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Studies on Superoxide Dismutase and Catalase in Different Tissues of Rat Infected with *Plasmodium berghei*

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Superoxide dismutase activity following Tsuchihashi extraction and catalase activity in different tissues were studied in the normal and the *P. berghei*-infected rats (with 80-90% parasitemia). Superoxide dismutase activity was significantly reduced in the liver of the infected rats. Catalase activity was found to be reduced in the liver but elevated in the spleen of the infected rats. Superoxide dismutase activity remained unaltered in brain and kidney of the infected rats. These results suggest that increased levels of superoxide anions and hydroxyl groups may occur in the liver and this may eventually lead to increased lipid peroxidation in rats infected with *P. berghei*.

Introduction :

Various pathological, biochemical and physiological changes are associated with malaria infection in experimental animals. Altered lipid profile is one of the biochemical changes observed in the liver and the spleen of the rodents infected with *P. berghei* (1-3). Sharma *et al.* (4) reported increased lipid peroxidation in biomembranes of the liver and the spleen of the mouse infected with *P. berghei* and this increase in lipid peroxidation was believed to be an index of cell injury in liver pathogenesis on infection. Superoxide anions and hydroxyl groups play a major role in the radical chain reaction leading to increased lipid peroxidation in biological systems (5). Moreover, the enzymes, superoxide dismutase (SOD) and xanthine oxidase involved in superoxide anion metabolism,

have been reported to be altered in the liver of the infected rodents suggesting thereby an increase in the generation of lipid peroxides on malaria infection (4, 6). There is a good deal of evidence suggesting the protective effects of SOD and catalase, the enzyme involved in hydroxyl group metabolism, on biological lipid peroxidation (5, 7, 8).

In view of the above reports on lipid peroxidation associated with rodent malaria and involvement of SOD and catalase in the formation of peroxide radicals, we have studied activities of these enzymes in different tissues of the rat infected with *P. berghei*.

Materials and Methods :

Animals and infection : Four week old albino rats obtained from the animal house of the Haffkine Biopharmaceuticals

Ltd. were used for the study. The strain of *Plasmodium berghei* was generously supplied by the National Institute of Communicable Diseases, Delhi and was maintained in rats by serial blood passage. For determining percent parasitemia, thin smears of blood drawn from the tail of the infected rat were stained with 5 per cent Giesma in the conventional way and the percentage of the infected red blood cells (RBC) was determined. The inoculum of the infected blood was prepared in citrated saline and each rat was injected intraperitoneally with an inoculum of one million parasitized RBCs.

Each group of the infected rats used consisted of six animals. When 80-90 per cent parasitemia had been established in them, which normally required 14-18 days from the day of inoculation, the animals were sacrificed by decapitation. Brain, liver, spleen and kidney were quickly removed, washed with ice-cold saline and weighed. A 10 per cent homogenate was prepared in 0.02 M phosphate buffer, pH 7.0, in all glass homogenizer.

Superoxide dismutase: An aliquot of the homogenate was treated with Triton X-100 at the final concentration of 1.0 per cent (W/V). It was then subjected to Tsuchihashi extraction (9) and centrifuged at 12000 x g for one hour. SOD activity was determined in the supernatant by the method of pyrogallol auto-oxidation as described earlier (10). One unit of SOD activity was defined as the volume of the supernatant required for inhibition of pyrogallol auto-oxidation by 50 per cent at 412 nm.

Catalase: Catalase activity was determined in 12000 x g supernatant (without Tsuchihashi extraction) by the method described by Bergmeyer (11). Decomposition of H_2O_2 by catalase was measured at 240 nm and one unit of the activity was defined as the volume of the supernatant required for change in optical density by 0.01 per minute.

Protein: Protein concentration was measured in the crude homogenate by the method of Lowry *et al.* (12).

The results are expressed as mean $\pm$ s.e.m. and the differences between mean values are assessed by Student's test (13).

Results :

The results of these studies are presented in Table 1.

The wet weight of the spleen was significantly increased in the infected rats, the increase being four fold over the control. However, there was no change in the wet weight of other tissues. Protein level was significantly lowered in the liver of the infected animals, the deficit being 13 per cent as compared to the controls. It was, however, not altered in other tissues.

SOD activity was significantly reduced in the liver of the infected rats, the deficit being 40 per cent as compared to the controls. It remained unaltered in other tissues. Catalase activity was also found to be reduced in the liver by 29 per cent as compared to the controls. However, catalase activity was elevated in the spleen by 116 per cent in the infected animals.

