ANTISCHISTOSOMAL ACTIVITY OF A NITRODIPHENYLAMINO ISOTHIOCYANATE (AMOSCANATE) AGAINST *SCHISTOSOMA JAPONICUM* (PHILIPPINE STRAIN) IN MICE AND RABBITS

Kazuo YASURAOKA and Yuji IRIE
Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki-ken 305, Japan

Although a number of drugs are available for the treatment of schistosomiasis, most, if not all, clinically used antischistosomal drugs are not satisfactory for large scale chemotherapy on account of toxicity or prolonged course of treatment. A recent critical review of the status of chemotherapy of schistosomiasis and of related research strategy (Hoffman, 1975) indicates that a drug suitable for the improved treatment of *S. japonicum* and for use in its mass chemotherapy needs to be developed to replace the antimonials. The results in recent years of treating experimental animals infected with *S. haematobium*, *S. mansoni* and *S. japonicum* (Striebel, 1976; Bueding *et al.*, 1976) indicate that 4-isothiocyanato-4'-nitrodiphenylamine (amoscanate) (subsequently referred to as amoscanate) has high antischistosomal activity when administered as a single oral dose. These findings stimulated the authors to test this compound in mice and rabbits infected with *S. japonicum*.

A liquid formulation of amoscanate (40 mg and 4 mg of the compound per ml of 1% Cremophor El and 25% glycerol) with small particles less than 1 micron in diameter was received through Dr. E. Bueding, the Johns Hopkins University. The Philippine strain of *S. japonicum* obtained from naturally infected *Oncomelania quadrasi* collected in Leyte Island, Philippines was used throughout this study.

Adult *S. japonicum* were not killed by 48 hrs' exposure to concentrations of the drug up to 15 μg/ml. It was found, however, that the drug produced marked damage to schistosomes at concentration as low as 0.15 μg/ml. A concentration of 0.015 μg/ml still had an inhibitory effect on the motility of worms after 72-96 hrs of incubation and produced complete immobilization after 120 hrs.

In *in vivo-in vitro* tests, all the serum of rabbits collected 1, 2, 4, 8, 24 and 48 hrs after drug dosage did not exhibit any inhibitory effect on the motility and pairing of adult *S. japonicum*. Although much more eggs were produced in most cases after 72-168 hrs' contact to media containing sera collected after treatment, those eggs were all dead and abnormal in appearance.

In the first series of experiment with 5-week infections in mice, a single oral dose of 20 mg/kg resulted in a complete parasitological cure. A single oral dose of 5 mg/kg reduced the number of worms by 98.1%.

To study possible differences in the efficacy of the drug on parasites of different age, two more series of tests were conducted with 1-, 3- and 5-week-old infections in mice. In the course of the second series of experiment, all the mice were weighed before and after treatment to see a sign of the side-effect. Although no schistosomicidal effect was seen against 1-week-old schistosomula, there was no apparent difference in the susceptibility to the drug between 3- and 5-week-old worms. Mice treated with a single oral dose of 5 mg to 20 mg/kg tended

to gain weight after treatment, while untreated control mice lost weight.

A further experiment was designed to observe the action of amoscanate when diluted with vehicle (1% Cremophor El and 25% glycerol). Groups of 8-11 mice each were treated with a single oral dose of 20 mg/kg in several dilutions, i.e. 40 mg, 20 mg, 4 mg and 0.8 mg of the active ingredient per ml of the vehicle. It seems likely that the effectiveness of the schistosomicide is enhanced to some extent when diluted with vehicle.

A trial similar to that designed for the study of amoscanate in mice was conducted in rabbits. Considering the degree of worm reduction, the antischistosomal activity of the drug in rabbits was almost identical with that in mice. Actually, a single oral dose of 20 mg/kg reduced the number of worms by more than 90% if treatment was given 4 to 5 weeks after infection. The sex ratio of surviving worms was comparable to that of untreated control worms.

Immunoserological changes in experimentally infected mice after treatment with amoscanate at a single oral dose of 20 mg/kg were studied. The antiboty titers of mice in complement fixation (CF), enzyme-linked immunosorbent (ELISA) and circumoval precipitin (COP) tests tended to decrease or not to increase rather immediately after treatment.

When worms were exposed to amoscanate by treating mice with the drug at a single oral dose of 20 mg/kg, certain ultrastructural changes were observed. The most clearly visible degeneration was seen in the tegument and vitellaria. However, even though the tegumental degeneration took place, the male worms did not show any notable change in the testes.

In order to be effective against schistosomes amoscanate must be present at sufficiently high concentrations for a sufficiently long period of time. Our *in vitro* experiments showed that the minimum concentration required in the serum was approximately 0.015 μg/ml. In *in vivo-in vitro* tests, however, the serum of rabbits collected 1, 2, 4, 8, 24 and 48 hrs after a curative drug dosage did not show any activity correspond to that observed at a concentration of 0.015 μg/ml of the drug *in vitro*. These conflicting results are possible due to a very rapid absorption, distribution and elimination of the drug, or to some unforeseen mismanagement in the test procedures used.

It was found in mice that 1-week-old schistosomula were markedly less susceptible to amoscanate than adult parasites. A similar result has been reported by Bueding *et al.* (1976). The efficacy of the drug was likely to be slightly greater with the diluted form than with the concentrated form. No observations using unisexual infections with male and female worms were made that could clarify the question whether amoscanate would act differently in male and female worms. It seemed likely, however, that in rabbits in particular but also in mice, there were no pronounced differences in the susceptibility of the drug between male and female *S. japonicum* worms.

Although many compounds possess appreciable schistosomicidal activity, only a few are presently suitable for large scale chemotherapy and these are generally effective against only *S. mansoni* and *S. haematobium* or are only of *S. haematobium* (Webbe and James, 1977). For example, metrifonate is virtually ineffective against *S. mansoni* yet gives consistently high cure rates against *S. haematobium*. Another example is the ineffectiveness of hycanthone against *S. japonicum* whereas it is highly active against *S. mansoni* and *S. haematobium* (Davis, 1972, 1974). The predicted prospects for successful mass chemotherapy using available compounds appear to be highest for *S. haematobium* and lowest for *S. japonicum*. The results of the present study show that amoscanate is effective against patent *S. japonicum* in mice and

rabbits in relatively low dosage regimens (a single oral dose of 20 mg/kg), as shown by reduction or complete elimination of adult flukes. Studies of Striebel (1976) have also revealed that this compound possesses potent activity against all three schistosome species pathogenic for man in various hosts including mice, hamsters, dogs and primates. Bueding *et al.* (1976) have likewise reported that in contrast to any other known antischistosomal compounds, 3 geographic strains (Philippines, Japanese and Chinese) of *S. japonicum* were at least as, if not more, susceptible than *S. mansoni* to this compound.

Curative treatment with this compound appears to be well tolerated. Mice treated with a curative dose showed no weight loss and gain weight rapidly afterwards, while untreated control mice lost weight. More detailed toxicological studies have demonstrated an excellent tolerance of the drug in animals (Striebel, 1976; Bueding *et al.*, 1976).

The results of the present study coupled with those obtained by other investigators are encouraging and it is felt that there would be a certain scope for its use in human schistosomiasis japonica.

Dr. F. Sy. (IPH, UP)

What are the possible mechanism of action of amoscanate?

Dr. Yasuraoka (Univ. of Tsukuba)

A complete schift of *S. japonicum* from the mesenteric veins to the liver sinuses was seen soon after the oral administration of amoscanate. Amoscanate may cause a reduction in the activity of glycogen phosphorylase phosphatase and thus induces a reduction of muscular activity of the worm. For further particulars, read Dr. Bueding's paper (Experientia, 32, 604-606).

Dr. A. T. Santos, Jr. (SCRS, Manila/MOH)

To what do you attribute increase in efficacy with the addition of a vehicle to the drug?

Dr. Yasuraoka (Univ. of Tsukuba)

The effectiveness of amoscanate was enhanced to some extent when diluted with vehicle (1% Cremophor El and 25% glycerol). The vehicle might make the absorption of the active ingredient through the wall of the digestive organs easier and faster.

