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

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

INTRODUCTION

In Republic of Guatemala, malaria patients are mainly monitored by passive case detection. There are more than 6,000 collaborative volunteers to take blood and make blood films from the patients who consult with them. Blood films are sent to Servisio Nacional de Erradication de la Malaria (SNEM) and are examined by experienced microscopists. The patients, in whom malaria was detected at SNEM, receive and take the drugs sent from SNEM by collaborative volunteers. The record of medication is written on individual cards. The patients with *Plasmodium falciparum* are given chloroquine and primaquine for three days and the patients with *P. vivax* are given chloroquine for three days and primaquine for eight days. Four to five hundred thousand films are collected, of which fifty to seventy thousand films are parasite positive every year. The slide positivity rate is 12 to 14%. Among

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the positive slides, 90 to 94% are *P. vivax* and the rest is *P. falciparum*. SNEM records the result of the slide examination on a card for each locality every month. Therefore, we can monitor the annual or monthly incidences of malaria in each locality. Besides those data, we needed the prevalence of malaria in a certain locality on a certain day to compare the incidence with the prevalence because we could not detect the latent cases of malaria by the present system of passive case detection.

We intended to obtain the prevalence rate of glucose-6-phosphate dehydrogenase (G6PD) deficiency in Guatemala. It is a hereditary abnormality which may result in haemolytic anemia after the administration of some kinds of drugs: *e.g.* 8-aminoquinoline antimalarials (primaquine *etc.*) (Carson *et al.*, 1956). Primaquine has been used for malaria patients in Guatemala without testing for G6PD. The information of the prevalence rate of G6PD deficiency was needed.

In Guatemala, there is also no datum on the Duffy blood group. Negative phenotype of the Duffy blood group, Fy (a−b−), is highly present in West Africans and American blacks. They are resistant to *P. vivax* but are susceptible to the other species of human malaria (Welch *et al.*, 1977; Miller *et al.*, 1978). There are many cases of *P. vivax* in Guatemala. We examined whether the Duffy positive population was dominant or not.

Materials and Methods

Study area

Based on the data of SNEM, we selected six localities (Figure 1, Table 1).

Figure 1 Map of Republic of Guatemala showing six villages where the survey was carried out.

Table 1 Slide positivity rate obtained by passive case detection in six localities in Guatemala (1983-1985)

Locality (Population)		No. of positive/slide examined (Slide positivity rate %) 1983		1984		1985	
Cubilguitz	(692)	101/258	(39.1)	36/99	(36.4)	23/57	(40.4)
Chicanuz	(1,184)	36/219	(16.4)	56/211	(26.5)	61/205	(29.8)
Aceituno	(1,139)	251/600	(41.8)	513/2,025	(25.3)	166/976	(11.9)
Milagro	(2,295)	106/320	(33.1)	79/292	(27.1)	34/319	(10.7)
Empalizada	(1,060)	139/478	(29.1)	130/458	(28.4)	105/459	(22.9)
Sipacate	(4,605)	482/1,406	(34.3)	523/1,738	(30.1)	597/2,164	(27.6)

Two villages of Kekchi tribe, Cubilguitz and Chicanuz were selected in Departamento Alta Verapaz in the northern part of Republic of Guatemala. The inhabitants produce corn, coffee beans, cocoa beans and cardamom. The annual rainfall is 1,800-2,500 mm. The altitude is 300-500 m. Many malaria patients were recorded in these two villages. Slide positivity rates in 1985 were over 30%.

Four villages of mestizos, Aceituno, Milagro, Empalizada and Sipacate were selected in Departamento Escuintla facing the Pacific Ocean. The residents grow suger cane and cotton and rear cattle. The annual rainfall is 2,200-2,900 mm. The altitude is 0-300 m. The slide positivity rates of the four villages three years ago were high; but they decreased to 11.9% and 10.7% in Aceituno and Milagro, respectively, while in Empalizada and Sipacate, they remained over 20% in 1985.

Collection of blood specimens and spleen examination

The surveys were carried out in the rainy season of November 1986 in Republic of Guatemala. We requested the residents of all ages to assemble in each village. Three drops of blood were taken by the finger prick method. The first drop of blood was for thick film to examine malaria parasite. The next drop of blood was placed on cellulose paper for G6PD test. The last drop was taken with a heparinized capillary tube and dropped in physiological saline for the determination of Duffy blood group. Spleen examination of the children under nine years old was done on the standing position. The spleen size was classified according to WHO criteria (1963).

Parasite examination

Thick films were stained with methylene blue and Giemsa solution (pH 7.4), and in each slide, 100 microscopic fields under oil immersion were examined. The parasite density was recorded as follows; (4+): over 201 parasites in one microscopic field, (3+): 21 to 200 parasites in one microscopic field, (2+): 2 to 20 parasites in one microscopic field, (+): one parasite in one microscopic field, (+/2): 41 to 60 parasites in 100 microscopic fields and (Number): one to 40 parasites in 100 microscopic fields.

G6PD test

G6PD test was done by the method of Fujii *et al.* (1984).

Table 2 Age group and malariometric survey in six localities in Guatemala

Age group	Spleen examination			Blood examination		
	No. of persons examined	Enlarged case	Spleen rate (%)	No. of slides examined	Positive case	Parasite rate (%)
0-11 months	83	0	0	83	1	1.2
12-23 months	82	0	0	82	2	2.4
2-4 years	228	1	0.4	228	6	2.6
5-9 years	366	0	0	366	6	1.6
10-14 years	—	—	—	210	7	3.3
15 and over	—	—	—	541	9	1.7
Total	759	1	0.1	1,510	31	2.1

Duffy blood group

The Duffy blood group was determined by the agglutination of erythrocytes by rabbit anti-human immunogloblin (MBL, Japan) after incubation of the red cells at 37°C with anti-Fy^a and anti-Fy^b sera (Biotest Diagnostics, West Germany). Gene frequencies of Fy^a, Fy^b and fy were estimated by a gene counting method of Yasuda (1968).

RESULTS

Malaria infections

Spleen examination was carried out in 759 children under nine years old. Only one child of two years old had a large spleen (class III by WHO criteria, 1963). *P. falciparum* gametocyte was positive in his blood with a parasite density five in 100 microscopic fields.

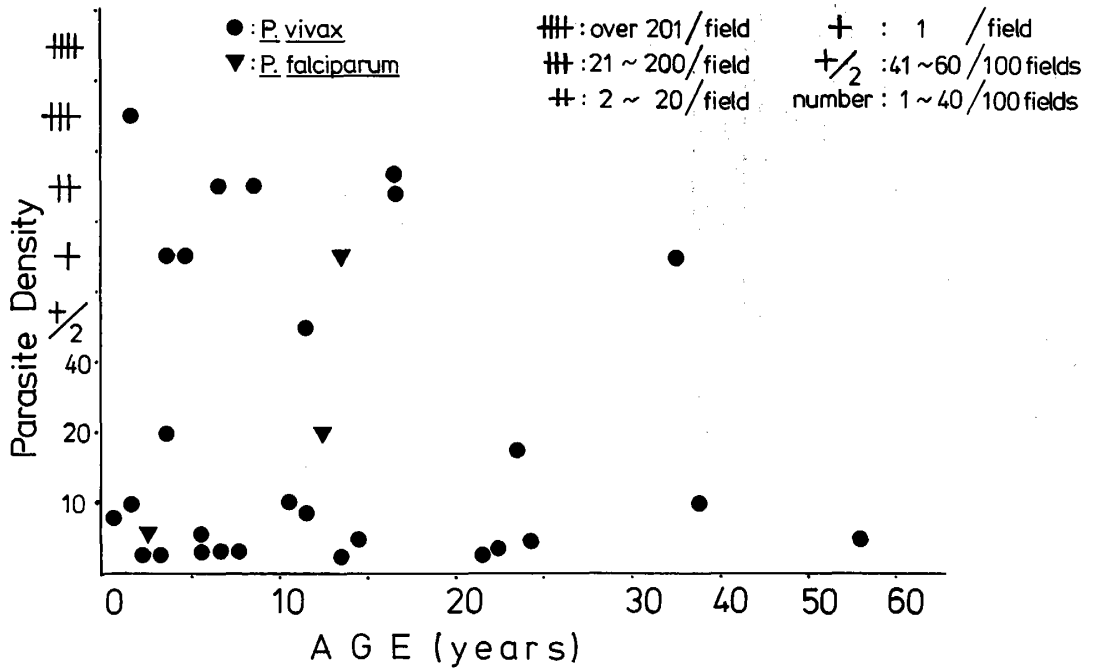

Figure 2 Correlation between age and parasite density.

The spleen rate of the children under nine years old was 0.1% (Table 2).

Thirty-one out of 1,510 blood specimens were found as malaria positive. The parasite rate was 2.1%. Three persons were infected with *P. falciparum* and 28 persons with *P. vivax*. The patients with high parasite density were observed in the young generation under 20 years old (Figure 2).

In 83 infants, one malaria patient was found. The infant parasite rate was 1.2%. There was no significant difference in the parasite rates between the age groups (Table 2). In six localities, the parasite rates ranged from 0 to 6.3%. The parasite rate was 0% in two localities where the slide positivity rate was relatively low in 1985. There was a slight correlation between the parasite rates and the slide positivity rates in the six localities ($r=0.568$) (Table 1, 3).

G6PD

In 567 males, three persons were detected to be G6PD deficient (0.5%). No person was G6PD deficient in 943 females (Table 4).