Table 1

Superoxide dismutase and catalase activities and protein levels in different tissues of the rat infected with *P. berghei* (80-90 per cent parasitemia).

Tissue	Group	Wet weight	Protein	SOD	Catalase
		g	mg/g	units/g	units/g
Liver	Control	(6) 2.873 ± 0.332	185.10 ± 7.70	433.50 ± 18.86	1962 ± 119
	Infected	(6) 2.600 ± 0.636	161.00* ± 5.10	256.91** ± 18.80	1383** ± 61
Spleen	Control	(6) 0.180 ± 0.015	129.80 ± 9.60	203.50 ± 3.50	12.60 ± 0.48
	Infected	(6) 0.899*** ± 0.143	127.40 ± 4.10	212.86 ± 10.88	27.20*** ± 3.10
Brain	Control	(4) 1.360 ± 0.100	180.06 ± 2.50	6.20 ± 0.28	ND
	Infected	(4) 1.285 ± 0.033	177.81 ± 11.81	5.80 ± 0.28	ND
Kidney	Control	(4) 0.401 ± 0.012	180.03 ± 9.31	9.50 ± 0.78	ND
	Infected	(4) 0.378 ± 0.044	204.27 ± 11.81	8.66 ± 0.64	ND

Figures in paranthesis indicate number of observations. ND, Not determined.

* P < .05, ** P < .01, *** P < .001.

Discussion :

The observed increase in spleen weight confirms the splenomegaly in experimental malaria infection (14). Four fold increase in the weight can be attributed to higher percentage of parasitemia.

The reduction observed in protein levels in the liver of the infected rats is in agreement with the other report (4). This reduction suggests an altered protein status in the liver of the rats infected with *P. berghei*.

The reduction observed in hepatic SOD activity in the present studies is in agreement with that of Sharma et al. (4). However, in view of the report that Tsuchihashi extraction removes mitochondrial SOD it is difficult to extrapolate the present observations to total SOD activity in the tissues studied in this investigation (15). Nevertheless the reduced activities of SOD and catalase observed in the liver may, perhaps, give rise to more of superoxide anions and hydroxyl groups (5) and thus may enhance the lipid peroxidation

in the liver of the rats infected with *P. berghei*. Moreover, SOD is also believed to be the natural defence mechanism against oxidative damage to the tissue (16) and hence reduction in SOD activity will make the tissue more vulnerable to the oxidative damage.

Although SOD activity remained unaltered in the spleen, catalase activity registered a significant increase. This differential effect on the two enzymes in the liver and the spleen is not surprising. *P. berghei* infection was found to be associated with increased levels of ascorbic acid and decrease in phospholipid levels in the liver but not in the spleen (4). Saxena *et al.* (17) reported the reduced activity of acetylcholine esterase in the liver and the increased activity of the same in the spleen of the rats infected with *P. berghei*. Moreover protein levels, in the present studies, were adversely affected only in the liver but not in the spleen. Thus the spleen does not probably undergo drastic catabolic process unlike the liver. A similar observation has also been made by Sharma *et al.* (4). The enlargement of the spleen could possibly indicate a compensating mechanism associated with the increase in catalase activity.

Our interest in studying SOD activity in brain stemmed from the report that mice infected with *P. berghei yoelii* 17 x strain exhibited symptoms of 'cerebral

malaria' (18). Therefore the effect of a higher percentage of parasitemia was investigated in the present studies with a view to achieve drastic effects, if any, on the brain. However, as can be seen from Table 1, none of the parameters studied in the brain were altered due to malaria infection. Moreover, brain acetylcholine esterase activity also remained unaltered (Kulkarni *et al.* unpublished). These results suggest that *P. berghei* infection in the rats as indicated by the present studies may not adversely affect the brain tissue.

Singer and Trager (19) reported altered levels of some of the metabolites in kidney of the young animals infected with *P. berghei*. However, in the present investigations protein levels and SOD activity were not altered in the kidney of the infected rats.

In summary, the present studies suggest that hepatic SOD and catalase activities are reduced in the rats infected with *P. berghei* (80-90 % parasitemia). This may, perhaps, result in the elevated levels of superoxide anions and hydroxyl groups which eventually may lead to increased lipid peroxidation in the liver.

