RESEARCH NOTES

SARCOCYSTIS FROM RATS OF CENTRAL JAVA†

During attempts to isolate infectious disease agents from rodents in Central Java, Indonesia, diaphragmatic muscles from rats were obtained and examined for the presence of *Trichinella spiralis* and other parasitic infections. The rats were trapped in villages and fields at elevations of 1100 and 1900 meters on the slopes of Mt. Merbabu, Bojalali Regency. At necropsy small portions of the diaphragms (5-10 mm) were excised, placed into 10% formalin and subsequently examined microscopically after sectioning and staining with hemotoxylin and eosin.

Diaphragm muscle was obtained from 185 *Rattus rattus diardi*, 187 *R. exulans*, 7 *R. sp. niviventer*, 3 *R. jalorensis* and 2 *R. argentiventer*. *T. spiralis* was not found but cysts similar to those of *Sarcocystis* were found in tissue sections of 8 *R. r. diardi* (4.3%), 12 *R. exulans* (6.4%) and one *R. argentiventer* (50%). All of the *Sarcocystis* infected animals were adults, 11 males and 10 females.

Sarcocystis has been reported from a variety of animals in Indonesia (Durfee *et al.*, 1972, *Southeast Asian J. Trop. Med. Pub. Hlth.*, 3 : 621) as well as from rats (*R. r. diardi*, *R.r. brevicadatus* and *R. norvegicus javanicus*) of West Java (Holz and Liem, 1964, *Z. Parasitenk.*, *25* : 405). To our knowledge this is the first report of *Sarcocystis* in rats from Bojalali Regency, Central Java.

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The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large, or of the Ministry of Health, Republic of Indonesia.

JOHN H. CROSS, JAMES W. FRESH, GWILYM S. JONES of the U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan and S. GUNAWAN of the Communicable Disease Center, Ministry of Health, Jakarta, Indonesia.

ANTAGONISM BY THE SPOROCYST STAGE OF *TRICHOBILHARZIA BREVIS* (TREMATODA: SCHISTOSOMATIDAE) AGAINST THAT OF *ECHINOSTOMA AUDYI* (TREMATODA: ECHINOSTOMATIDAE)†

The interaction between *T. brevis* and *E. audyi* has been studied previously (Lie *et al.*, 1965, *Nature, 206* : 422; Bash and Lie, 1966; *Z. Parasitenk, 27* : 252 and 260; Ow-Yang *et al.*, 1970, *Southeast Asian J. Trop. Med. Pub. Hlth., 1* : 160). The echinostome is the dominant species, and snails harbouring *E. audyi* could not be superinfected with the schistosome. Snails with well-developed *T. brevis* infection could be superinfected with the echinostome, whose predatory rediae eventually eliminated the former species from the snail host; however, it was suspected that complete development of the echinostome might take longer in snails already harbouring the schistosome (Basch and Lie, 1966, *Z. Parasitenk., 27* : 260). The present communication reports indirect antagonism exerted by the subordinate *T. brevis* on the early developmental stage of *E. audyi.*

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Lymnaea rubiginosa snails 5 ± 1 mm long were each exposed to 10 *T. brevis* miracidia. Thirty-four days later, snails shedding cercariae were each exposed to 10 *E. audyi* miracidia. One week thereafter the snails were dissected and the heart examined for *E. audyi* sporocysts and rediae using the compound microscope, under the slight pressure of a 13-mm circular number O cover glass.