Table 3 Spleen rate and parasite rate obtained by malariometric survey in six localities in Guatemala

Locality	Spleen examination			Blood examination		
	No. of persons examined	Spleen enlarged	Spleen rate (%)	No. of slides examined	Positive case	Parasite rate (%)
Cubilguitz	129	0	0	253	10	4.0
Chicanuz	165	0	0	271	2	0.7
Aceituno	117	0	0	241	0	0
Milagro	148	0	0	323	0	0
Empalizada	95	1	1.1	221	14	6.3
Sipacate	105	0	0	201	5	2.5
Total	759	1	0.1	1,510	31	2.1

Table 4 Detection of glucose-6-phosphate dehydrogenase deficiency in six localities in Guatemala

Locality	Male		Female	
	No. examined	No. G6PD deficient (%)	No. examined	No. G6PD deficient (%)
Cubilguitz	104	0 (0)	149	0 (0)
Chicanuz	82	0 (0)	189	0 (0)
Aceituno	91	1 (1.1)	150	0 (0)
Milagro	138	2 (1.4)	185	0 (0)
Empalizada	83	0 (0)	138	0 (0)
Sipacate	69	0 (0)	132	0 (0)
Total	567	3 (0.5)	943	0 (0)

Table 5 Distribution of phenotypes of Duffy blood group in six localites in Guatemala

Locality	No. of cases examined	Phenotype Fy(a+b−)	Fy(a+b+)	Fy(a−b+)	Fy(a−b−)
Cubilguitz	100	63	13	5	19
Chicanuz	100	73	10	8	9
Aceituno	100	70	18	3	9
Milagro	100	54	13	18	15
Empalizada	100	38	19	21	22
Sipacate	100	34	35	15	16
Total	600	332 (55.3%)	108 (18.0%)	70 (11.7%)	90 (15.0%)

Table 6 Distribution of phenotypes of Duffy blood group in malaria positives

Species	No. of cases examined	Phenotype Fy (a+b−) (%)	Fy (a+b+) (%)	Fy (a−b+) (%)	Fy (a−b−) (%)
P. vivax	28	16 (57.1)	5 (17.9)	7 (25.0)	0 (0)
P. falciparum	3	1 (33.3)	0 (0)	2 (66.7)	0 (0)
Total	31	17 (54.9)	5 (16.1)	9 (29.0)	0 (0)

Duffy blood group

In each locality, 100 selected persons were examined for the Duffy blood group. In each locality, the distribution of four phenotypes of the Duffy blood group was slightly different (Table 5). As a whole, 85.0% of the residents were Duffy positive and 15.0% were Duffy negative. The gene frequencies of Fy^a, Fy^b and fy were estimated to be 47.3%, 15.8% and 36.9%, respectively.

The Duffy blood group was determined in all persons who were malaria positive (Table 6). No person belonged to the phenotype, Fy (a−b−). It was statistically significant that all 28 persons with *P. vivax* were Duffy positive ($p < 0.025$).

Discussion

There was a slight correlation between the parasite rates and the slide positivity rates in the six localities. The higher the slide positivity rate, the higher was the parasite rate. We can estimate malaria endemicity using the slide positivity rates. In Aceituno and Milagro, no parasite carrier was found, whereas the slide positivity rates were over 10% in 1985. It is difficult to believe that malaria had been eradicated within one year in those villages. We concluded that malaria endemicity differed in each village and the blood samples were collected in the area where malaria was not endemic at the period. Kanbara and Panjaitan (1983) reported in North Sumatra, Indonesia that the parasite rates were different among sub-villages in the same village with 6,000 population; they ranged from zero to 26% in the same month.

The Duffy blood group system consisting of four phenotypes, Fy (a+b−), Fy (a+b+), Fy (a−b+) and Fy (a−b−) is defined by two antisera, anti-Fy^a and anti-Fy^b. Duffy negative erythrocytes, Fy (a−b−), are not agglutinated by either antiserum. The Duffy negative phenotype frequently occurs in blacks. The blacks with Duffy negative erythrocytes cannot be infected by the human malaria parasite *P. vivax* (Miller *et al.*, 1976). Duffy negative human erythrocytes are resistant to invasion *in vitro* by monkey malaria parasites (Miller *et al.*, 1975). It has been postulated that the molecule serving as the antigen in the Duffy positive individuals serves the malaria parasite as a site of attachment or penetration on the surface of the erythrocyte.

In the fields, the parasite rate of *P. vivax* was reported to be zero or lower in the Duffy negative group than in the Duffy positive group (Welch *et al.*, 1977; Spencer *et al.*, 1978; Mathews and Armstrong, 1981). Our results suported those reports mentioned above. In 28 patients with *P. vivax,* all were Duffy positive. It was extremely unlikely that all the 28 patients would be Duffy positive by chance alone ($p < 0.025$). This fact is similar to that reported by Miller *et al.* (1978). They determined blood group phenotypes of 13 American blacks who were infected with *P. vivax* in Vietnam. All were Duffy blood group positive as compared to 40 to 50% Duffy positive in American blacks.

The Duffy type is different in distribution among races. Gene frequencies of Fy^a, Fy^b and fy are 5.3%, 12.2% and 82.5%, respectively in New York Negros (Sanger *et al.*, 1955); 43.5%, 56.5% and 0%, respectively in Caucasians (Chown *et al.*, 1965). They are 89.8%, 10.3% and 0.9%, respectively in Japanese (Nakajima, 1971). When we compare these data with our result (47.3%, 15.8% and 36.9%), Guatemalan dose not belong to any of the races mentioned above.

G6PD deficiency is said to be few in Central America (WHO, 1967). We confirmed this tendency since the percentage of G6PD deficiency in male was 0.5%. The prevalence of G6PD deficiency is very low in Caucasians (WHO, 1967) and Japanese (Nakatsuji and Miwa, 1979), however, it is high in Tropical Asia (Panich, 1981; Matsuoka *et al.*, 1986) and Africa (WHO, 1967). Guatemalan is similar to Caucasians or Japanese so far as the prevalence of G6PD deficiency is concerned.

Primaquine, which is widely used for malaria patients in Guatemala, has a side effect of hemolysis in some of G6PD deficiency (WHO, 1981). We have no experience of the patient with hemolytic crisis caused by primaquine in Guatemala. Probably it is due to the low prevalence of G6PD deficiency. However, we should continue to monitor the side effect of primaquine through collaborative volunteers of malaria teams, health centers and hospitals.

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

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

1,510名から採血を行い，31名に原虫陽性者を得た（2.1%）。*P. falciparum* 3例，*P. vivax* 28例であった。村落別では原虫陽性率は最高6.3%であり，2カ村で0%であった。新生児原虫陽性率は1.2%であった。年齢層別では小児・成人とも原虫陽性率に差を認めなかった。血液中の原虫濃度の高い者は若年層で多くみられた。脾腫は10歳未満の小児759名中1例のみであった（0.1%）。G6PD欠乏者は男子567名中3名(0.5%)にみられたが，女子には認めなかった。Duffy血液型はFy (a+b−)55.3%，Fy (a+b+)18.0%，Fy (a−b+)11.7%，Fy (a−b−)15.0%であった。gene frequencyは，Fy^a 47%，Fy^b 16%，fy 37%であった。三日熱マラリア陽性者28名は全員Duffy血液型陽性であり，これは統計的に有意であった（$P<0.025$）。

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A NEW METHOD TO DETECT *STRONGYLOIDES STERCORALIS* FROM HUMAN STOOL

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Abstract: A new method for the detection of *Strongyloides* larvae was established. A small amount of stool was placed in the center of an agar plate and was incubated at 37°C for 24 hr. Characteristically aligned bacterial colonies or furrows left by crawling *Strongyloides* larvae appeared on the agar surface are the positive findings. The larvae gathered in a well made on positive plate were identified. By using this method, *Strongyloides* was detected in 46 cases (4.5%) out of 1,017 healthy adults. Whereas, it was detected in 0 and 3 cases (0 and 0.3%) by direct stool smear method and filter paper technique, respectively. Examination of 246 cases by this agar plate method and formalin-ether method (MGL) revealed that 14 cases (5.7%) were positive by the former and 2 cases (0.8%) by the latter. Agar plate method is not laborious nor expensive, and recommendable for mass examination and for the detection of asymptomatic carriers.

INTRODUCTION

Strongyloides stercoralis is a well known parasitic nematode as the etiologic agent of human strongyloidiasis. This parasite is mainly distributed in the tropical and subtropical areas, and infection rate up to 20% or more among inhabitants has been reported (Beaver *et al.*, 1984). In Japan, the endemic areas are located in southern part of Kagoshima and whole Okinawa Prefecture (Tanaka, 1966) where fatal cases are occasionally found. There has been no satisfactory method for detection of *Strongyloides* larvae from human stool (cf. Asato *et al.*, 1984). For diagnosis of strongyloidiasis, the test tube culture method (Harada and Mori, 1955) has been recommended (Tanaka *et al.*, 1958). However, recent works have claimed that this method is not so satisfactory as has been believed (Asato *et al.*, 1984).

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Although some immunological techniques have been developed (Neva *et al.*, 1981; Sato *et al.*, 1984), their value is still limited to screening test.

