学　寄生虫学教室　3 エチオピア帝国中央研究所医動物学部門

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SIMULIUM DAMNOSUM, NATURALLY INFECTED WITH *ONCHOCERCA VOLVULUS* IN SOUTH-WEST ETHIOPIA[1]

IKUO TANAKA[2], YOSHISATO INOUE[3], ISAO TADA[4], ISAO IWAMOTO[5] and TEFERRA WONDE[6]

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Abstract: The existence of onchocerciasis in Ethiopia, especially in its South-Western Region was known. The naturally infected *Simulium* has not been reported in the region, though there has been reported the existence of *S. damnosum, S. woodi* etc. which were identified as the vectors of this disease in other parts of Africa. The authors obtained a number of *Onchocerca volvulus* from *S. damnosum* collected by biting-catch method in the field in August and November, 1971. Dissection was made on the 975 *Simulium* out of about 1000 caught at the riverside of Gojeb and those caught at Didessa riverside. The infection rate ranged from 10.2 to 12.9% in the former and 20.0 to 40.6 in the latter. The sausage type was most frequently found and the late stage type was detected in 11% of *Simulium*. An evidence was given that *S. damnosum* is the main vector of onchocerciasis in Ethiopia, since this species formed an absolute majority of the flies collected by biting catch and no filaria was detected in the other species.

The existence of onchocerciasis in Ethiopia was initially ascertained in 1939 at Bonga, Kefa Province, and the collection record on *Simulium damnosum* was made by the same author (Giaquinto, M. 1939).

Oomen, A. P. (1969) collected *S. damnosum* and *S. woodi*, and suggested that *S. damnosum* might be the vector of this disease in the south-west Ethiopia surveyed.

Although *S. damnosum* has been incriminated to be one of the potential vectors of onchocerciasis in Ethiopia, naturally infected *S. damnosum* has not yet been reported, so far as we are aware.

In order to clarify the vector species of this disease, we visited the endemic area of South-west Ethiopia in 1971, and made attempts to check infected adult black flies.

This paper is a brief report on the discovery of naturally infected *S. damnosum* with *Onchocerca volvulus* in the area.

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1 The contents of this paper was preliminarily presented at the 24th Annual Meeting of the Japan Society of Sanitary Zoology, Okayama, 3 April 1972. 2 Division of Pest Control, Japan Environmental Sanitation Center, Kawasaki, Japan. 3 Dept. of Medical Zoology, National Institute of Health, Tokyo, Japan. 4 Dept. of Medical Zoology, Kanazawa Medical University, Uchinada, Ishikawa, Japan. 5 Dept. of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan. 6 Dept. of Medical Zoology, Imperial Central Laboratory & Research Institute, Addis Ababa, Ethiopia.

Materials and Methods

(a) Date of the survey and collection sites (Fig. 1)
March 18–25, 1971: riverside of Gojeb, Kefa Province
July 31-August 15: riverside of Didessa and adjacent plateau 1 km from the riverside, Ilubabor Province
November 15–18 : riverside of Gojeb and Didessa

(b) Catching method for the adult black flies
Collection was made by two or three volunteers seated on a rock or the ground at the individual sites. All the black flies settled on the naked parts of the body were caught using a sucking tube every 30 minutes from sun rise to sun set.

(c) Treatment and dissection of the flies
Collected flies were anesthetized with ether and, in every 60 minutes, were gathered in batches in one small glass tube. After identification, the flies were dissected in a droplet of physiologic saline solution on a slide glass under a dissecting microscope in order to find the parasite.

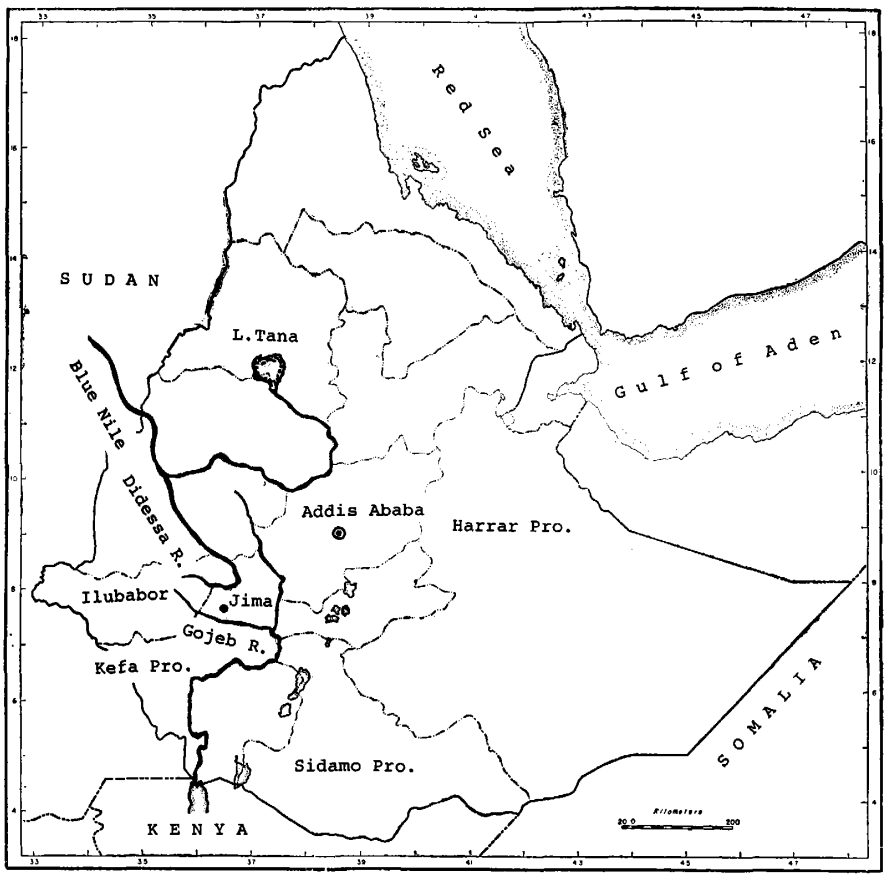

Fig. 1 Map of Ethiopia showing the rivers where *S. damnosum* were collected.

Results

As shown in Table 1, the infection rate of *S. damnosum* with the larvae of *O. volvulus* ranged from 20.0 to 40.6% at the several collection sites in Didessa area and ranged from 10.2 to 12.9% at the Gojeb riverside. The other species of black fly had not been found to be infected with filarial worms.

Table 1 Number of the naturally infected *Simulium damnosum* with the filarial worm, *Onchocerca volvulus*

		Gojeb riverside		Didessa riverside		Didessa plateau
		Mar. 24	Nov. 18	Aug. 13	Nov. 15	Aug. 15
No. flies collected		85	127	726	5	32
No. flies infected		11(—)	13(8)	237(96)	1(0)	13(4)
Infection rate %		12.9	10.2	32.6	20.0	40.6
No. of infected flies harbouring several stages*	m	—	2	12	0	2
	s	—	3	116	1	6
	l	—	7	64	0	3
	m+s	—	0	13	0	1
	m+l	—	0	2	0	0
	s+l	—	1	29	0	0
	m+s+l	—	0	1	0	1

* Stage type of the larvae
m: microfilaria type
s: sausage type
l: late stage type
—: each stages not checked
Parenthesis: total flies possessing late stage type worm

In adult *S. damnosum* dissected, all the three developmental stages of *O. volvulus* i.e., microfilaria type, sausage type and late stage type were found. Of these three types, the sausage type was most abundant and this was followed by the late stage type and then microfilaria type. Finding the black flies possessing the late stage type is especially important from the epidemiological viewpoint.

In August 1971, at the Didessa riverside, out of 237 infected *S. damnosum*, 96 harboured the late stage worms. The body length measured ranged from 300μ to 700μ.

Regarding the frequency of harbouring worms in one dissected fly, usually only one type was recognized. In a few case, however, two or three types of filarial larvae were simultaneously found.

The highest number of the sausage type larvae recognized was 51, while that of the late stage type, 17.

Some smeared specimens were identified as the larval stage of *O. volvulus* by Dr. R. L. Muller, of London School of Tropical Medicine and Hygiene.

Discussion

It has been already known that onchocerciasis is endemic in South-west Ethiopia (Cohen, L. B. 1960, Oomen, A. P. 1969, Iwamoto, I. et al. 1973). There is a general agreement that *S. damnosum* and *S. neavei* complex are the main vector of onchocerciasis in many tropical regions in Africa (De Meillon, B. 1957). Oomen, A. P. (1969), in particular, collected *S. damnosum* and *S. woodi*, and postulated that *S. damnosum* might be the vector of onchocerciasis in Ethiopia. Ogata, K. et al. (1970) reported that they collected seven anthropophilic *Simulium* species in Ethiopia and *S. damnosum* was recognized as the most common one.

Through our study, naturally infected *S. damnosum* with *Onchocerca volvulus* was abundantly found in natural population, whereas the other black fly species were found free from filarial larvae.

Thus, the present study brings an evidence that *S. damnosum* is the main vector of onchocerciasis in South-west Ethiopia, yet no possibility is denied if onchocerciasis could also be transmitted by the other *Simulium* species in this area. It is hoped that further studies will throw more light on this problem.

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We also wish to thank to Dr. R. L. Muller, of the London School of Tropical Medicine and Hygiene, for confirming the identification of the filarial larvae in our black fly specimens.

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エチオピア南西部において *Onchocerca volvulus* 自然感染の見られた *Simulium damnosum*

田中生男[1]・井上義郷[2]・多田　功[3]・岩本　功[4]・テフェラ・ウオンデ[5]

エチオピアにおいては，とくに，その南西部にオンコセルカ病が存在することが知られ，また，他のアフリカ地域で，そのベクターと判明している，*Simulium damnosum*, *S. woodi* などのブユが生息することが確認されていたが，自然感染ブユは発見されなかった。筆者らは1971年同国に滞在し，8月と11月に行なった biting catch によって得た，野外の *S. damnosum* から，多数の *Onchocerca volvulus* を得た。

Gojeb, Didessa 両河岸から得た個体約1000のうち，975個体について解剖を行なった結果，前者で10.2～12.9%，後者で20.0～40.6%の陽性個体を得た。ブユ体内における *O. volvulus* の発育段階別では，sausage type のものが最も多かったが，約11%の個体が発育終期のものを保有していた。

biting catch によって採集されたブユは，ほとんどが *S. damnosum* であったこと，および，他種のブユからは全くフイラリアが発見されなかったことから，エチオピアにおいても，*S. damnosum* がオンコセルカ病の主要媒介種であるという確証を得た。

1　日本環境衛生センター　環境生物部　2　国立予防衛生研究所　衛生昆虫部　3　金沢医科大学医動物学教室　4　長崎大学熱帯医学研究所　寄生虫学部門　5　エチオピア帝国中央研究所　医動物学部門

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QUANTITATIVE STUDIES ON THE EMERGENCE OF *ONCHOCERCA VOLVULUS* MICROFILARIAE FROM SKIN SNIPS

ISAO TADA[1], ISAO IWAMOTO[2] AND TEFERRA WONDE[3]

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Abstract: The authors made studies on the emergence of *O. volvulus* microfilariae from skin snips in order to assess accurate MFD in onchocercal infections from quantitative view point. The results obtained are as follows: Skin snips should not be teased into small pieces, but the intact snips should be incubated for a longer period than the teased ones. The distribution of microfilariae in a minute skin area is quantitatively even in most cases. This finding suggests the usefulness of this method to compare MFD of the adjacent skin regions to each other. However, the comparison of MFD with extremely different-sized snips should be avoided. There were no significant changes in MFD by warming skin surface.

For the diagnosis of human onchocerciasis, the skin snip method has been widely used as an essential and standard method. During a period of epidemiological survey of onchocerciasis in Ilubabor Province, Ethiopia, the present authors attained the conclusion that the microfilaria density (MFD) of skin snips obtained was highly affected by the teasing process and incubation time. For example, when the biopsies were teased by the technique recommended by several previous workers, there were some microfilariae which were still migrating out from the newly cut surface of the fragmented tissue 15 to 20 minutes after incubation. Small numbers of microfilariae newly released were observed from time to time by additional incubation. Furthermore, many microfilariae were found torn into small pieces so that they were immobile. These findings suggested the possibility that the teasing process caused mechanical damage to the microfilariae in the biopsies. This process naturally might lead to an inaccurate MFD. It was inconvenient for the authors to assess the densities of microfilariae under several chemical stimulants, unless a standard method was established. For this reason, the authors made quantitative studies on the emergence of *Onchocerca volvulus* microfilariae from skin snips in order to establish a standardized method for the skin snips which could be of epidemiological

1 Dept. of Medical Zoology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan (Present address: Dept. of Medical Zoology, Kanazawa Medical University, Uchinada, Ishikawa, Japan). 2 Dept. of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan (Present address: Dept. of Internal Medicine, in same Institute). 3 Dept. of Medical Zoology, Imperial Central Laboratory & Research Institute, Addis Ababa, Ethiopia.

The present work was supported by a research grant from the Imperial Central Laboratory & Research Institute of Ethiopia and the Overseas Technical Cooperation Agency of Japan.

and experimental importance.