EVALUATION OF QUANTITATIVE SAMPLING FOR *ONCOMELANIA QUADRASI* BY FILTER PAPER

Hiroshi TANAKA[1], Manuel J. SANTOS[2], Hajime MATSUDA[1], Kazuo YASURAOKA[3] and Alfredo T. SANTOS, Jr.[2]

1) *Department of Parasitology, Institute of Medical Science, University of Tokyo, Tokyo 108 Japan*
2) *Schistosomiasis Control and Research Service, Ministry of Health, Philippines*
3) *School of Medicine, Tsukuba University, Tsukuba 305 Japan*

For the purpose of determining the population density of *Oncomelania quadrasi*, methods using ring and tube samplers with a 13.5 cm diameter for both, were established by Pesigan *et al.* (1958) and have been widely used for determining the snail density on dry, moist and wet soil surface. It was, however, difficult to collect snails in the submerged areas, therefore, the horizontal and vertical distribution of the snails in the waters has not yet been clarified. On the other hand, snails in the ditches or in the bank of irrigation canal or water-logged area have been collected by placing fallen dry banana leaves as attractant.

The present study deals with establishment of a quantitative collection method of snails in the waters using filter paper as a simulated material of banana leaves.

Attractancy was compared between filter papaer (Toyo Roshi No. 131) and dry banana leaves. Both materials cut at different sizes of rectangular strips were placed in the wet bank. The number of snails collected per 100 cm^2 was 2.6, 16.6 and 2.8 for filter paper and was 2.5 for banana leaf showing more attractancy in the filter paper. In the 2nd experiment, filter paper attracted 1.6, 3.3, 4.9, 4.4, 1.6, 2.4, 2.7 and 3.6 snails/100 cm^2 while banana leaves 1.0, 1.2, 2.8, 2.7, 5.7 and 1.0. The mean densities were 3.06 and 2.40 in the filter paper and banana leaf, respectively, and the difference of two means was not significant by *t*-test. In comparison of both materials at the same size of 30 cm x 15 cm snails captured per stripe were 67, 64, 51, 56, 65 and 90 by fitler paper and 46, 47, 108, 30, 75 and 45 by the banana leaf at means of 65.5 and 58.5, respectively. There was no statistical difference inbetween either.

It is believed that the tube sampling provides the best quantitative collection of snails. The efficiency of quantitative sampling by filter paper was compared with tube sampling by collecting 30 samples each by both methods in 4 areas. Significant correlation of the snail densities between both sampling methods existed at 3 areas of 4 and the coefficient of those 3 areas was 0.6502 (Table 1). Although the correlation existed, it was not so high. Therefore, the quantification of snail sampling was not so accurate by the filter paper method. Sex and age composition of snails collected by filter paper was not so different from that by the tube sampler.

The relation between the number of snails at 85 cm^2 area of the cross-section of tube sampler (Y) and that on a sheet of filter paper at an area of 20 x 20 cm (X) could be obtained by the following two formulas:

$Y = 0.77\ X + 1.57$
$X = 0.55\ Y + 1.35$

The type of distribution, whether snails can be regarded to scatter uniformly or unevenly and what type of theoretical distribution the snail are distributed, was examined by the collection data by both sampling methods. The distribution of snail did not fit Poisson distribution, a type of uniform distribution at any sampling data. However, all data fitted the negative binomial distribution, a type of uneven distribution, as shown in Table 2. The degree of clustering of snails is represented inversely by the constant k. The value of k varied from 0.69 to 1.49 by the filter paper and 1.07 to 5.92 by the tube and they were always smaller by filter paper than by tube method. Since it is generally believed that the type of distribution in the field reflects more directly to the result of sampling by the tube, it can be said that snails sometimes were distributed closely following the Poisson type, a uniform distribution, in the natural environment. And the uneven distribution of snails becomes more conspicuous by the filter paper sampling.

The method of quantitative sampling by filter paper was established by this study and the method made the study of distribution of snails in the water possible.

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Table 1 Correlation of the number of snails collected by tube method and filter paper method

Exp. No.	Place	Tube (Y)* / Filter paper (X)	Correlation coefficient (r)	*t* value	d.f.	Probability	Formula of regression line
1	Ricefield along canal	1.3571	0.3830	2.375	28	0.05 >	Y=0.31x+0.49
2	Tibak depression 1	0.6064	−0.0028	0.014	25	> 0.9	–
3	Tibak depression 2	1.0548	0.5976	4.830	27	0.001 >	Y=0.65x+1.03
4	Tibak depression 3	1.2047	0.4842	3.347	28	0.01 >	Y=0.57x+5.36
Total	1) + 3) + 4)	1.1789	0.6502	10.506	87	0.001 >	Y=0.77x+1.57

* Ratio of the number of snails by tube method (Y) to that by filter paper method (X).
Area of cross-section of a tube sampler 85 cm^2. Area of filter paper 20 cm x 20 cm = 400 cm^2.

Table 2 Distribution of snails at Tibak depression (No. 3) observed by filter paper and tube sampling methods

No. of snails per sample	Method of sampling			
	Filter paper		Tube	
	Fo	Fnb	Fo	Fnb
0	5		1	
1	0		3	
2	4	10.72	2	7.87
3	2		3	
4	3		1	
5	1		3	
6	1	6.77	2	
7	1		1	8.25
8	2		2	
9	3		3	
11	0		2	
12	2	4.83	0	
14	2		0	
15	0		1	5.82
16	0		1	
20	0		2	
23	2		0	
26	1		0	
30	0		1	
36	0		1	
43	1		0	
50	0	7.68	1	8.06
Total	30	30.00	30	30.00
$\bar{x}$	8.4667		10.2000	
s^2	89.1822		123.9600	
k	0.8881		0.9146	
corrected k	0.6946		–	
p	12.1893		11.1524	
x^2	2.73		2.16	
Pr.	0.1–0.05		0.2–0.1	

Fo, frequency observed.

Fnb frequency calculated following the negative binomial distribution.

DISTRIBUTION OF *ONCOMELANIA QUADRASI* IN WATERS IN THE PHILIPPINES

Manuel J. SANTOS[1], Hiroshi TANAKA[2], Hajime MATSUDA[2], Rogelio HAMBRE[1], Yuzuru IWANAGA[3], Hiroshi SHIMOMURA[4], Bayani L. BLAS[1] and Alfredo T. SANTOS, Jr.[1]

1) *Schistosomiasis Control and Research Service, Ministry of Health*
2) *Institute of Medical Science, University of Tokyo*
3) *Hiroshima University School of Medicine*
4) *Miyazaki Medical College*

The distribution of *Oncomelania quadrasi*, the intermediate snail host of *Schistosoma japonicum* and its habitats have so far been studied in the Philippines. It is well known that *O. quadrasi* lives on wet or moist soil surface in ricefields, banks of streams, creeks and huge water logged areas and in pockets of medium or large rivers.

The present paper deals with the horizontal and vertical distribution of *Oncomelania quadrasi* in both standing and running water habitats using filter paper sampling method. Filter paper with size of 20 x 20 cm was used in the most collection sites. The filter paper inserted in wire mesh folder was placed at the bottom of the water and the bamboo poles of 7 cm diameter covered with filter paper were stood at the bottom to examine the horizontal and vertical distribution of snails in water, respectively. In the studies made in the three streams surveyed, that is, in Vicob-Malaigang Stream, Juber Creek and Gacao South Main Canal, snail density was found to be highest at the margin of the water in the sloping bank and in the near-by wet bank. The density of snail became lesser at the higher portion of the moist bank. Snails were also found in the water plants growing from the bank and shallow places close to the bank. In the running water habitat only a few snail were noted on the surface or at the bottom of water.

Dr. K. Makiya (Nagoya Univ.)

I noticed in your slides that in some portion of the water, there are some snails at the bottom. What are the limiting factors to determine the vertical distribution of snail?

Mr. M. Santos (SCRS, Manila/MOH)

Snails at the bottom of the water come mostly from the vegetation which were either knocked down by mechanical or physical means and floats on the surface for a short time. So those snails found at the bottom is closely related to the condition of vegetation therein.

