STUDIES ON *STRONGYLOIDES RATTI* WITH A SPECIAL REFERENCE TO THE SCREENING TEST FOR *STRONGYLOIDES* ANTHELMINTICS

BY

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**Introduction**

*Strongyloides ratti* Sandground, 1925 is a parasite of *Rattus norvegicus* and has many similarity to the human *Strongyloides* (*S. stercoralis*). It has been often used as a material for ecological studies on genus *Strongyloides* since the discovery of this parasite. Sandground (1926) and Graham (1936–38) applying *S. ratti* attempted to determine the nature of the females of parasitic generation and found important approach to its resolution. Furthermore Sheldon’s findings (1937) presented the course of parasitism of this worm in experimentally infected albino rats.

On the other hand, the treatment of human strongyloidiasis is still of problem. Though gentian violet and dithiazanine are valuable anthelmintics, the side reaction of those drugs restricts their wide use and the discovery of more safety and more satisfactory anthelmintic is expected. Little information has so far been available about the application of *S. ratti* for the screening test of anthelmintics besides an examination made by Erhardt & Denecke (1939).

The present study attempted to construct the method of the screening test with *S. ratti*, because of such advantages as the collection of the material was thought simple, the experiment could be made with laboratory rats and consequently whole process was befitting to the laboratory study. And also in the life cycle of this parasite, differing from *S. stercoralis*, autoinfection is not thought to take place, for little egg hatches in the intestine of a host animal. A lack of autoinfection makes the valuation of anthelmintics simple because administered drugs affect only females of the parasitic stage and the results of the vermicidal and/or vermilugial action can be directly obtained.

The present study, purposing the screening test, began with a survey of the distribution of this parasite among house rats and all observations were measured quantitatively as possible and attention was much payed for a choice of the simple procedures of technics for the practical use. The epidemiological study of this parasite simplified the collection of material for...
experiments and the sensitivity of the cultivation and floatation technics were tested for the quantitative detection of the discharged number of eggs in feces. After the natural course of the egg discharge in experimentally infected albino rats was examined, standard method of test of drugs was established. Following the testing procedure, several kinds of anthelmintics were tested and the practical evaluation of this testing method was studied. From the results of a series of present studies, Strongyloides ratti could be applied to the screening test of anthelmintics under certain conditions. A part of the present study has been already published by the authors (1959) in Japanese and the present paper deals with revised results of the whole studies.

METHODS

Details of the experimental technics were presented in each chapter and the technics common to the present study were as follow:

Fecal examination

As the rats discharged feces at night, collection of fecal samples was made at 9 A.M. in the morning and approximately 5 grams of soft portion were used. The fecal pellets were smashed between two pieces of slide glass to distribute the eggs homogenously in a fecal sample, and the eggs were detected from thus prepared feces.

1. Smear technic.: Fecal film was prepared for the purpose of the examination of the establishment of parasitism in infected rats and also the quantitative measurement of egg counts. In quantitative measurement, 10 mg of fecal samples were weighed using a torsion balance and divided into three thin smear specimens. Egg count was presented as an average of three hold observations of 10 mg fecal samples.

2. Floatation technic.: The floatation technic used was the routine procedure with saturated saline solution. In quantitative observations, 100 mg of fecal samples were weighed applying a torsion balance and the test tube used was 8 cm in height and 1.2 cm in diameter. The tubes filled with saturated brine solution were kept for 45 minutes and the surface layer of the solution was removed with a piece of cover glass. In the kept period, those test tubes were settled in cold water, bottom half of the tube dipped, in order to fix the current of the solution in the tube because the current was the main factor to disturb the quantitative measurement. (Tanaka, Asako and others, 1957). The average was calculated from the counts of three tubes made from a fecal sample.

3. Cultivation technic.: The test-tube cultivation was used for detection because of its simple procedures and readily application to the quantitative
measurement. On a half part of a filter paper tape for chromatography (15 cm × 2 cm) 0.5–0.3 gram of feces was smeared. And the filter paper was introduced into a test tube (14 mm × 15.5 cm) with 2.5 cc of tap water, advancing the intact side of the filter paper to the bottom. The opening of a test tube was covered with polyethylene sheet and rubber band. This technic was originated by Harada & Mori (1951) for the purpose of the detection of the hookworms and was developed for widely practical use by Sasa, Hayashig, Tanaka et al. (1957, 58), Sasa, Tanaka, Abe et al. (1958), Tanaka (1957, 58) Tanaka, Amano et al. (1958), Tanaka, Tokuriki et al. (1958). For the cultivation of S. ratti, the tubes were incubated at 26°C during 6 days for detection and 5 days for quantitative counting. The larvae appeared in the test tubes were collected into a watch glass and were counted under a stereomicroscope, sucking the larvae into a micropipette. Counts of larvae were determined from an average of three tubes prepared from a fecal sample.

Rearing of albino rats

Albino rats, weighing approximately 200 g were used and fed on the dry blocks of rat food and water in an individual wire cage of metal net. Between neighboring cages, metal diaphragm was fixed to prevent the contamination of fecal pellets of other rats (Fig. 1). In metal dishes under rat cages a sheet of newspaper with a little amount of water was placed to keep the humidity for the prevention of loss of water content from fecal pellets.

Counting of parasitic females

As the site of maintenance of S. ratti is in the intestinal mucosa, the

Fig. 1. Rearing cages of rats.
quantitative count of this parasitic females is difficult. Comparing several techniques, the purpose was approximately satisfied using the autolysis technic of the intestine kept in an ice box, originated by HUNNINEN (1935) and SHELDON (1937). The removed intestine was cut longitudinally, washed in water and placed on a Petri dish. After water was introduced into the Petri dish, as the surface of water barely covering the intestine, the dish was kept in an ice box overnight. In those preparation, intestinal mucosa was autolysed and scattered in fragments when the intestine was shaken in the water. The intestinal mucosa was absolutely removed from the wall by replacing the water of the Petri dish and washing the intestine for several times. In the water used for washing, worms were collected isolating from the intestinal mucosa. When the large fragments remained, they could be divided into small size by the repeat of suddenly sucking and spouting of the water with a pipette.