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Reinvestigation of an old Focus of Schistosomiasis in Gimvi Village, District Ratnagiri, in Maharashtra State India

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The focus of schistosomiasis in Gimvi Village of Ratnagiri was re-investigated. Six hundred and seven urine and stool samples were examined for the presence of schistosome ova. Stool samples did not show any ova. However, the terminal spined ova of schistosome were present in four samples of urine indicating persistence of the focus of schistosomiasis.

Introduction :

In 1952, Gadgil and Shah (1) had reported a focus of schistosomiasis in Gimvi Village in Ratnagiri District of Maharashtra State. In their study, 205 infected persons were detected. Eight snails were found infected with schistosome cercariae. The predominant species of snails was *Ferrissia tenuis*. The focus was considered 60 years old by these investigators at that time.

In 1958, Dave and Dhage (2) resurveyed the area and found that the incidence of infection had remained static. These workers postulated that *F. tenuis* was probably the intermediate host and feared that the focus may spread from there to other parts of the country.

Therefore the area was later subjected to molluscicidal treatment by Public Health Department of Maharashtra.

Today, the threat of introduction of schistosomiasis infection in India has increased manifold because of swiftness of modern travel and movement of local population to and from schistosome endemic areas. This is mainly because of the fact that one of the snail species could serve as a vector of *Schistosoma* sp. as shown by the focus situated in Gimvi. Although the focus of schistosomiasis is restricted to the Gimvi Village, one cannot rule out the possibility of its spread with expansion in irrigation facilities. Measures are needed to prevent the spread of the focus and to keep the existing focus under surveillance. There-

fore, it was decided to reinvestigate the focus with following objectives :

- (i) To investigate whether the focus still exists by carrying out a total survey of the population for schistosomiasis ;
- (ii) To study its effect on the population by clinical examination of cases ;
- (iii) To study the snail population to identify possible vectors.

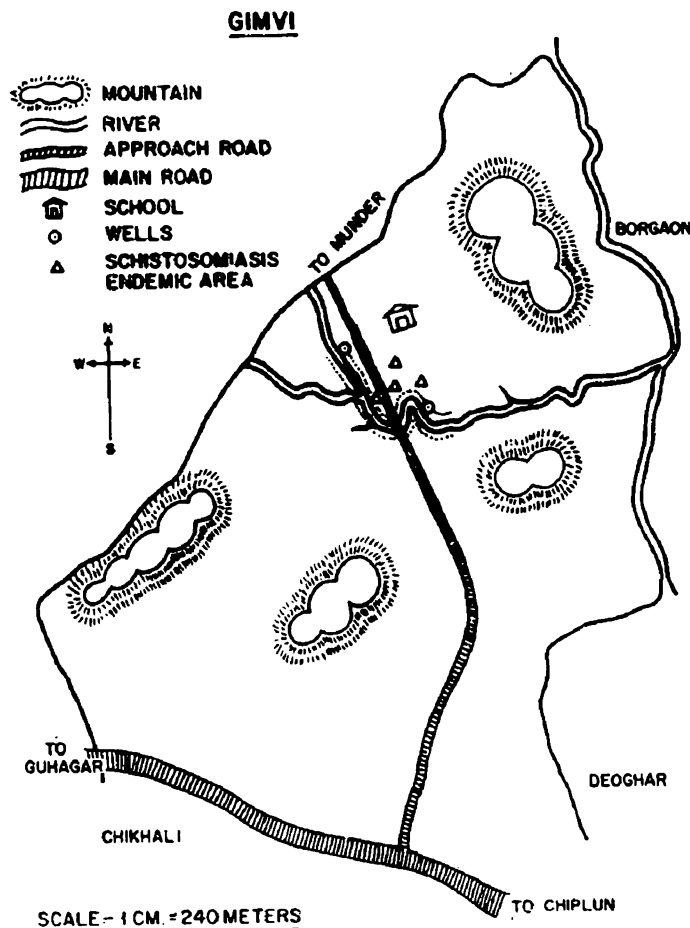
Materials and Methods

Study Area

Gimvi is a village situated in Guhagar Taluka of Ratnagiri District (Fig. 1). The village is located in the hilly ranges of the

Konkan coast, about 16 kilometers from the sea-shore. The average temperature of the place varies from 20°C to 35°C during different seasons of the year. The average rainfall ranges between 250-300 cm per year.