Materials and Methods

In Abdella, Ilubabor Province of Ethiopia, the experiments were performed, the first one in August and the second in November, 1971. Our preliminary survey with skin snippings revealed that the microfilaria rate was 80.7% in male adults from this village. Volunteers were then picked among these adults and brought to the Dabana Missionary Station for the experiments.

All of the experiments were undertaken at room temperature; in August, it ranged from 18.0 to 23.0 C., and in November, from 17.5 to 21.5 C.

Every skin snip was taken from the left buttock of volunteers with a needle and a surgical blade. The detailed technique to obtain the skin snip was described by Duke (1962). When multiple snips were needed from one volunteer, every snip was taken 1 cm apart from the others. Thereafter, skin snips were placed into drops of physiological saline on slides.

The slide was then placed in an optical apparatus which magnified and projected the shape of the skin snip on a piece of paper put on the top of the apparatus. The area of the skin snip was obtained by counting the number of smallest sections (1 mm^2) encircled by the outline of the projected biopsy. One square millimeter of the actual skin snip area was equivalent to 49 mm^2 on the paper. The precise area of the biopsy was obtained by calculation in mm^2. The measured snips in physiologic saline were incubated at room temperature for a determined time and were transferred to the saline on the next slide carefully with a small forceps. This process was repeated continuously. The number of microfilariae which were left behind was immediately counted under 50× magnification of a binocular microscope. The microfilaria density (MFD) was calculated by dividing the total number of microfilariae which were released from one snip by the individual snip area in mm^2.

The microfilariae found from these volunteers were identified as those of *Onchocerca volvulus* from the morphological feature of the stained specimens and from the measurement of its anatomical landmarks (Iwamoto et al., 1972).

Results

1. The effect of teasing the skin snip on the emergence of microfilariae

Three snips were taken from each of the 6 volunteers (from T–1 to 6). The first snip was torn into small pieces with needles for about 30 seconds (snip P), the second one, coarsely torn into two pieces (snip M) and the third one (snip G) was not teased. Skin snips were then incubated for approximately 22 hours and the MFD obtained is shown in Table 1. The result of this experiment has been already reported in a brief article by the present authors (Tada et al., 1973). The highest MFD is seen almost in snip G. On the other hand, the snips P, which were torn into small pieces due to the previously recommended technique released less microfilariae than the others. When the highest MFD from 3 snips is described as 1.00, the relative MFD of the other two snips are calculated by proportions.

The relative MFD in average is as follows: 0.92 in snip G, 0.79 in M and 0.50 in P, respectively. In contrast to the method recommended by various previous researchers, the present data clearly show that skin snips should not be torn into pieces to assess the accurate MFD.

TABLE 1 The effect of teasing the skin snips on the MFD

Case No.	MFD		
	Snip type* G	M	P
T-1	16.6 (0.61)***	27.3** (1.00)	17.4 (0.64)
T-2	11.8 (1.00)	10.7 (0.91)	6.4 (0.54)
T-3	21.2 (1.00)	11.7 (0.55)	9.1 (0.43)
T-4	4.0 (0.89)	4.5 (1.00)	1.6 (0.36)
T-5	111.9 (1.00)	84.7 (0.76)	89.6 (0.80)
T-6	46.1 (1.00)	23.7 (0.51)	10.8 (0.23)
Average of the relative MFD (6 cases)	0.92	0.79	0.50

* Snip type: G, non-teased; M, coarsely teased; and P, teased into small pieces

** The under-lined count shows the highest MFD among the 3 snips from the same individual

*** Relative MFD: When the highest MFD of a snip among the 3 snips is determined as 1.00, the relative MFD of the others is obtained by proportional calculations

In this experiment, as shown in Table 2, the number of microfilariae released were counted right from the beginning every 20 minutes to 120 minutes, and every 60 minutes from 120 to the end of the incubation. The end of incubation ranged from 8 to 22 hours depending on the emergence of microfilariae from individual snips. The table shows the cumulative percentage of microfilariae from 3 types of snips in association with the incubation time while cumulative percentage of microfilariae was apparently the lowest in snip G at any incubation time. For example, snip P released almost 90% of microfilariae in average at 80 minute incubation, while only 67.6% of microfilariae emerged from snip G in average. This fact does not contradict the above mentioned conclusion that skin snips should not be teased, but it may indicate that the living microfilariae would emerge easily and quickly within a short time from the teased snips. But it should be also beared in mind that teased skin snips could lead to a wrong conclusion. The teasing would hinder the accurate MFD of each skin snip by causing mechanical damage to the microfilariae in the skin.

TABLE 2 The effect of teasing on the recovery rate* of microfilariae in individual snip types arranged by incubation time

Incubation time (min.)	Cumulative percentage of microfilariae released from skin snips (average of 6 cases)		
	Snip type**		
	G	M	P
20	36.2	50.4	61.4
40	50.0	65.5	76.1
60	60.1	77.0	84.0
80	67.6	81.8	89.7
100	72.0	85.6	92.3
120	75.6	88.9	94.2
180	84.6	93.8	96.0
240	86.9	96.5	99.1
300	91.7	97.1	99.4
360	93.4	99.1	99.9

* Recovery rate (in percent): The ratio of microfilaria count at individual incubation time to the total count of microfilatiae obtained from the identical snip

** Snip type: as shown in Table 1

From this experiment, it is concluded that skin snips should not be teased, in particular for the quantitative assessment of MFD in human skin.

2. The distribution of microfilariae in the skin

To assess the changes in MFD for some quantitative studies, the distribution of microfilariae should be even in some small skin regions. Based on this viewpoint, the authors examined if the microfilariae were evenly distributed in minute skin regions or not by comparing the MFD of 3 skin snips from each of 8 volunteers (from N–1 to 13). The skin snips were taken in a triangular shape, 1 cm apart from each other from the left buttock of volunteers. Those snips were then incubated for 24 hours and the individual MFD obtained and the average MFD and percentage deviation of the individual MFD of three snips from the average one are shown in Table 3. The MFD of 3 different snips highly coincided with each other in most of the cases examined. Fig. 1 clearly shows that in case of the subjects whose MFD are below 10, the maximal measurement error of MFD is 20% or more. On the other hand, the error is markedly reduced in proportion to the increase of the average MFD. For this reason, it is quite appropriate to use volunteers whose MFD is 20 or more for the purpose of quantitative studies in order to minimize the measurement error within 10%. In this experiment, however, the MFD of case N–8 unexpectedly fluctuated notwithstanding its proper MFD in its average. This finding may suggest the rare presence of uneven distribution of microfilariae even in closely adjacent skin regions. However, generally speaking, in onchocercal infections, it may be concluded that the MFD is uniform in small skin regions.

TABLE 3 Comparisons of the MFD in minute skin regions

Case No.	Snip No.	Snip area in mm²	Mf* count per snip	MFD (x)	Average MFD (M)	Difference of individual MFD from the average one	
						Difference in MFD ($x-M$)	Difference in percentage**
N-1	1	7.37	256	34.74	36.34	−1.60	−4.40
	2	6.49	238	36.67		+0.33	+0.91
	3	6.94	261	37.61		+1.27	+3.49
N-4	1	8.43	189	22.42	22.08	+0.34	+1.54
	2	7.47	164	21.95		−0.13	−0.59
	3	6.08	133	21.88		−0.20	−0.91
N-5	1	5.59	127	22.72	21.25	+1.47	+6.92
	2	6.45	123	19.07		−2.18	−10.26
	3	6.24	137	21.96		+0.71	+3.34
N-6	1	5.49	230	41.89	42.83	−0.94	−2.19
	2	3.94	172	43.65		+0.82	+1.91
	3	5.33	229	42.96		+0.13	+0.30
N-8	1	5.02	201	40.04	54.69	−14.65	−26.79
	2	3.86	192	49.74		−4.95	−9.05
	3	5.02	373	74.30		+19.61	+35.86
N-9	1	5.16	278	53.88	55.20	−1.32	−2.39
	2	4.18	237	56.70		+1.50	+2.72
	3	6.16	339	55.03		−0.17	−0.31
N-10	1	6.18	53	8.58	9.01	−0.43	−4.77
	2	3.61	27	7.48		−1.53	−16.98
	3	3.65	40	10.96		+1.95	+21.64
N-13	1	7.29	174	23.87	27.28	−3.41	−12.50
	2	7.55	221	29.27		+1.99	+7.29
	3	6.69	192	28.70		+1.42	+5.21

* Mf: microfilaria

** Difference in percentage: $\frac{x-M}{M} \times 100(\%)$. The highest absolute value of the percentage among the 3 snips was regarded as the measurement error and graphically shown in Fig. 4

3. Relation between incubation time and the emergence of microfilariae

The authors tried to examine the relation between incubation time and the emergence of microfilariae in non-teased skin snips, which were taken from 4 persons whose MFD was as follows: 272.0 in Tm-1; 63.2 in Tm-2; 160.6 in Tm-3; and 22.0 in Tm-4 cases, respectively. Each fresh skin snip was quickly transferred successively to the next slide at intervals of 1 minute during the incubation period ranging from 1 to 20 minutes, and then at 60 minute intervals from 1 to 6 hours. The size of the skin snip was measured after 20 minute incubation in this experiment. The skin snips were incubated for 19 to 30 hours until no more microfilariae were observed. In the experiment which was performed in August, only a single skin snip was examined which was taken from 4 volunteers. Fig. 2 shows the emergence of microfilariae from a single skin snip during an incubation time ranging from one to 360 minutes. In November, however, a similar experiment was repeated

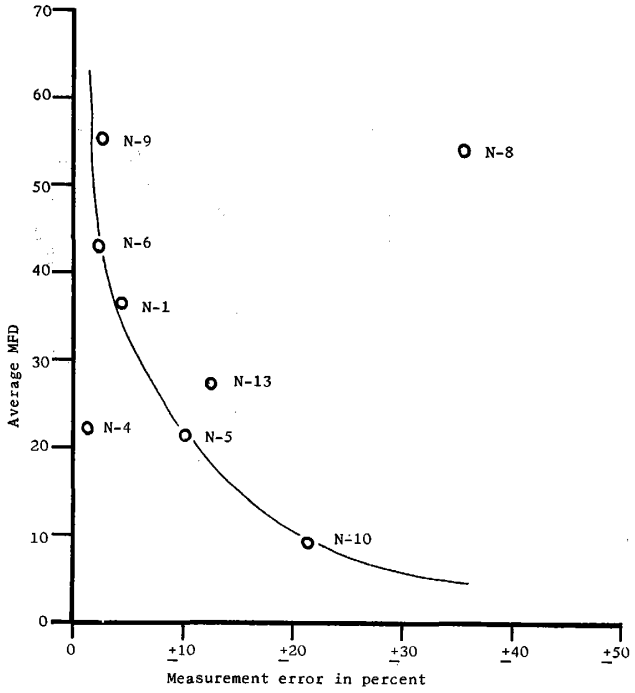

Fig. 1 Relation between the measurement error and the average MFD of 3 biopsies from the same subject.

Measurement error: $\frac{a-M}{M} \times 100$ (%)

(M; arithmetic mean of MFD from 3 snips, a; the farthest MFD from M)

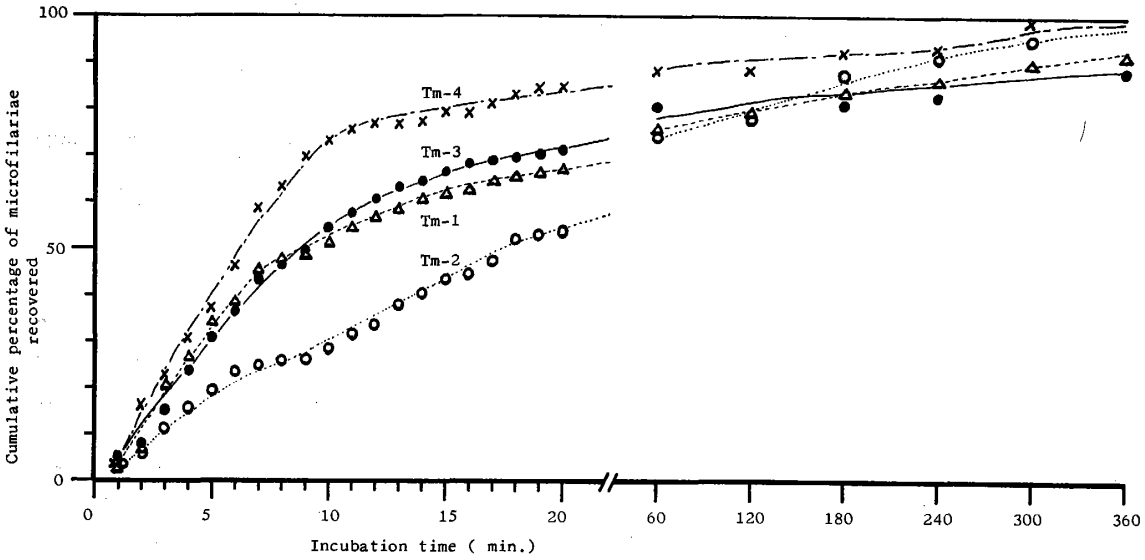

Fig. 2 The emergence of microfilariae from the skin snips of 4 volunteers (fromTm-1 to 4).

by using 3 snips from one volunteer to check the previous result. The result of the latter experiment is shown in Table 4 (group N), in which the transfer of skin snips was made 7 times in the whole incubation only to see the general tendency of the

TABLE 4 The emergence of *O. volvulus* microfilariae from skin snips

Incubation time (min.)	The average cumulative percentage of microfilariae released	
	group T* (6 cases)	group N** (8 cases)
15	—	21.4
20	36.2	—
30	—	35.3
40	50.0	—
60	60.1	46.2
80	67.6	—
100	72.0	—
120	75.6	56.5
180	84.6	66.8
240	86.9	72.6
300	91.7	78.3
360	93.4	—

* group T: volunteers examined in experiment 1
** group N: volunteers examined in experiment 2

microfilarial emergence. This table also shows similar observations made in the experiment 1. All of these experiments revealed similar results. In contrast to the results reported by Duke (1962) and to the recommendation by WHO (1966), the emergence of microfilariae from non-teased skin-snips was more prolonged than expected. In the first experiment, the recovery rate of microfilariae at 20 minute incubation revealed that 66.9% in Tm–1, 53.6% in Tm–2, 71.5% in Tm–3 and 84.9% in Tm–4, respectively. In the experiment carried out in November, however, only 35.3% of the total microfilariae were released by 30 minute incubation. On the contrary, the third series of experiments were quite similar to the results of the first one. From these findings, it can be concluded that skin snips should be incubated for at least 5 to 6 hours at a temperature of about 20 C to assess the accurate MFD. From a practical view point, however, it is inconvenient to incubate skin snips for such a long time for the mass examination of human onchocerciasis.