**Optimum Conditions for Cultivation**

The efficiency of the test-tube cultivation technic was much influenced by the volume of the feces on the filter paper, temperature and the period of incubation. Optimum conditions for the test-tube cultivation were obtained changing those elements written above.

1. **Temperature:** The test tubes each containing 0.5 g. of feces on the filter paper were incubated at 20°, 26°, 30°, and 37°C. for 6 days, 5 tubes at each temperature, and the largest counts of larvae were gained at 26°C.

2. **Volume of feces:** The volume of feces on the filter paper was varied 0.1, 0.3, 0.5, 0.7 and 1.0 g for comparison and 5 tubes with each volume were incubated at 26°C. for 6 days. The water in the test tubes was kept clear when less volume of feces was used and the adequate volume for the filter paper (2.5 × 15 cm) was between 0.3 and 0.5 g. From the results, 0.5 g of feces was smeared for detection and 0.3 g was used for the purpose of the quantitative measurement.

3. **Period of days:** Incubation period was determined making two different experiments. In one method of experiment, 10 cultivation tubes with 2.5 cc of water were prepared and kept at 26°C. In every 24 hours, the water was changed and the larvae appeared within the latest 24 hours were counted. The counts of larvae were the most in number at the 2nd and 3rd 24 hours as shown in Fig. 2. From the cumulated number of counts in every 24 hours, as presented in Fig. 2, the larvae appeared 37.4 per cent by 2 days to the value of the total counts in the period of 16 days, 59.6% by 3 days, 76.9% by 4 days, 85.5% by 5 days, 89.7% by 6 days, 93.2% by 7 days and 97.8% by 10 days. In this observation, approximately 90 per cent of larvae were
known to appear within 6 days.

In another experiment, 64 cultivation tubes of the same preparation were incubated and 8 tubes were daily taken out and observed for 8 days. The average of the counts of larvae in Fig. 3 reached the maximum within 3 to 4 days. As a control group, larvae were counted at every 24 hours as similar to the former experiment and the average of 8 tubes was presented in the same graph (Fig. 3). In those comparison, more larvae could be obtained in the cultivation of which water was daily renewed.

From those two experiments, the larvae in a cultivation tube could be regarded as to appear to the maximum value within 6 days. On the other hand, the water increased turbidity and larvae was often hardly observed by the incubation for longer than 6 days. Consequently the adequate period of days for incubation was determined as 5 to 6 days. And as the water of almost all tubes was kept clear within 5 days, the period was fixed as 5 days for the purpose of quantitative observation.

**Propriety of Cultivation and Floatation Technics for Quantitative Measurement**

For the detection of *S. ratti* from the feces of rats, the floatation technic was also applicable besides smear or cultivation technics. The floatation technic and cultivation technic were more sensitive for the detection than smear technic and if they were applicable to the quantitative observation, the egg counting makes ease from the stool with less number of eggs. In the

![Graph](image_url)

**Fig. 2.** Daily occurrence of larvae of *S. ratti* in test tube cultivation technic.
(cultivated at 26°C, water renewed every 24 hours, average of 10 tubes)
following experiments, correlations of the detected number by the flotation or cultivation technics to those by the smear technic were tested. Prior to the study of the correlation, the stage of *S. ratti* in the feces was observed to know if many rhabditoid larvae hatched in the evacuated feces. As the rhabditoid larvae could not be floated in the saturated brine and if they existed in feces, the quantitative observation was impossible by flotation technic.

1. The stage of *S. ratti* in feces: The fecal samples immediately after evacuation were obtained from 5 rats and the stage of *S. ratti* was observed. The observed form was all eggs counting 100 in each sample. The ratio of eggs to larvae in evacuated feces was estimated by the indirect method of the observation of the hatching time. The feces just evacuated was emulsified with water and incubated at 29°C. The small portion of them was observed making smear specimens at every 2 hours and the regression line of the time-hatching rate was calculated as presented in Fig. 4. The formula of the regression line is \( Y = 0.16X + 2.87 \) where \( Y \) is probit of the hatching ratio and \( X \) is hours elapsed from the evacuation of feces. From this formula,

![Image of graph showing the comparison of the occurring courses of larvae of *S. ratti* cultivated with and without replacing water in the test-tube cultivation technic. (at 26°C)]

(a) Replacing water at every 24 hours, average of 5 tubes.
(b) Average of 8 tubes which were taken from 64 tubes prepared at the beginning.
Fig. 4. Regression line of time-hatching ratio of *S. ratti* at 29°C.

Fig. 5. Correlation of egg counts measured by *smear* technique and flotation technique. 

$r = 0.78$  \hspace{1cm} $F_0 = 60.52 > F_{0.01} = 7.17$
the percentage of the hatch was estimated as 1.7% (Probit 2.87) at the beginning and the larva in the feces was also regarded as negligible. The result indicates that the floatation technic is expected to be applicable when the feces is used within a short period of time after evacuation.

2. Correlation between egg counts of smear and floatation technics: From a sample of feces, 10 mg was taken three times for smear specimens and 100 mg three times for floatation technic. The averages of egg counts of three observations of both smear and floatation technics were calculated and regarded as the corresponding values. The observation were repeated in 52 fecal samples and the result was illustrated in Fig. 5. The coefficient of correlation was calculated as 0.78 and the formula of the regression line was $Y = 4.3X + 353.5$ where $X$ was count in 10 mg feces by smear technic and $Y$ was that in 100 mg by floatation technic.

![Graph showing correlation between smear and cultivation techniques](image)

Fig. 6. Correlation of egg counts measured by smear technic and cultivation technic. $r = 0.83$, $F_8 = 232.51 > F_{10,0.01} = 6.89$
3. Correlation between egg counts of smear and cultivation technics: The correlation between smear and cultivation technics was tested in the similar method to the former experiment. Feces was used \(10\) mg by smear technic and \(0.5\) g by the cultivation. The observation of 107 fecal samples gave the result shown in Fig. 6. The coefficient of correlation was 0.83 and the regression line was \(Y = 40.3X + 653.0\) where \(X\) was smear technic and \(Y\) cultivation technic.