DISPERSAL OF *ONCOMELANIA QUADRASI* A REVIEW

Eraklio A. BAÑEZ
Schistosomiasis Control and Research Project,
Ministry of Health, Philippines

Oncomelania quadrasi is known to occur in 22 provinces in the Philippines and covers an estimated area of 162,851 hectares. Its distribution is related to topography. Flatness of the snail inhabited places is an outstanding features which provides the retention of water, a vital factor to an animal with an aquatic stage of its development.

Among the places inhabited by *O. quadrasi* are the following: (1) flood plain forest and swamps, (2) ricefields, (3) streams, (4) small swamps or pockets, (5) road ditches and borrow pits and (6) irrigation canals.

The movement of *O. quadrasi* was observed to ranged from 0.160 to 0.935 cm in 10 minutes depending on time of the day. The shortest movement was at 2 o'clock in the afternoon and the farthest was at 10 o'clock at night. Climbing of snails on vegetation was recorded at an average height ranging from 0.75 to 5.06 cm.

Humidity, temperature, light and atmospheric pressure apparently had a significant influence on snail movement. Decrease in humidity decreased movement. On the other hand, increased in temperature and light increased movement.

Snail movement is further influenced by the presence or absence of barriers. The role of natural force as water current can cause passive snail movement.

The replacement of individual snails in a habitat was largely due to migration (inward and outward movement) which in turn was influenced by changes of water level along the banks of stream. The inward movement of snails increased with the lowering of water level, while the outward movement increased with the elevation of the water level.

In conclusion, the dispersal of *O. quadrasi* should be taken into consideration of snail population study, especially so, that migration may play an important role in the repopulation of snails after snail control measures have been instituted.

DISTRIBUTION PATTERN OF *ONCOMELANIA QUADRASI*, EVALUATION METHOD FOR SNAIL CONTROL AND DETERMINATION OF NECESSARY SAMPLE SIZE FOR THE SNAIL SURVEY

Kiyoshi MAKIYA[1], Hiroshi TANAKA[2], Eraklio A. BAÑEZ[3], Bayani L. BLAS[3] and Nobuo KUMADA[1]

1) *Department of Medical Zoology, Nagoya University School of Medicine*
2) *Department of Parasitology, Institute of Medical Science, University of Tokyo*
3) *Schistosomiasis Control and Research Project, Ministry of Health, Philippines*

Accumulated knowledge on schistosomiasis control in the Philippines indicates that the control of the intermediate host snail is the most effective and essential control measure for this disease.

When any kind of snail control is carried out, it is necessary to evaluate the control measure by comparing snail density before and after the control work. However, difficulty has always been found in comparing the snail density because of uneven distribution of the snail over the infested field.

Pesigan *et al.* (1958) noticed that the snail was distributed unevenly in the field. In processing the snail survey data, however, they discarded all negative (zero snail) samples that did not fall adjacent to a sample containing snails to fit their survey data with the negative binomial, and used the transformation method of $\sin h^{-1}\sqrt{(x+0.375)/(k-0.75)}$.

Thereafter, it became clear from the studies by Tanaka *et al.* (1975, 1976) that the parameter k of the negative binomial lowered less than the limit of 0.75 for snail populations in the field, therefore the above-mentioned transformation method became of limited use in the field.

As an approach to this problem, an ecological study was carried out to elucidate the distribution pattern of the host snail, and thereby to improve the method of data transformation and determination of necessary sample size for the snail survey.

(1) Statistical analysis was made on the existing survey data of snail density in the pilot areas of Dagami, Matagob and Tacloban, Leyte. Snail counts per sample were tested for the best fit to the theoretical distribution models, i.e., (i) Poisson, (ii) negative binomial, (iii) double Poisson and (iv) Neyman type A. As a result, the double Poisson, the Neyman type A and the negative binomial distributions fitted 21 populations (71%), 17 (57%) and 13 (43%), respectively. The Poisson model fitted only 2 cases (7%) among the 30 populations.

From these results, the snail is found to be distributed not randomly but contagiously over the field and the populations consist of unevenly distributed small clusters in many cases.

(2) When sampled from such uneven snail populations, the variance of snail counts varies with change of mean density, with the result that the statistical comparison can not be applied to the survey data. In order to stabilize the variance of mean snail counts in many surveys, several transformation methods so far reported were compared for their efficiency and readiness of the practical use:

(i) $log\ (x + 1)$, (ii) $k^{-1/2}\ sin\ h^{-1}\ \sqrt{kx}$, (iii) $k^{-1/2}\ sin\ h^{-1}\ \sqrt{k\ (x + 1/2)}$, (iv) $log\ (x + k/2)$, (v) $sin\ h^{-1}\ \sqrt{x\ (\beta - 1)\ /\ (\alpha + 1)}$ and two additional transformations (vi) $log\ (x + 1/10)$ and (vii) $log\ (x + 1/100)$ (see Table).

Transformation by $y = log\ (x + 1/100)$ was determined to be the most reliable and practical one for this purpose. Using this transformation, the statistical comparison such as the t-test and the analysis of variance was made possible without omitting zero-snail samples. This transformation can readily be used without calculating the parameters like k, α and β in the other parametric transformations (ii), (iii), (iv) and (v).

(3) The sample size and the distance between sampling sites were tentatively determined in SCRP without theoretical background. In order to make an effective plan for snail survey, it is convenient to know in advance the minimal sample size necessary for obtaining a required degree of reliability and for saving time and manpower.

The minimal necessary sample size (q) can be calculated using the formula based on the $I\delta$ dispersion index (Morisita, 1962)

$$q = \frac{t^2}{\in^2}\ (I\delta - 1 + \frac{1}{\bar{x}})$$

where t is the Student's t value, $\bar{x}$ mean snail density and $\in$ the relative error calculated by $ts_{\bar{x}}\ /\ \bar{x}$ ($s_{\bar{x}}$: standard error).

The $I\delta$ dispersion index is defined as

$$I\delta = n\frac{\Sigma x\ (x - 1)}{N(N - 1)}$$

where n is number of samples, x snail count per sample and N the total number of snails.

As can be understood from the formula, the necessary sample size depends not only upon the mean density and its variance but also upon the degree of contagiousness. The more contagiously the snail is distributed, the more sample is necessary for obtaining a reliable density.

A nomograph was given on the various snail densities and contagiousness encountered in the infested field ($t = 1$, $\in = 0.3$). Using this nomograph, the necessary sample size can easily be determined in advance by the preparatory sampling.

The reliability of the existing snail sampling data in the three pilot areas was examined in terms of the relative error of the mean density. The relative errors ranged below 20% in most sampling data, that is, the snail survey in the pilot areas was considered to be highly reliable as snail census in the field.

By adopting the relative error of 30% as a permissible level, a comparison was made between the actually employed sample size and the minimal necessity determined theoretically. The result indicates that the sample size actually taken was more than twice the minimal necessity for most surveys in the pilot areas. Accordingly, the sample size can be minimized depending upon the required reliability, if it is necessary to save manpower.

However, the snail density and the degree of contagiousness of the same population may change by year depending upon the environmental factors and the control measures applied. When frequent preparatory survey is difficult to carry out for determination of the minimal sample size, it is acceptable to continue the on-going procedure of snail sampling with an interval of 5 m.

Table Comparison of several transformation methods in the efficiency of stabilizing variance (Digwasay A)

Transformation		1974	1975	1976	a [1]	F [2]
No transformation	Mean $(\bar{x})$	0.911	0.196	0.113	2.348	880.2
	SD (s)	2.260	0.623	0.356		
$k^{-1/2} \sin h^{-1}\sqrt{kx}$ [3]	Mean $(\bar{y})$	0.361	0.137	0.101	1.619	228.4
	SD (s)	0.737	0.392	0.307		
$k^{-1/2} \sin h^{-1}\sqrt{k(x+1/2)}$ [3]	$\bar{y}$	0.932	0.711	0.746	1.832	388.1
	s	0.506	0.225	0.157		
$\sin h^{-1}\sqrt{x(\beta-1)/(\alpha+1)}$ [4]	$\bar{y}$	0.491	0.224	0.179	1.333	103.8
	s	0.965	0.626	0.290		
$\log(x + k/2)$ [3]	$\bar{y}$	-0.648	-0.830	-0.861	1.443	143.9
	s	0.631	0.382	0.316		
$\log(x + 1)$	$\bar{y}$	0.139	0.047	0.032	1.758	330.8
	s	0.291	0.139	0.099		
$\log(x + 1/10)$	$\bar{y}$	-0.676	-0.861	-0.892	1.435	140.8
	s	0.645	0.392	0.325		
$\log(x + 1/100)$	$\bar{y}$	-1.468	-1.749	-1.796	1.273	85.2
	s	1.042	0.701	0.614		

1) Regression coefficient in $s = a\bar{y} + b$ (or $s = a\bar{x} + b$).
2) F value in Cochran's stability test of variance.
3) Common $k\,(= 0.2162)$ was used as a parameter.
4) α, β: Parameters in the mean crowding-on-mean density regression (Iwao, 1968).