On bacteriological examination of the stools in Okinawa, the authors have occasionally noticed a network of furrows with bacterial colonies on the surface of agar plate media. Subsequently it was proved that these furrows were left by the crawling *Strongyloides* larvae. This phenomenon suggested a new diagnostic method for strongyloidiasis using agar plate. This paper describes the value of the agar plate method for the detection of *Strongyloides* in comparing with some traditional methods.

Materials and Methods

Stool specimens: Stools collected from 1,017 adults visited Center for Preventive Medicine, Okinawa, for medical examination were used. The donors of stool specimens were healthy at the time of sampling without any remarkable diseases. The stools were examined within the day of sampling, but the stools for examination with MGL (formalin-ether sedimentation technique) were stocked in a refrigerator for 1 to 4 weeks before examination.

Detection of larvae: All specimens were examined by the agar plate method, traditional direct smear and test tube culture methods. Randomly selected 246 samples were also examined with MGL method.

Agar plate method: Meat extract agar plates media (E-MC01, Eiken Co., Tokyo) for bacteriological examination were used. Finger head-sized stool was placed at the center of a plate, and was incubated at 37°C for 24 hr or more. After incubation, the plates were examined. The plates with aligned bacterial colonies and/or furrows were searched for crawling larvae under low magnification microscope (40×).

Traditional methods: The examinations with direct smear, test tube culture and MGL method were carried out by routine procedures (Harada and Mori, 1955; Ritchie, 1948).

Results

Forty-six (4.5%) out of 1,017 stool samples were found positive for *Strongyloides* by the agar plate method. Characteristic alignment of bacterial colonies and furrows left by crawling rhabditoid and/or filariform larvae were clearly observed on agar plates (Fig. 1). On the other hand, no larva was demonstrated by the direct smear method, and only 3 samples (0.3%) were positive for *Strongyloides* by the filter paper cultures. Among 246 samples examined with both agar plate and MGL method, only 2 (0.8%) were positive by MGL method, whereas *Strongyloides* was detected in 14 samples (5.7%) by the agar plate method (Table 1). The agar plate method detected *Strongyloides* larvae from all the samples which were positive by the other methods. When a well was made on the agar plate with positive findings (aligned bacterial colonies or furrows) and filled with water or physiological saline solution, the larvae gathered around the well and entered the water. The larvae moving in the water were easily collected by using a pipette to be identified. In several cases, adult free-living worms were observed on the agar plate. In such cases, oviposition and hatching were seen.

In 2 out of positive 46 cases, aligned bacterial colonies and/or furrows were observed at 48 hr although they were not discernible at 24 hr. Incubation more than 48 hr did not result

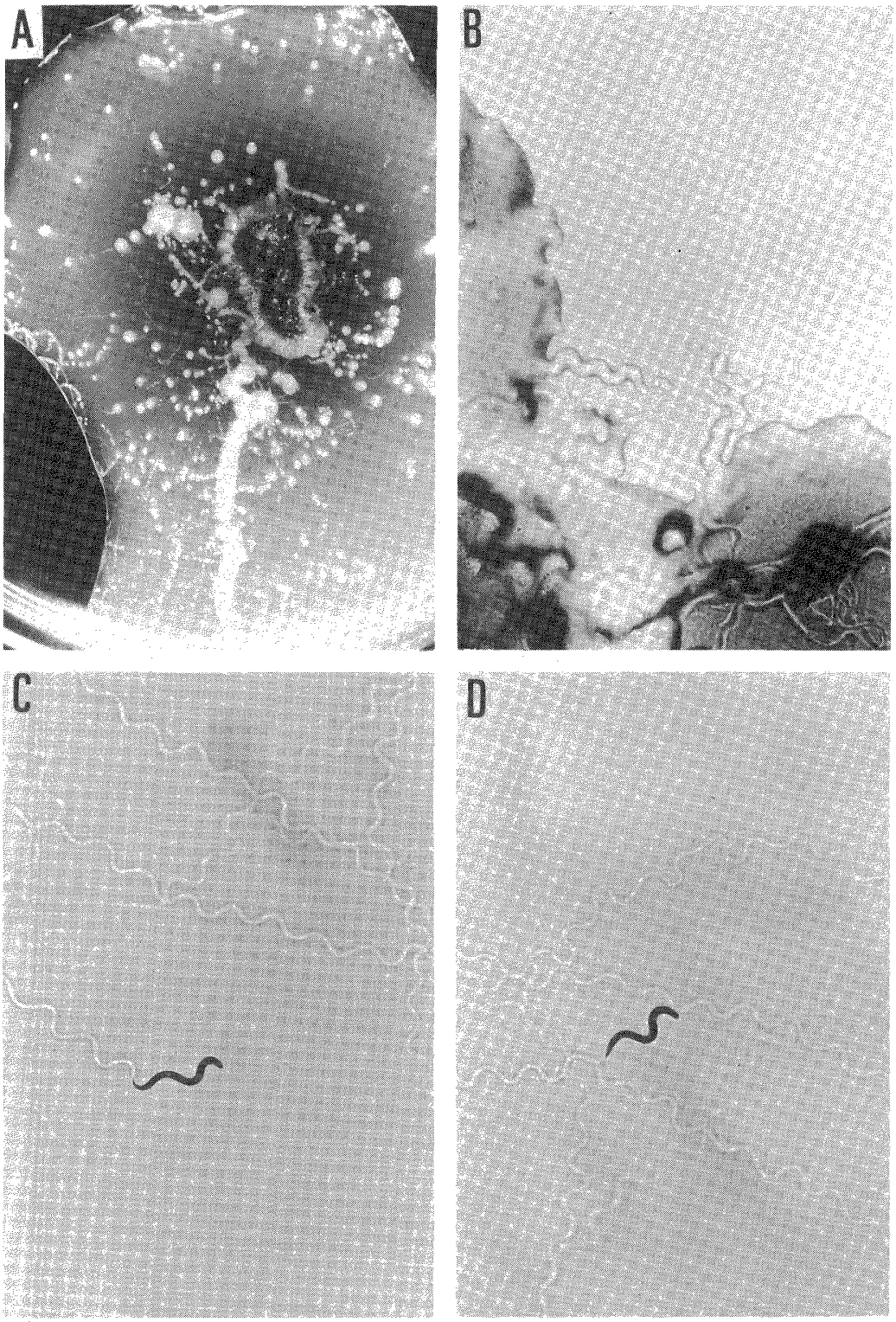

Figure 1. Positive findings found on agar plate culture of stools contaminated with *Strongyloides stercoralis* larvae.
A : Aligned bacterial colonies formed around the stool.
B : Enlarged view of the aligned bacterial colonies.
C, D : *Strongyloides* larvae and furrows left by them on the agar plate.

Table 1 Detection rate of *Strongyloides*

Methods	Number of stools examined	Number of positive cases (%)
Direct smear	1,017	0
Filter paper	1,017	3 (0.3)
Agar plate	1,017	46 (4.5)
MGL	246	2 (0.8)
Agar plate	246	14 (5.7)

in the increased detection rate. No parasite other than *Strongyloides stercoralis* was found by any of the methods employed in the present survey.

Discussion

The results of the present study have clearly shown that the detection rate of *Strongyloides stercoralis* from human stools by the agar plate method was markedly higher than those by traditional methods. It is apparent that the detection of *Strongyloides* larvae depends on the number of worms in the stool specimens primarily. When the number of worms in the stool sample was very few (for example only one worm), it must be so difficult to find it out by the traditional methods (cf. Asato *et al.*, 1984). But only one worm left a network of furrows by crawling on the agar surface and developed aligned bacterial colonies. Therefore, it is possible to find a very small number of worms by the agar plate method as long as the worms are alive. Considering this phenomenon, most of the stools examined in the present study were supposed to be only slightly contaminated with *Strongyloides*, because the detection rate by the traditional methods was very poor. If the number of worms in the stool was so many as easily detectable even by direct smear method, the detection rate by any methods is supposed to be similar. Therefore, the agar plate method is especially significant in the examination of asymptomatic carriers.

It has been well known that many soil and plant parasitic nematodes are readily cultured on agar plate (Yokoo, 1959). In this viewpoint, it is quite reasonable that *Strongyloides*, which is phylogenetically close to the free-living rhabditoids, is developed on agar plate. Panosian *et al.* (1986) found *Strongyloides stercoralis* on agar plate media in the routine laboratory work of bacteriological examination. They suspected the presence of *Strongyloides* because aligned bacterial colonies displaced from the streak marks were developed. However, as far as the authors are aware of, no attempt has been made to utilize agar plate for the diagnosis of strongyloidiasis.

In Okinawa Prefecture, the test tube culture method has been mainly used for *Strongyloides* detection, and the infection rate has been believed to be less than 2% (Center for Preventive Medicine, Okinawa, 1983). However, Asato *et al.* (1984, 1985) demonstrated, by using a combination of MGL and test tube culture methods, that the rate was much higher. Since the present results proved that the agar plate method is more efficient than MGL and test tube culture methods, it is suggested that the exact prevalence may be surprisingly higher than that believed so far. As agar plate method is not laborious nor expensive, its application

should be recommended in epidemiological survey.

Recently, strongyloidiasis has attracted special interests as opportunistic agent in immunocompromised condition such as AIDS (Ndayiragije and Matheron, 1985). Moreover, its special relationship with adult T-cell leukemia (ATL) has been stressed (Nakada *et al.*, 1984, 1987). The unstable results of fecal examination with traditional methods might have disturbed these researches. The agar plate method may also contribute to laboratory research of strongyloidiasis.