Though the correlations exist between smear and floatation technics and also smear and cultivation technics, variances were great in Fig. 5 and 6. Consequently the quantitative measurement by means of floatation or cultivation technics should be applied only when the great changes or differences of egg counts were measured and the accuracy of the measurement of both indirect methods were incomparable to the Stoll’s egg counting technics. The advantage of the measurements by the floatation and cultivation is, however, to be applied to the feces with less count of eggs, of which counting could not be readily made by Stoll’s method because the latter is principally the dilution method.

**Distribution of *S. ratti* among House Rats**

The detection of *Strongyloides ratti* has usually been made obtaining the parasitic females from the intestinal mucosa of rats. However, the detection was attempted to be made from the fecal examination of rats by means of cultivation technic in the present study. And the parasitized incidences among rats were epidemiologically observed. As the eggs which appeared in high incidence in the fecal pillets of the house rats were those of *Nippostrongylus brasiliensis* (Travassos, 1914) and *S. ratti*, identification of both eggs and larvae was needed for the detection.

1. Identification: Eggs of *S. ratti* has thin shell and contains developed embryo, almost being in tadpole stage. The size of 50 eggs measured 53.0 \(\mu\) in an average with a standard deviation of 3.89 \(\mu\) in length and average 27.4 \(\mu\), standard deviation 1.57 \(\mu\) in width. On the other hand, the size of the eggs of *Nippostrongylus brasiliensis* was 65.8 \(\mu\) in an average with 4.10 \(\mu\) of standard deviation in length and 36.6 \(\mu\) in an average with 2.23 \(\mu\) of standard deviation in breadth. The shell is comparatively thick and the content is the mollula stage. *S. ratti* could be identified from *N. brasiliensis* observing eggs in feces by the morphological characters mentioned above. (Fig. 7)

The infective larvae of *S. ratti* look similar to those of another species of genus *Strongyloides* and is characterized by the long esophagus and divided tail tip. The larvae of *S. ratti* are shorter in length and more slender than
Fig. 7. Eggs immediately after evacuation
A.C. *Strongyloides ratti*, B.D. *Nippostrongylus brasiliensis*

Fig. 8. Infective larvae A. *St. ratti* B. *Nip. brasiliensis* N.R. nerve ring,
G.P. genital primordium, An. anus, T. tail, E.P. excretory pore
Fig. 9. Infective larvae A. *St. ratti*  B. Tail of *St. ratti*  C. *Nip. brasiliensis*  D. its anterior portion  E. its tail.

Table 1. Measurements of larvae of *Strongyloides ratti* and *Nippostrongylus brasiliensis* (averages)

<table>
<thead>
<tr>
<th>Species of larva</th>
<th><em>Stron. ratti</em></th>
<th><em>Nippo. brasiliens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouth cavity</strong></td>
<td>/</td>
<td>10.2 μ</td>
</tr>
<tr>
<td><strong>Nerve ring</strong></td>
<td>99.1 μ</td>
<td>110.9</td>
</tr>
<tr>
<td><strong>Excretory pore</strong></td>
<td>/</td>
<td>128.1</td>
</tr>
<tr>
<td><strong>End of esophagus</strong></td>
<td>256.9</td>
<td>155.3</td>
</tr>
<tr>
<td><strong>Genital premordium</strong></td>
<td>358.7</td>
<td>379.3</td>
</tr>
<tr>
<td><strong>Anus</strong></td>
<td>521.1</td>
<td>607.4</td>
</tr>
<tr>
<td><strong>Tail tip</strong></td>
<td>584.0</td>
<td>665.2</td>
</tr>
<tr>
<td><strong>Length of Genital premordium</strong></td>
<td>15.0 μ</td>
<td>10.9 μ</td>
</tr>
<tr>
<td><strong>Body breadth</strong></td>
<td>17.9</td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Length of tail</strong></td>
<td>63.0</td>
<td>53.0</td>
</tr>
<tr>
<td><strong>Length of sheath</strong></td>
<td>/</td>
<td>781.5</td>
</tr>
<tr>
<td><strong>ant. part of body</strong></td>
<td>/</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>post.</strong></td>
<td>/</td>
<td>113.9</td>
</tr>
<tr>
<td><strong>% to body length</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mouth cavity</strong></td>
<td>/</td>
<td>1.9 %</td>
</tr>
<tr>
<td><strong>Nerve ring</strong></td>
<td>17.0 %</td>
<td>15.7</td>
</tr>
<tr>
<td><strong>Excretory pore</strong></td>
<td>/</td>
<td>19.9</td>
</tr>
<tr>
<td><strong>End of esophagus</strong></td>
<td>43.3</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>Genital premordium</strong></td>
<td>61.6</td>
<td>57.1</td>
</tr>
<tr>
<td><strong>Anus</strong></td>
<td>89.2</td>
<td>92.1</td>
</tr>
<tr>
<td><strong>Body breadth</strong></td>
<td>3.3 %</td>
<td>4.6 %</td>
</tr>
<tr>
<td><strong>Length of tail</strong></td>
<td>10.8</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>% of genit. prem. in intestine from ant.</strong></td>
<td>38.4</td>
<td>50.3</td>
</tr>
<tr>
<td><strong>Nos. of specimens</strong></td>
<td>20</td>
<td>11</td>
</tr>
</tbody>
</table>
N. brasiliensis and readily distinguishable from the latter under stereomicroscope with low power (Fig. 8. 9.). The size and the proportion of the main structures of both larvae are presented in Table 1.

2. Distribution: The house rats, Rattus norvegicus and Rattus rattus were caught by snap traps in urban areas of Tokyo. S. rattii was detected from the contents of large intestine and coecum by means of the test-tube cultivation technic. Rats caught were 280 R. norvegicus and 98 R. rattus during the period from Dec. 1959 to June, 1958. The parasitized ratio in R. norvegicus was 62.5 per cent and much higher than that of R. rattus (13.3 per cent).

The incidences were compared among the localities of collection as presented in Table 2, but little difference was observed.