Dr. Blas (SCRP, Palo/MOH)

At the SCRP, we do snail sampling at intervals of 5 meters taking no less than 30 samples. In bigger snail areas, will taking of samples every 10 meters be acceptable?

Mr. Makiya (Nagoya University)

As I showed the reliability in various cases, sampling at every 5 meters is more reliable in the pilot areas in most cases. When snail areas are wide and have high snail density as well as low contagiousness, taking of samples at every 10 meters can be permissible. However, the on-going sampling procedure with 5-meter interval is much more recommended, if saving of labor is not necessary.

EVALUATION OF THE SNAIL CONTROL PROJECTS IN THE PILOT AREAS AND THE EFFECT OF DRAINAGE ON THE SNAIL DENSITY

Kiyoshi MAKIYA[1], Hiroshi TANAKA[2], Eraklio A. BAÑEZ[3], Bayani L. BLAS[3], Nobuo KUMADA[1] and Antonio PEREZ[3]

1) *Department of Medical Zoology, Nagoya University School of Medicine*
2) *Department of Parasitology, Institute of Medical Science, University of Tokyo*
3) *Schistosomiasis Control and Research Project, Ministry of Health, Philippines*

This report is concerned with a practical application of the theoretical considerations on data processing of snail sampling to the analysis of data in special references to (1) evaluation of the snail control projects in the three RP-Japan pilot areas in Leyte, and (2) a change of the distribution pattern of the snail population before and after drainage canal construction, and evaluation of the effect of drainage on the snail density in an experimental area at Tibak depression.

(1) The schistosomiasis control study was carried out in three areas in Leyte since 1974. Snail survey was made by the ring method in accordance with the control measures such as removal of vegetations, construction and improvement of drainage canals and application of molluscicides.

Snail density was compared between the pre-control survey and the yearly census after the control measures. After stabilizing variance of the snail count by the transformation of $y = log(x + 1/100)$, change of density was examined by the t-test.

The results indicated that snail density reduced significantly in most of the populations after the control measures. In some populations, however, snail density increased again one or two years after the significant reduction, indicating that continuation or improvement of the control measures is necessary for preventing repopulation of the snail.

(2) In order to evaluate the control effect of drainage on snail population, a 200 m - long drainage canal was constructed in a swampy depression (ca. 1,300 m^2) in Tibak, Leyte. Snail survey was carried out weekly by the ring method as well as the measurement of water content of the surface soil.

As the first step, the distribution pattern of the snail count per sample was examined. The negative binomial and the double Poisson distributions fitted well the pre-control population and the former model became fitted better after the construction of the drainage canal. An interpretation of this result is that the distribution pattern changed from an uneven distribution of snail colonies into the uneven distribution of individual snails without forming the clusters.

Such a change of the distribution pattern was also confirmed by analysis of the mean density – mean crowding relationship (Iwao, 1972). The result showed that snail density per colony decreased from about 20 to 1 simultaneously with the advance of drainage effect. This meant that the snail population consisted of colonial components before the control

measure and changed into the non-colonial distribution consisted of individual snails.

The reduction of snail density after the drainage was highly significant by the t-test after stabilizing variance of the snail count using the transformation formula mentioned above (see Table).

To evaluate the effect of drainage on snail density, the relationship was analyzed among the successive change of the ratio of dead snail, population density and water content of the soil. As a result, water content of the soil diminished to one third of the initial level along with a distinguishable change of the wet soil into dry condition with a cracked surface. In parallel with drying of the soil, the ratio of dead snails increased over fifteenfold and population density reduced by about 97% in the fifth week after the drainage work (Table).

The reduction of snail density in this experimental area can be ascribed largely to loss of soil moisutre, judging from the above-mentioned results and the following observations : (i) No molluscicide was applied during the survey, (ii) most of the dead snails were newly died ones with shrunk bodies inside, and (iii) the correlation coefficient between the soil moisture index and the mean density was highly significant ($r = 0.960$, $p < 0.01$).

In view of the experimental evidence indicated here, it may be concluded that water loss of surface soil is very effective in reducing snail density in swampy areas. Construction of drainage canal, accordingly, is an effective control measure of snail populations so long as the drainage canal is well maintained and functioning.

Table The reduction of water content of the soil and snail density after the construction of the drainage canal

Week	Soil moisture index (h)[1]	Ratio of dead snail (%)	Mean snail density[2]		t-value[3]
			$A_{\bar{y}}$	$\bar{y}$	
Mar. IV	1.386	1.8	0.0732	-1.0800	—
		Drainage work completed			
Apr. I	1.057	6.5	0.0176	-1.5599	3.515 ***
II	0.674	12.0	0.0116	-1.6652	4.472 ***
III	0.615	29.6	0.0036	-1.8664	6.639 ***
IV	0.563	11.1	0.0063	-1.7881	5.702 ***
May I	0.451	29.2	0.0020	-1.9222	7.358 ***

1) Soil moisture index was calculated using $h = (w - d)/d$ (w: wet weight of the soil, d: dry weight after 24 hours of desiccation under 150°C).

2) Mean snail density was calculated after transformation by $y = \log(x + 1/100)$.
$\bar{y} = \Sigma \log(x + 1/100)/n$, $A_{\bar{y}} = \text{antilog}\,\bar{y} - 1/100$

3) Significance test of the snail density between the pre-drainage survey and each consecutive survey after the drainage work.

*** significant at 0.1% level.

MOLLUSCICIDAL EFFECT OF "GOGO" BARK AGAINST *ONCOMELANIA QUADRASI* IN THE LABORATORY

M. J. SANTOS[1] and K. YASURAOKA[2]

1) *Schistosomiasis Control and Research Service, Ministry of Health, Philippines*
2) *Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki-ken, 305, Japan*

The "Gogo" bark showed mild molluscicidal property at a concentration of 1:4,000 grams/liter with a exposure period of 24 hours among young and juvenile *O. quadrasi.*

With an ethanol extract, 90 to 100% mortality was obtained among young and juveniles with a concentration of 50 to 100 ppm. Using adult snails, 70 to 100% mortality was obtained with a concentration of 100 and 800 ppm.

The LC_{50} against adult *O. quadrasi* was 58 (30.9 - 109.0) ppm. and was less than 12.5 ppm for juveniles for 24 hours exposures (immersion testing).

The potency of the "Gogo" bark extract was almost constant even after exposure to sunlight for 6 hours. The LC_{50} was 58 (32.2 - 104.4) ppm in the exposed and 41 (21.6 - 77.9) ppm in the non-exposed solution.

The potency of the extract was not remarkably affected by the hardness of water. The LC_{50} was 57 (29.2 - 111.2) ppm in hard and 50 (27.8 - 90) ppm in distilled water.

LABORATORY ASSESSMENT OF THE MOLLUSCICIDAL ACTIVITY OF THE PLANT *CROTON TIGLIUM* AGAINST *ONCOMELANIA* SNAILS

Kazuo YASURAOKA[1], Junichi HASHIGUCHI[1] and Eraklio A. BAÑEZ[2]

1) *Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki-ken 305, Japan*
2) *Schistosomiasis Control and Research Project, Palo, Leyte, Philippines*

Although plant molluscicides may not compete with other existing and expensive synthetic molluscicides, they may be useful in the control of schistosomiasis in rural areas on a "self-help" basin. From such a viewpoint, 23 species of local plants which grow in the Philippines were collected and tested in the laboratory for its molluscicidal activity against *Oncomelania quadrasi.* Some of these plants are used by the local folks as fish poisons. The results showed that the seed of *Croton tiglium* (Tuba in Tagalog) and *Jatropha curcas* (Tubang-bakod in Tagalog) have some molluscicidal potentials against *O. quadrasi.* To put the seeds into practical use as a molluscicide, laboratory tests with the crude extracts from the seed of *C. tiglium* were carried out against *O. quadrasi.*

The seeds were shelled and 100 mg of the inner fruits was mashed in a mortor with 200 ml of distilled water. After allowing the mixture stand for 24 hrs it was centrifuged twice each at 2,000 rpm for 10 minutes and the supernatant was regarded as 500 parts per million (ppm), for which a series of appropriate dilutions was prepared.