Since living larva in the stool sample is indispensable for detection by agar plate method, stool samples should be stored in an appropriate condition until examination. It has been well documented that some free-living rhabditoid nematodes such as *Rhabditis hominis* are occasionally contaminated in human feces. Asato *et al.* (1985) found *R. hominis* in 2 cases (0.09%) of 2,176 inhabitants examined in Itoman-City, Okinawa. It is probable that such species may also developed on agar plate. Therefore, species identification is necessary to certify the diagnosis. Instead of making wells in agar plate to collect the worms, we usually used small amount of agar media (10 m*l* or less per plate). The Pettri-dish was not completely covered with this amount of agar, and some agar-defect-area (hollow) appeared in the plate. The water was poured into that hollow of the plate with positive findings.

On proceeding the present study, some interesting facts were noted. When fungal colonies developed on the agar plate, the larvae of *Strongyloides* did not come close to the fungi. Although the fungus was not identified, there is a possibility to get some anti-*Strongyloides* agent from this fungus. Some cases with numerous furrows but without any bacterial colonies were occasionally seen. This is probably due to antibiotics taken prior to stool sampling.

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糞便中からの糞線虫の新しい検出法

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普通寒天平板培地を用いた糞線虫の新しい検出法を開発したので，その有用性を報告する。指頭大の糞便を寒天平板培地の中央に置き，37℃で24時間培養する。糞線虫陽性例では，寒天平板上に糞線虫が這った後に残された轍や，その後に増殖した細菌コロニーの特徴的な線状配列を認めることができる。幼虫の同定に関しては，陽性所見のある平板に穴(well)を作製し，水を満たすと幼虫は水中に集まってくるので，それを吸い上げて鏡検し確かめた。検査結果の内訳は，人間ドック受診者1,017人中，陽性例が寒天平板法46人（4.5%），直接塗抹法0人（0%），濾紙培養法3人(0.3%)であった。そのうち，ランダムに抽出した246人についてMGL法を用いて検査したが，陽性率は寒天平板法14人（5.7%），MGL法2人（0.8%）であった。以上の結果から，寒天平板法が従来の方法に比し，極めて高い検出率を示すことが知られた。本法は，手技が容易でかつ安価であり，特に健康保虫者のスクリーニングに有用であると思われる。

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HEPATIC HYDATIDOSIS IN MAN AND HIS LIVESTOCKS IN SOUTHERN IRAQ

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Abstract: Human hepatic hydatidosis was studied in surgically operated 61 cases at Basrah Hospitals in 1985. The age of the patients varied from 6 to 75 years. The ratio of rural to urban patients was 42 to 19. There were 31 females and 30 males.

A survey on animal hepatic hydatidosis at Basrah abattoirs during the first 6 months of 1985 was carried out. A total of 57,255 slaughtered animals was inspected and the results revealed that 8.4% of 38,398 sheep, 3.7% of 16,229 cattle and 5.6% of 2,628 buffaloes were found to be infected.

Prevalence of hydatidosis is considered to be high in Middle East countries (Mattosian *et al.*, 1977). Many workers have proved that the disease is endemic (Al-Jeboori, 1976; Mahmoud, 1980; Al-Sakkal, 1982) and enzootic (Senekjie and Beattie, 1940; Imari, 1954; Babero *et al.*, 1963; Mubarek, 1978; Mahmoud, 1980; Al-Abbassy *et al.*, 1980) in nature in the central and northern parts of Iraq.

Sixty one cases of human hepatic hydatidosis operated during 12 months period of 1985 were studied in Basrah Hospitals, southern Iraq (Table 1). Age of patients varied from 6 to 75 years with mean of 31.2 ± 16.9 years. Male patients were slightly younger (30.3 ± 15.7 years) than females (32.0 ± 18.4 years). There were 31 females and 30 males. Examinations and background of 61 cases are shown in Table 2.

Animal hepatic hydatidosis was determined by the inspection of 57,255 livers belong to 38,398 sheep, 16,229 cattle and 2,628 buffaloes slaughtered at Basrah abattoirs during the first 6 months of 1985. The infection retes were 8.4%, 3.7% and 5.6% for sheep, cattle and buffaloes respectively (Table 3).

The present study indicates that the hepatic hydatidosis is endemic and enzootic in natural environment in the southern Iraq.

The maximum prevalence observed was among patients in ages 30s' and 40s'. In males, the prevalence was highest in age 40s', while in females it was noticed in age 30s'. Almost 25% of all cases occurred in patients under the age 19. But 47.5% of cases were detected among patients of age class of 20 to 39. These findings are in agreement with results of other

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Table 1 Age distribution of patients which were operated on for hepatic hydatidosis and confirmed surgically in 1985

Age (years)	No. of patients		Total
	Females	Males	
0 - 9	4	3	7
10-19	2	6	8
20-29	11	4	15
30-39	5	9	14
40-49	2	4	6
50-59	4	3	7
60-69	1	1	2
70-79	2	0	2
Total	31	30	61

Table 2 Surgically confirmed 61 cases of human hepatic hydatidosis in 1985

Residency	Uraban	19	Rural	42
Liver lobe	Right	44	Left	10
	Both	7		
Casoni test	Positive	23	Negative	21
	Not done	17		
Ultra sound test	Positive	22	Not done	39
Dog ownership	Yes	41	No	20

Table 3 Prevalence of hepatic hydatidosis among slaughtered animals at Basrah abattoirs during the first 6 months of 1985

Species	No. examined	Livers infected	% of total	% of cyst fertility rate
Sheep	38,398	3,221	8.4	67.7
Cattle	16,229	598	3.7	10.6
Buffaloes	2,628	147	5.6	12.9

workers (Al-Jeboori, 1976; Mahmoud, 1980; Al-Sakkal, 1982). They concluded that the old aged people are more prone to this type of infection. It has also been reported that most hydatid cysts are acquired in the childhood (Beard, 1978). This may be due in part to greater susceptibility of man to develop the infection as compared with animals (Schwabe *et al.*, 1959).

In this investigation, females and males are about equally infected. Similar sex distribution was observed in Yougoslavia (Suice, 1957) and Lebanon (Schwabe and Abo-Daoud, 1961). This is possibly due to the existence of some epidemiological factors such as socio-cultural and occupational risks.

It is well known that liver is most frequently affected. In this study, about three quarters of hepatic cysts are found in the right lobe of the liver (Table 2).

Casoni test was positive in 52.3% of examined cases (Table 2). However, test sensitivity may depend on the location and condition of the cyst (Mahmoud, 1980).

Results also showed that the most frequent occurrence of animal hepatic hydatidosis was in sheep (8.4%), followed by buffaloes (5.6%) and cattle (3.7%) (Table 3). Similar observations were recorded in the central and northern parts of Iraq (Imari, 1954; Babero *et al.*, 1963; Mahmoud, 1980; Al-Abbassy *et al.*, 1980). In contrast, Senekjie and Beattie (1940) and Mubarek (1978) have reported higher prevalence in cattle than in sheep. This distribution probably correlates with the degree of keeping dogs within the herds of sheep and cattle.

The high prevalence detected among aged slaughtered animals (2-7 years) can be attributed to the slow development of cysts (Schantze *et al.*, 1977).

From the epidemiological point of view, sheep are considered to be a potential source of canine infection as they harbour mostly fertile cysts (Benyan and Mahdi, 1987).

These results together with those observed in our previous work (Benyan and Mahdi, 1987) contributes to a better understanding of the epidemiological situation of hepatic and pulmonary hydatidosis in southern Iraq.

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ハブ毒による血圧降下と2，3の薬物の抑制効果

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緒　言

環境衛生の発達と都市化にもかかわらず奄美大島や沖縄県下には現在なお毎年数百名のハブ咬傷者の発生をみ，そして抗血清の開発と普及にもかかわらず今なお約1％の犠牲者を出している（井上ら，1982)。

その死因についてはすでに諸家によりさまざまな検討がなされているが，今なお不明な点が多い（沢井，1960；館野ら，1960)。

ハブ咬傷による局所変化は出血，腫張，筋壊死であり，全身性には重篤な場合には死に至る。逢坂らは出血因子と致死因子とは別のものと考え，2種の出血因子を分離し（逢坂ら，1968；Omori Satoh and Ohsaka, 1970)，腫張作用とその因子については山川らにより報告され（山川ら，1973; Nozaki *et al.*, 1974)，筋壊死については鎮西によりハブ粗毒より筋壊死因子（myonecrotic factor : MNF)の分離が報告された(Chinzei, 1974；鎮西，1987)。

今回はこのMNFを用いてその生体反応に及ぼす影響について検討した。

材料および方法

1．動物および薬物

体重7-15kgの雌雄雑種成犬，および体重400-500gのWistar系雄性ラットを用いた。作用物質および薬物としては奄美大島産ハブ毒と，これから分離したMNF，抗ヒスタミン薬としてジフェンヒドラミンとプロメサジン，カリクレイン阻害薬(トラジロール，三共)，タマサキツヅラフジから抽出されたビスコクラウリン型のアルカロイド（セファランチン，化研生薬）を用いた。

MNFの分離：奄美大島で1969年採毒の凍結乾燥ハブ粗毒を0.15M NaClに1％溶液とし，これに等量の冷アセトンを加えて，−20°C，4時間放置し遠心（8,000×g，−10°C，10分）し，その上清に再び冷アセトンを最終濃度60％量に加え，一晩−20°Cに放置後，遠心(13,700×g，−10°C，30分）この沈殿物を0.15M NaClで溶解し，脱イオン水で透析後凍結乾燥。粗毒1gから，約150mg得られた。