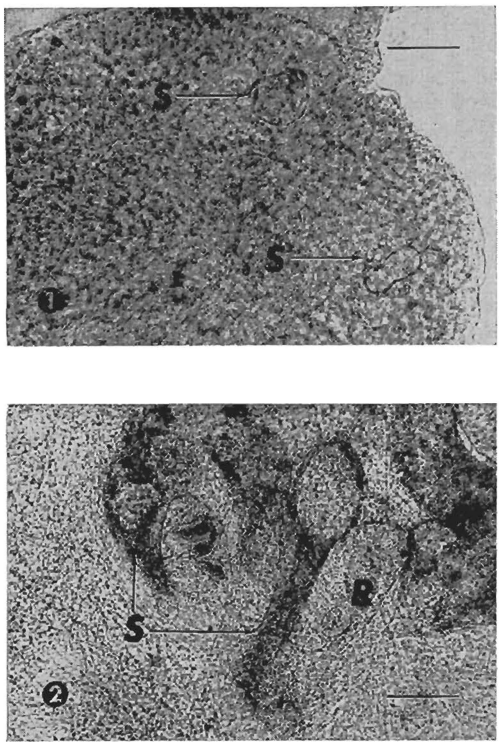
Table 1 shows that development of *E. audyi* was markedly inhibited in snails

Table 1

Development of early stages of *E. audyi* in *L. rubiginosa* with and without earlier *T. brevis* infection.

	Snails with earlier *T. brevis* infection*	Previously uninfected snails
No. snails dissected	7	10
No. snails with *T. brevis* sporocysts-cercariae	7	-
No. snails with *E. audyi* sporocysts/rediae	7	10
Total no. *E. audyi* sporocysts	17	35
No. *E. audyi* sporocysts per snail, range	1-5	2-5
Mean size of *E. audyi* sporocysts (microns)	98.6×68.2	554.2 × 95.8
Size range of *E. audyi* sporocysts (microns)	65.0×57.2 to 143.0 × 67.6	288.4 × 82.4 to 834.3 × 113.3
Total no. *E. audyi* rediae outside sporocyst	1	39
Mean no. *E. audyi* rediae per snail	0.15	3.9

* Snails harbouring 34-day *T. brevis* infection were exposed to *E. audyi* miracidia (10 miracidia per snail) and dissected 1 week later.



Figs. 1-2—Ventricle of *Lymnaea rubiginosa* snail with one-week-old sporocysts (S) and rediae (R) of *Echinostoma audyi:* (1) in double infections with *Trichobilharzia brevis* and (2) in single infection controls. Scale — 100 microns.

harbouring *T. brevis*, as indicated by the following: (1) *E. audyi* sporocysts were much smaller in doubly infected snails than in singly infected controls, and (2) the number of rediae outside the sporocysts was significantly lower in doubly infected snails.

E. audyi sporocysts were small and rounded in *T. brevis*-infected snails (Fig. 1) but large and elongated in single infections (Fig. 2). One-week-old *E. audyi* sporocysts in single infections were usually pigmented; in double infections, they remained translucent, and flame cells were easily seen.

Indirect antagonism exerted by the subordinate trematode species against development of the dominant species has been reported in various combinations (cf. Lie, 1969, *Proc. Fourth Southeast Asian Seminar Parasit. Trop. Med.* p. 131; Lim and Heyneman, 1972, *Advances Parasit.*, 10 : 191). The mechansim of this interaction is not yet understood.

HOK-KAN LIM, KIAN-JOE LIE and CHEE KONG OW-YANG. Institute for Medical Research, Kuala Lumpur, Malaysia, and University of California, ICMR, San Francisco, California, U.S.A.

INABILITY TO TRANSMIT *BREINLIA SERGENTI* TO A MARSUPIAL, *TRICHOSURUS VULPECULA*

Yorke and Maplestone erected the genus *Breinlia* in 1926 for the species *Filaria trichosuri* Breinl, 1913 from the brush - tailed possum, *Trichosurus vulpecula*, in Queensland. Since that time, seven additional species of *Breinlia* have been described from marsupials in Australia and New Guinea (Mackerras, 1962. *Aust. J. Zool., 10* : 400; Wahid, 1962. *J. Helminth., 36* : 207).