In August, 1971, the authors examined the inhabitants in Dedessa, Ilubabor Province of Ethiopia. Thirty-three microfilaria positives were found out of 54 cases examined with 15 minutes of incubation using the non-teased snip method. Then the skin snips from 21 negatives left were incubated again for 45 minutes longer. Three new positives with a low MFD were found from these negative cases. This fact may also suggest the importance of a longer incubation even for the epidemiological and routine examinations of onchocerciasis to pick up positives with low MFD. Incubation for 1 hour may satisfy the practical purpose of mass examinations when unteased snips are used.

4. Relation between skin snip size and MFD

From each of 5 volunteers (from S–1 to 5), 3 adjacent skin snips of different size were taken to compare the MFD. The authors called the largest snip L, the smallest one S, and the medium one M from their relative difference in size. In this experiment, L, M and S meant the relative size of biopsies taken from the same person. The actual size in mm^2 is seen in Fig. 3. The skin snips were incubated for 8 to 29 hours depending on the emergence of microfilariae. The MFD of each skin snip among the different sizes obtained is also shown in Fig. 3. This experiment clarified that the highest MFD was usually seen in the snip whose area ranged from 5 to 8 mm^2. This finding suggests that one should not compare the MFD of skin snips of extremely different size with each other.

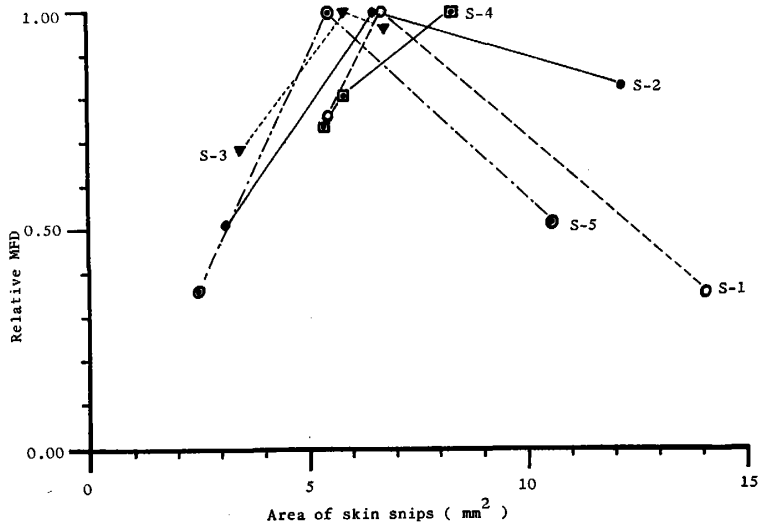

Fig. 3 Relation between skin-snip size and MFD.

5. The effect of warming the skin surface on MFD

Rodger (1957) reported that when the surface temperature fell, microfilariae tended to move more deeply into the dermis. This phenomenon will apparently cause the reduction of MFD in the epidermis. Nelson (1970) stated that in East Africa it had been common practice to apply hot water bottles to the skin to encourage microfilariae to migrate to the superficial layer. These reports seem to indicate that the microfilariae are sensitive to changes of environmental temperature. However, according to the latter author, there are no data to substantiate the validity of this technique. When MFD of the skin is actually increased by warming, the warming technique may have diagnostic validity. In order to assess this point, the present authors examined the MFD of positives before and after warming the skin surface.

The left buttock of volunteers (from N–14 to 20) were covered with a sheet of black-colored polyvinyl immediately after the first snip was removed to exclude the effect of light. The sheet was then heated indirectly by an electric bulb (500 W) about 50 cm apart. The temperature of the sheet was kept at 41 C by adjusting the distance between the bulb and the buttock. The second snip was taken adjacent

to the first one after 15 minutes of warming. The MFD of the two skin snips obtained before and after heating were calculated and compared. The result is shown in Table 5. In cases N–14 and 15, MFD was markedly reduced after warming. On

TABLE 5 The effect of warming the skin surface on the MFD

Case No.		N–14	N–15	N–16	N–17	N–18	N–19	N–20
MFD	First snip* (MFDa)	34.69	21.13	84.28	13.14	31.67	37.61	24.76
	Second snip** (MFDb)	22.99	12.18	88.93	21.69	40.11	38.54	30.41
Difference in MFD		−11.70	−8.95	+4.65	+8.55	+8.44	+0.93	+5.65
Difference in percent***		−33.7	−42.4	+5.5	+65.1	+26.7	+2.5	+22.8

* before warming

** This snip was taken 1 cm. apart from the first one, 15 minutes after the beginning of the incubation.

*** $\frac{\text{MFDb}-\text{MFDa}}{\text{MFDa}} \times 100(\%)$

the other hand, the MFD significantly increased after warming in cases N–17, 18 and 20. No significant changes were observed in cases N–16 and 19. From this brief experiment, it is not easy to reach a final conclusion whether the behavior of the microfilariae was constant or not after warming the skin.

DISCUSSION

In onchocerciasis, teasing of the skin snips has been adopted by most of the previous workers for the quick detection of microfilariae. According to Duke (1962) it was thought essential to tear the snips with needles so that all the contained microfilariae could be freed. He observed about 90% of microfilariae had come out from skin snips after 5 minutes and constant total counts had been obtained at 10–15 minutes. In case of animals infected with *Onchocerca gutturosa,* Nelson et al (1966) showed that many of the microfilariae failed to emerge into the saline unless the skin snips were teased. Lagraulet et al (1967) also lightly teased the biopsies with 2 needles and examined them after 10 minutes of incubation. In contrast to the above quoted results, as the result of the present study shows, the teasing procedure mechanically damaged microfilariae in the skin and caused a reduction in the MFD. This suggests that when the unteased skin snips are incubated for 5 to 6 hours, an accurate MFD will be obtained. Therefore, the present authors conclude that as far as the recovery rate of microfilariae is concerned, torn snips reveal poor and inaccurate MFD.

Some workers did not consider the individual MFD as reliable. Rodger and Brown (1957) used IDF (Individual density figure) and DQ (Density quotient) based on the known anatomical distribution of the microfilaria population and not on microfilaria counts. Because the MFD was shown to be subject to wide variations

in the same site from day to day. Duke (1968) used multiple weighed skin snips to assess the concentration of microfilariae. As microfilariae are not evenly distributed all over the body surface, it seems preferable to use those methods to assess the density of infections. At the same time, it will be necessary to assess the accurate MFD of small skin regions depending on the nature of experiments, such as the effects of treatment, physical and chemical stimulation. Experiment 2 shows that the microfilariae are evenly distributed in small skin areas. This finding may help in the future to perform quantitative studies on the changes in MFD under different conditions.

According to Duke (1962), although the MFD of adjacent snips is usually approximately constant, the assessed densities might vary by as much as 1 : 3 because of the occurrence of pocketing of microfilariae. This is reasonable because the presence of nodules and/or adult worms would disturb the even distribution of microfilariae in the skin. An uneven distribution of microfilariae was also reported in the case N–8 by the present authors. This fact, however, might not indicate the unreliability of MFD because of its rare occurrence. The use of multiple unteased snips will diminish the possible errors of this kind and reveal accurate MFD.

In the present study, the authors did not weigh the skin snips, but measured their surface area. There are apparently some advantages in measuring the area of skin snips: 1) The snip is easily measured in a few drops of saline on a glass slide. This enabled us to avoid time elapsing which frequently causes snips to dry before weighing. 2) The apparatus is easily transferred. On the other hand, in case of weighing, it needs electricity and some adjustment. This is impractical in rural areas. 3) When skin snips are taken almost in a constant thickness, it is considered that the MFD reflects exclusively the 2–dimensioned distribution of microfilariae. This solved one of the inconveniences which are frequently shown in weighing snips that a thin broad biopsy might weigh the same as a narrow deep one which was stated by Rodger and Brown (1957). Lagraulet and Bard (1969) also preferred an estimation of density based upon surface area, because according to them, this method has a theoretical advantage over a method based upon weight of a biopsy which may include some tissue beneath that part of the upper dermis. According to Rodger and Brown, the weighing of biopsies of such small pieces of tissue, the rate of drying in tropical climates and the time elapsing before weighing might all be probable sources of error. The measurement of the surface of skin snips with irregualr outline is somewhat more difficult than weighing them. However, as shown in the experiment 2, this method enabled us to get almost equivalent MFD of some biopsies in minute skin regions. For these reasons, mentioned above, in order to be able to follow the changes of MFD in several experiments, the present method is considered to be of value.

As to the size of skin snips, Duke (1962) used bloodless circular or oval snips about 3–5 mm in diameter and weighing 1–4 mg from volunteers for the skin snip method. Further, Duke et al (1967) concluded that the concentration of mf/mg was independent of the weight within the range from 1 to 3 mg snips based on the examinations of 360 skin snips. In the experiment 4 of the authors, who examined the relation between the size of skin snips and their MFD, the skin snips whose sur-

face area ranged from 5 to 8 mm^2 were considered best. It can be speculated that all the microfilariae will not migrate out in an extremely large snip due to mechanical factors. In extremely small snips, the relative area of the epidermis where microfilariae are usually present might be reduced in comparison with the whole area of the skin snip. Furthermore, the vertical distribution of microfilaria population and the thickness of skin snips seem to have affected MFD of biopsies in different sizes. To overcome this kind of obstacle, the use of a skin punch method (Lagraulet and Bard, 1969) might solve the problem.

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皮膚切片からの *Onchocerca volvulus* マイクロフィラリアの遊出に関する定量的研究

多田　功[1]・岩本　功[2]・テフェラ・ウオンデ[3]

エチオピア南西部のオンコセルカ症患者について皮膚切片からのマイクロフィラリア(mf)遊出を定量的に検討した。これは従来のSkin snip法が定量的でないため，精密なMFD(皮膚内mf密度)を必要とする実験のための検討である。その結果，皮膚片は細切すべきでないこと，更に生理的食塩水中でのインキュベート時間は十分長く行なうべきことが明らかにされた。この方法で近接皮膚領域のMFDを計測した結果，きわめて満足すべき値を得た。唯この際，大きさの極端に異なる皮膚片相互のMFDは多少異なることが判明した。この定量的方法を用いて，皮膚を温めた場合のMFDを測定したが加温による変動はまちまちで一定の成績を示さなかった。

1 鹿児島大学医学部　医動物学教室　2 長崎大学熱帯医学研究所　寄生虫学部門　3 エチオピア帝国中央研究所　医動物学部門

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ONCHOCERCIASIS, A POSSIBLE ETIOLOGY OF ELEPHANTIASIS IN SOUTH-WEST ETHIOPIA

TEFERRA WONDE[1], ISAO TADA[2] and ISAO IWAMOTO[3]

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Abstract: A comparative study on dermal tissue and enlarged inguinal lymph nodes from subjects infected with *O. volvulus* is presented and the histo-pathological findings are discussed. The present data indicate that there could be causal relationship between onchocerciasis and elephantiasis in South-west Ethiopia, endemic focus of the onchocercal disease. The authors drew particular attention to the non-filarial etiology reported by other investigators in Ethiopia and assessed the two contradicting views known as the non-filarial and filarial etiology of elephantiasis.