2．方　法

a．麻酔犬の血行動態に及ぼす影響

塩酸ケタミン（ケタラール，三共）12mg/kgを筋肉内に注射し，更にペントバルビタール（ネンブタール，山之内)20mg/kgを静脈内に投与して麻酔した後，気策内挿管を行い人工呼吸器に接続した。

収縮期血圧（SBP)，拡張期血圧（DBP）および平均血圧（MBP）は右股動脈にカテーテルを挿入し，圧トランスデューサー（MPU-0.5，日本光電）を介して電気血圧計（RP-5，日本光電）を用いて測定した。また左腎動脈に電磁流量計のプローブを装着して電磁流量計（日本光電MF 25）で腎血流量を測定した。薬物は右股静脈に挿入したポリエチレンチューブ（PE-60）より注入した。

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b．ラットの血圧に及ぼす影響

薬物の反応を血圧の変動を指標にしてとらえるためにラットを用いて検討した。ペントバルビタール(20 mg/kg)を腹腔内に投与し麻酔した後両側股動静脈を露出しポリエチレンチューブ(PE-160)を右側動脈および左側静脈に挿入し動脈からはイヌの場合と同様にして血圧を測定し，静脈側からは薬物の注入を行った。

c．セファランチンの薬物作用

撲殺したラットの回腸および子宮を用いた。浴槽内の温度は32°C—35°Cに保ち，栄養液はTyrode液を使用し絶えず通気した。反応記録はMagnus法に従い各収縮薬をcumulation法で投与して薬量—反応曲線を得た。

結　果

1．イヌにおける血行動態

イヌにハブの粗毒(2 mg/kg)を静脈内投与した場合の血行動態を図1に，MNF 100 μg/kg(Folin法)を静脈内投与した場合の血圧および腎血流量の変動を図2に示した。血圧の下降と共に腎血流量はわずかに増加しているが，数分間で血圧，腎血流とも投与前のレベルにもどった。この濃度での降圧効果はタキフラキシーを呈し，同一の個体での同一条件による実験結果は再現性に乏しいことが認められた。

2．ラットにおける生体反応

a．図3に示すようにラットにMNF 100 μg/kg(Folin法)を二度にわけて静注するとイヌの場合と同様に急激な血圧下降が惹起されまた脈圧の減少が認められた。血圧がMNF投与前のレベルにもどるにはイヌの場合よりも長時間を要した。ラットの場合にもタキフラキシーが認められた。この血圧降下は図4に示すようにジフェンヒドラミンやプロメタジンのような抗ヒスタミン薬により抑制された。

b．血圧降下時のキニン系の関与を検討する目的で，カリクレイン阻害剤(Trasylol)を投与後，同量のMNFを投与した。図5に示すように血圧降下は抑制されなかった。

c．台湾で古くから毒蛇咬傷の治療に民間療法として用いられてきた防己科の1種(タマサキツヅラフジ)から抽出されたアルカロイド(セファランチン)を5 mg/kg静脈内投与した時の血圧変動を図6に示す。MNF投与によって起こる血圧降下時にセファランチンを投与すると下降した血圧が速やかに正常レベルに回復することが認められた。

d．セファランチンの薬理作用

ラットの子宮およびモルモットの腸管を用いて，ブラジキニン，ヒスタミン，セロトニンの薬量反応曲線を図7—9に示した。いずれも薬量に応じた収縮がみられた。セファランチン1 μg/kgの投与後ブラジキニン，ヒスタミン，セロトニンを投与すると，図10—12に示すようにいずれも収縮が抑制されている。即ちセファランチンには非特異性の抗ブラディキニン，抗ヒスタミン，抗セロトニン作用があることが認められた。

考　察

ハブ毒による死の原因については心筋に対する直接の抑制作用や乳酸性アシドーシスや低血流状態などいろいろな問題が指摘され検討されているがまだ不明の点が多い(寺泉，1958)。今回の実験は強力な筋壊死をひきおこすハブ毒中のある成分が壊死のみでなく強力な血圧降下作用を有することを示した。ハブ毒のイヌにおける50%致死量は筋肉内投与で8.2 mg/kgとされているが(貫ら，1963)本実験では極微量(100 μg/kg)の筋壊死因子で著しい血行動態の変化が認められ，この血圧降下は抗ヒスタミン剤の前処置で抑制されることから肥満細胞からのヒスタミンの遊離によるのではないかと思われた。

貫ら(1963)はイヌにおけるハブ毒の静脈内投与実験の結果から投与量に応じて2つの病態のあることを報告している。すなわち①2—10 mg/kgでは一過性の血圧低下を来たすが一度回復しその後数時間で死亡する。②15 mg/kgでは急速な血圧低下とともに呼吸麻痺により短時間で死に至る。図1，2に示すように今回の実験ではおそらく①に相当するのであろうが死に至るものは1例もな

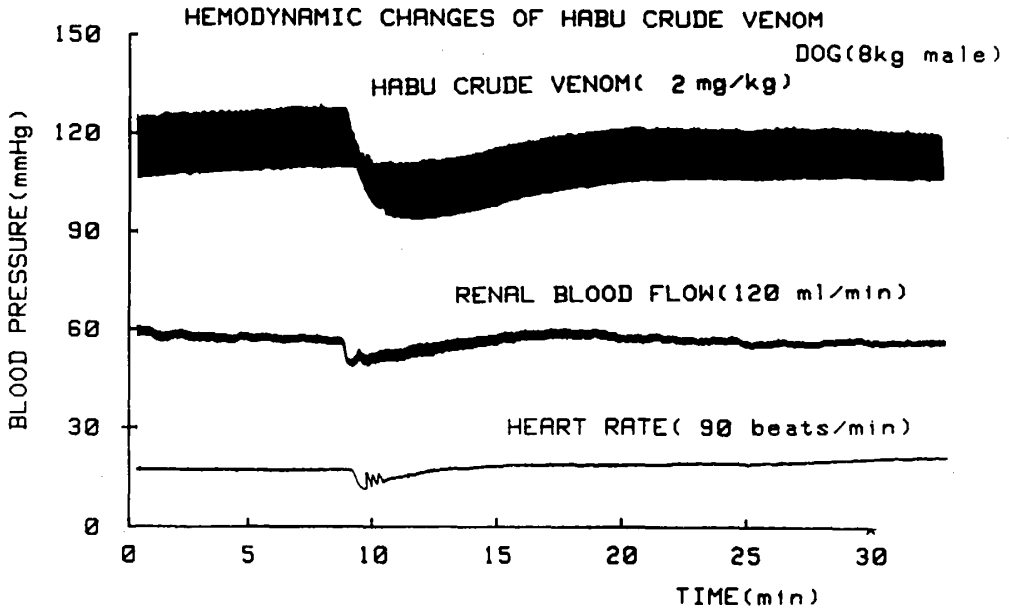

Figure 1 Hemodynamic changes caused by intravenous injection of crude Habu venom (2 mg/kg) in a dog.

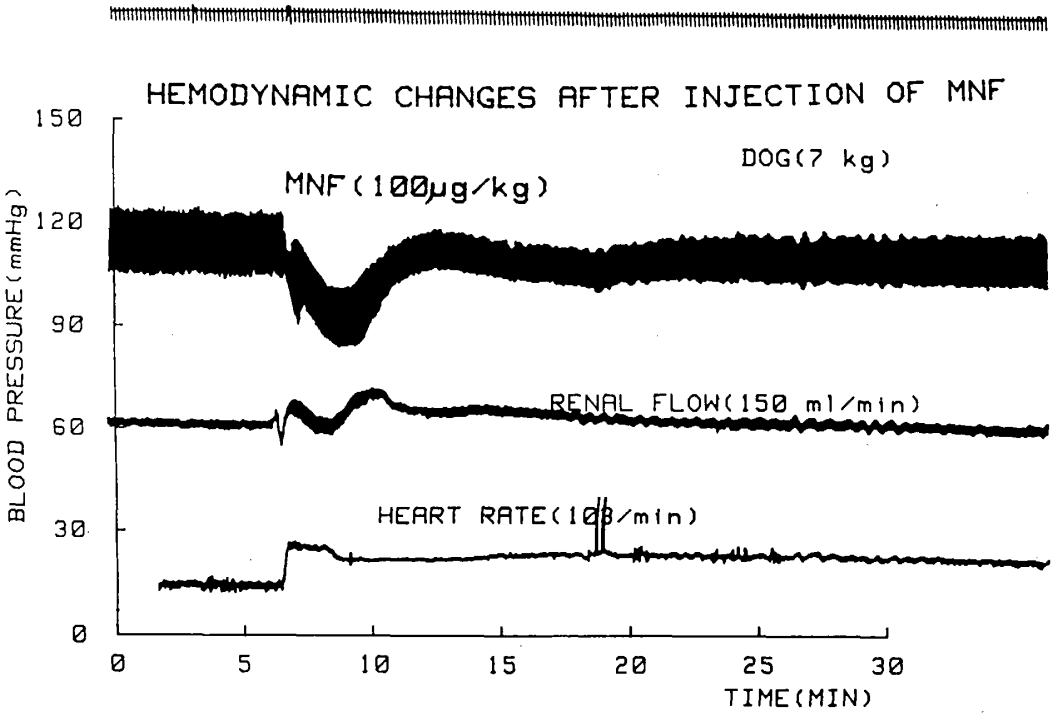

Figure 2 Hemodynamic changes of a dog after intravenous injection of myonecrotic factor (100 μg/kg). Blood pressure, renal blood flow and heart rate are shown.