For the test 10 mature snails (3.5 to 5 mm in length) were exposed to 100 ml of dilution of the desired concentration in aged tap water at temperatures of 28 - 30°C. The testing procedure was essentially the same as described by us (Komiya *et al.*, 1962), using 30 snails for each dilution, with 10 snails per dish. Snails were exposed to test dilutions for 48 hrs. Following the exposure period the snails were rinsed and placed in aged tap water for a 48 hrs recovery period, after which the reading of dead and living snails was made. The data were analyzed by probit analysis method (Litchfield and Wilcoxon, 1949).

Test were made in triplicate each using the twofold dilution series. The computed LC_{50} and its 95% confidence limits are as follows:

1st	0.70 (0.51 – 0.96) ppm
2nd	0.66 (0.47 – 0.93) ppm
3rd	0.87 (0.50 – 1.51) ppm

Japanese killifish, *Oryzias latipes,* in groups of 8 were exposed for 48 hrs to appropriate 1.5 - fold dilution series of the crude extract. The LC_{50} and its 95% confidence limits were 0.07 (0.053 - 0.112) ppm. It was thus found that the concentration of *C. tiglium* which kills *O. latipes* is about 1/10 of that needed to kill snails.

The acute oral toxicity of the crude extract to mice (DDD strain) was determined, and

the oral LC_{50} was 1,620 mg/Kg of body weight. Skin-irritation tests were performed with an aqueous paste of the crude extract placed in some quantities. When an aqueous paste of the crude extract was applied on the forearm of human volunteers, it induced transient irritation and small reddish papules within 12 hrs after application but disappeared in 24 hrs.

Analytical work on the seed and testing of the components was then carried out to find the active ingredient. The seed was chopped fine and ground in a mortor. Extracts were first prepared from 300 g of the ground materials in 6 litres of hot methanol. The methanol extract was condensed and dried by evaporation in vacuo, from which (1) ether soluble, (2) benzene soluble, (3) ethyl-acetate soluble, (4) acetone soluble, (5) isobutanol soluble and (6) water soluble fractions were successively prepared.

Each fraction was dissolved in its respective solvent which had been used for extraction and a tenfold dilution series of 0.5, 5 and 50 ppm was prepared in distilled water. Preliminary tests consisted of using only the ether, acetone and water fractions of *C. tiglium* and the ether, benzene and water fractions of *J. curcas*.

Comparing the results of the six materials tested it seemed that the ether fraction was more effective than the rest.

Further trials were carried out with the ether fractions in triplicate each using the twofold dilution series. The computed LC_{50} and its 95% confidence limits are as follows:

	1st test	2nd test	3rd test
C. tiglium seeds ether extract	0.105 ppm (0.081 – 0.054)	0.088 ppm (0.06 – 0.129)	0.102 ppm (0.079 – 0.132)
Niclosamide	0.044 ppm (0.036 – 0.054)	0.055 ppm (0.05 – 0.06)	0.059 ppm (0.05 – 0.068)

The ether soluble fraction of the seed of *C. tiglium* contained and efficient molluscicidal activity against *O. quadrasi*. This plant is a shrub and is found in the Philippines from northern Luzon to southern Mindanao. The seeds are borne in capsules which are usually three-angled and from 1.5 to 2 cm in length.

According to Lewkowitsch (1915), the seeds of this plant yield 53 to 56 percent of croton oil. It was formally used as a counterirritant and a purgative. Therefore, the molluscicidal activity of croton oil (Tokyo Kasei, Co.) against *O. quadrasi* was studied.

Tests were made in triplicate and the computed LC_{50} with 95% confidence limits are as follows:

1st ..... 0.30 (0.19 – 0.47) ppm
2nd ..... 0.54 (0.33 – 0.87) ppm
3rd ..... 0.46 (0.29 – 0.74) ppm

For a crude natural product such as *C. tiglium*, a LC_{50} of about 0.7 ppm is noteworthy. Limited field trials revealed that a dose of 4 g/m^2 of the aqueous crude extract killed more

than 90% of snails under field conditions. Comparing with the results of Gogo (*Entada phaseoloides*) (Yasuraoka *et al.*, 1977), it seems likely that *C. tiglium* is much more efficient than *E. phaseoloides* against *O. quadrasi*. The main disadvantage of *C. tiglium* is that it is highly toxic to fish. The importance of a molluscicide of this kind cannot be overestimated in those areas where schistosomiasis is endemic and where fish is an important source of protein for the inhabitants. Additional disadvantage is the fact that the seed of *C. tiglium* contains croton oil which has been used as a cocarcinogen in cancer research. It is well known that dimethylbenzanthracene or some other carcinogenic hydrocarbon induces latent tumors under the influence of croton oil (Berenblum, 1941; Berenblum and Shubik, 1947). More recently, Pound (1966, 1970) reported that croton oil enhanced the tumor-initiating ability of urethan and ultraviolet light. The question then arises as to the usefulness of the seed as a practical molluscicide.

Miss L. L. Talaue (Dept. of Zool, UP)

Were other parts of the plant considered - those which are more luscious such as the roots and leaves, which may not contain croton oil?

Dr. Yasuraoka (Univ. of Tsukuba)

We examined only the seed.

FIELD TRIALS WITH TUBA (*CROTON TIGLIUM*) SEED AND POLYNACTIN AGAINST *ONCOMELANIA QUADRASI*

Eraklio A. BAÑEZ
Schistosomiasis Control and Research Project, Ministry of Health

Two small scale field trials were conducted to assess the molluscicidal activity of tuba seed (*Croton tiglium*) and polynactin with Bayluscide (niclosamide) as the reference molluscicide. Results showed that at 8 grams per 2 liters of water per square meter of tuba seed powder can compare favorably with Bayluscide at 1 gram per 2 liters of water per square meter. Polynactin, however, showed poor results when applied at 1 mililiter in 2 liters of water per square meter.

Preliminary assessment on the effect of tuba seed powder on the miracidia and cercariae of *S. japonicum* showed 100% immobilization for both at 8 ppm within one hour. Polynactin and Bayluscide were also effective against these larvae at 2 and 1 ppm, respectively.

With the promising results, it is recommended that further studies and field trials be made while at the same time a practical method of preparing the compound from tuba seeds for field application both as a molluscicide and/or cercariacide be worked out.

LABORATORY ASSESSMENT OF THE MOLLUSCICIDAL ACTIVITY OF THE PLANT *JATROPHA CURCAS* AGAINST *ONCOMELANIA* SNAIL

Kazuo YASURAOKA[1], Junichi HASHIGUCHI[1] and Bayani L. BLAS[2]

1) *Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki-ken 305, Japan*
2) *Schistosomiasis Control and Research Project, Palo, Leyte, Philippines*

In the course of our studies in plant-origin molluscicides, the seeds of both *Croton tiglium* (Tuba in Tagalog) and *Jatropha curcas* (Tubang-bakod in Tagalog) which grow indigenously and abundantly in the Philippines showed a promising molluscicidal activity against *Oncomelania quadrasi*.

The seed of *C. tiglium* was molluscicidal, but piscicidal and cocarcinogenic as well. Our endeavour, therefore, is being made to assess the molluscicidal properties of *J. curcas*.

Tests with Crude Extract against *O. quadrasi*:

The seeds were shelled and 50 mg of the inner fruits was thoroughly mashed in a mortor with 50 ml of distilled water, to which approximately 950 ml of distilled water was added to give a 50 ppm emulsion. A two-fold dilution series was prepared from the emulsion in order to compute the LC_{50} values. *O. quadrasi* used in the experiment were collected from Leyte, Philippines. Only snails which were actively moving when placed in aged tap water were used.