It is a well established fact that onchocerciasis is endemic in South-west Ethiopia (Cohen, 1960, Oomen, 1969, Iwamoto et al, 1972 and Tada et al, 1972). The authors observed many cases of elephantiasis, in particular that of the lower legs, in people suffering from onchocerciasis. Although Oomen (1969), Price (1972) and Heather and Price (1972) concluded that in Ethiopia filarial infection could not be the cause of elephantiasis, the present authors tried to investigate the possible causal relationship between onchocerciasis and elephantiasis. Then the inguinal and femoral lymph nodes of many people whose skin-snips released *O. volvulus* microfilariae were found palpable and quite often enlarged in most cases. In this paper, the histo-pathological findings of the dermis and inguinal lymph nodes containing onchocercal microfilariae are presented and onchocerciasis as a potential etiology of elephantiasis is discussed.

MATERIALS AND METHODS

Enlarged inguinal lymph nodes were taken from 10 subjects whose skin snips from the buttocks and legs freed numerous onchocercal microfilariae. Inguinal nodes from affected limbs 6, palpable inguinal lymph nodes from clinically normal scrotum and limbs 3, and one inguinal node from a patient with scrotal elephantiasis. All surgically removed inguinal nodes were fixed in 10% formalin, embedded in paraffin

1 Dept. of Medical Zoology, Imperial Central Laboratory & Research Institute, Addis Ababa, Ethiopia. 2 Dept. of Medical Zoology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan (Present address: Dept. of Medical Zoology, Kanazawa Medical University, Uchinada, Ishikawa, Japan). 3 Dept. of Parasitology, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan (Present address: Dept. of Internal Medicine, in same Institute).

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and cut at 5 to 8 μ. Slides from each were stained with hematoxylin and eosin. Skin snips from the same patients with microfilariae of *O. volvulus* were also sectioned for histo-pathological and comparative studies as described above.

Results

Out of 10 inguinal lymph nodes surgically removed from subjects with onchocerciasis infection, 9 were positive for the larval filariae of *O. volvulus*. Lymph node material with microfilariae is shown in Figs. 1 and 2.

The criteria for the identification and differentiation of onchocercal microfilariae from those of other filarial spp. are described by the authors in another paper which is under preparation. The tissue section of the lymph nodes shows an extensive fibrosis entangling a large part of the medullary cords and a portion of peripheral lymphoid follicles with remarkable and wide obliteration of sinusoid spaces.

In the fibrotic lesion, blood capillaries are markedly congested and small arteries are surrounded by dense lamellar fibrosis known as "onion-skin" appearance. Diffuse infiltration of chronic inflammatory cells such as lymphocytes and plasma cells as well as eosinophiles and occasional multinucleated giant cells are seen. There are a considerable number of microfilariae embedded within the perivascular areas of the fibrotic lesion. The remaining part of the medulla being not severely collagenized reveals enlarged reticulum cells and many plasma cells. Lymphoid follicles show enlarged germinal centers.

Despite numerous microfilariae in the dermal tissue as seen in Figs. 3 and 4, no typical inflammatory reaction around the parasites is present. Perivascular lymphocytic infiltration is observed while eosinophiles are absent.

Discussions

Cohen (1960), Oomen (1969), Price (1972) and Heather and Price (1972) excluded the possibility of filarial etiology of elephantiasis in Ethiopia. They reported that both adult and larval filariae were absent from the areas investigated. Furthermore, Heather and Price (1972) reached the conclusion that silicates "play a significant part in establishing conditions favourable for the development of swollen leg, even possibly providing the trigger mechanism for the onset of filarial elephantiasis".

In contrast to the hypothesis quoted above, as indicated in Figs. 1 and 2, the present authors detected a number of onchocercal microfilariae in inguinal nodes. The extensive fibrosis, diffuse infiltration of lymphocytes, plasma cells, fairly large number of eosinophiles and multinucleated giant cells seem to be caused by the microfilarial invasion. In other words, the cellular reaction could be interpreted as a response to the parasitic infection. The absence of the inflammatory reaction of cellular elements in the dermal tissue around the microfilariae is also a common phenomenon in other filarial infections which is not yet clearly understood. Connor et al (1970) observed also only scattered eosinophilic leucocytes in the pretreated skin specimens.

There is a general consensus that the larval and adult stages of *O. volvulus* are only restricted to the dermal and subcutaneous tissue. The presence of numerous

onchocercal microfilariae in the inguinal lymph nodes with and without clinical manifestations shows that this opinion needs a revision.

It is quite evident that the organisms migrate from the dermis and subcutis to the lymphatic system and cause histo-pathological changes described by other researchers (Connor et al, 1970) and present authors. The finding of microfilariae in the inguinal lymph node of a patient with scrotal elephantiasis indicates also the causal relationship between onchocerciasis and elephantiasis.

According to the anatomical landmarks of onchocercal microfilariae described by Iwamoto et al (1972), those of *W. bancrofti* are excluded in the present study. Furthermore, the examination of the peripheral blood taken by day and night interval gave also repeatedly negative results. It is worthwhile to note that McConnel (personal communication) recently found *W. bancrofti* infection in Gambella, one of the endemic foci of onchocerciasis. This finding, however, does not contradict the present one. Ouzilleau (1913), Dubois (1916) and Dubois and Forrow (1939) already reported elephantiasis of scrotum and legs in association with onchocerciasis. Sharp (1926) described also hydroceles, enlarged testes and lymphatic enlargement of thes crotum in patients infected with *O. volvulus*. Further, Connor et al (1970) reaffirmed the probable interaction between onchocerciasis and elephantiasis in Bussinga Ubangi territory of Zaire.

Our studies discussed above with those of previous authors suggest that *O. volvulus* could be regarded as one of the potential causative agents of elephantiasis in South-west Ethiopia, especially where onchocerciasis is mesoendemic.

We also do not deny that silicate particles observed in macrophages of inguinal nodes by Heather and Price (1972) might possibly cause elephantiasis, although we did not yet pay attention to the problems concerning the trace elements. In other words, nonfilarial elephantiasis in Ethiopia could not yet be excluded. We are also aware of the fact that detailed investigations such as lymphangiography and others should be performed in the near future before reaching a final conclusion that *O. volvulus* is the etiology of elephantiasis in the regions surveyed.

Acknowledgements

The present work would have been impossible without the help of many people. The authors express their deep gratitude to Dr. Solarov for removing the lymph nodes surgically. We are also indebted for reviewing the histo-pathological changes and comments to Dr. Tokuoka, Faculty of Medicine, Kagoshima University. The medical and paramedical personnels of Ras Desta Damtew Hospital deserve also our thanks.

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エチオピア南西部における象皮病の一成因としてのオンコセルカ症

テフェラ・ウオンデ[1]・多　田　　功[2]・岩　本　　功[3]

エチオピア南西部には下肢象皮病患者が多く，その地域はまたオンコセルカ症の流行地でもある。従来より象皮病の成因については種々の説があるが，著者らは本症とオンコセルカとの関連を追求するため，イルバボール州に住む下肢象皮病患者10名のそけい部リンパ節の組織学的検索を行なった。その結果，9例のリンパ節についてオンコセルカのマイクロフィラリアを見出した。更に，リンパ節にはマイクロフィラリアないしはオンコセルカ感染に対するものと考えられる組織学的所見を認めた。こうしてリンパ系の閉塞が起こり，二次的に下肢の象皮病が成立したと考えうる所見であった。この結果から，従来東アフリカの象皮病の成因について，非フィラリア性のものとする説に対し，再検討を要すると結論する。

1 エチオピア帝国中央研究所　医動物学部門　2 鹿児島大学医学部　医動物学教室　3 長崎大学熱帯医学研究所　寄生虫学部門

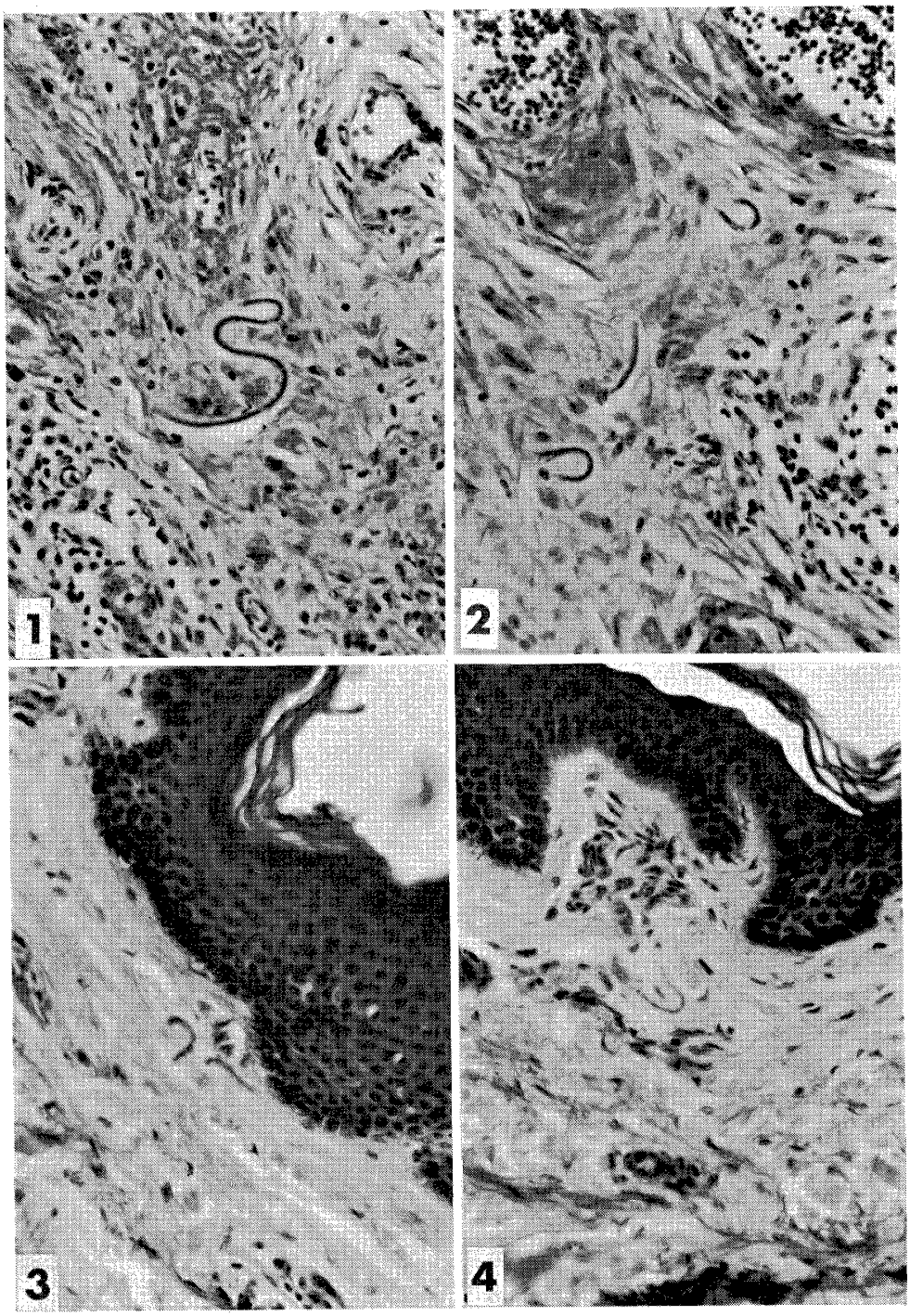

Fig. 1 and 2 Microfilariae shown in the inguinal lymphnodes from 2 elephantiasis patients (Abdella, Ilubabor Province, Ethiopia).

Fig. 3 and 4 Microfilariae shown in the dermal tissue from 2 onchocerciasis patients (Abdella, Ilubabor Province, Ethiopia).