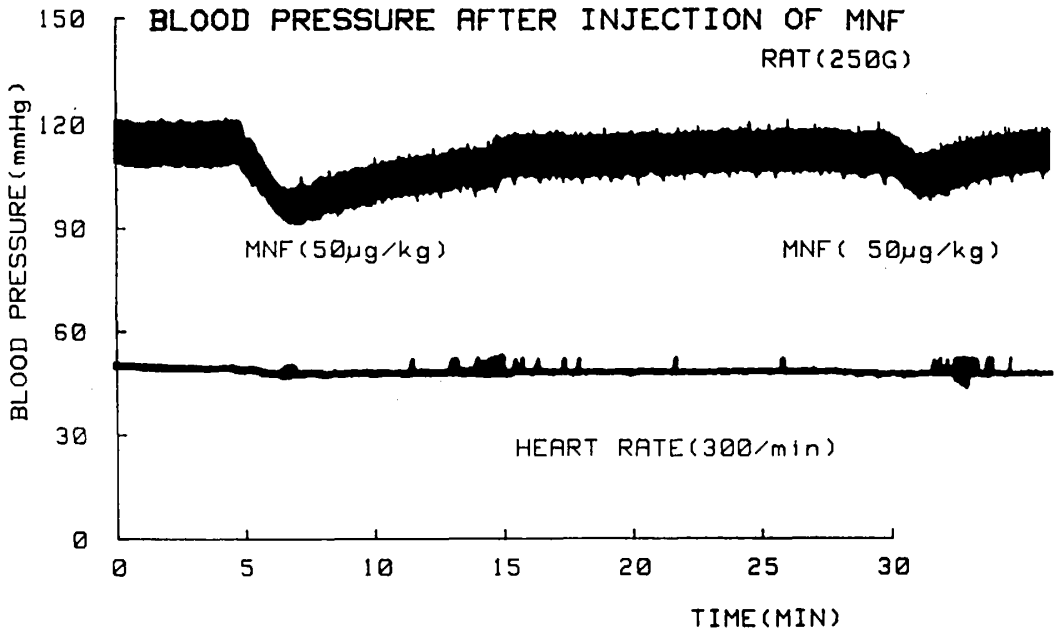

Figure 3 Changes of blood pressure and heart rate of a rat after intravenous injection of MNF. Blood pressure abruptly fell by the first injection, but the degree of hypotension was slight by own second injection.

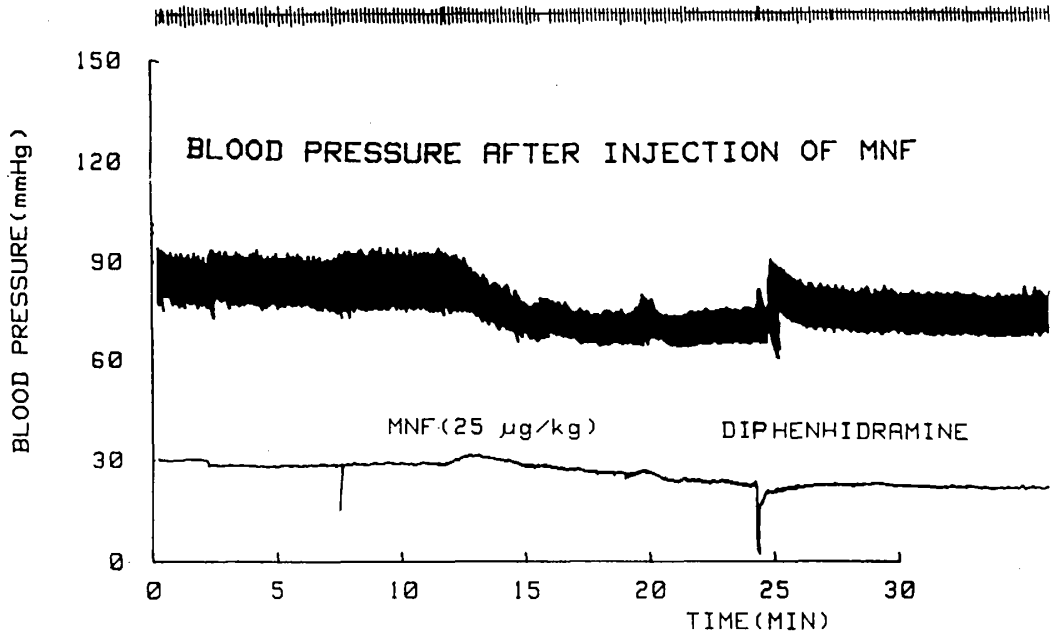

Figure 4 Blood pressure and heart rate of a rat. Hypotension caused by the MNF injection was recovered by the intravenous injection of diphenhidramine (21.5 mg/kg).

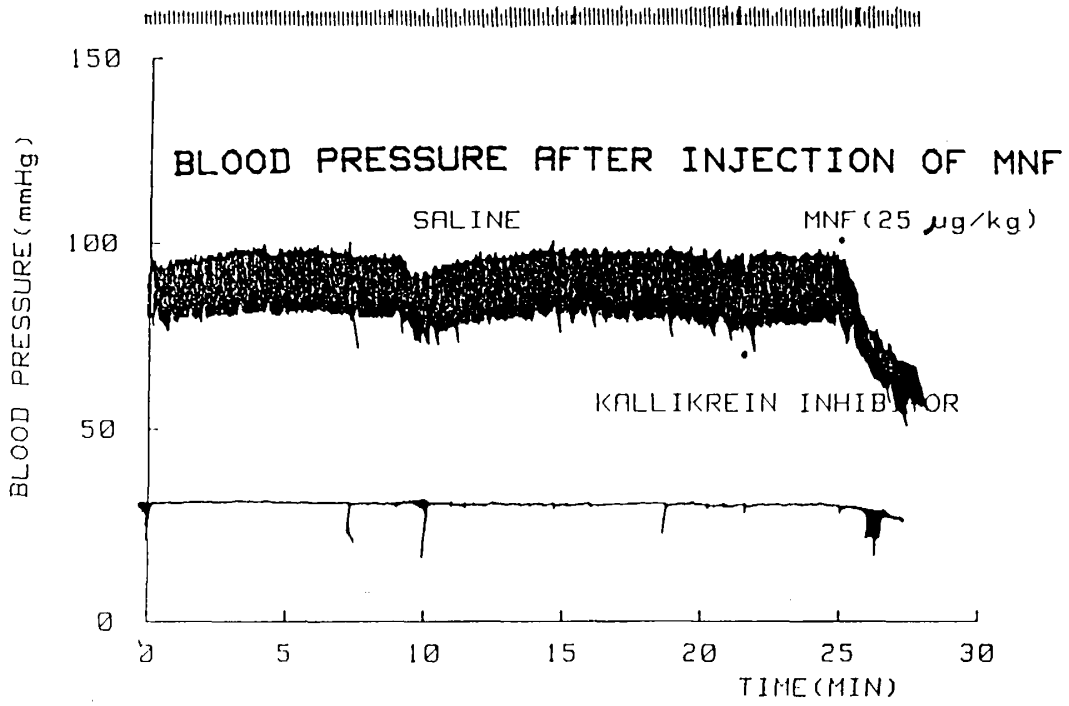

Figure 5 Changes of blood pressure and heart rate of a rat. Kallikrein inhibitor (900 unit/kg) did not inhibit the hypotensive action of MNF.

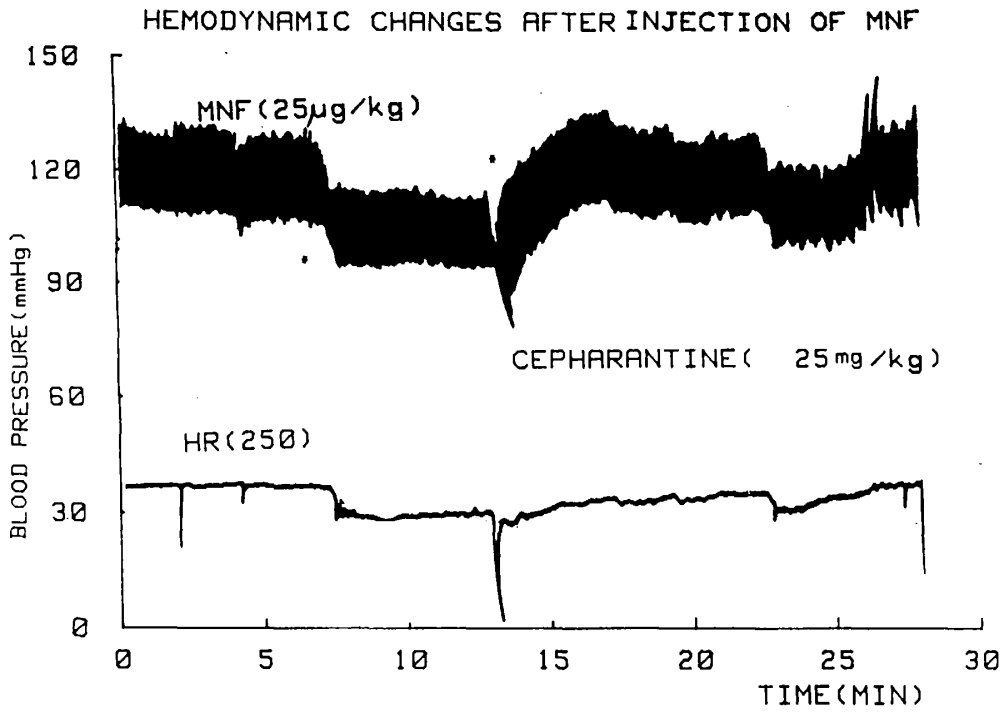

Figure 6 Effect of cepharantine on the hypotensive action of MNF. Blood pressure rose back to the normal level after intravenous injection of cepharantine (25 mg/kg).