There are two rivulets ; one of this, runs through the village and is used by the inhabitants of the place. The rivulet assumes the size of a river during monsoon i.e., from June to September and early part of October. During this period the rate of flow of water of the stream is considerable and the snail are sparsely dispersed. As the wells are full, this rivulet is not used by the inhabitants during monsoon. From the month of November onwards, the stream settles



down and the water is hardly knee-deep. The population of snails increases from this month onwards upto the month of May. The snails are observed in the rivulet submerged in the water and attached to stones, mango leaves and decaying vegetation.

Recently, piped water supply and sanitary facilities have been introduced in the village to reduce the schistosome infection. In spite of this, villagers, particularly those residing near the rivulet, mainly use the water from this rivulet for washing clothes and cleaning utensils. It gets heavily contaminated with urine and faeces of the inhabitants staying in the vicinity because of their disinclination to use the sanitary arrangements which are available in the village. The children use the rivulet for bathing and swimming. There are about 65 houses in the vicinity of the rivulet. It is in this area that Gadgil and Shah (1) had detected schistosomiasis infection. Our study also showed the presence of schistosomiasis infection with terminal spine ova, only in this part of village Gimvi. The inhabitants affected with this infection serve as the source of supply of schistosome ova and miracidia to the snails.

In all, 607 individuals out of a total of 912 were examined for presence of schistosome ova by collecting their urine and stool samples. The examination urine and stool for schistosome ova was done by the methods recommended by WHO (1967, 1965) (3,4). The snails collected from this village were dissected and examined for the presence of schistosome cercaria.

Results

Four inhabitants were found to excrete terminal spined schistosome ova in their

urine. The stool samples, however, did not show any presence of schistosome ova. The infected persons belonged to different age groups. One individual was 8 years, two persons were 22 and 34 years old and the fourth person was 50 years old. The infection was mild, the number of ova was low and centrifugation was necessary for their detection. The number of ova per sample ranged between 2-5 per ml. The presence of infection in a young, 8 year old child, indicates recent transmission as this inhabitant has never moved out of this village. This also indicates persistence of the focus in the area even today. Microscopic hematuria was present in all four cases.

Clinical examination

Clinical examination of all the inhabitants was undertaken. Eighty per cent of the inhabitants were seen to suffer from anaemia and malnutrition. None of the 607 persons examined showed any gross abnormality.

Old persons (above fifty) who had suffered from schistosomiasis in the past did not excrete any schistosome ova. They had not taken any treatment. According to them, the infection regressed spontaneously.

Study of snails

During this preliminary survey two species of snails were observed viz., *F. tenuis* and *Paludomus obesa*. Snails were collected by using traps and with forceps. In all 500 snails of each species were examined for cercaria. Only five *F. tenuis* were found to harbour bifurcate cercaria. Their identification as schistosome cercaria needs further confirmation and as such more snails are being examined.

Study of other animals

Cattle, dogs and rodents (10 each) were examined for animal schistosomiasis. None of these animals showed presence of schistosome infection.

Discussion

On the basis of the study carried out we conclude that the focus of schistosomiasis still exists. Epidemiologically, the following features peculiar to the infection in this area are worth mentioning :

- (1) The infection is present in the population staying in the vicinity of the rivulet.
- (2) The snail vector present in the area is unknown to any other endemic areas of the world.
- (3) The rivulet is indiscriminately used by the population resulting in the continuation of infection cycle.
- (4) The infection does not produce any external symptoms except mild hematuria. Therefore, the infected person becomes a source of infection and continues to contaminate the rivulet.
- (5) Only four cases were detected during the study. This shows that

the infection is considerably reduced in Gimvi village. To what extent it has spread to other areas, remains to be assessed. These studies emphasise a need for a detailed investigation of the focus.

Investigations have been continued to assess various factors relating to persistence of infection, nature of infection and species of *Schistosoma* existing in the area.

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Study of a focus of *Angiostrongylus cantonensis* in Greater Bombay

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Two suspected cases of human angiostrongyliasis occurred in Greater Bombay area. These cases developed eosinophilic meningitis after the consumption of raw slugs. The symptoms, in brief, consisted of pleocytosis of CSF with 75% eosinophilia, paralysis of lower limbs, severe headache and retention of urine. The CSF, however, did not show any evidence of presence of *Angiostrongylus cantonensis* larvae. Histopathological studies revealed presence of worms and acute inflammation in the brain tissue of experimentally *A. cantonensis* infected rats. The intermediate host slugs were found to harbour *A. cantonensis* larvae. The infection was persistent in the slugs throughout the year. Preadult and adult worms obtained from experimentally infected rats confirmed that these worms belonged to the species *A. cantonensis*. This report demonstrates the presence of *A. cantonensis* in Greater Bombay area for the first time.