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沖縄における溶連菌の疫学

塩川優一[1]・村上正中[1]・只野寿太郎[2]・山田俊彦[2]

昭和48年6月6日　受付

はじめに

熱帯医学においては気候風土と疾病の関係が大きな研究対象である。われわれはリウマチ熱とその原因となる溶血性レンサ球菌（以下溶連菌と略す）の関係について長年研究を行なっているが最近沖縄において調査を行なったところ内地と明らかな差違を認めた。これは興味ある事実であると考えるので以下報告する。

溶連菌と疾患の関係

溶連菌は広く生物間に分布しているグラム陽性菌であり，ヒトでも咽頭，皮膚などにしばしば見出される。この菌によっておこる疾病は大別して二種類に分けられる。第1はその毒素によって直接発症する炎症性疾患であって一次症とよばれ，咽頭炎，中耳炎，副鼻腔炎，膿皮症などはもっともしばしば見られるものである。第2は二次症とよばれ，リウマチ熱，急性糸球体腎炎，紫斑病，結節性紅斑などがこれに属する。これらの疾患の発症には溶連菌の菌体，または毒素に対する免疫学的な機序が関係するとされ，実際これらでは病巣より溶連菌が証明されることはない。リウマチ熱について考えてみるとまず上気道における溶連菌感染があり，ついでその細胞壁に対する抗体が産生されるが，この抗体はヒトの心臓に対しても作用する。すなわち溶連菌細胞壁とヒト心臓組織は共通成分を有し交叉免疫という機序により心臓組織とも結合するのである。その結果心臓に炎症を発生するがこれがリウマチ熱である。リウマチ熱の約半数はそのまま治癒するが残りは弁膜に傷害を残す。これが心臓弁膜症である。すなわち溶連菌感染症，リウマチ熱，弁膜症という一連の疾患単位があり，従って弁膜症の発生を防ぐためにはさかのぼってリウマチ熱，溶連菌に対する対策が必要となる。われわれは以上の考え方に基づいてリウマチ熱と溶連菌の調査を行なっているのである。

溶連菌は各種の抗生剤に対して感受性を有し，とくにペニシリンに対してはいまだ耐性菌が発生していない。そこで溶連菌は至ってコントロールしやすい菌であるとの考えが拡がっている。実際一次症には罹患してもただちに化学療法により治癒せしめることが出来るのである。しかし二次症についてはそうではない。これは菌が直接関与せず，それに対する免疫学的な機序が原因となっているからである。この点を考える時溶連菌に対して一層の関心を持ち対策を講ずる必要があると考えるのである。

溶連菌の検索とその性状

溶連菌は健康者の咽頭にしばしば証明される。すなわち滅菌綿棒により扁桃上の粘液を強くこすって採取し，これを血液寒天上に培養し，β溶血を示す集落を釣菌する。最近は採取した綿棒をシリカゲル入りの試験管に入れ，密封すれば，数週間は保存されることが分った。そこで遠隔の地で採取しても航空機などで検査機関迄送付することが出来る。われわれの沖縄における調査もこの方法によったのである。

溶連菌は上述のように健康者の咽頭からも証明されるがその頻度は年令により地域により種々である。一般に学童では成人より保菌率が高い，また一年では夏には低く冬には高いことが知られている。また密集した集団では陽性になりやすい。

1 順天堂大学医学部　内科学教室　2　同　臨床病理学教室

しかし従来行なわれた検索は小中学校の学童，およびしょう紅熱や腎炎の発生した集団のもので一般人の健康者集団については研究されていない。

溶連菌はさらに群と型により細かく分類されている。まずその細胞壁の成分である多糖類の性状により A, B, C, D, G などの18の群に分けられる。そのうちでヒトで感染症をおこすものは主としてA群である。A群溶連菌は沈降反応を用いてM型に分類される。これは Lancefield により細胞壁のM蛋白質の性状を以て分類する。ただしこの方法は免疫血清が得にくいところに問題があった。ところが最近凝集反応を用い，細胞壁のT蛋白質により分類する方法が広く用いられるようになった。これは Griffith によるT型である。この方法は抗血清が得やすいがその特異性に問題があった。しかし最近宮本によりその特異性が確認され，T型を応用した国際共同研究が行なわれている[2]。

溶連菌の型と疾患の関係については以前より研究が行なわれている。M型に関してはリウマチ熱はいずれの型でもおこる。急性糸球体腎炎は12，1，4，49型などでおこることが知られ，皮膚感染症にも特殊な型が知られている。型についてとくに興味あるのはその地域的，年次的分布である。これは1964年以来行なわれている国際共同研究ではじめて明らかになった[2]。1964年において世界各国よりの材料では12型がもっとも多くついで4型が多かった。日本ではこの時点では4型がもっとも多く次いで6型であった。なお日本では1938～39年には4型が優位，1955～57年にも4型が主であったがやがて1958年以後6型が優位となりついで1964年の4型の優勢に至っている。第2回の国際共同研究は1968～69年に行なわれたがこの時期には多くの国で12型が優勢となった。そして日本もこれと同調するごとく12型が主となり現在に至っている。そして12型が腎炎の原因菌であることを考える時この動向は注目に値するものである。

リウマチ性心疾患の頻度

リウマチ熱は小中学校の学童に多い疾患である。またこの年令層では先天性心疾患がかなりの数を占めており，リウマチ性心疾患の増加が始まる。従って小中学校において心臓検診を行ない心疾患を発見し，あわせてリウマチ熱について注意をうながすことは意義あることである。ただしリウマチ熱は急性の疾患であるから1年に1回の検診ではその実態を把握することは困難である。そこで溶連菌二次症の指標としては間接ではあるが成立したリウマチ性心疾患を取り，リウマチ熱の既往歴をあわせて調査することになる。

日本循環器学会を中心とする調査では1971年，日本全国で 80万人の学童のうちリウマチ性心疾患は0.01% に証明された（なお先天性心疾患は0.24% であった)[3]。この出現率は1960年には0.18%であったからリウマチ性心疾患はこの10年間に95%という著しい減少をみたことになる。なお先天性心疾患は当然のことであるがこの間に大した変動を示していない。リウマチ熱の既往歴を有する学童は1971年において0.49% を占めていたがこの数字もこの15年間あまり変動していない。

リウマチ性心疾患が激減した理由はリウマチ熱が著しく軽症になったためと考えられる。それではリウマチ熱が軽症になった原因は何かといえばそれは医療が発達し，リウマチ熱の原因となる溶連菌感染症が起るとただちに抗生物質が投与されるためと考える。また経済的発展により栄養がよくなり，疾患に対する抵抗力がついたことも重要と考えたい。しかしそれにもかかわらず溶連菌は依然として広く分布し，いつでもリウマチ熱，リウマチ性心疾患が起り得る状態にあるのである。

東京都府中市の調査

つぎに述べる沖縄の調査成績と比較するために対照として，われわれが1969年以来行なっている東京都府中市の某小学校の成績を述べて置く。ここでは咽頭溶連菌の検索を行なったが，1969年には溶連菌 38.2%，そのうちA群15.2%，1970年にはおのおの25.0%と19.2%，1971年には25.0%と19.9%であった。すなわち溶連菌の陽性率はかなり高い。そしてそのうちA群溶連菌の型別をみると12型が優勢を占めておりその他1型，3型，4型などがみられ，世界および日本の趨勢と一致して

いた。また血清 ASO 値を検査し，その 333 Todd 単位以上を陽性とすると，1969年 10.0%，1970 年 6.8%，1971 年 15.7% を示しており，これは溶連菌による感染の存在を示すものである。

この集団におけるリウマチ性心疾患は 1969 年，70年，71年の3カ年にわたり0.01%であり，これは同期間の日本全国の成績と一致していた。またリウマチ熱の既往者は 1969 年は 0.53% であったが 1970, 71 年はおのおの 0.18 および 0.11% と減少を示した。

沖縄波照間島における調査

以上のような日本内地の状況，とくに東京都府中市の成績と比較するために沖縄において1971年以来調査を試みた。まず沖縄の最南端にある波照間島を選び調査を行なった。

この島は石垣島より海上約 70 km の位置にある孤島で行政上は沖縄県八重山郡竹富町に属する。面積 15 km² 人口約 1,000，本土との交通は 1 週 2 回の定期船によるのみである。

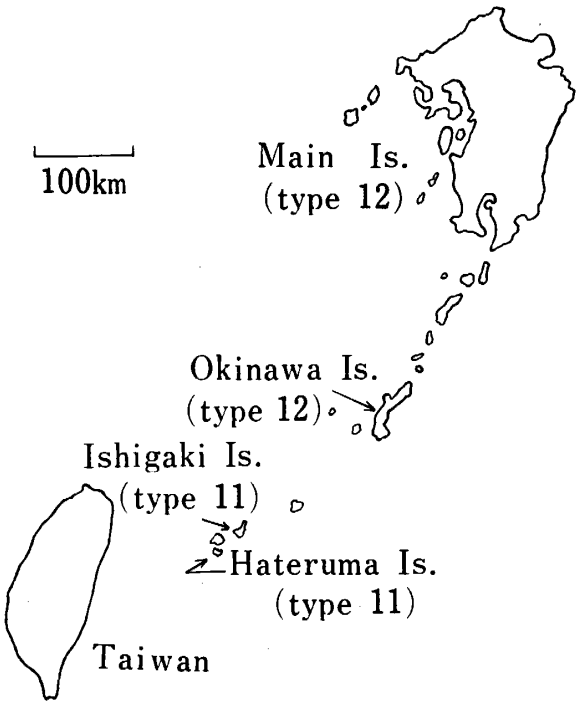

Fig. 1 T types of hemolytic streptococci isolated from main island and Ryukyu islands in Japan.

TABLE 1 Incidence of hemolytic streptococci from throat culture among primary and secondary schoolchildren

	Year	Number Exmined	Group A (%)	Total Str. (%)	Dominant T-Type
Fuchu (Tokyo)	1969	145	22 (15.2)	38 (38.2)	12
	1970	188	36 (19.2)	47 (25.0)	12
	1971	176	35 (19.9)	44 (25.0)	12
Hateruma (Ryukyu)	1971 March	222	90 (40.5)	135 (60.8)	11
	1971 July	332	83 (25.0)	145 (43.7)	11
	1972 March	112	58 (51.7)	70 (62.5)	11
Ishigaki (Ryukyu)	1972 March	110	39 (35.4)	56 (50.9)	11
Koza (Ryukyu)	1972 March	77	41 (53.2)	55 (71.4)	12

TABLE 2 Incidence of heart disease, rheumatic fever history, and high ASO titer among primary and secondary schoolchildren

	Year	Number Examined	CHD (%)	RHD (%)	RF History (%)	*High ASO (%)
Fuchu (Tokyo)	1969	16.162	50 (0.31)	2 (0.01)	86 (0.53)	14/140 (10.0)
	1970	18.187	47 (0.25)	3 (0.01)	32 (0.18)	9/132 (6.8)
	1971	19.300	44 (0.23)	2 (0.01)	22 (0.11)	25/159 (15.7)
Hateruma (Ryukyu)	1971	334	2 (0.60)	0	?	10/273 (3.7)
Koza (Ryukyu)	1971	2.200	2 (0.09)	4 (0.18)	?	?

* over 333 Todd Unit

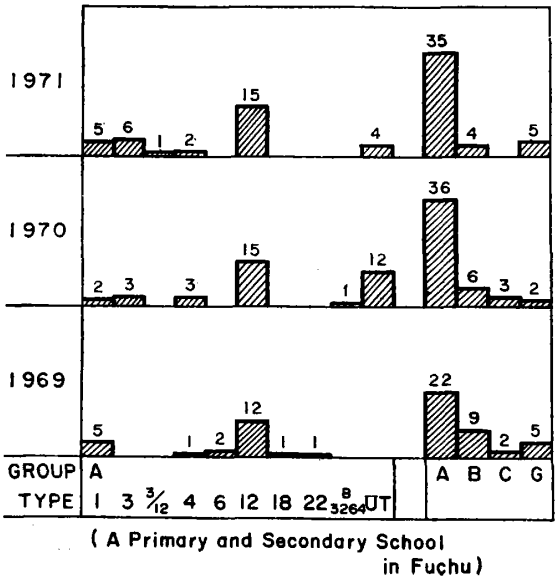

Fig. 2 Group and type of hemolytic streptococci from throat culture in Fuchu (Tokyo).

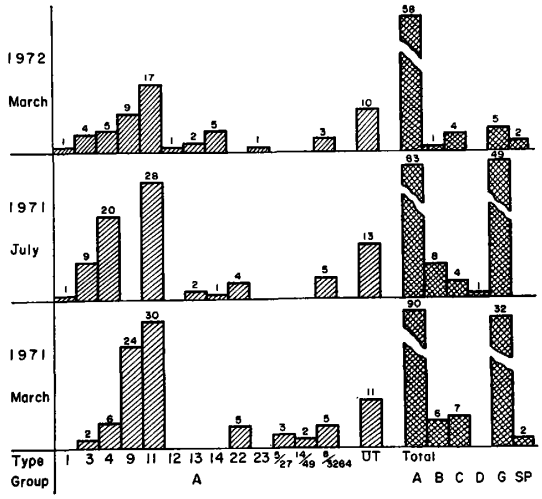

Fig. 3 Group and type of hemolytic streptococci from throat culture in Hateruma (Ryukyu).

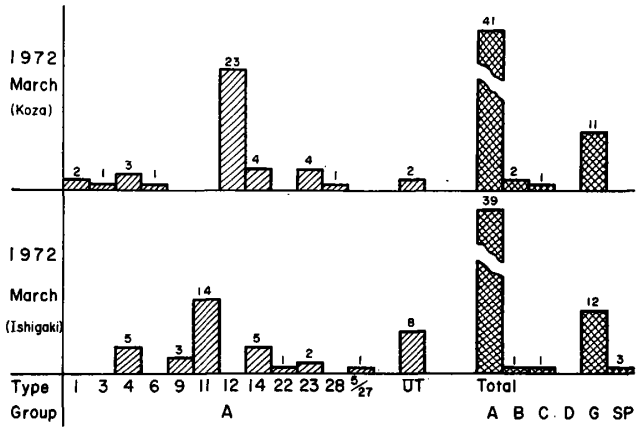

Fig. 4 Group and type of hemolytic streptococci from throat culture in Ryukyu Is.