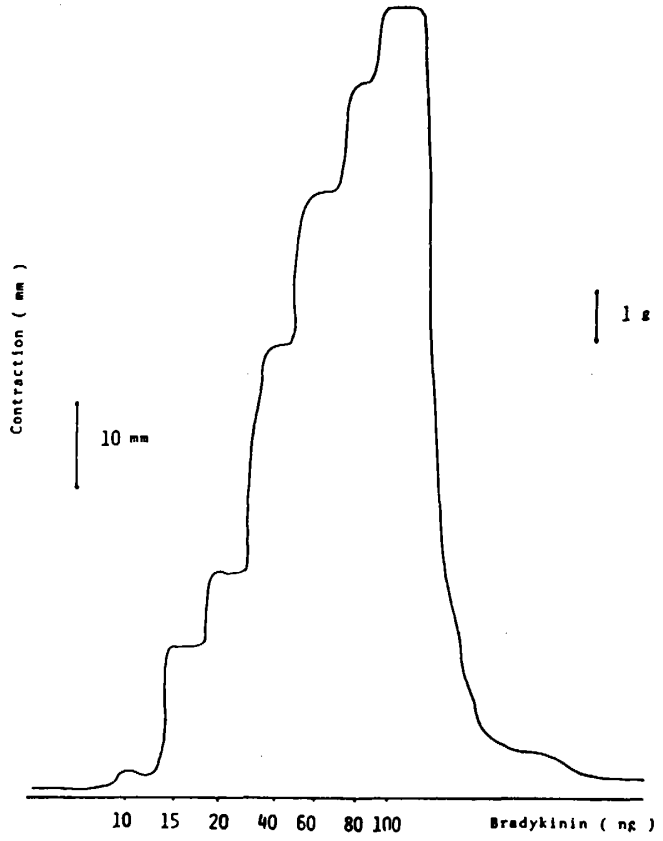

Figure 7 Dose-response curve of bradykinin. Rat uterus showed contraction after bradykinin application in Magnus apparatus.

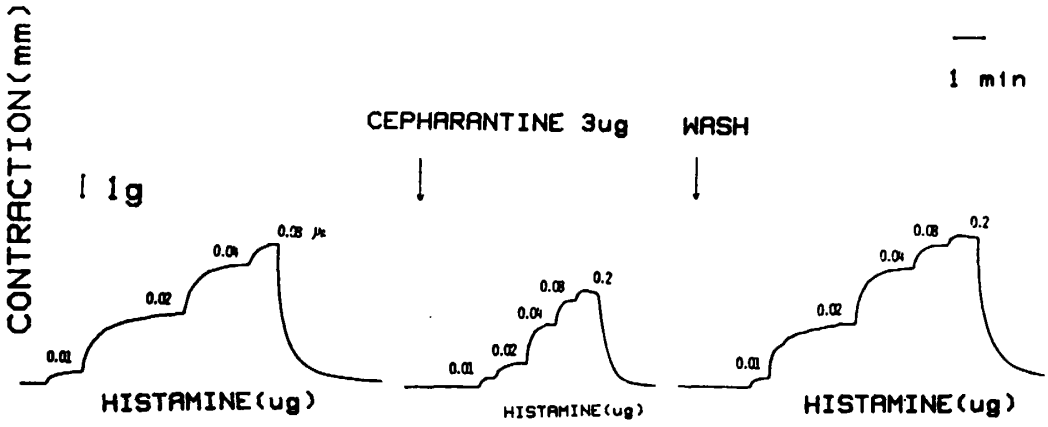

Figure 8 Histamine-induced contraction in the guinea-pig ileum.
Left: Histamine showed dose-dependent contraction.
Middle: Cepharantine (5 μg) was given before histamine administration.
Right: After washing several times, the same dose of histamine was given.

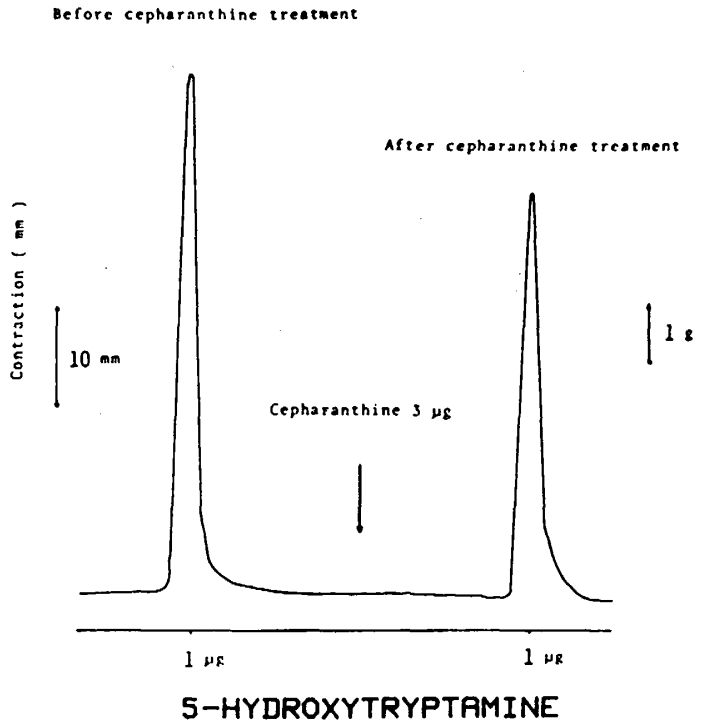

Figure 9 Contraction of guinea-pig ileum by 5-hydroxytryptamine. After cepharantine administration the contraction was suppressed.

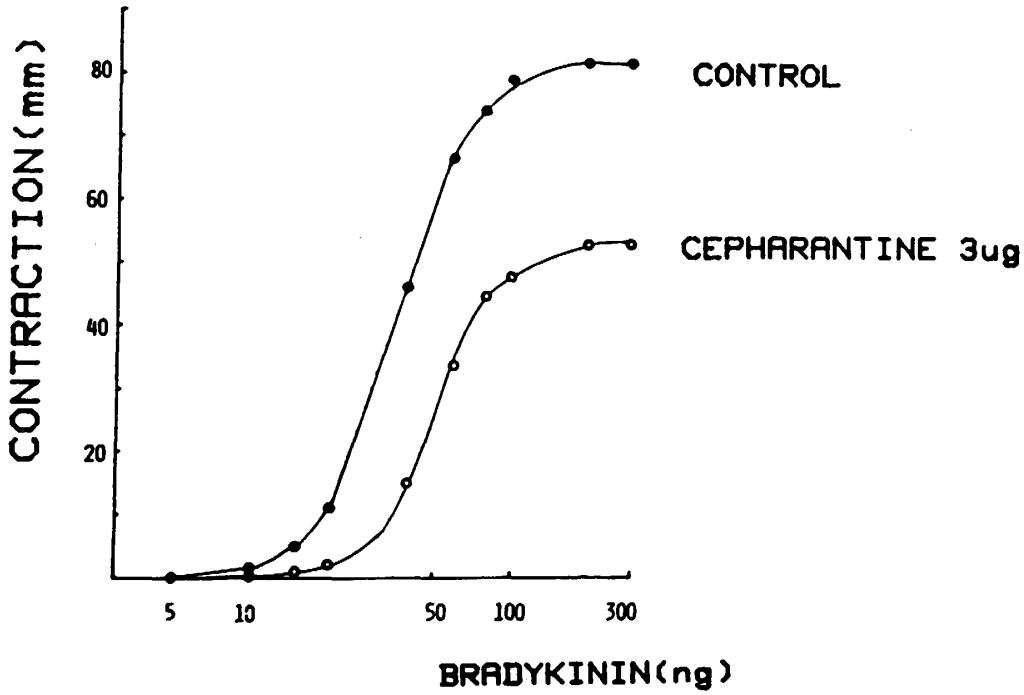

Figure 10 Inhibitory effect of cepharantine on bradykinin-induced contraction of rat uterus.
● : control, ○ : 3 μg of cepharanthine administration.