Introduction

Angiostrongylus cantonensis, a metastrongyloid parasite of rats, is now recognised as the causative agent of a common form of eosinophilic meningitis (cerebral angiostrongyliasis) in the Pacific and South East Asia. Although this parasite was first found accidentally by Nomura and Lin (1945) in the cerebrospinal fluid of a case of meningitis in Taiwan, the full significance of the finding was recognised only in the early part of 1961 when *A. cantonensis* was incriminated as a possible cause of the epidemic of eosinophilic meningitis in Tahiti. Investigations in Tahiti demonstrated the parasite among rats and the presence of infective larvae of the parasite in fresh water prawns which served as a major source of infection in that area. Studies

showed that this occurrence of parasite among rats was observed in all the areas of the Pacific Islands where human disease was reported. Geographically the parasite has been found, among rodents largely within the tropical belt, extending from Madagascar eastward to the Society Islands, and from Okinawa South to Queensland, Australia. Human angiostrongyliasis has been found in the same area where murine infection occurs, and upto the present time it has been recognised only in parts of South East Asia and the Pacific Islands (Alicata, 1969).

To our knowledge, no human angiostrongyliasis has been reported in India so far except for one suspected case (Gujar et al 1969). Survey of rodents reported from various parts of the country

have not recorded the presence of murine angiostrongylis infection except for Madras City where a focus was demonstrated in *Bandicota indica* (Parmeter and Chowdhury, 1966; Anantraman 1969). The occurrence of two suspected cases of human angiostrongyliasis presented a possibility of existence of a focus of this infection in Greater Bombay area and encouraged us to undertake the present investigations.

Materials and Methods

Human cases: The first case was a male, 33 years old, hailing from Nepal but living in Bombay since 15 years. He was transferred to the Neurology Department of J. J. Group of Hospitals, Bombay, on 3rd July 1980 from St. George's Hospital, Bombay.

On 15th June 1980 he ingested 3 live slugs found near the ditch water in the vicinity of Dockyard. He took these slugs as a treatment for backache from which he had been suffering for several years.

The patient developed nausea and pain in the afternoon immediately after the ingestion of the slugs. Later he developed moderate cough and pruritis. On the next day the patient experienced a dull aching pain all over the body. On the fourth day he experienced some difficulty in walking which progressively increased during the next 6 days. He also experienced tingling sensation in the lower limbs, beginning with soles and ascending to the level of the chest during subsequent days.

On the eighth day the patient could barely walk. On the day of admission patient felt tingling of both hands with a

slight weakness of grip. There was retention of urine (9th day). There was no fever, but he had continuous headache.

The sensory examination revealed hyperesthesia to superficial pain upto the level of D₄, while thermal sensation was impaired till D₈. Vibration sensation was impeded upto the lower ribs. Other modalities of sensation were normal. Deep reflexes in the upper limbs were normal while those in the lower limbs were absent. Abdominal reflexes were absent and plantar reflexes were extensor. The clinical impression was therefore one of the acute transverse myelitis related to the ingestion of slugs.

Haematological and Clinico-pathological studies: Haemoglobin level and RBC count were within the normal range. Total leucocyte count on 9th July was 6000 Cmm with eosinophilia showing 18 per cent. Urine showed some pus cells. Blood sugar levels and liver function tests were normal. CSF showed pleocytosis with eosinophilia reaching a peak of 75 per cent. There was also a protein rise ranging between 80 mg 5 and 120 mg per cent. There were no nematode larvae in CSF. The CSF sugar was low on one occasion. Mantoux test was mildly positive at 14 mm after 72 hours of admission. EMG revealed no denervation of the thenar and hypothenar muscles.

The patient was given symptomatic treatment. Recovery took place in about 50 days. The second patient also demonstrated similar manifestations. They were, however, of milder nature. Various symptoms exhibited by the patient are summarised in Table 1.

Examination of slugs and experimental infection of rats: Slugs were collected at different intervals for a period of one year, from August 1980 to August 1981, from the area situated in the vicinity of patient's residential site and were brought to the laboratory. In all approx. 800 slugs were examined for the presence of *Angiostrongylus* larvae. All slugs were identified as *Laevicaulis alatae*.