Petter (1958. *Bull. Soc. Zool. France, 83* : 423) described *B. sergenti*, formerly *F. sergenti* Mathis and Leger, 1909, from the slow loris, *Nycticebus tardigradus*, and considered that it was closely related to *B. thylogali* a parasite of the pademelon, *Thylogale wilcoxi*, in Queensland.

From a zoogeographical point of view, it would be of interest to know if marsupials were susceptible to infection with *B. sergenti.* Attempts to transmit *B. sergenti* to common laboratory animals and other primates found in Southeast Asia have been unsuccessful (Zaman, 1972. *Southeast Asian J. Trop. Med. Pub. Hlth., 3* : 143) and it was concluded that this species was highly host specific.

The cardiac blood of three brush-tailed possums caught at Wahroonga, N.S.W. were found to be free of microfilariae on two occasions before challenge. *Aedes aegypti* were infected with *B. sergenti* in Singapore in November, 1970 by feeding on a slow loris which had a heavy microfilaraemia. These mosquitoes were flown to Sydney, dissected 14 days after infection and infective larvae inoculated subcutaneously into the animals, as shown in the Table, by the method of Edeson and Wharton (1957. *Trans. Roy. Soc. Trop. Med. Hyg., 51* : 366).

Cardiac blood from each animal was examined for microfilarae three, four, twelve and eighteen months after challenge with negative results.

This investigation indicates that *T. vulpecula* is not susceptible to infection with *B. sergenti* and further demonstrates the high host specificity of *B. sergenti.*

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B. McMILLAN, J.C. WALKER and V. ZAMAN. School of Public Health and Tropical Medicine, University of Sydney, N.S.W. 2006, Australia, and Department of Parasitology, Faculty of Medicine, University of Singapore, Singapore 3.

Possum No.	Sex	Weight kg.	Site of inoculation	No. infective larvae inoculated
1	♀	2.5	Right front leg	50
			Right hind leg	60
			Left hind leg	50
2	♂	2.2	Right front leg	35
			Right hind leg	70
3	♂	2.9	Right front leg	60

DIFFERENCE BETWEEN G-6-PD B AND MAHIDOL :

Differential Stimulation by Magnesium Chloride

G-6-PD deficiency is very common in Thailand, occurring on the average in about 12 per cent males (Tuchinda *et al.*, 1968. *Biochem. Genet.*, *2*:253). Twelve enzyme variants have been found among these G-6-PD deficient subjects (Panich, 1973, unpublished data) and the most common one being G-6-PD Mahidol (Panich *et al.*, 1972. *Southeast Asian J. Top. Med. Pub. Hlth.*, *3*: 624). Using conventional parameters, partial purified G-6-PD Mahidol could not, however, be differentated from G-6-PD B in normal persons. It was thus not known whether G-6-PD Mahidol was actually the normal enzyme present in lower level in red blood cells (Panich *et al.*, 1972. *J. Med. Ass. Thailand*, *55*:576). An evidence that G-6-PD Mahidol is a distinct enzyme variant is presented in this communication.

Partially purified erythrocyte G-6-PD type B and Mahidol were obtained by the method described earlier (Panich *et al.*, 1972. *J. Med. Ass. Thailand*, *55*:576) and were kept in 43.6 per cent ammonium sulphate at either -20°C or 4°C. Both types of enzyme were dialyzed together in three changes of 0.05 M Tris HCl, pH 8.0 containing 10 μM EDTA, 10 μM TPN, and 7 mM 2-mercaptoethanol and then subjected to $MgCl_2$ stimulation test.

The enzyme was adjusted to give activity of 0.02-0.05 OD unit per minute per 50 μl of enzyme solution as measured by the WHO method at 25°C (WHO Scientific Group, 1967. *W.H.O. Techn.Rep. Ser.*, No. 366). Enzyme activity was then measured in three systems each containing 0, 0.005, and 0.01 M $MgCl_2$ respectively and 0.1 M Tris HCl, pH 8.0, 0.0002 M TPN, and 0.0006 M glucose 6-phosphate. Optical density change was measured at 340 nm every 30 seconds for 5 minutes using Beckman DB-G Spectrophotometer at 25° C. G-6-PD activity was calculated for each system of each type of enzyme by the least-square method. The result is presented in Table 1.