For the test, 10 mature snails (3.5 to 5.0 mm in shell length) were put into a Petri-dish (10 cm in diameter) with 100 ml of test dilution at 25°C. The testing procedure was accomplished as reported previously (Komiya *et al.*, 1962), using 30 snails for each dilution with 10 snails per dish.

Snails were exposed to test dilutions for 48 hrs. Following the exposure period the snails were rinsed and placed in aged tap water for 48 hour recovery period, after which the reading of dead and living snails was made. The data were analysed by probit analysis method (Litchfield and Wilcoxon, 1949).

Tests were made in triplicate each using the two-fold dilution series. The LC_{50} and LC_{90} are as follows:

	LC_{50} (95% confidence limit)	LC_{90}
1st	25.5 (19.5 – 33.4) ppm	48.5 ppm
2nd	24.5 (22.5 – 26.6) ppm	47.0 ppm
3rd	18.0 (14.9 – 21.7) ppm	27.5 ppm

Acute Oral Toxicity of the Seed in Mice:

The ground seed was given to 5-week-old mice (DDD strain) with the aid of a stomach tube attached to a syringe in a single dose of 0.1, 1.0 and 10 g/kg of body weight. All the

mice treated could stand even a dose of 10 g/kg. A 100% mortality, however, was obtained following a dose of 10 g/kg for 3 consecutive days.

Toxicity to Fish:

Japanese killyfish, *Oryzias latipes* in group of ten (10) were exposed to appropriate two-fold dilution series of mashed fruit for 48 hrs at 25°C. Only 40% mortality was obtained even at 500 ppm, the maximum dilution tested and no LC_{50} was computed. It was thus found that the concentration of the fruit which kills *O. latipes* is far higher than that needed to kill snails.

Comparison of the Molluscicidal Activities of Different Fractions from the Seed:

In order to assess the relative molluscicidal activity of various fractions of the seed, 6 grams of the ground innerfruit was extracted with 20 ml of methanol at 25°C for 20 hrs. The methanol phase was centrifuged at 3,000 rpm for 5 min. and then 10 ml of the supernatant was brought to dryness in vacuo, yielding 254 mg of semifluid material upon evaporation. Extraction was also performed in a similar procedure with each of n-butanol, chloroform and benzene and yielded 1,457 mg, 1,896 mg and 1,457 mg of viscous form, respectively.

A 100 ppm dilution was prepared by emulsifying 20 mg of each fraction in 1 ml of N-methyl-2-pyrrolidone and then by bringing it up to 200 ml by the addition of water, from which two-fold dilution series were made with stirring. Parallel tests with niclosamide were run. The testing procedure was essentially same as described above. The LC_{50} values are as follows:

Methanol Fraction	6.7 ppm
n-Butanol Fraction	45 ppm
Chloroform Fraction	65 ppm
Benzene Fraction	40 ppm

of the fractions tested, methanol fraction was found to possess molluscicidal properties against *O. quadrasi.*

Influence of Strong Acids and Alkalines upon the Molluscicidal Activity of the Methanol Fraction:

A 100 mg of the methanol fraction was dissolved in 2 ml of N-methyl-2-pyrrolidone, of which 0.1 ml was diluted with 50 ml of N/100 HCl with stirring at 60°C for 20 minutes.

The dilution was then adjusted to pH 5.6 by the addition of N/100 NaOH, from which a 50 ppm dilution was made. Treatment with N/100 NaOH was performed in a similar manner to that with N/100 HCl. All experiments involved appropriate controls, i.e. water, N-methyl-2-pyrrolidone and N/100 NaCl treated either with N/100 HCl or with N/100 NaOH.

All the snails were killed in 50 ppm methanol extract pretreated with N/100 HCl or N/100 NaOH. No remarkable degradation of the molluscicidal activity of the methanol extract was thus observed when treated with acid and alkaline.

Preliminary Field Trials:

Two separate field trials with the crude extract were conducted during months, October to November 1979, one in Caibaan and another in Gacao in Leyte. Two sets of ten quadrats (1 square meter each) were measured, delineated by wooden boards to prevent snails crawling in and out. Five hundred snails were released into each quadrat and made to acclimatize for 3 days prior to application of the chemical. Measured doses of the extract were diluted in 2 liters of water and sprayed in each quadrat. Samplings were made by scraping the top soil surface of 9 rings (13.5 cm in diameter) after a period of 1, 2, 3, and 4 weeks from the application. A satisfactory kill, more than 90 percent mortality, was obtained at a dose of 4 grams per m^2 as early as 2 weeks after application of the extract.

Comments:

Limited field trials revealed that *J. curcas* was as efficient as *Croton tiglium*, whereas laboratory trials showed that the former was less efficient than the latter. *J. curcas* is much more aboundant than *C. tiglium* in the Philippines. Additional advantage to be considered is that *J. curcas* is less toxic to fish than *C. tiglium*.

The results of the present study are very encouraging and it is felt that there would be a certain scope for its practical use as a molluscicide in the Philippines.

SNAIL CONTROL BY MEANS OF MOLLUSCICIDING

Rogelio HAMBRE
Schistosomiasis Control and Research Project, Ministry of Health

In the screening of chemicals made by Schistosomiasis Control and Research Project (SCRP), it was reported that Bayer 73 or Bayluscide 70% and sodium pentachlorophenate appeared to be the best among the top nine chemicals with more than 90% snail mortality rate when applied at a concentration of 10 ppm. Of these two chemicals, Bayluscide is less toxic to the handler. Therefore, this is the chemicals of choice in the control program of the Schistosomiasis Control & Research Project (SCRP) until such time that other relatively effective and non-toxic compound to the handler either, synthetic or of plant origin can be developed.

Bayluscide is applied at 0.5 to 1 g per square meter either by broadcasting or hand spraying. When the latter method is used, the chemical is mixed with sawdust or rice bran to attain a more or less even application in the area per square meter.

Application can be carried out in 6 months interval in a cleared area. Clearing is necessary before chemical application will be carried out. Without clearing, snail repopulation goes beyond 5% as early as 1 month and 20 days. This would indicate that, in an area which has not been cleared, repopulation can start much earlier.

Due to ineffectiveness of Bayluscide in uncleared areas plus its high cost, Bayluscide molluscicide is limited to areas which have been controlled by agro-engineering measures, as a terminal measure in pockets that are difficult to drain or in areas where snails still persist.

The present method of evaluation is by percentage snail reduction in an area based on the estimated total snail population.

SCHISTOSOMIASIS CONTROL PROGRAM: HEALTH COMPONENT NATIONAL IRRIGATION SYSTEMS IMPROVEMENT PROJECT – I

Julian S. NOSEÑAS
Schistosomiasis Control and Research Service, Ministry of Health

The Schistosomiasis Control Program in Leyte under the National Irrigation Systems Improvement Project – I (NISIP-I) of the National Irrigation Administration (NIA) came about as an offshoot of the government policy to develop the country side with emphasis on agriculture and other infrastructure with the end in view of uplifting the social and economic well-being of the people in the rural areas. However, provision of irrigation facilities by itself sometimes spread the disease as snail vectors which otherwise are confined in one area are carried by irrigation canals to formerly unaffected areas. It is for this reason, among others, that NISIP-I has one of its main objectives, a Schistosomiasis Control Program.

There are two components of the schistosomiasis control program, namely: the infrastructure and the health components. The infrastructure component consists of snail control through construction of proper drainage in water-logged areas and construction of footbridges, while the health component includes treatment of human cases, sanitation campaign, chemical control of snails (synchronized with the agro-engineering control), health education, and disease evaluation. This plan actually involves implementation of the four-pronged approaches in schistosomiasis control which are intended to break the weak links in the life cycle of the parasite.