Isolation of larvae from the slugs was done by using standard parasitological procedures like homogenisation of molluscan tissue, digestion in artificial gastric juice and recovery of larval nematodes by a Baerman apparatus. Experimental infection of rats was undertaken by infecting orally the Haffkine strain of white rats using a dose of 80 larvae/ml/rat. In all 11 rats were infected. The rats were sacrificed at different intervals and progress of infection was recorded.

Sections of the brain of rats infected with *A. cantonensis* worms after staining with haematoxylin and eosin were examined for evidence of damage to the nervous system.

Worms were collected in warm digestive fluid (for collection from CSF) or in warm saline (from lungs and heart) and were preserved for identification.

Results :

Molluscan intermediate host: The infection was found to be prevalent in the slugs throughout the year as all batches of slugs examined during different parts of the year produced infective larvae.

Experimental infection: Table 2 shows recovery of *Angiostrongylus cantonensis* worms from albino rats after oral infection with 3rd stage larvae recovered from the slug, *Laevicaulis alatae*. Preadult worms are seen in the CNS, 21 days after the infection. No worms were, however,

found in the brain after 30 days of infection. Adult male and female worms were recovered from the heart and lung tissues after 45 days. Adult male and female worms from these tissues were recovered even after 390 days. First stage larvae of *A. cantonensis* were recovered from faeces of experimentally infected rats after 50 days of infection.

Various stages of *A. cantonensis* (Fig. 1-6) were recovered from the slugs (Fig. 7) and experimentally infected rats. Average dimensions (in mm) of *Angiostrongylus cantonensis* worms recovered from experimentally infected albino rats are recorded in Table 3. Morphological features presented by the worm at different stages confirm that these worms belong to the species *Angiostrongylus cantonensis*.

Histological examinations: Histological examinations of brain tissue of experimentally infected rats demonstrate the presence of worm in the brain tissue (Fig. 8) showing acute inflammation of the leptomeninges. Profiles of a few nematode worms cut in longitudinal and transverse section could be seen in the subarachnoid space over the cerebral cortex. The worms were surrounded by haemorrhagic and neutrophilic infiltrate. The cerebral cortex showed intensive congestion. Occasionally worm was observed burrowing into the surface of the cerebral cortex causing focal superficial necrosis. Most of the cortex revealed oedema and congestion. Foci of necrosis with polymorphonuclear infiltrate, well within the substance of the cerebral cortex, unrelated to the surface worms were also observed (Fig. 9, 10).

Discussion :

The investigations carried out following the occurrence of two suspected cases

Figures 1-6 :