It is beyond doubt that stimulation effect by $MgCl_2$ is more pronounced in G-6-PD B than in G-6-PD Mahidol. This would be the first detected biochemical properties which differentiate G-6-PD Mahidol from G-6-PD B.

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PHORNSRI PHUTRAKUL and VICHARN PANICH.
Faculty of Medical Technology and Faculty of Graduate Studies, Mahidol University, Bangkok 7, Thailand.

Table 1

Stimulation effect of 0.005 M and 0.01 M $MgCl_2$ on G-6-PD B and Mahidol.

No.	G-6-PD	$MgCl_2$	No. Subjects	G-6-PD Activity*
1.	B	0.005 M $MgCl_2$	6	107.7 ± 4.0
2.	Mahidol	0.005 M $MgCl_2$	5	103.6 ± 1.3
3.	B	0.01 M $MgCl_2$	6	113.7 ± 5.4
4.	Mahidol	0.01 M $MgCl_2$	5	106.5 ± 3.6

* Expressed in per cent of activity measured without $MgCl_2$, the values given are mean ± S.D.
p value between 1 & 2 < 0.05, 3 & 4 < 0.025, 1 & 3 < 0.0005, 2 & 4 < 0.0125.

MEASUREMENT OF THE RED CELL ATP LEVELS IN THAI BLOOD DONORS USING THE LUCIFERASE ENZYME AND THE LIQUID SCINTILLATION COUNTER†

It is well established that in the human red cell, adenosine triphosphate (ATP) is essential for maintenance of cation balance and for the couple sodium-potassium active transport mechanism (Hoffman, 1962. *J. Gen. Physiol.*, *45*: 837). Its major functions are glycolysis at the hexokinase and phospho-fructosekinase steps. ATP is also essential for maintenance of red cell shape and for normal *in vivo* survival of erythrocyte. If blood is stored for some time, it will have a significant decrease in ATP levels which correlates with its shorter survival *in vivo* (Akerblom *et al.*, 1967. *Transfusion*, 7:5). Determination of ATP content of erythrocytes is also of considerable interest from the view point of hereditary abnormalities of red cell metabolism, such as pyruvate kinase deficiency and certain genetic disorders characterized by increased or decreased red cell ATP levels. We report here the red cell ATP levels in normal Thais determined by the luciferase enzyme using the liquid Scintillation counter.

Red cell ATP levels were determined in 142 Thai blood donors, 124 males and 18 females. The ages ranged between 18 and 56 years of age. They were blood donors at the Thai Red Cross with haemoglobin concentration between 11.2 and 16.7 gm%. Blood samples were collected into heparin, 20 units per ml of blood. One volume of blood was precipitated without delay with three volumes of ice-cold 6% (w/v) perchloric acid. After thorough stirring which was necessary for good extraction of ATP, aliquots of the supernatant obtained by centrifugation were used for the estimation of ATP by the luciferase enzyme using the liquid Scintillation counter as described by Stanley and Williams (Stanley and Williams, 1969. *Anal. Biochem.*, *29* : 381).

An average value of red cell ATP levels in 142 Thai blood donors was found to be 116.87 ± 30.63 μM/100 ml RBC (range 50.2-195.9 μM/100 ml RBC) or 3.47 ± 0.91 μM/gm Hb (range 1.54-6.01 μM/gm Hb). There is no significant difference between the mean values of red cell ATP levels in male and female, therefore these values are mixed together. The comparison of the red cell ATP levels from the present studies with the previous results obtained by various methods is shown in Table 1 and the frequency distribution of these values is illustrated in Fig. 1.