The seasonal prevalence in R. norvegicus was observed applying chi square test and the rate in the winter showed significantly higher than in the

<table>
<thead>
<tr>
<th>Localities of Tokyo</th>
<th>Rattus norvegicus</th>
<th>Rattus rattus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exam. nos.</td>
<td>Posit. nos.</td>
<td>Posit. %</td>
</tr>
<tr>
<td>Ohta-ku</td>
<td>41</td>
<td>28</td>
</tr>
<tr>
<td>Shinagawa-ku</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Minato-ku</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Kohto-ku</td>
<td>59</td>
<td>46</td>
</tr>
<tr>
<td>Chiyoda-ku</td>
<td>102</td>
<td>45</td>
</tr>
<tr>
<td>Others</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>280</td>
<td>175</td>
</tr>
</tbody>
</table>

Table 3. Seasonal difference of the infesting rates of S. rattii in Rattus norvegicus

<table>
<thead>
<tr>
<th>Season</th>
<th>Posit. nos.</th>
<th>(%)</th>
<th>Neg. nos.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>84 (70.6)</td>
<td>35</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>91 (56.5)</td>
<td>70</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175 (62.5)</td>
<td>105</td>
<td>280</td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2=5.77$, $0.02 > Pr > 0.01$ Significant

Table 4. Sexual difference of the infesting rates of S. rattii in Rattus norvegicus

<table>
<thead>
<tr>
<th>Sex</th>
<th>Posit. nos.</th>
<th>(%)</th>
<th>Neg. nos.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>86 (59.3)</td>
<td>59</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>89 (65.9)</td>
<td>46</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175 (62.5)</td>
<td>105</td>
<td>280</td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2=1.30$, $0.30 > Pr > 0.20$ Non-significant
summer (Table 3). In the summer season, fermentation of the intestinal contents was fast progressed, the embryo in the eggs might be killed and the efficiency of the detection by the cultivation technic was supposed to be reduced.

![Graph 10](image1)

**Fig. 10.** Comparison of the infesting rates of *S. ratti* by body weight difference among *Rattus norvegicus* in the winter (bars indicate 98% confidence intervals).

![Graph 11](image2)

**Fig. 11.** The similar comparison to Fig. 10 among *Rattus rattus*.

![Graph 12](image3)

**Fig. 12.** Comparison of the infesting rates of *Nipponstrongylus brasiliensis* by body weight difference among *Rattus norvegicus* in the winter (bars indicate the 98% confidence intervals).
The difference of the incidences between sexes was also compared among *R. norvegicus* in the similar method and no significant difference was clarified (Table 4).

Corresponding to the incidences among age groups in man, the incidences by body weight groups were calculated. *Rattus norvegicus* caught in the winter season, was classified into 50 g body weight groups and the incidences of those groups with 98 per cent reliability were presented in Fig. 10 and the incidences of *R. rattus* in the similar calculation in Fig. 11. The incidences of *S. ratti* among rats by the body weight show somewhat similarity to those of hookworm infection by age difference of man.

Comparing to the incidences of *N. brasiliensis* infection by body weight difference (Fig. 12), the incidence of *S. ratti* at the lightest body weight group is much higher than that of *N. brasiliensis* and the difference seems to exist between modes of infections of *Strongyloides* and *Nippostrongylus* among rats. From the results of the study on the distribution, the material of *S. ratti* for the experiment is known to readily available from a large size of *R. norvegicus* caught on the ground.

**THE COURSE OF THE INFECTED WORMS IN ALBINO RATS**

The albino rats are susceptible to the infection of *S. ratti*. The infected albino rats began to discharge eggs of *S. ratti* within a short period of days and lasted the evacuation for long period. The course of the evacuation was quantitatively observed. And paralleling with the observation of the eggs, the period of maintenance of the parasitic females of this worm was examined. The evacuation of eggs was measured applying quantitative test-tube cultivation technic which, as mentioned above, roughly correlating to the measurement of E.P.G. (egg per gram). As it has been generally known that the value of E.P.G. is much influenced by the ratio of water content in stool, the water proportion of the feces of albino rats was measured.

1. Water proportion of feces of rats: A small portion of the feces

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Max.</th>
<th>Min.</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75.7</td>
<td>64.2</td>
<td>70.0</td>
</tr>
<tr>
<td>2</td>
<td>73.2</td>
<td>64.4</td>
<td>68.6</td>
</tr>
<tr>
<td>3</td>
<td>77.3</td>
<td>60.3</td>
<td>69.6</td>
</tr>
<tr>
<td>4</td>
<td>78.7</td>
<td>65.0</td>
<td>71.0</td>
</tr>
<tr>
<td>5</td>
<td>75.6</td>
<td>65.3</td>
<td>70.0</td>
</tr>
<tr>
<td>Total</td>
<td>78.7</td>
<td>60.3</td>
<td>70.0</td>
</tr>
</tbody>
</table>
(approximately 100 mg) of a rat was smeared on a piece of dried filter paper (2 × 10 cm) and kept in an incubator at 37°C for 10 days and loss of weight of the feces was measured using a torsion balance. The samples were taken 99 times from 5 rats and the average of water proportion among all samples was 70.0 per cent, ranging 60.3 to 78.7 per cent, as presented in Table 5. As the water proportion was almost fixed around 70 per cent and the volume of daily output of the feces was controlled under the same rearing conditions, E.P.G. observed was regarded as directly to indicate the proportional value to the whole daily output of eggs.

2. The course of the output of eggs: Five albino rats were subcutaneously injected with 500 infective filariform larvae of *S. ratti* and the evacuation of eggs was observed applying test-tube cultivation technic. The evacuation began 6 days after infection in 3 rats and after 7 days in 2 rats, and the egg counts increased rapidly and reached to the maximum

![Graph](image-url)

**Fig. 13.** Daily occurrence of egg counts of *S. ratti* in 0.3g of feces measured by test tube cultivation technic in 3 albino rats with experimental infection.
count on the 10 to 15 days. The output of eggs gradually reduced in number after the maximum till approximately 5 per cent of the maximum after 100 days and furthermore lasted for more than 250 days. The courses of the output number of eggs in three rats with successful observation were illustrated in Fig. 13, indicating the period of 34 days. And the observation of a course of a rat in long period was presented in Fig. 14.