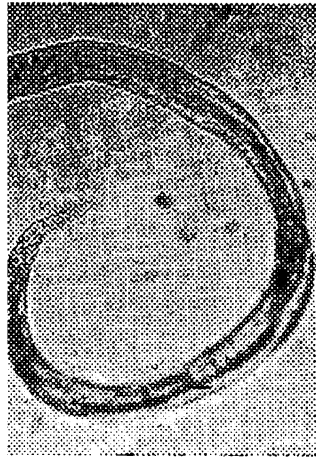


Fig. 1 Larva

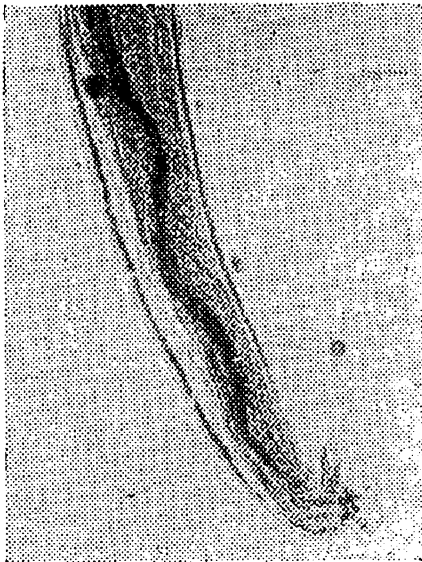


Fig. 5 Bursa of adult male worm

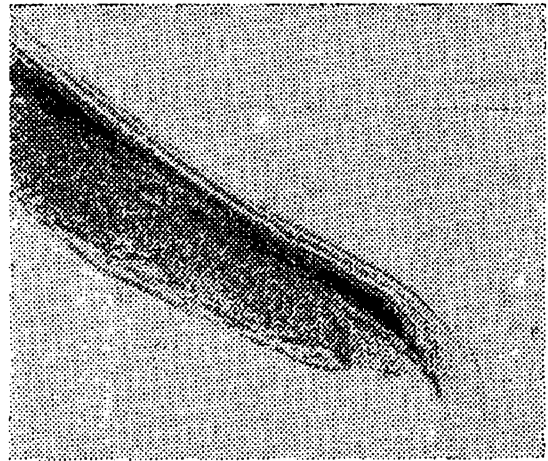


Fig. 6 Caudal end of adult female worm

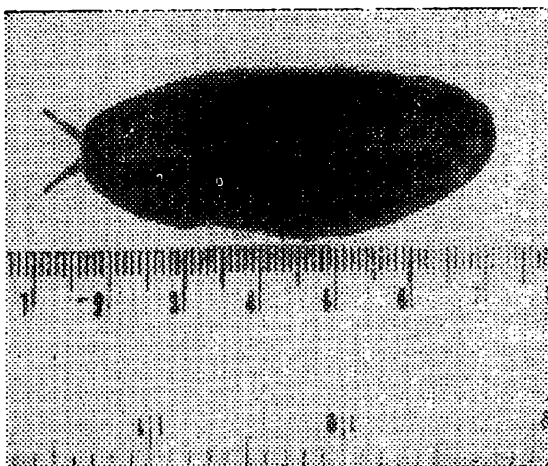


Fig. 7 Slug — *Laevicaulis altae*



Fig. 8 Cross section of the worm in the brain (L.P.)



Fig. 9 Cross section of the worm in the brain (H. P.)

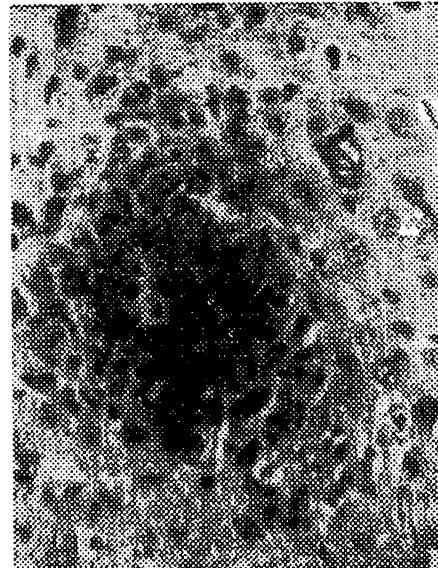


Fig. 10 Focal necrosis in cerebral cortex.

of human angiostrongyliasis show the existence of a focus in Greater Bombay area. We observed that the slug, *Laevicaulis altae*, is a known intermediate host for *A. cantonensis* elsewhere and, in this area also, we found that the slugs harboured infection. The infection was found to occur throughout the year. The slugs are very widely distributed all over the city area and it remains to be seen now as to how widespread is the infection in these slugs. The vertebrate reservoir has been shown to be *Rattus norvegicus* (Renapurkar et al., in press). *Rattus norvegicus* is one of the prevalent rodent species in Greater Bombay area. The infection is likely to be fairly widespread in these rodents also. It is also likely that the reservoirs of infection may be present in other invertebrate and vertebrate animals.

While commenting on modes of transmission to man, Alicata (1969) observed that the ingestion of uncooked food containing infective larvae of *A. cantonensis*, or food containing an infected intermediate or a carrier host, appear to be the chief source of human infection. Other less likely methods consist of contamination of drinking water with the infective larvae and contact of the broken skin with soil containing such larvae.

With the knowledge of the presence of reservoir of infection in Greater Bombay area, we hope that it will be possible by further studies to reveal detailed information on the extent of distribution of this focus in these reservoirs and other animals and enable us to assess the possible risk of human infection.

Table 1
Course of disease after consumption of slugs

Symptoms	Period after ingestion of slugs h(hour)/d(day)						
	3 h	24 h	2 d	3 d	4-6 d	9 d	14 d
Nausea and abdominal pain	++	+	—	—	—	—	—
Vomiting	+	—	—	—	—	—	—
Fever	—	—	—	—	—	—	—
Pruritis without rash	—	+	+	—	—	—	—
Dullness and pain all over the body	—	—	+	+	—	—	—
Weakness of lower limbs	—	—	—	—	++	++	+++
Weakness of upper limbs and tingling sensation.	—	—	—	—	+	+	+
Retention of urine	—	—	—	—	—	++	—
Flexor spasms	—	—	—	—	—	+	+
Headache	—	—	—	—	+++	+++	+++

Remarks: (i) Patient admitted on day 9 with paralysis of lower limbs and painful retention of urine, severe headache without vomiting.