Several methods for the measurement of red cell ATP levels have been developed recently e.g. paper and column chromatography, measuring glucose-6-phosphate formation in the hexokinase reaction with glucose-6-phosphate dehydrogenase (G-6-PD) and TRN, employing glyceraldehyde phosphate dehydrogenase reaction to measure the oxidation of DPNH and the firefly technique. In the present studies, only 0.1 ml of whole blood was used for the estimation of ATP by the luciferase enzyme after extraction with perchloric acid. It is a simple and rapid method which can determaine ATP over a wide range of 10^{-9} to 10^{-12} mole. The results in the present studies show that red cell ATP levels in normal Thais determined by this procedure are in the same order of magnitude of values obtained by various different methods. Results from the present studies

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Table 1

Red cell ATP levels obtained by various methods*.

Method of extraction	Method of ATP assay	Normal		Reference
		As given	Calculated as μM/gm Hb	
Whole blood TCA extract	GAPD back reaction	1.13±0.27 μM/ml RBC	3.32±0.08	Minikami *et al.*, 1965. *J. Biochem.*, *58*:543.
Whole blood perchloric acid extract	Hexokinase	3.86±0.13 μM/gm Hb	3.86±0.13	Gross *et al.*, 1963. *Blood*, *21*:755.
Perchloric acid extract of washed cells.	Paper chromatography	106 μM/100 ml RBC	3.12	Greenwalt & Ayers,1960. *Blood*, *15*:698.
TCA extract of washed cells	Hexokinase	2.75 μM/gm Hb	2.75	Brewer and Powell,1966. *J.Lab.Clin.Med.*, *67*:726.
PCA extract of washed cells	Column chromatography	82.4 μM/100 ml RBC	2.42	Mandel *et al.*, 1962. *Folia Haemat.*, *78*:525.
TCA extract of washed cells	Paper chromatography	0.68 μM/gm RBC	2.19	Gerlach *et al.*, 1958. *Pfleuge. Arch. Ges. Physiol.*, *266*:528.
PCA extract of washed cells	Column chromatography	69.2 μM/100 ml RBC	2.04	DeLuca *et al.*, 1962. *Anal. Biochem.*, *4*:39.
TCA extract of washed cells	Column chromatography	2.7-3.7 μM P/ml RBC	2.03 - 3.63	Barlett, 1959. *J.Biol.Chem.*, *234*:449.
Whole blood	Firefly	5.2 μM/gm Hb	5.2	Beutler and Baluda,1964.
Whole blood	Firefly	5.45±1.36 μM/gm Hb	5.45±1.36	*Blood*, *23*:688.
Whole blood	Firefly	138 μM/100 ml RBC	4.05±0.11	Beutler and Mathai,1967. *Blood*, *30*:311.
Whole blood PCA extract	Firefly and liquid scintillation counter	116.87±30.63 μM/100 ml RBC	3.47±0.91	Present study

*This table was adapted from Beutler and Mathai (Beutler and Mathai, 1967. *Blood*, *30*:311).

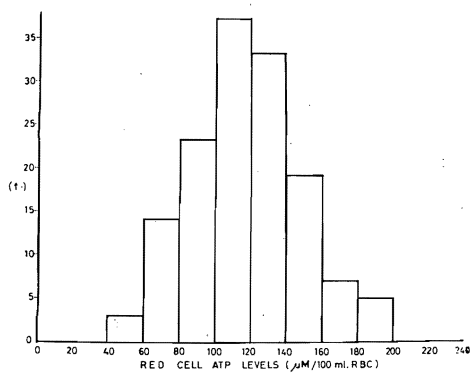

Fig. 1—The distribution of red cell ATP levels in 142 Thai blood donors.

are being served as base line data for subsequent comparison with values obtained from hereditary abnormalities of red cell metabolism, some genetic disorders and subjects with malarial infection (Areekul *et al.*, 1973. In preparation).