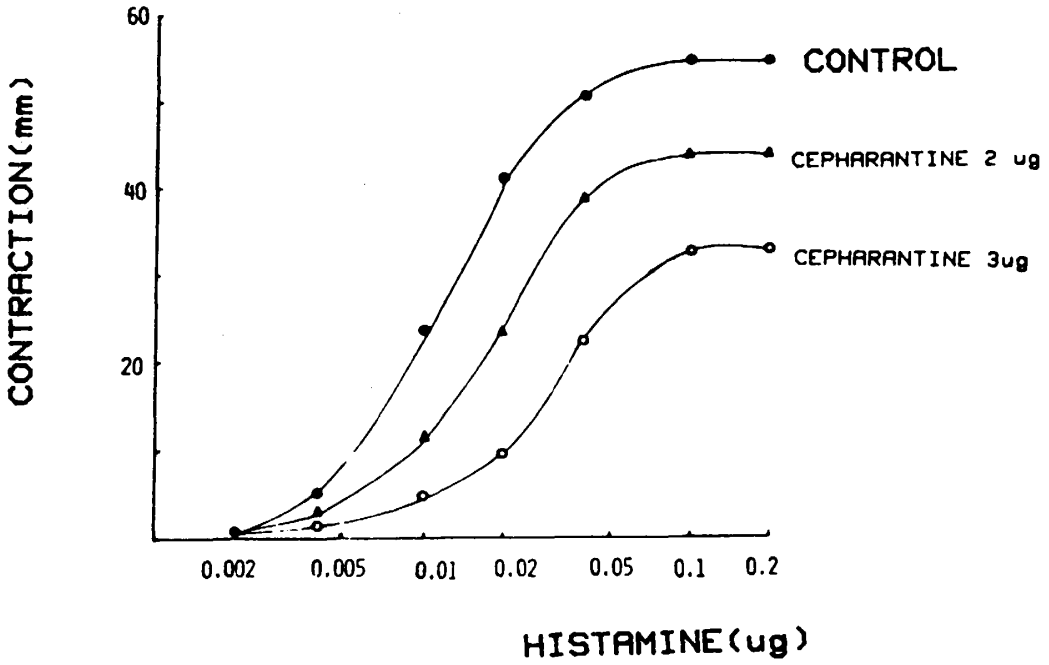

Figure 11 Inhibitory effect of cepharanthine on histamine-induced contraction of guinea-pig ileum.
● : control ▲ : 2 μg of cepharantine ○ : 3 μg of cepharanthine

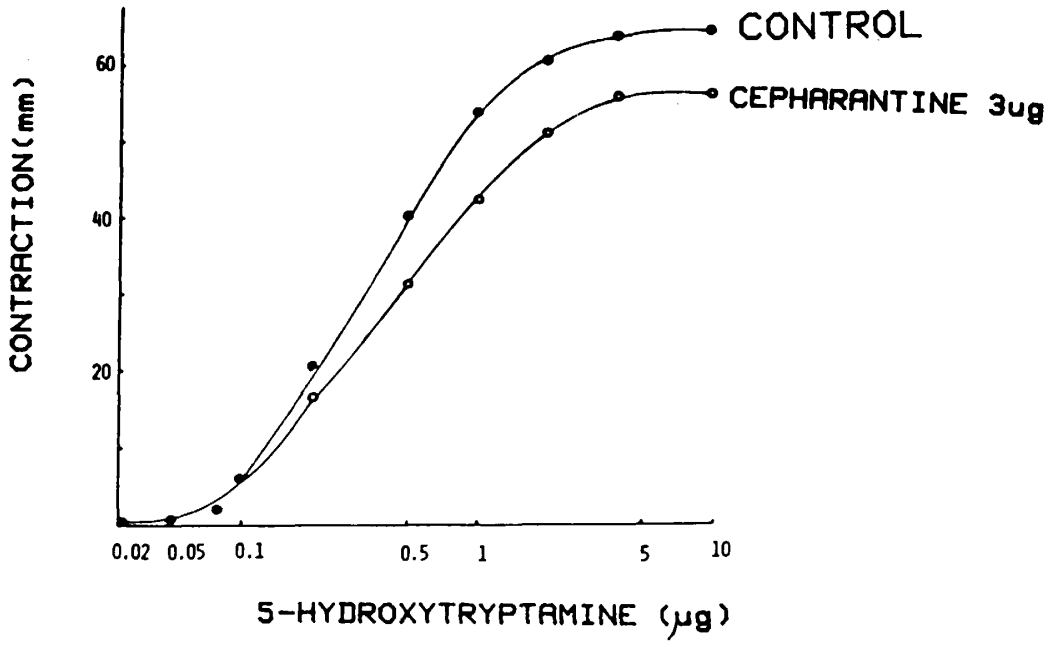

Figure 12 5-hydroxytryptamine-induced contraction of rat uterus.
● : control ○ : 3 μg cepharanthine

かった。死をひき起こすにはもっと大量の粗毒かMNF以外の要因が必要ではないかと思われる。

図2に見るような血圧効果をKurihara (1964)，貫ら (1963) は一過性のハブ毒による直接の心臓抑制作用とみなしているが，今回の実験では心臓に対する直接作用はみていないが，抗ヒスタミン薬の前投与によってこの血圧下降は抑制されることから，MNFによりヒスタミンなどの物質が遊離されこれが血管壁に作用して細動脈を拡張させることにより血圧降下をひき起こすのではないかと思われた。井上ら (1982) もイヌでの観察から中心静脈圧の上昇がみられずむしろ下降したことから通常の心原性ショックとはことなる可能性が示唆している。さらに田所ら (1961) はハブ毒を用いたカエル摘出心の実験で適当量では振幅増大を起こすことを報告しており，ハブ毒の作用が心臓に対して抑制作用のみではないことを示している。

桜川ら (1978) は家兎にハブ毒5㎎を静注すると血管内凝固症候群 (D.I.C.) が起こることを報告したが，今回のイヌ，ラットでの実験では1例も認めなかった。種差による違いなのか粗毒とMNFの違いなのか今後検討を要する問題の1つである。

セファランチンはStephania cepharanthaの根茎から抽出されたbiscoclaurin型アルカロイドの1つであり，蛇咬傷の治療をはじめ種々の疾患に使われている(前田ら，1986)。セファランチンのマムシ咬傷に対する効果については，マムシ毒を用いて小菅らによりすでに詳細な検討がなされている (小菅ら，1965) が，*in vitro* 実験では有効性が認められるものの *in vivo* では直接の中和作用は認められていない。*in vivo* で蛇毒を中和するものは今のところ抗血清しかなく，治療に際してはこれが第一選択であることは明らかであり，セファランチンは対症療法としてあくまで補助療法の1つに過ぎないわけで臨床家による奏効例についての報告を過信，過大評価して，安易にセファランチンのみの投与でことたれりとするべきではない。なお最も実用的な応急療法としては，*in vivo* に近い実験として毒牙痕に直接タンニン酸溶液を注入洗浄すれば受傷直後ならば著効を奏することが専門家の間では知られている。

セファランチンの薬理作用についてはまだ不明な点が多いが，今回は抗ヒスタミン，抗ブラジキニンおよび抗セロトニン作用について検討した。

杉山ら (1976) は蛇毒 (*Agkistrodon blomhoffi*) によるヒスタミン遊離を認め，これは蛇毒のホスホリパーゼ A_2 の作用によってリゾレシチンが形成されることによることを示し，これらのヒスタミン遊離がセファランチンによって抑制されることを示した。ハブ毒についての検討はなされていないが，今回の実験から，同様の作用がハブ毒にもあるのではないかと思われる。

血圧降下はさまざまな原因で起こりうるが，MNF投与後に生体内の遊離ヒスタミンやセロトニンを測定した報告はみあたらない。MNFの作用を検討するにあたり，この遊離ヒスタミンをはじめセロトニンやブラジキニンの測定は重要であり，現在検討中である。なお，MNFについては図1，2に示す如く粗毒に比べきわめて微量で血行動態に変化を及ぼすきわめて興味ある成分であると思われる。その生理的・薬理的研究と共に出血因子や腫脹因子にみられるような更に詳細な生化学的検討がなされるべきであり，現在生化学者により検討中である。

結　論

1．筋壊死因子をイヌに静脈内投与すると直ちに血圧の降下が認められ，これは徐々に平常域にもどった。同様の変化はラットでも認められたが，血圧降下の持続時間はイヌよりも長かった。

2．筋壊死因子による血圧降下はプロメタジンやジフェンヒドラミンなどの抗ヒスタミンによる抑制された。

3．ビスコクラウリン型アルカロイドのセファランチンには抗ヒスタミン，抗セロトニン及び抗ブラディキニン作用があり，筋壊死因子による血圧降下作用を抑制した。

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HYPOTENSION CAUSED BY HABU VENOM AND THE ANTIHYPOTENSIVE EFFECTS OF SOME DRUGS

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A patient bitten by a venomous snake Habu often shows blood pressure fall.

Also in experimental animals an abrupt fall of blood pressure is observed after the injection of Habu venom, and the hemorrage, edema and muscular necrosis are also seen on the site of the injection.

In the course of the study of the venom, it has been noticed that the venom consists of various factors, such as bleeding factor, swelling factor and necrotizing factor.

Myonecrotic factor (MNF) which causes necrosis of muscle was isolated from the crude Habu venom of Amami by precipitating with cold acetone.

In this study we showed the effects of this factor on the hemodynamic changes and also some drugs which inhibit the action of this factor.

When the myonecrotic factor (100 μg/kg) was intravenously injected into dogs, blood pressure fell immediately and gradually returned to the initial normal level.

Similar hemodynamic changes were observed in rats after MNF injection but the duration of hyotensive period was longer. The hypotensive effects of MNF were inhibited by the following injection of antihistamines such as promethazine and dihenhydramine.

Cepharantine, biscoclaurin type alkaloid which is clinically used for the treatment of Habu bite showed antihistaminic, antiserotonic, and antibradykinic action and suppressed the hypotensive action of MNF. Although this drug has been used for the treatment of Habu bite the mechanism is still obscure, and these actions may be involved to some extents.

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