The course of the discharge of eggs was the same in the observation with the floatation technic in three rats infected with 1,000 larvae.

3. Maintenance period of the adult: From the course of the eggs, the maintaining period of the parasitic females was thought to be short. In order to observe directly the falling process of the parasitic females, 20 rats were infected with 4,000 infective larvae and 3 or 4 among them were sacrificed on the 3, 6, 10, 20 and 40th days respectively. The parasitic females were isolated from a part of the intestine, 10 cm from the ending of stomach, using the technic described in the chapter of method. The result shown in Table 6 and Fig. 15 indicates that the invasion of the worm into the intestinal mucosa begins between 3 to 6 days after inoculation, numbers of worm reach to the maximum after 10 days and the worms

![Graph](image)

Fig. 14. Long period observation of change of evacuated egg counts of St. ratti in an albino rat with experimental infection, measured by test-tube cultivation technic from 0.3 g feces.

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Nos. of rat</th>
<th>Nos. of females</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>2193</td>
<td>1595</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>1680</td>
<td>2275</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>805</td>
<td>1760</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>240</td>
<td>960</td>
</tr>
</tbody>
</table>

Table 6. Daily change of the maintaining number of the parasitic females of S. ratti in albino rats
begin to leave after 10 days, reducing the number to one third of the maximum on the 40th day.

The half of the number of parasitic females leaves within 35 days and this period corresponds to the average longevity of parasitic stage. In comparison of the both courses of parasitic females and output of eggs (Fig. 15), eggs began to appear and increased 2 or 3 days later than that of the adult, reached to the maximum value approximately on the same day and reduced faster than the adult. The faster reduction of eggs after maximum value seems to be caused by the fall of the oviposition of the adult worms.

4. Relationship between numbers of infected larvae and parasitized

Figs. 16-24. Comparison of changes of egg counts of St. ratti between treated and untreated rats. The course of a rat is shown as a line and egg counts are presented as percentages to the average of measurements before treatment in each rat.

![Graph showing egg counts over time and relationship between infected larvae and parasitized individuals.]

Fig. 15. (Left) Relationship between course of remaining number of females in parasitic generation of St. ratti and that of evacuated egg counts. Numbers of females were averages of 3 to 4 rats infected with 4000 larvae and egg counts were averages of 5 rats infected with 500 larvae.

Fig. 16-1. (Right) Gentian violet #1. 2 mg/100 g body weight daily for 10 days
worms: The numbers of the establishment of parasitism in the infections of the different numbers of infective larvae were observed. The larvae injected were 500 to 7,000 and the number of the adult was estimated from the eggs in feces 14 to 16 days after infection. Eggs in this period were measured quantitatively using test-tube cultivation, 3 tubes from a sample for 3 days in a rat and the average of 9 tubes was regarded as the corresponding value to the numbers of adults. The ratio, nos. of eggs detected/nos. of larvae infected, was used for comparison in place of the establishing ratio of parasitism, nos. of adult detected/nos. of larvae infected. The result (Table 7) shows that the ratio of establishment of parasitism is not much influenced by the numbers of infected larvae between 500 to 7,000 i.e. almost the same rate of the infected larvae gains successful parasitism.

SCREENING TEST OF ANTHELMINTICS

1. Choice of method: Albino rats weighing approximately 200 g were subcutaneously inoculated with 2,000 to 3,000 infective larvae and the experiments were made in the period 15 to 32 days after infection. Those conditions were selected considering natural course of the maintenance of this parasite already described in the preceding chapters. The counting of eggs in feces was made applying test-tube cultivation technic. As in this technic, the work is divided into two different days, preparation of the cultivation in a certain day and observation of the larvae after 5 days, a worker is able to measure more samples in a day than in the other technics. Eggs in feces were counted using three tubes from a sample in a day on the 15, 17, 19th days and the average of 9 tubes was determined as the evacuating value of eggs before treatment. After treatment, eggs were counted in the similar method, three tubes from a sample in a day on the three alternated days.

The anthelmintics tested were Gentian violet, Dithiazanine, Stibophen,

<table>
<thead>
<tr>
<th>Nos. of larvae infected</th>
<th>Nos. of rat</th>
<th>Average of eggs evacuated</th>
<th>Nos. of eggs Nos. of larvae</th>
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<tr>
<td>500</td>
<td>5</td>
<td>713.2</td>
<td>1.43</td>
</tr>
<tr>
<td>1000</td>
<td>9</td>
<td>1649.0</td>
<td>1.65</td>
</tr>
<tr>
<td>2000</td>
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<td>1778.9</td>
<td>0.89</td>
</tr>
<tr>
<td>3000</td>
<td>47</td>
<td>4360.7</td>
<td>1.45</td>
</tr>
<tr>
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<tr>
<td>7000</td>
<td>14</td>
<td>7363.9</td>
<td>1.19</td>
</tr>
</tbody>
</table>

* Average egg counts were determined using the test-tube cultivation technic. Fecal samples were collected 3 times from a rat 14,15 and 16 days after infection and 0.3 g was used for cultivation.
Diethylcarbamazine, Thiocarbarson, Nematolyt, Piperazine malate, Tetra-chloroethylen and Quinacrine hydrochloride. The dose per body weight administered was more than 5 times of human therapeutic dose and the dose and duration of treatment in each experiment are presented in Fig. 16-24 and Table 8. Stibophen was injected intramuscularly, the others perorally. Tetrachloroethylen, Gentian violet in the form of solution and Piperazine malate syrup were introduced with an long metal needle with vinyl tube at an advancing end. Dithizaníne, Diethylcarbamazine, Thiocarbarson and Quinacrine hydrochloride were admixed in a boiled solution of 3 per cent starch and intubated perorally. Nematolyt was smashed, mixed into flour paste and the rats were fed on this paste mixture.