医療施設として薬局1軒，医師はなく，医介補1名である。従って住民は重病に罹ると石垣島その他に行かなければならない。そして抗生物質もほとんど使用されていない。

この島にある唯一の小中学校の学童について溶連菌の検索を行なった。第1回は1971年3月，第2回は同7月，第3回は1972年3月に施行した。その成績を述べると，溶連菌およびA群溶連菌は第1回60.8%および40.5%，第2回43.7%および25.0%，第3回62.5%および51.7%に陽性であった。すなわち対照である東京都に比すれば2倍前後の高い率であった。

つぎにA群の型をみると著しい所見として優勢なのは11型であってこれはこの3回を通じて同じであり，12型が優位である日本内地および東京都とは明らかに異なっていた。また12型は第3回以外にはみられず，東京都にはない9，14型をみることがあるなどの差違を認めている。さらに群においてG群が多いことも注目すべきであろう。

このように咽頭溶連菌の出現率が高いのに拘らず血清ASO値の333 Todd単位以上は3.7%と低い。すなわちこのように保菌率は高いが感染は少ないことを示している。

それではここでリウマチ性心疾患の頻度はどの位かが問題になる。1971年心電計，心音計を用いてこれらの学童の心臓検診を行なった。その結果は先天性心疾患0.6%，リウマチ性心疾患は0という成績であった。ただし対象学童の数は334名であるからこれで内地より低いということは推計学上いえないが，しかし著しくリウマチ性心疾患が多いであろうという予想は裏切られたのである。すなわち血清ASO値が上昇していないことと相まってこの島の溶連菌は健康保菌者の状態で存在している。その理由が11型という特殊な型のためであるかどうかはなお未解決である。

その他の沖縄における調査

内地と波照間島の明らかな差違が知られたのでその中間はどうなっているかがつぎの研究課題となる。まず石垣島の石垣市における小学校で調査を行なった。その成績は例数は少ないが溶連菌50.9%，A群35.4%とやはり著しい高率を認めた。そしてその型分類では11型が優勢であった。また9，14型をみること，12型をみないこと，G群がかなり多いことなど，波照間島によく似た傾向を示した。なお心臓検診や血清ASO価の検査はここでは行なっていない。

つぎに沖縄本島について調査を行なった。コザ市の小学校では1972年の調査の結果，溶連菌71.4%，A群53.2%という成績であった。これも著しい高値である。そしてその型別をみると12型が優勢であった。ここでは11型をみないし9型もみられない，など東京都に近い傾向があるが一方14型がありG群が多いことは波照間，石垣島に近い。そしてここでは23，28型などもみられた。

コザ市における心臓病については渡慶次寛氏の御教示を受けた。その結果はリウマチ性心疾患0.18%ということで内地に比して明らかな増加をみている。なおコザにおいては1973年4月にふたたび検診を行なったがその最終的な成績は得られていないがリウマチ性心疾患は0.1%と推定され，やはり内地より多いようである。また血清ASO価，リウマチ熱既往も今回は調査しているので次の機会に報告する予定である。

以上の調査よりの結論

われわれは，日本内地と沖縄各地を溶連菌の面より比較しこれによりその後遺症であるリウマチ熱とリウマチ性心疾患の発生要因を探ろうという考えで以上のような研究を行ないつつある。その結果得られたことはまず日本内地と沖縄の咽頭溶連菌の陽性率における大きな差違である。すなわち溶連菌は日本内地よりも沖縄にはるかに出現率が高い。その原因は衛生状態，医療とくに抗生物質使用の普及状況などにあると考えるが，その他に沖縄の高温，高湿も関係するであろう。ただし波照間島における3回の調査では3月の方が7月より陽性率が低かった。そしてそれに伴ってリウマチ熱，リウマチ性心疾患の陽性率も高いわけであるが，確かにコザでは府中よりも高率であった。

しかしこの咽頭に高率に存在する溶連菌はすべて感染しているわけではない。まず咽頭菌陽性で

あってもかならずしも血清ASO値は上昇していない。そしてこの点は府中に比べて波照間の方が血清ASO値ははるかに低値であったことでも明らかである。しかしわれわれは陽性率が高いことはそれだけ感染の機会が多いと考えている。

最後に興味のあるのは証明された咽頭溶連菌の群，および型である。まずA群溶連菌のT型において内地は12型が優勢であるのに沖縄では波照間島，石垣島では11型が優勢であり，さらに沖縄本島では内地と同じく12型が優勢であった。すなわち石垣島と沖縄本島の間には溶連菌の型が変化する所があると考えられる。われわれはさらにこの両島の間の小島を調査しこの変換する線を明らかにしたいと考えている。この型の変化の原因は分らない。しかしおそらく住民の移動により溶連菌が運搬されそれによりこのような変化が生じたものと考えたい。最近沖縄の急速な発展と観光客を含めた人口の移動は著しいものがあり，この情勢は当然溶連菌の型の分布に影響を与えていると思われる。われわれはこの研究を続行しているがその中で，とくに波照間島の菌型が内地と同じになる時点はいつか，その時もっとも大きな要因は何かということに興味を集中している。すでにこの島でも1972年にはじめて12型が出現しており，1973年に予定される調査の結果が期待されるのである。

沖縄における奇病の報告

この研究に従事している途中で東京大学医科学研究所沢井芳男教授よりひとつの重要な示唆を受けた。それはかつて沖縄に奇病の流行がありそれは溶連菌感染症によるということである[1)]。文献によると大正3年（1914年）7～8月より沖縄本島において一種の奇病が流行した。本症は潜伏期約3日，悪感戦慄で発病し，高熱，頭痛，四肢痛，数カ所の皮膚に蜂窩織炎を生じ，その他筋炎，関節炎，リンパ節炎，腹膜炎，胸膜炎，眼球炎，顔面丹毒などをみる。そして敗血症または心臓麻痺で急速に倒れた。患者数は1,000，死亡300余名に及んだ。翌1915年二木，宮川の出張研究によりレンサ球菌を分離し，その感染症が流行したものとされた。当時のことであるから抗生剤がなく，このようなはげしい流行をみたものと思われる。さらに沖縄の高温，高湿な気候，風土がこのような流行を起すに関与したものであろう。

この奇病の記録は非常に興味深いものであるが当時この菌の群，型を分類することはなく終っており，現在の溶連菌の保菌状態といかなる関係にあるかは分っていない。しかし沖縄と溶連菌の関係が古いものであり，また密接であることを物語るものである。

おわりに

1．沖縄の数カ所において小中学学童の咽頭粘液より溶連菌を分離した。そしてその出現率，群，型（T型）を内地（東京府中市）のそれと比較した。また同時にリウマチ性心疾患の罹患率，血清ASO価，リウマチ熱既往などを調査した。

2．沖縄の学童では内地のそれに比し咽頭溶連菌の出現率は高く，リウマチ性心疾患の頻度も高いが血清ASO価の異常率は低い。大部分は保菌状態にあるもので何らかの機会に感染しリウマチ熱が発症すると考える。

3．沖縄の波照間島，石垣島ではA群溶連菌のうちで11型が優勢であった。一方沖縄本島は内地と同じく12型が主力を占めていた。この型の分布状態は興味ある問題であろう。

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EPIDEMIOLOGY OF STREPTOCOCCI IN RYUKYU ISLANDS

YUICHI SHIOKAWA[1], MASANAKA MURAKAMI[1],
JUTARO TADANO[2] and TOSHIHIKO YAMADA[2]

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Primary and secondary school children in Ryukyu Islands were subjected to throat culture for hemolytic streptococci (HS) to study the incidence of HS as a whole, group and type distribution, and the prevalence of rheumatic heart disease. The results were compared with those obtained in school children in Fuchu City of Tokyo Metropolis by the similar procedure.

The incidence of HS as a whole was much higher in Hateruma, Ishigaki and Koza of Ryukyu Islands than in Fuchu City. In typing of group A streptococci by T-agglutination it was interesting to see that the dominant type in Hateruma and Ishigaki was T-11, while in Koza it was T-12, which was similar to the dominat type in Tokyo and other part of Japan. The incidence of rheumatic heart disease in Koza, Ryukyu, was 10 times as high as Fuchu, Tokyo Metropolis, as expected. The discrepancy of types of streptococci in Ryukyu Islands is worth mentioning, and further investigation is continued by us.

1 Dept. of Internal Medicine, 2 Dept. of Clinical Pathology, Juntendo University, School of Medicine, Hongo, Bunkyo, Tokyo, Japan.

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SEASONAL VARIATION IN HEMOGLOBIN CONCENTRATION AND HEMATOCRIT VALUE[1]

SHOTARO NERIISHI[2], KAZUKO FUKUSHIMA[3] AND LEONARD A. SAGAN[4]

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Abstract: The observation that hemoglobin concentration and hematocrit value vary with the season has not been generally accepted but studies made in Japan support this.

Reported here are the results of analysis on the month-specific mean hemoglobin concentration and hematocrit values from 29,482 blood samples collected during the 5 years from 1958 to 1963 from ABCC-JNIH Adult Health Study sample members in Hiroshima and Nagasaki. The values show definitely that seasonal variation is present. Both hemoglobin and hematocrit values showed a pronounced negative correlation to temperature and humidity.

It was found on grouping the subjects by sex, age, and relative weight that the range of seasonal variation was greater in older persons than in younger, and in obese persons than in those of light weight. It was characteristic that the range of seasonal variation was small in the groups with high levels and, contrarily, large in the group with low levels. The two extremes were presented in males under 40 years of age of the light relative weight group, and in obese males aged 40 and over.

There have been a number of reports that hemoglobin concentration varies seasonally, but this conclusion has not been generally accepted. Wintrobe (1967), in his text book of hematology, denies the existence of seasonal variation.

Engelbreth-Holm and Videbaeck (1948) reported in 1948 that determinations of hemoglobin concentration and red blood cell counts in 69 medical students in January, March, June, and October showed seasonal differences with somewhat lower values noted in June. According to Wilson (1953) who made monthly examinations for 2 years between 1949 and 1952 of 15 members of an Antarctic Exploration Party, no variation was seen in red blood cell counts, but there was an evident seasonal variation in hemoglobin concentration. Further, Christie (1958) observed in six members of a party exploring the Central Greenland Icecap a rapid decline in hemoglobin level during the first 2 weeks, and thought it was probably attributable to physical labor.

In a 1-year follow-up study of the hematocrit value in 24 young Japanese men

1 Adult Health Study, 1958-63, Hiroshima and Nagasaki by Atomic Bomb Casualty Commission and Japanese National Institute of Health. 2 Dept. of Clinical Laboratories, ABCC, Sakurababa, Nagasaki, Japan. 3 Dept. of Statistics, ABCC, Hijiyama Park, Hiroshima Japan. 4 Dept. of Medicine, ABCC (Present address: Palo Alto Medical Clinic, 300 Homer Av., Palo Alto, California, U.S.A.).

and women, Watanabe (1958) observed a decline in the summer, and ascribed it to probable blood dilution. The results of Wadsworth (1954) did not support the inference made by Watanabe. He could not demonstrate any statistical difference in hemoglobin value between inhabitants of tropical Singapore and of temperate Europe.

These reports indicate that opinions are divided regarding seasonal variation of hemoglobin and hematocrit values. Numerous factors might induce seasonal variation. For example, the amount of physical activity fluctuates during a year according to occupation, which could be reflected in seasonal variations in these values. They could be affected by seasonal changes in food variety and intake.

The presence or absence of seasonal variation in hemoglobin, hematocrit, and other blood constituents and the relationship of such variation to sex, age, and somatotype are examined here. Analysis was made of monthly variation in approximately 30,000 blood samples obtained during the 5 years from 1958 to 1963 from a large fixed sample of adults in two middle-sized Japanese cities.

Materials and Methods

The individuals on whom blood tests were regularly conducted are voluntary participants in the joint ABCC–JNIH Adult Health Study. This population sample comprises A-bomb survivors and nonexposed comparison subject residents in Hiroshima and Nagasaki Cities (Freedman et al.). This fixed population is divided into 24 subgroups so that each month one subgroup will undergo medical examination in a biennial cycle. The actual visit of the subjects in each subgroup for examination may deviate 2 or 3 months from the scheduled month, but the subjects undergoing examination each month can be considered to be randomly representative of the entire sample.

Analysis was made of five items of the regular examination: hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), white blood cell count, and specific gravity of urine.

The number examined each month is approximately 350–400 in Hiroshima and 100–150 in Nagasaki, and the total blood samples obtained in examinations during 1958–63 was 22,404 in Hiroshima and 7,078 in Nagasaki; a total of 29,482 for the two cities combined. Since the results of blood tests employed in this study showed no significant difference in mean values between the exposed groups and the nonexposed groups (Hoshino, 1964), data for all groups were combined in this study.

First, the year-specific mean values for each item classified by city, sex, and age were studied to determine the levels for each group and the presence or absence of a specific trend during the 5 years. The month-specific mean values were then examined by group to determine the presence or absence of seasonal variation. Month-specific mean values were obtained by pooling results for the 5 years.

Relative body weight was used as an index of somatotype. Those of the same sex, age, and stature were classified into three groups according to body weight: those under the 25th percentile were designated as the light weight group (L-group), those between the 25th and 75th percentile as the middle weight group (M-group),

and those at the 75th percentile or more as the heavy weight group (H-group) (Siegel et al, 1958–60).

Standard methods were used in the blood tests (Freedman et al.). The cyanmethemoglobin method was employed for hemoglobin determination; for hematocrits the Wintrobe tube was used in Hiroshima and the capillary tube in Nagasaki.

The month-specific mean values for temperature and humidity were based on records of the weather stations of the two cities.