Of the 24 known endemic towns in Leyte, at least 19 will directly be benefited by the project. The 19 endemic towns are found within the service areas of seven irrigation systems located in Leyte's northeastern coastal plains. The climate and topography of the coastal plain are particularly suitable to the snail vector of the parasite, while the way of life of the rural population, with its constant exposure to water, and the low level of public hygiene all work together to produce optimum combinations needed for high infection rate. The proposed control program would make the habitat less favorable to the vector snails by improved drainage, reduce the number of snails by use of molluscicides, lessen the population exposure with the provision of footbridges, improved general hygiene by environmental sanitation and health education, and reduce the parasite reservoir by chemotherapy. This combination of public health and engineering measures is based on a pilot work previously done in Leyte and similar campaigns carried out in other schistosomiasis affected countries.

In accordance with a Memorandum of Agreement between the National Irrigation Administration (NIA) and the Ministry of Health (MOH) signed on February 2, 1977, the NIA, as principal or head agency in the execution of the schistosomiasis control program shall undertake the infrastructure or engineering component, procure necessary materials and equipment for the MOH, and provide reasonable logistic support and incentives to personnel fielded by the MOH for schistosomiasis control within the project area, and the MOH takes responsibility in the implementation of the health component. The MOH, among others, shall prepare and submit, in consultation with the NIA, detailed plan of implementation for the health component and assist the NIA in the preparation of plans for drainage and for operation and maintenance of the canals in the area in order to increase their impact on schistosomiasis

control. The schistosomiasis control in Leyte is programmed for a 5-year period from 1977 to 1981.

The estimated total cost for the health component is ₱ 26,025,000. Of this amount, ₱ 15.90 M shall be financed by the World Bank and the remaining ₱ 10.125 M by the Philippine Government as local counterpart.

The main emphasis of the health component of the control program is to provide for a health education campaign based principally on the schools and barrio health centers in 19 disease-affected municipalities. The health education program has been designed to have a more immediate impact on the implementation of the project on the local level by orienting a large number of opinion leaders in the basic problems of schistosomiasis and the required preventive behavior. This is backed up by an environmental sanitation drive to furnish about 15,000 unequipped households in the area with watersealed toilets and to improve the water supply with the installation of at least 5,000 jetmatic hand pumps. Following the provisions of drainage, the reclaimed areas would be treated with molluscicides for effective snail control, limited initially to the fringes of newly drained irrigated areas and later carried out on a continual basis on selected habitat which have not been drained and which are in close proximity to population clusters. The elementary school population in the endemic areas would be monitored yearly for disease incidence and drugs will have to be provided for the treatment of 60,000 cases.

SCHISTOSOMIASIS CONTROL PROGRAM – INFRASTRUCTURE COMPONENT: NATIONAL IRRIGATION ADMINISTRATION

R. BONROSTRO
National Irrigation Administration, Ministry of Public Works

The National Irrigation Administration (NIA) in her unending support to the country's objective of achieving self-sufficiency in basic food and to correct regional economic and social imbalances is presently implementing the National Irrigation Systems Improvement Project, Package I (NISIP-I) with loan assistance from the World Bank.

Included as one of the important objective of the project would be to carry out a Schistosomiasis Control Program in the area of high endemicity of the disease in the island of Leyte in collaboration with the Ministry of Health (MOH).

Schistosomiasis is endemic in 24 out of the 49 municipalities of Leyte and considered to be a major health problem in the area. Seven of the ten irrigation systems included under the project are located on Leyte's eastern coastal plains, one of the worst schistosomiasis endemic region. The total command area of the seven irrigation systems is 11,800 hectares. The mean annual rainfall is about 2,112 millimeters but even in April, the driest month in the year, rainfall averages 113 millimeters. The combination of constant rain, flat relief and poor drainage has made the area particularly dense in snail habitat. The project area is characterized by poor drainage. The natural drainage channels are shallow in depth and water flow to the coastal reaches are retarded due to the flat topography and constraints at the outlet to the sea.

The main engineering emphasis of the program would be to open up all existing drainage ways, to increase the intensity of drainage works in cropped areas with the basin as a whole and to have provisions for good access to drainage work so as to permit regular and efficient maintenance. The drainage network would be planned to include all existing drainage ways above and adjacent to the irrigation service area which are contributing to the spread of the disease. Gravel surfaced Operation and Maintenance (O & M) roads will be constructed on majority of the main drains including some of the secondary drains. About 250 kilometers of existing creeks and natural channels would be enlarged, realigned, deepened and properly shaped as design to serve as drainage canals.

To reduce contact of the population with water in irrigation and drainage canals, about 800 foot bridges will be constructed. Foot bridges made of T-shaped precast elements would be erected at 250 meters intervals on major ditches, drains and the lower portion of secondary laterals or drains. On major irrigation and drainage canals, there would be concrete bridges located on various crossings to facilitate the easy movement of both equipment and the populace.

To effectively implement the schistosomiasis control program, a Memorandum of Agreement between MOH and NIA has been executed to set out the responsibilities of the two agencies. NIA would carry out all the engineering portion while the MOH would be responsible for the health aspect. NIA would be responsible for O & M of the drainage facilities and MOH would be responsible for including provision for a continuous molluscicide maintenance program and monitoring of snail population and clinical infestation.

The schistosomiasis control program is estimated to cost a total of US$15.7 million, including a foreign exchange component of US$7.4 million. The engineering aspect would amount to US$12.2 million and the health aspect US$3.5 million.

The schistosomiasis control program would reduce the prevalence of the disease among the entire population of Leyte and would increase agricultural yield mostly on unquantifiable benefits derived from the project.

SUCCESS OF SCHISTOSOMIASIS CONTROL IN JAPAN

Kazuo YASURAOKA
Department of Medical Biology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki-ken 305, Japan

Five endemic areas of schistosomiasis have long been known in Japan, four on the main island of Honshu and one on the island of Kyushu. Although some small snail colonies are still found in the Kofu Basin, schistosomiasis in Japan is now on the wane and it is perhaps the only country where the stage seems set for the ultimate eradication of the disease. Such a great success can be attributed to well-planned, persistent control measures and over-all rises in economic and social standards.

It may be said without much exaggeration that in Japan we have already started the snail control work for schistosomiasis several hundred years ago. It was, of course, far before Drs. Fujinami, Miyagawa, Miyairi and Suzuki made clear the life cycle of this parasite in the early part of this century. There is a large-scale embankment of the Kamanashi river flowing through the Kofu Basin, a largest endemic area of schistosomiasis in Japan. The embankment is called "Shingen-tsutsumi" since it was constructed by the order of Takeda Shingen, a famous commander in this area in the mid-sixteenth Century. The socioeconomic necessities and geographical conditions in Japan induced the people to work out river improvement, drainage in swamps, land reclamation and extensive cultivation of land. About 70 years ago when how to control this disease was known, there were few or no flood-plain forests and swamps, and as a result, the vector snails had already been shut up only in irrigation ditches in rice fields. We were able to concentrate our attention on the snails only in irrigation ditches. It was, therefore, not painstaking task for us to control the vector snails by the use of molluscicides and the lining of irrigation ditches in rice field with concrete.

As early as 1914, Miyagawa recommended the use of unslaked lime and calcium cyanamide as a molluscicide. Other snail control measures such as borning and burying snails were adviced by Tsuchiya (1916) and Nagao and Kato (1917), respectively.

On the other hand, Yamanashi Prefectural Government carried out a snail hand-picking campaign in 56 municipalities for a period of 8 years, 1917-1924. Well-trained 3 technicians in each municipality were engaged in the campaign. The Government gave a bounty of Ca. US 50 cents for the first 1 *go* (=0.18 liter) of snails and 10 cents for each additional 0.18 liter. However, it was not long before they noticed that the snail hand-picking itself was not effective means in snail control.

In accordance with the Parasitic Diseases Control Law enacted in 1931, snail control with molluscicides has been carried out as a national programme. In its early stages, calcium cyanamide was used extensively as a molluscicide in almost all the endemic areas. The use of calcium cyanamide was discontinued for several years after the war and replaced successively by sodium pentachlorophenate (NaPCP) in 1951-1954, Yurimin (3,5-dibromo-4-hydroxy- 4' nitrozaobenzene) in 1965-1971and then B2 (sodium 2,5-dichloro-4-bromophenol) in 1975.

The time of application of molluscicides must be taken into consideration. In Japan, where there is a cold season of 3 to 4 months, and where irrigation starts in May and ends in

September, applications have been made twice a year, in spring and autumn when irrigation is not in progress.