2. Estimation of anthelmintic effect: As the discharge of eggs reduced after a short period of days in its natural course, the experimental treatment had to be made comparing the natural course of the control group of 2 or 3 rats. The egg counts of each rat in the course of experiment were presented by a percentage to the average count of each rat before treatment measured by 3 tubes from a sample for 3 days. In Figs. 16 to 24, the changes of

![Graph showing egg count over days after infection]
egg counts of each rat are illustrated with a line in logarithmic expression. And when the lines of treated rats are under those of the control group, the treatment is regarded as effective.

Fig. 16-3. Gentian violet #3.
2 mg/100 g body weight daily for 7 days
In another method, the reduction rate in a percentage was calculated from the both averages of egg counts before and after treatment. The reduction rates in treated and control groups are compared. When the reduction rates are larger at the experimental group than at the control group in an experiment the anthelmintic used is valued as effective.

3. Result: The results show a little difference between repetition of the experiments with a same drug. Observing the graphs, the lines of treated group with Stibophen are far below the control group and the drugs

Fig. 17. Dithiazanine iodide.
10 mg/100 g body weight daily for 5 days
Fig. 18-1. Sibophen #1.
1.7 mg/100 g body weight daily for 7 days
with which egg counts seem to be reduced are Gentian violet #2, Stibophen #2, Nematolyt, Piperazine malate and Tetrachloroethylen. On the contrary, little reduction is seen with Gentian violet #1, #3, Dithiazanine, Diethylcarbamazine, Thiocarbarsone and Quinacrine hydrochloride.

In the comparison of reduction rates (Table 8), enormous reduction is seen with Stibophen #1, slightly with Stibophen #2, Nematolyt, Piperazine malate and Tetrachloroethylen #2 and none with Dithiazanine, Diethylcarbamazine, Thiocarbarsone, Tetrachloroethylen #1, and Quinacrine hydrochloride.

After those comparisons the drug which showed the remarkable reduction of egg output was Stibophen in the screening test of the present study. A little possibility of affection was seen in the experiment with Nematolyt,
Piperazine malate and Tetrachloroethylen. And little therapeutic effect was attained with Gentian violet, Dithiazanine, Diethylcarbamazine, Thiocarbamide and Quinacrine hydrochloride.

**DISCUSSION**

In the screening test of the present study, Stibophen was the most effective but little effect was shown in Gentian violet or Dithiazanine, both of which have been proved suitable anthelmintics on human strongyloidiasis. In the experiments with Gentian violet, Dithiazanine and Quinacrine hydrochloride, rats became to take little food because of toxicity of those drugs to the alimentary tract. The reduced intake of food caused the concentration of egg counts in feces and E.P.G. sometimes increased as presented in the experiment with those drugs. In consequence of above consideration, it is inadequate that the anthelmintic effect is attempted to test with the drugs which lose the appetite of rats in the screening test with *S. ratti*. If the evacuation of eggs of *S. ratti* is stationial for certain period, the effect

![Graph](image)

*Fig. 19. Diethylcarbamazine.*

40 mg/100 g body weight daily for 7 days
of drugs is simply measured by the observation of the reduction of the egg count. But as the egg count of this worm reduces enormously in natural course within a short period of days, approximately 2 weeks, the reduction of eggs caused by the administration of drugs is observed with difficulty.

The accurate density of eggs in feces is usually indicated by EPG value, applying the Stoll's dilution method. Erhardt & Denecke (1939) observed the course of the output of eggs of S. ratti using the similar technic to the Stoll's technic. And for the same purpose Sheldon (1937) and Graham (1938) cultivated rat's feces in fecal emulsion and extracted larvae with Baermann's apparatus and regarded the number of larvae as the corresponding value of EPG. In the present study estimation of egg count was made applying the test-tube cultivation technic. In those different methods of measurement, the course of the egg output observed were almost similar.

Fig. 20-1. Thiocarbamide #1.
12.5 mg/100 g body weight daily for 10 days
In those observations, daily fluctuation of EPG were comparatively wide. The daily alteration of EPG are generally believed to be arisen by principally lack of uniformity of the oviposition of the worm, and secondary by the fluctuation of water proportion of feces, that of volume of food taken, inaccuracy of the measurement and the unequal distribution of eggs in feces (Ishizaki, 1953, Sato, 1953, 56). Those elements, which disturb the uniformity of daily EPG were discussed adapting the authors' experiment with S. ratti. The distribution of eggs of Ascaris and hook worms was studied by Ishizaki (1953) and Sato (1953, 56) and they reported eggs scattered in Poison's distribution. Tanaka, Tokuriki and others (1958) examined the larval distribution of S. stercoralis in the stool and found it was Poison's or normal type in the restricted part of stool. From those results the distribution of eggs of S. ratti was thought to be uniform. Furthermore the fecal pill was smashed and the uniformity of the egg dis-

![Graph](image-url)

*Fig. 20-2. Thiocarbarsone #2, 12.5 mg/100 g body weight daily for 6 days*
tribution was artificially made in the present study.

The accuracy of the quantitative egg counting by means of cultivation technic was examined and the technic was found to be applicable in stead of smear technic which was thought to correspond to Stoll's technic (Ishizaki, 1953). And in fact the daily fluctuation of egg count observed in the present study was narrower than that measured by Sheldon (1937) but somewhat wider than that in Erhardt & Denecke (1939). Ono (1958) applying a cultivation technic proved existence of correlation between LPG (larvae per gram) and EPG of hook worms. Considering those data, the accuracy of measurement with cultivation technic is not thought so worse.

If the daily oviposition is constant, EPG is reduced when the water proportion of the stool is large, because the eggs are diluted in feces. Ishizaki (1953) observing whether the reduction of EPG was proportional to the increase of the water content of stool, the daily fluctuation of EPG of Ascaris in man was flattend by the adjustment with water proportion. Sato

![Graph showing egg count over time with and without treatment.]

Fig. 21. Nematolyt.
0.8 g/100 g body weight daily for 7 days
(1953, 56) partially agreeing with Ishizaki's result, reported that EPG of hook worms of man was more influenced by the other unknown factors than water proportion of the stool. In the present study, the water in fecal pills was measured, proved always approximately constant and exerted little influence to the fluctuation of egg counts.