Results

The mean hemoglobin and hematocrit values by city, age (<40 vs 40+), and sex in each year during the 5 years demonstrate a slight but not significant increase from 1958 to 1963 (Table 1). However, large differences are noted by sex and age.

Table 1 Number examined and mean values of Hb and H-crit by year, sex and age (1958–63, Hiroshima and Nagasaki)

	Year	<40			40+			Total N
		N	Hb (g/100m*l*)	H-crit (%)	N	Hb (g/100m*l*)	H-crit (%)	
Hiroshima								
Male	1958–59	601	14.1	43.9	888	13.6	42.5	1489
	59–60	680	14.5	44.6	1157	13.8	42.5	1837
	60–61	603	14.5	44.7	990	13.8	42.9	1593
	61–62	561	14.7	44.5	1035	13.9	42.6	1596
	62–63	595	14.7	45.2	1027	14.1	43.4	1622
	Total	3040	14.5	44.6	5097	13.8	42.8	8137
Female	1958–59	919	12.0	37.7	1327	12.0	37.8	2246
	59–60	1597	12.2	37.7	1900	12.1	37.5	3497
	60–61	1115	12.2	38.1	1632	12.2	38.0	2747
	61–62	1162	12.4	38.1	1742	12.4	38.1	2904
	62–63	1056	12.3	38.4	1817	12.4	38.4	2873
	Total	5849	12.2	38.0	8418	12.2	38.0	14267
							Hiroshima total:	22404
Nagasaki								
Male	1958–59	167	14.2	44.1	144	13.6	42.5	311
	59–60	467	14.5	44.1	464	14.0	42.7	931
	60–61	194	14.7	45.3	181	14.0	43.2	375
	61–62	378	14.5	44.7	465	13.9	43.2	843
	62–63	428	14.6	44.2	452	14.1	42.7	880
	Total	1634	14.5	44.5	1706	13.7	42.9	3340
Female	1958–59	263	11.9	37.6	165	11.8	37.2	428
	59–60	815	12.2	37.6	464	12.2	37.5	1279
	60–61	321	12.2	38.0	206	12.3	38.2	527
	61–62	787	12.1	38.0	503	12.2	38.0	1290
	62–63	690	12.3	37.5	524	12.4	37.6	1214
	Total	2876	12.1	37.7	1862	12.2	37.7	4738
							Nagasaki total:	7078

Therefore, the study of monthly changes was made by sex and age. Data were maintained by city since seasonal temperature and humidity variation are somewhat different in each city.

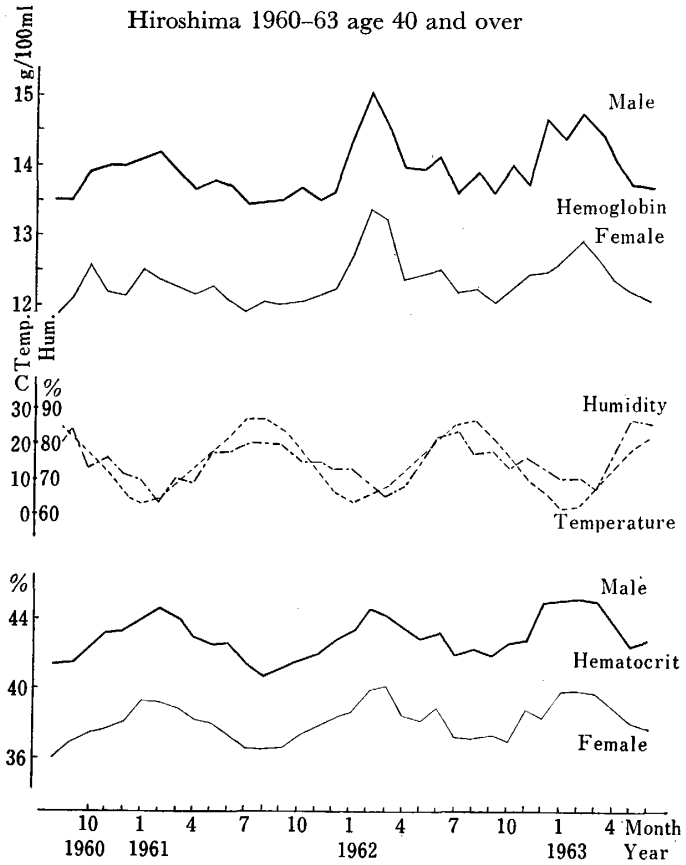

Fig. 1 Monthly changes in hemoglobin, hematocrit, temperature and humidity.

As shown in part in Fig. 1, the curves of the mean hemoglobin and hematocrit values for the 5-year period and the mean curves for temperature and humidity vary inversely. That is, the hemoglobin and hematocrit curves are elevated during the cold season between December and February and are depressed in the warm season between July and September. This phenomenon, though differing in extent, is noted in all sex and age groups, and the monthly variations are all significant at the 1% level by the F-test.

Since the yearly increment was negligible for the hemoglobin and hematocrit values, an index of seasonal variation could be obtained by merely averaging all results over the 5-year period for each specific month, and it was not necessary to employ more sophiscated methods, such as link relatives.

The levels for each month are shown in Fig. 2. The range of annual variation (the difference between the highest and the lowest values) together with the annual mean derived from the mean values for the 5-year period are shown in Table 2.

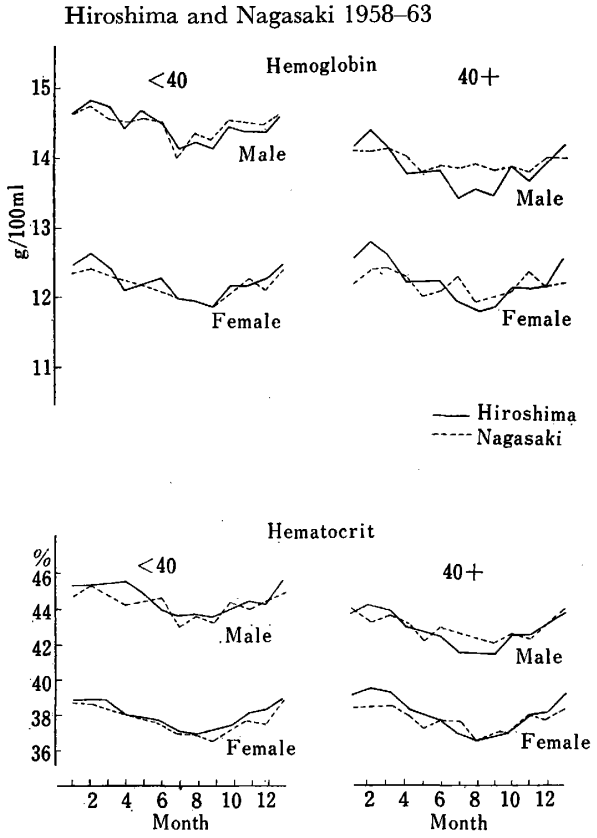

Fig. 2 Seasonal variation in hemoglobin and hematocrit by age and sex.

TABLE 2 Mean and range* of variation in year of Hb and H-crit by sex, age and relative weight groups (Average of 1958–63, Hiroshima and Nagasaki)

		Hb (g/100ml)				H-crit (%)			
		Male		Female		Male		Female	
		<40	40+	<40	40+	<40	40+	<40	40+
Hiroshima									
Total	Mean	14.5	13.8	12.2	12.2	44.6	42.8	38.0	37.9
	Range	0.7	1.0	0.8	1.0	1.9	2.8	2.2	3.1
L-group	Mean	15.0	14.4	12.4	12.5	45.7	44.3	38.3	38.7
	Range	0.5	1.0	0.9	0.9	2.0	3.1	2.6	2.8
M-group	Mean	14.4	13.8	12.2	12.2	44.4	42.7	37.9	37.8
	Range	0.7	0.8	0.7	1.0	2.3	2.3	2.3	3.1
H-group	Mean	14.2	13.5	12.2	12.1	43.9	41.8	37.9	37.6
	Range	0.9	1.4	0.9	1.1	3.3	4.0	2.8	3.9
Nagasaki									
Total	Mean	14.5	13.9	12.2	12.2	44.4	42.9	37.7	37.7
	Range	0.7	0.4	0.6	0.5	2.5	2.0	2.3	1.9
L-group	Mean	14.9	14.4	12.3	12.6	45.4	44.0	38.0	38.8
	Range	1.0	0.7	0.5	0.9	2.4	2.3	2.4	3.0
M-group	Mean	14.5	13.9	12.2	12.1	44.4	42.9	37.7	37.3
	Range	0.6	0.5	0.5	0.6	2.4	2.1	2.1	2.2
H-group	Mean	14.2	13.6	12.2	12.1	43.5	41.9	37.6	37.5
	Range	1.1	0.9	0.7	0.9	4.4	3.1	3.0	2.0

* Difference between the highest and lowest

Between the summer and the winter season a difference of 0.5–1 g/100 m*l* in hemoglobin and 2%–3% in hematocrit is seen, and this difference appears more marked in the age group of 40 years or over in both sexes in Hiroshima. The trend was not as consistent in the Nagasaki data which are based on smaller numbers.

The hemoglobin and hematocrit values not being of the same level for the two sexes and age groups, they have been presented in the form of relative month-specific index numbers with 100 as the annual average (Table 3). The month-specific index numbers for hemoglobin and hematocrit are very close and the range of variation of each is about 6%.

TABLE 3 Seasonal index number of Hb and H-crit by sex and age (Annual average = 100, Hiroshima and Nagasaki)

		Hb				H-crit			
		Male		Female		Male		Female	
		<40	40+	<40	40+	<40	40+	<40	40+
Hiroshima	1	101.2	102.3	101.9	102.7	102.0	102.4	102.3	103.2
	2	102.6	104.0	103.5	104.5	102.2	103.6	103.2	104.1
	3	101.9	102.1	101.8	102.8	102.2	102.9	102.3	103.7
	4	99.7	99.6	99.1	100.1	100.4	100.6	100.3	101.2
	5	101.3	99.6	99.7	99.9	101.1	99.7	99.7	100.1
	6	100.2	99.9	100.5	100.0	98.9	99.3	99.3	99.3
	7	97.6	97.0	98.2	97.7	98.0	97.2	97.9	97.2
	8	98.3	97.9	97.8	96.6	98.2	97.1	97.3	96.0
	9	97.8	97.3	97.2	97.0	97.9	97.0	97.5	96.8
	10	99.9	100.2	99.5	99.3	98.9	99.2	98.6	97.8
	11	99.4	98.8	99.6	99.1	99.8	99.3	100.2	99.7
	12	99.3	100.6	100.4	99.3	99.5	101.0	100.9	100.3
Range*		5.0	7.0	6.3	7.9	4.3	6.6	5.9	8.1
Nagasaki	1	100.9	101.1	101.7	99.9	101.0	102.8	102.7	101.8
	2	101.7	101.0	102.1	101.5	102.4	100.9	102.5	102.0
	3	100.6	101.6	101.4	101.9	100.9	101.8	101.7	101.9
	4	100.2	100.7	100.5	100.8	99.8	100.7	100.7	100.9
	5	100.5	98.8	100.1	98.7	100.3	98.4	100.2	98.7
	6	100.2	99.5	99.2	99.1	100.6	100.2	99.6	99.8
	7	96.6	99.4	98.5	101.0	96.9	99.4	98.1	100.0
	8	98.9	99.7	98.2	97.8	98.6	98.7	97.6	97.0
	9	98.4	99.1	97.4	98.5	97.4	98.2	96.7	97.9
	10	100.2	99.5	98.9	99.1	100.1	99.3	98.3	98.5
	11	99.9	99.0	100.8	101.5	99.3	98.5	99.9	100.9
	12	99.7	100.5	99.4	99.7	100.2	100.6	99.6	99.4
Range*		5.1	2.8	4.7	4.1	5.5	4.6	6.0	5.0

* Difference between the highest and lowest in the year

Further, to consider the factor of somatotype, the mean hemoglobin and hematocrit levels during the 5-year period were compared by sex, age, and relative weight group, and the results are shown in Table 2. Generally the H-group shows lower values for both hemoglobin and hematocrit than the L-group in all sex and age groups. Except for Nagasaki females under 40 years of age, the difference is significant at the 1% level. The difference between the two groups is more marked in males and become more pronounced with age in both sexes. The M-group presents values intermediate between the L- and H-groups. Accordingly, the highest hemoglobin and hematocrit values were observed in males under 40 years of age in the L-group and the lowest values in females of 40 years of age or over in the H-group.