Cement lining of irrigation ditches is expensive but it has several advantages. The majority of snails are buried in soil during the process of the construction of new ditches, and cemented ditches not only make it difficult for snails to get food but also interfere with their oviposition and reproduction. The relatively high velocities possible in lined ditches bring unfavorable conditions for snails. In addition, the application of molluscicides is easier, and more effective in lined ditches than in earth ditches.

In Japan, cement lining of irrigation ditches has been carried out in practice since 1950. The status, as of 1976, of cement-lined ditches in the endemic areas in Kofu Basin attained a length of 1,690 km, i.e. more than the distance between the northern tip of Luzon Island and the southern tip of Mindanao Island. Lined ditches must, however, be maintained clear of muddy vegetation, sand, pebbles, algae and aquatic vegetation which frequently tend to accumulate in them.

Environmental sanitation, safe water supply backed up by socioeconomic development in all the endemic areas played a large role in our control campaign. It should be emphasized, however, that such a success of schistosomiasis control in Japan would have been impossible without intimate collaboration with inhabitants in the endemic areas. They volunteered without any recompense to undertake such practical works as snail survey, weed clearance, spraying molluscicides, and the like.

Mr. Sales (MIRDO-NIA)

Dr. Yasuraoka, can you give us an idea of how much is the annual budget for schistosomiasis Control in Japan?

Dr. Yasuraoka (Univ. of Tsukuba)

The local government at Kofu spent US$2 M in 1978 for the consolidation of the effect of schistosomiasis control. The budget spent by other components dealing with Public Works or River Water Management was excluded from the above amount.

LIST OF GUESTS, OFFICIALS AND PARTICIPANTS

GUESTS

Dr. Jesus Azurin
Deputy Minister of Health
Republic of the Philippines

Mr. Kiyohisa Mikanagi
Japanese Ambassador
to the Philippines

Mr. Nakajima
Regional Director
World Health Organization
Western Pacific Regional Office
Manila, Philippines

OFFICIALS

Dr. Alfredo T. Santos, Jr.
Schistosomiasis Control and
Research Service
Ministry of Health

Dr. Ernesto J. Zerrudo
Schistosomiasis Control Council

Dr. Bayani L. Blas
SCRS, Palo, Leyte
Ministry of Health

Dr. Julian Noseñas
SCRP, Palo, Leyte
Ministry of Health

Dr. Benjamin Cabrera
Institute of Public Health
University of the Philippines

Dr. Edito Garcia
Institute of Public Health
University of the Philippines

Dr. Ernesto Domingo
College of Medicine
University of the Philippiens

Dr. Yoshio Hirose
First Secretary
Embassy of Japan, Manila

Dr. Masaharu Ito
Japan International Cooperation
Agency, Tokyo

Mr. Toshikazu Miura
JICA, Manila Office

Dr. Hiroshi Ogonuki
Japanese expert in Bureau of
Research and Laboratories

Dr. Kazuo Yasuraoka
Department of Medical Biology
Institute of Basic Medical Sciences
University of Tsukuba

Dr. Hiroshi Tanaka
Department of Parasitology
Institute of Medical Science
University of Tokyo

Dr. Masataka Hayashi
Department of Neurology
Kofu City Hospital

Prof. Rolando Garcia
College of Arts and Sciences
University of the Philippines

Engr. Vicente de Veyra
Schistosomiasis Control Council

Dr. Gerundio Portillo
SCRP, Palo, Leyte
Ministry of Health

Mr. Cesar de los Reyes
University of the Philippines
College Tacloban

Dr. Perla Velasco
SCRS, Manila
Ministry of Health

Miss Anastacia Daya-on
Schistosomiasis Control Council

Mr. Kiyoshi Makiya
Department of Medical Zoology
Nagoya University School of Medicine

Mr. Koichi Goto
JICA, Manila Office

Miss Mitsuyo Kamijo
JICA, Tokyo

Dr. Arturo Reyes
World Health Organization
Western Pacific Regional Office
Manila, Philippines

Mr. Manuel J. Santos
SCRS, Manila
Ministry of Health

Dr. Lilian Tormis
SCRP, Palo, Leyte
Ministry of Health

PARTICIPANTS

Schistosomiasis Control and Research Service, Manila, Ministry of Health

Dr. Ofelia Alialy
Miss Arlita Aquitania
Miss Merle Macarañag
Miss Agripina Cudia
Miss Blesida Racimo
Miss Emelita Gatmaitan
Mrs. Lili P. Bautista
Miss Dolores Enriquez
Mr. Kenneth Bolante
Mr. Cresente Creer
Mr. Alfonso Malicdem
Mr. Celerino Cruz
Mr. Absolon Guia
Mr. Jun Inosencio
Mr. Escolastico M. Fabella
Dr. Ruel Villarete
Dr. Roger Abear
Miss Eliza Felipe
Mrs. Veronica P. San Agustin
Ms. Norma Natividad

SCRS, Irosin, Sorsogon
Dr. Felix Gillego
Dr. Roque Burio

SCRS, Victoria Oriental Mindoro
Dr. Marcela Papasin
Dr. Rosalinda Boada

SCRS, Bonifacio, Misamis Occidental
Dr. Guadalupe Flores

SCRS, Catbalogan, W. Samar
Dr. Rizal Velasco

SCRS, Digos, Davao del Sur
Dr. Lorna Ranada

SCRP, Palo, Leyte
Mr. Eraklio A. Bañez
Mr. Rogelio Hambre

SCRS, Tagum, Davao del Norte
Dr. Ofelia Poliquit

Schistosomiasis Control Council

Engr. Edgardo Bautista
Engr. Edgardo Castañeda
Mr. Rosalito Nucum
Miss Lydia Competente
Mrs. Felicitas S. Sajona
Miss Corazon Ingles
Miss Delia S. Mata
Miss Leonida Alo
Mr. Manuel I. Rosales

Japanese Participants

Dr. Kunioki Araki
Department of Microbiology
The Institute of Public Health

Dr. Hajime Matsuda
Department of Parasitology
Institute of Medical Science
University of Tokyo

Dr. Haruo Kamiya
Department of Parasitology
Faculty of Veterinary Medicine
Hokkaido University

Dr. Masanori Kawanaka
Department of Parasitology
National Institute of Health, Japan

Dr. Mitsuo Ishige
Hokkaido Prefectural Institute of Health

Mr. Masato Uwagawa
Japanese Expert in NIA

Dr. Atsuo Sato
Faculty of Medicine
Kagoshima University

Dr. Hisatake Nojima
Faculty of Medicine
Kagoshima University

Institute of Public Health
University of the Philippines

Dr. Francisco S. Sy
Dr. Nelia Salazar
Mr. Amante S. Cruz
Ms. Bernadette Ramirez
Ms. Helen Masangcay
Mr. Ronaldo Trias
Miss Lilian de las Llagas
Miss Helen Y. de Asis

Department of Zoology
University of the Philippines

Prof. Reynaldo Yago
Prof. Ruben C. Umaly
Miss Liana L. Talaue

Up Student

Dr. Syed Sultan
Miss Elizabeth Tadeña
Miss Jocelyn Nicolas

Malaria Eradication Service
Ministry of Health

Dr. Delfin Rivera

Office of Health Education and
Personnel Training, Ministry of Health

Mrs. Aida Soldevilla
Miss Rosario de Vera

Mr. Val Loyola
Information, MOH

Mindoro Integrated Rural Development Office

Mr. Carlos Sales

National Irrigation Administration

Engr. R. T. Bonrostro
Engr. Serafin Palteng
Engr. Mariano Leuterio

Bureau of Animal Industry
Ministry of Agriculture

Dr. Pedro Dumag

University of Chicago, USA

Dr. Robert M. Lewert
Dr. Mariano Yogore, Jr

University of California, Los Angeles, USA

Dr. Ralph A. Barr

U.S. Naval Medical Research Unit II, Manila

Dr. John Cross
Dr. Kurt Sorensen

Swiss Tropical Institute

Dr. A. Degremont

Department of Biology,Mahidol University
Bangkok, Thailand

Dr. Suchart Upatham
Dr. Vithoon Viyanant

JICA