The course of egg count observed in the experiment was proved to be the real and natural course from the consideration developed above. Furthermore, the rapid reduction of egg count within a short period was found to be unavoidable from the examination of the maintenance period of the parasitic females.

The eggs were found in feces from 6 days after infection in the present study but Sheldon (1938) from 4 days. Sheldon reported the duration of the egg evacuation was 98.2 days in an average by the infection with

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**Fig. 22-1.** Piperazine malate #1.

130 mg/100 g body weight daily for 7 days.
1,000 larvae and Tanabe more than 125 days. However, in the present study the duration was longer than 250 days with the infection of 500 larvae. The maximum of the egg count was observed from 10 to 15 days after infection by those workers but the decrease of the count was the most acute in Erhardt & Denecke's observation in which the count was minimized and reached to the stationary value within 25 days. In the present study the same situation was attained after 50 to 100 days. Erhardt & Denecke (1939) tested the drug at the period after 25 days when the egg count was comparatively constant. But in this period, daily fluctuation existed and sometimes no egg was detected in authors' experience. By those reasons, the authors tested the effect of drugs in the period from 15 to 34 days after infection and the effect of the drug was signified comparing that of the natural courses in control rats.

Through the present study, it is difficult to test the effect of drugs

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**Fig. 22-2.** Piperazine malate #2.
130 mg/100 g body weight daily for 7 days
with *S. ratti* because its maintenance in host animals was short and the evacuation of eggs has a rapid reducing trend. But the test could be successfully made as in the present paper under certain condition. The test is not to apply to such drugs as to give a side reaction to the alimentary tract of rats. And the test should be finished in short period and always compared to the control group.

Gentian violet was proved to be an effective anthelmintic on human strongyloidiasis by De Langen (1928), Faust (1930, 32) and Yoshino (1939). Recently vermifugal action of Gentian violet was revealed by Shiroma & Tanaka (1959), Shiroma (1959) and Tanaka & Shiroma (1960). Swartzwelder and others (1957, 58) applied Dithiazanine iodide to the treatment of human strongyloidiasis and Tanaka & Shiroma (1960) also observed vermifugal action of this drug. Sato (1939) tested the effect of several drugs in dogs experimentally infected with *Strongyloides stercoralis* of man and reported Gentian violet was the only valuable anth-

![Graph showing egg count over days for treated and untreated groups](image)

**Fig. 23-1. Tetrachloroethylene #1.**
0.1 cc/100 g body weight daily for 7 days
elmatic. The result of the test of drugs with dogs shows close coincidence with the effect to the human strongyloidiasis but little with rats. The test with rats seems to be restricted by the conditions already mentioned. But the drug picked out by the test with rats is worthy of examining human application.

Detection of *S. ratti* in rats has been usually made by finding the females in the parasitic stage from the intestinal mucosa. However, the complicated technic is needed in this method and the efficiency of the detection is not so satisfied. In the present study, *S. ratti* was detected from the feces using the test-tube cultivation technic. By the former technic, Tanabe & Takeishi (1936) found *S. ratti* in 66.9 per cent among *Rattus norvegicus* in Tokyo, Nishimura (1946) in 44.0 per cent in Kagoshima Prefecture. The present authors detected it in 62.5 per cent among *Rattus norvegicus* through all seasons and in 70.6 per cent in the winter. Comparing the other data,

![Graph](image)

**Fig. 23-2.** Tetrachloroethylene #2.
0.1 cc/100 g body weight daily for 7 days.
detection by the cultivation technic was thought to be as susceptible as another method and also efficient. Prior to the fecal examination, the stage of this worm in fecal pellets was examined. Sandground (1925) observed both eggs and hatched larvae in feces but Tanabe (1938) found only eggs in the feces immediately after evacuation. In the present study, though only eggs were found from the feces in coincidence with Tanabe's observation, the larvae hatched in feces were estimated as in 1.7 per cent of eggs from the observation of time-hatching rates and were negligible in practice. Less occurrence of larvae in feces gained the sensibility of the floatation technic. In the detection of S. ratti from feces of house rats, eggs and infective larvae had to be differentiated from those of Nippostrongylus brasiliensis. So the morphological characteristics of both species were studied and identification could be successfully made in each technic of detection. The structure of the infective larvae of S. ratti was compared with that of S. stercoralis, but remarkable difference was not found except for the larger size of body length and fairly conspicuous bifurcation of tail tip in S. ratti.

The optimum temperature for cultivation of S. ratti was determined

Fig. 24-1. Quinacrine hydrochloride #1. 10 mg/100 g body weight daily for 5 days

Fig. 24-2. Quinacrine hydrochloride #2. 10 mg/100 g body weight daily for 5 days
Table 8. Comparison of the effect of drugs by the difference of reduction rates (percentage) of eggs between treated and untreated rats

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<th>Kinds of drugs</th>
<th>Exp. no.</th>
<th>dose per day per 100g b.w.</th>
<th>Days for admin.</th>
<th>Reduction rates in percentage</th>
<th>Untreated group</th>
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<td></td>
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<td></td>
<td>Treated group</td>
<td>average</td>
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<td>Gentian violet</td>
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<td>2</td>
<td>2.0</td>
<td>7</td>
<td>0   0   82.0 67.4</td>
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<td>7</td>
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<td>**97.0</td>
</tr>
<tr>
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<td>7</td>
<td>79.0 95.9 84.3 99.4</td>
<td>*90.7</td>
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<td>7</td>
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<td>7</td>
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<td>5</td>
<td>0 0 0 0 0</td>
<td>*68.6</td>
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</table>

* Eggs were reduced more in treated group than in untreated group
** " extremely more
as 26°C to 28°C by authors, at 24°C to 25°C, Graham (1936) at 24°C, Sheldon (1937) and at 24°C to 28°C, Tanabe (1936). The optimum temperature for the cultivation of *S. ratti* seems to be lower than that of *S. stercoralis* in which 30°C is suitable (Tanaka, Tokuriki et al. 1958). The existence of *S. ratti* in lower temperature is a cause of infestation on the wider and cooler areas than *S. stercoralis*. The other elements for test-tube cultivation technic, volume of feces and the period for incubation, were almost similar to the results of Shirasaka (1959) who studied with hook worms and *Trichostrongylus*.