SUVIT AREEKUL and YUPA CHANTACHUM. Department of Radioisotopes, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

RELATIONSHIP BETWEEN LIVER WEIGHT AND BODY WEIGHT IN NORMAL MONKEYS AND DOGS

In many research and experimental investigations, sometimes it is essential to know the weight of some internal organs *in vivo* of the experimental animals. Thus, there is a need for a formula that correlates the weight of internal organs with parameters that could be easily determined. The present paper described a method for estimating the weight of the liver from the body weight in normal monkeys and dogs.

It has been shown in many instances that the organ weight in animal would be related to its body weight if a simple assumption was made that the specific growth rate of that organ was a function of the specific growth rate of the body. That is:-

$$\frac{dy}{y} = m \frac{dx}{x} \quad \ldots\ldots\ldots\ldots (1)$$

where y and x are the weights of the organ and the body respectively, and m is the equilibrium constant. This equation is integrated to:-

$$\ln y = m \ln x + \ln C \quad \ldots\ldots\ldots (2)$$

where C is a constant.

Then $y = Cx^m$ $\ldots\ldots\ldots\ldots\ldots (3)$

This equation is known as an allometry formula and C is called the initial growth index. It follows from eq (2) that the graph of log y against log x is a straight line such that log C is the intercept on the log y axis and m is the slope of the line.

Using the data from Tanticharoenyos (Tanticharoenyos, 1972, pers. comm.), we have plotted the log (base 10) of liver weights (y) as a function of the log (base 10) of body weights (x) from 178 normal monkeys. The results showed a close relationship between these two parameters as illustrated in Fig 1. The least square solution was found to be

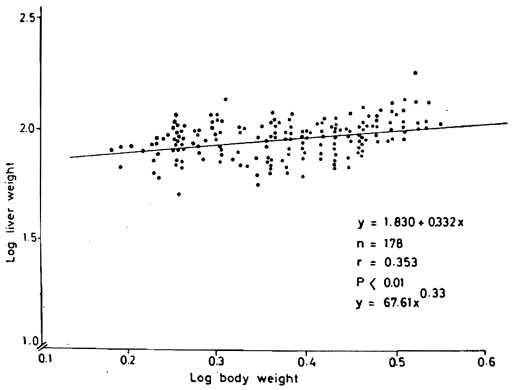

Fig. 1—Relationship between log liver weight and log body weight in 178 normal monkeys.

$y = 1.830 + 0.332\ x$, $r = 0.353$ and $P < 0.01$. This indicates that x and y obey a simple allometry law and the final solution corresponding to eq (3) was $y = 67.61\ x^{0.33}$.

A relationship between the values of log liver weights and log body weights of 58 normal dogs from data of Areekul *et al* (unpublished data) is shown in Fig. 2. A derived least square equation was $y = 1.686 + 0.925\ x$, $r = 0.755$ and $P < 0.01$. An allometry formula for this relationship was $y = 47.86\ x^{0.92}$. Thus we can work on the

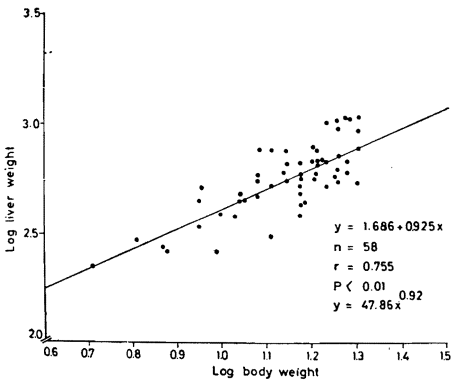

Fig. 2—Relationship between log liver weight and log body weight in 58 normal dogs.

assumption that the liver can be described as the function of 0.33 and 0.92 power of the body weight in normal monkeys and dogs respectively. These findings could therefore be used to estimate the weights of the liver *in vivo* from the known body weights of the normal monkeys and dogs without sacrificing the animals.

SUVIT AREEKUL. Department of Radioisotopes, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.