The monthly changes in mean values for the 5-year period in these relative weight groups are shown in Figs. 3–1, 2. The range of annual variation is given under each mean value in Table 2. These show for both sexes and age groups, excluding Nagasaki females under 40 years of age, a smaller range of annual variation in the L-group, who have high hemoglobin and hematocrit levels, and a greater range of variation in the H-group, with low levels. This tendency is particularly marked in Hiroshima, where males under 40 years of age in the L-group with the highest levels presented the smallest range of variation annually (0.5 g/100 m*l* and

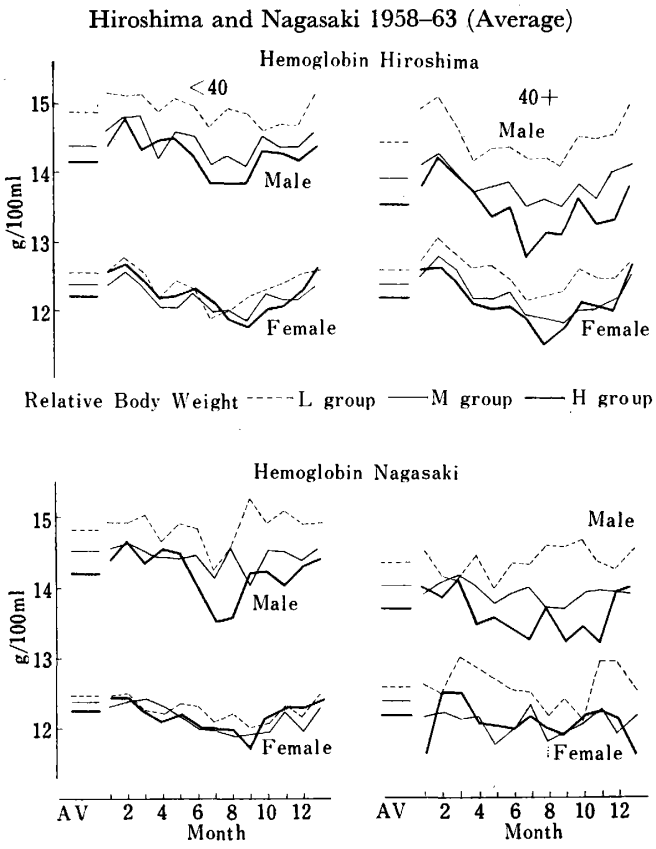

Fig. 3–1 Seasonal variation in hemoglobin by age, sex and relative weight group.

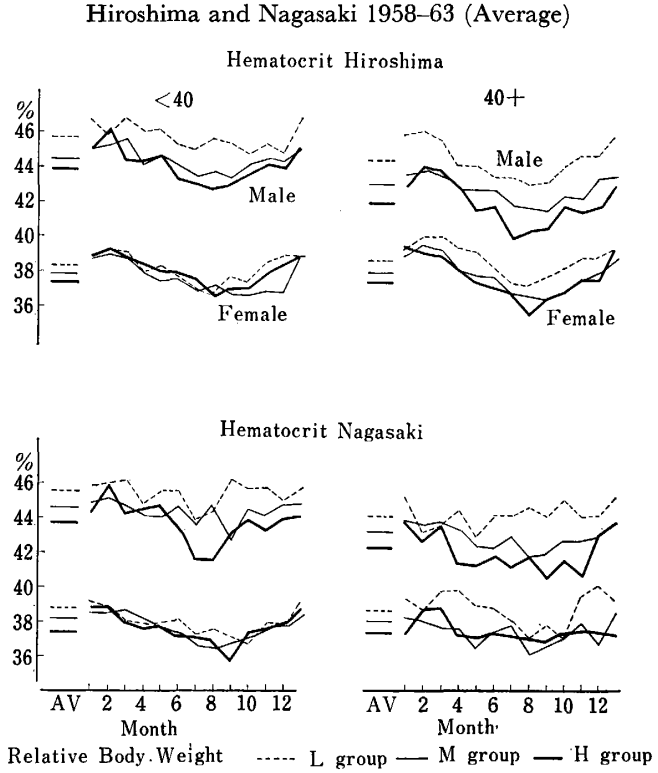

Fig. 3–2 Seasonal variation in hematocrit by age, sex and relative weight group.

2.1%). Contrarily, in those 40 years of age or over in the H-group, in whom the levels are quite low, both males and females present ranges of annual variation exceeding 1 g/100 m*l* for hemoglobin and 3% for hematocrit, and the hemoglobin

TABLE 4 Range* in seasonal index number of Hb and H-crit by sex, age and relative weight group
(Annual average=100, Hiroshima and Nagasaki)

Relative weight group	Hb				H-crit			
	Male		Female		Male		Female	
	<40	40+	<40	40+	<40	40+	<40	40+
Hiroshima								
L-group	3.6	6.9	7.2	7.0	4.5	6.9	6.7	7.3
M-group	5.0	5.6	5.9	7.9	5.2	5.4	6.1	8.1
H-group	6.5	10.5	7.7	9.5	7.6	9.6	7.3	10.2
Nagasaki								
L-group	6.8	4.9	4.0	7.4	5.2	5.1	6.2	7.8
M-group	4.3	3.4	4.4	4.8	5.5	4.9	5.6	5.8
H-group	8.0	6.5	5.8	7.6	10.0	7.4	7.9	5.4

* Difference between the highest and lowest

TABLE 5 Correlation* of Hb and H-crit to temperature or humidity by sex, age and relative weight group (1958–63, Hiroshima)

Sex, Age and RW Group			To Temperature			To Humidity		
			ρ	$\hat{b}$	Test for Ho: $b=0$	ρ	$\hat{b}$	Test for Ho: $b=0$
Hb								
Male	<40	Total	−.41	−.021	**	−.30	−.022	*
		L-group	−.01	−.009	N.S.	−.01	−.014	N.S.
		M-group	−.39	−.020	**	−.27	−.021	*
		H-group	−.42	−.028	**	−.30	−.030	*
	40+	Total	−.63	−.032	***	−.47	−.036	**
		L-group	−.57	−.034	**	−.31	−0.28	*
		M-group	−.48	−.025	**	−.31	−.024	*
		H-group	−.52	−.035	**	−.49	−.049	**
Female	<40	Total	−.59	−.023	***	−.39	−.023	**
		L-group	−.53	−.028	**	−.28	−.023	*
		M-group	−.52	−.019	**	−.39	−.022	**
		H-group	−.54	−.029	**	−.39	−.032	**
	40+	Total	−.63	−.030	***	−.46	−.033	**
		L-group	−.50	−.025	**	−.35	−.027	**
		M-group	−.59	−.030	**	−.41	−.031	**
		H-group	−.59	−.033	***	−.49	−.041	**
H-crit								
Male	<40	Total	−.61	−.078	**	−.41	−.079	**
		L-group	−.26	−.038	*	−.26	−.056	*
		M-group	−.57	−.077	**	−.38	−.078	**
		H-group	−.54	−.096	**	−.33	−.090	*
	40+	Total	−.31	−.115	***	−.61	−.131	**
		L-group	−.76	−.131	**	−.50	−.130	**
		M-group	−.66	−.089	**	−.43	−.088	**
		H-group	−.69	−.137	**	−.62	−.185	**
Female	<40	Total	−.82	−.090	***	−.54	−.089	**
		L-group	−.69	−.099	***	−.37	−.082	**
		M-group	−.74	−.082	**	−.54	−.090	**
		H-group	−.71	−.097	**	−.44	−.091	**
	40+	Total	−.82	−.115	***	−.59	−.125	**
		L-group	−.71	−.102	**	−.27	−.058	*
		M-group	−.76	−.114	**	−.51	−.116	**
		H-group	−.79	−.123	***	−.63	−.148	**

* Linear correlation: $Y=a+bX$

X: monthly records of temperature or humidity

Y: monthly average of Hb or H-crit

and hematocrit levels become especially low in the summer.

The range of annual variation becomes more impressive if expressed as a range of monthly index numbers with 100 as a base (Tables 3, 4). In groups in which variation is marked the range reaches 10% of the annual average.

Lastly, the correlation of monthly mean hemoglobin and hematocrit values with mean temperature and humidity values, by relative weight group is shown in Table 5. For both hemoglobin and hematocrit high negative linear correlations with temperature and humidity were noted (with the exception of Hiroshima males under 40 years of age of L-group, all groups presented a correlation significant at 1%–5% level with Ho: b=0). Generally the correlations tend to be low in the L-group and high in the H-group. The group with especially low correlation is the L-group of Hiroshima males under 40 year of age, in which there apparently is hardly any correlation with seasonal change. Further, Fig. 1 suggests a time-lag of about 1 month between the observed values of hemoglobin and hematocrit and those of temperature and humidity. If this was taken into account, the correlation would probably have been higher, but calculations have not been made.

With reference to MCHC, WBC, and specific gravity of urine, no correlation of their monthly mean values with temperature and humidity was noted in any of the groups.

Discussion

Analyses made in this study of the numerous data collected over many years confirm that hemoglobin and hematocrit values in Japanese tend to be high in the winter and low in the summer.

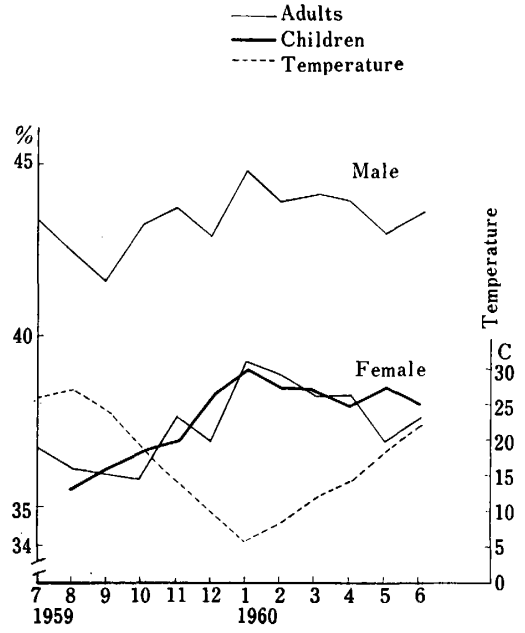

Fig. 4 Monthly changes in hematocrit for children and adults Nagasaki 1959–60.

The presence of seasonal variation of hemoglobin and hematocrit has not generally been accepted in foreign countries. However, in Japan the presence of such variation has been noted in studies made by Japanese scientists over a period of some 20 years from around 1940, including those of Watanabe (1958), Kuroda (1953), Nunoyama (1956), Nishimura (1961) and others.

The result of the present study support these previous reports. This has been confirmed also by an observation of the seasonal variation of hematocrit in a separate population of children made in the same laboratory at the same time as this study (Neriishi S., 1969, Fig. 4). Due to multiple reasons no attempt will be made to explain the mechanism that produces the seasonal variation which is so characteristically observed in Japan.

The presence of seasonal variations in hemoglobin and hematocrit values itself supports and confirms the findings of a number of other studies, but further observation by sex, age, and relative weight group revealed a number of interesting facts. Though the seasonal variation of the different sex, age, and somatotype groups may be of a common pattern, each differs in extent. Thus, it may be interpreted that the influence of season does not affect all people uniformly. The most characteristic example is seen in males under 40 years of age in the light weight group. In this group, showing the highest hemoglobin and hematocrit levels, the range of seasonal variation is the smallest, and contrary to the general tendency for the levels to lower with age, the range of seasonal variation becomes greater. Further, the H-group as compared with the L- and M-groups has lower levels of both hemoglobin and hematocrit, and moreover the range of variation is greater. An extreme example is seen in males 40 years of age and over in the H-group. In this group, both hemoglobin and hematocrit levels are markedly lower than those in the L-group and the range of seasonal variation is greater than that of any other group, extending to as much as 10% of the annual average. Thus, each age group was shown to have its own hemoglobin and hematocrit levels and range of seasonal variation.

It is highly probable that the results of clinical laboratory tests may be affected by such natural conditions as temperature and humidity. However, the fact that the seasonal variation differed between the young and old and, especially, between subjects of relatively light or heavy weights, implies that seasonal laboratory variability cannot possibly represent the whole explanation.

No detailed analysis of the health condition of the participants was attempted, but as most of them were ambulant the observations made can be considered to have general applicabilities. Recently, the relationship between obesity and health has become the subject of much discussion (Fredman et al, 1964), and also a study on seasonal variation in cardiovascular functioning was reported (Schneider and Costiloe, 1972).

The finding of definite differences in blood parameters such as hemoglobin and hematocrit is felt to be significant.

This study was based on data collected during 5 years from 1958 to 1963, but the mode of living of the Japanese people is rapidly changing (Kagan et al. 1972), so that it would be interesting to continue observations hereafter to see whether this

phenomenon of seasonal variation will tend to disappear.

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ヘモグロビン濃度およびヘマトクリット値の季節的変動

錬石昇太郎[1]・福島和子[1]・Leonard A. Sagan[2]

ヘモグロビン濃度ならびにヘマトクリット値が季節的に変動するという観察は一般には容認されていないが，日本で行なわれた研究はこれを肯定している。

ここに報告するものは，広島市および長崎市における成人健康調査対象者について1958-63年の5年間に収集した29,482件の血液標本からヘモグロビン濃度ならびにヘマトクリット値の月別平均値を求め解析した成績である。これらの値が季節的に変動することが明確に認められた。ヘモグロビン濃度もヘマトクリット値も，気温および湿度に対して明らかな負の相関を示した。

調査対象を性，年齢および相対的体重値別に分類すると，高年齢層は若年齢層に比べ，また，肥満群は軽体重群に比べ，季節的変動の幅が大きいことが認められた。季節的変動の幅は，検査で高値を示す群では小さく，低値を示す群では逆に大きいことが特徴的であった。その両極端は，男子における40歳未満の軽体重群と40歳以上の肥満群とに認められた。

1 原爆傷害調査委員会 長崎 2 同 広島

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