The incidences of *S. ratti* among the house rats were much higher in *Rattus norvegicus* than in *Rattus rattus*. This difference was thought to be arisen by the difference of the residing places of both species. As *Rattus norvegicus* lives in the drain or on the soil ground, those places are considered as the foci of the infection. The incidence of *S. ratti* increased paralleling with the increase of the body weight among *Rattus norvegicus*. In Sheldon’s experiment (1937), the rats repeatedly infected with *S. ratti* gained the immunity or resistance to the succeeding infection. But this acquired immunity did not much influence the incidences of rats in natural environment because large rats harboured the worm in high rates, each having many worms. The establishment of the infection seems to be much influenced by the chance of infection overwhelming the immunity. The difference of the incidences of *S. ratti* by body weight has close similarity to that of hook worms in man. The age difference of *S. stercoralis* of man was not obvious in the observation of Faust (1931) in Panama and of Tanaka (1958) on Amami Oshima Island but on the other hand Sasa, Teruya et al. (1958) and Shiroma (1959) observed in Okinawa that the incidence increased gradually at the older age groups, somewhat relating to the difference of body weight groups of rats.

**Summary**

1) The main purpose of the present study was the establishment of the screening test of anthelmintics on strongyloidiasis with *S. ratti*. And also the distribution of this parasite among rats was examined. Peculiar to a series of studies, detection of *S. ratti* was made applying test-tube cultivation technic.

2) In application of the test-tube cultivation technic, optimum conditions of the temperature, period of cultivation and the volume of feces on the filter paper were studied. The adequate temperature of incubation was 26°C for the detection of the parasite, the cultivation of 0.5 g of feces for 6 days presented the best result. The cultivation was successfully made
with 0.3 g for 5 days for the purpose of the quantitative measurement and
0.5 g for 3 or 4 days for obtaining the materials of experimental infection.
3) Correlations of counting of eggs with the cultivation technic or with
the flotation technic and that with smear technic were tested. The co-
efficient of correlation between the cultivation of 300 mg and the smear
of 10 mg was 0.83 and the formula of the regression line was Y (cultiva-
tion) = 40.3X (smear) + 653.0. Those of the flotation were 0.78, Y (floata-
tion) = 4.3 X + 353.5. Those indirect methods of detection were applicable
to the quantitative measurement when the large difference of the egg counts
was attempted to examine.
4) As the rhabditoid larvae is not found with the flotation technic, the
proportion of larvae in feces much influences the susceptibility of the floata-
tion technic. In the feces immediately after evacuated, none of larvae was
found among 500 eggs from 5 fecal samples. In another examination, the
rate of the larvae was estimated as 1.7 per cent from the regression line of
time-hatching ratio and has no influence to the sensibility of the floatation
technic.
5) Using the test-tube cultivation technic, S. ratti was found 62.5 per cent
(70.6 per cent in the winter) among 280 Rattus norvegicus and 13.3 per
cent of 98 Rattus rattus in Tokyo. No significant difference of infecting
rates was observed among localities and sexes. The incidence increased in
the groups of rats with large size. The material of S. ratti could be readily
obtained from the large Rattus norvegicus caught on the ground especially
near the ditch.
6) The course of the output of eggs in albino rats infected with S. ratti
was followed using the quantitative measurement of the test-tube cultiva-
tion technic. Though the density of eggs in feces is much influenced
by the change of water proportion in the feces, no attention was needed
to pay concerning with rat’s feces because the water proportion was fixed
approximately 70.0 per cent (60.3 to 78.7 per cent). Eggs appeared 6 days
after infection, reached maximum counts 10 to 15 days, gradually reduced
in small number within 50 days and lasted to be discharged till more than
250 days.
7) The parasitic females invaded into the intestinal mucosa between 3 to
6 days after infection, increased till maximum in 10 days and began to
leave from the intestine. The number of the worm reduced one third on
the 40th day and the period of days of the half reduction was 35 days.
This period of days is regarded as the average of the maintenance of S. ratti
in the parasitic stage. The reduction of the egg count was faster than
that of the parasitic females and this seemed to be caused by the fall of
the oviposition of the adults. The number of the parasitized worms corre-
sponded to that of the infected larvae in the infection with 500 to 7,000.

8) Considering with the natural course of the maintenance of this parasite, the reduction of the egg count after the experimental treatment should be always compared to the untreated control groups and the experiment should be accomplished in a short period, 15 to 35 days after infection. The egg counts of both groups were measured three times each before and after treatment, and the course of egg counts was observed in each rat. The reduction of egg counts were compared between both groups on the graphs, the average of the reduction rates in a percentage of treated rats was compared with that of untreated rats and the evaluation of a drug was determined.

9) From those experiments, the most active anthelmintic effect was attained with Stibophen. A little effect was supposed with Nematolylt, Piperazine malate and Tetrachloroethylphen and no affection was seen with Gentian violet, Dithiazanine, Diethylcarbamazine, Thiocarbarbione and Quinacrine hydrochloride. The anthelmintic evaluation about drugs in rats is different from that of human strongyloidiasis because Gentian violet and Dithiazanine are valuable in the human cases but invaluable in rats. When rats take such drugs as Gentian violet, Dithiazanine and Quinacrine, intake of food was enormously reduced for their toxicity to the alimentary tract, the eggs in feces was concentrated, egg counts were consequently less reduced than in untreated rats and the drug was regarded as no value. The drugs with toxicity to the alimentary tract are inadequate to test their anthelmintic effect in rats.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Prof. Rokuro Kano and Asst. Prof. Takeshi Oshima of the Department of Public Health, Tokyo Medical & Dental University for their constant guidance given in the present studies, to Mr. Hiroyasu Kasai for rearing rats and to staffs of the Division of Prevention, Bureau of Public Health, Tokyo for the collection of house rats.

LITERATURE

* Japanese text

(Ab) Abstract of the meeting.


*Amano, R., Mizuno, H., Tange, H. & Tanaka, H. (1959) The daily course of the infesting
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