(ii) Haematological investigations showed normal blood picture with total leucocyte count 6000, Cmm — Eosinophilia, 18 per cent.

(iii) C.S.F. Pleocytosis with eosinophilia 75 per cent, Protein 80 — 120 mg per cent.

(iv) All features of acute transverse myelitis present.
+mild; ++ moderate; +++ severe

Table 2

Recovery of *Angiostrongylus cantonensis* worms from albino rats after oral infection of 3rd stage larvae

No. of animals infected	Day post infection	No. of animal killed	Worm recovery		
			CNS	Heart	Lungs
11	14	2	—	—	—
	21	2	6,10	—	—
	46	2	—	6,3	2,1
	80	1	—	—	5
	172	1	—	—	5
	250	2	—	2,5	3,2
	390	1	—	2	1

Table 3

Average dimensions in mm of *Angiostrongylus cantonensis* worms recovered from experimentally infected albino rats

Size	Preadult		Adult	
	Male	Female	Male	Female
Total length	10.0	10.0	20.0	23.66
Total width	0.107	0.130	0.19	0.266
Oesophagus length	0.254	0.230	0.28	0.30
Oesophageal bulb	0.037 x	0.04 x	0.05 x	0.046 x
	0.035	0.04	0.05	0.040
Tail length Bursa	0.04	0.15	0.05	0.163
Spicule length	—	—	0.980	
Gubernaculum length	—	—	0.35	

Acknowledgements

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Effects of Postweaning Prolonged Protein Deficiency on Rat Brain Enzymes

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Weanling albino rats were reared on protein deficient diet for a period of 63 weeks. Brain protein, DNA and activities of malic enzyme, citrate cleavage enzyme and glucose-6-phosphate dehydrogenase were measured at the end of the experiment. The activities of all the three enzymes showed significant deficits suggesting eventual reduction in brain NADPH + levels.

Introduction

Protein deficiency has been shown to impair glucose tolerance in pigs (1) and also in human population (2). Impaired carbohydrate metabolism has been reported in protein deficiency (2) and hypoglycemia is a frequently observed feature in Kwashiorkor (3). Studies with brain tissue slices of low and high-protein animals and have revealed significant differences in glucose utilization (4). Moreover protein deficiency has been associated with deficits in brain glutamate dehydrogenase (5) which is NAD dependent and the synthesis of NAD is affected in protein deficiency (6). Neonatal protein deficiency has also been associated with deficits in brain pyruvate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase and malic enzyme (7). However in the same studies postweaning protein deficiency was found to decrease rat brain isocitrate dehydrogenase and malic enzyme only.

The experimental work reported here addresses itself to the effects of post-

weaning prolonged protein deficiency on rat brain enzymes. The enzymes investigated were selected as representatives of different metabolic pathways associated mainly with carbohydrate and to some extent with lipid metabolism.

Materials and Methods

Weanling albino rats, acquired from the animal house of Haffkine Biopharmaceutical Co. Ltd., Bombay, matched for sex, body weight and age, were fed 5 or 20% protein diet for a period of 63 weeks. Diet composition remained essentially same as reported earlier (8). At the end of regimen, rats were sacrificed by decapitation and whole brains including olfactory lobes were quickly dissected out.

Brain DNA (9) and protein (10) were estimated in an aliquote of whole-brain homogenate prepared in 0.02 M phosphate buffer, pH 7.0. The remaining homogenate was treated with Triton X-100 (0.1% w/v) for 30 minutes, centrifuged at 10,000 g for 30 minutes at

4°C and supernatant was used for estimations of activities of malic enzyme (11), citrate cleavage enzyme (12) and glucose-6-phosphate dehydrogenase (13). One unit of the enzyme was defined as change in optical density by 0.01 at 340 nm under experimental conditions. All results are expressed as Mean $\pm$ S.E.M. (14).

Results :

Protein deficiency during post-weaning period was found to result in significant body weight deficit of 41% (Table 1). Brain weight deficit in the deficient animals was of the order of 12%. However, the value for brain weight to body weight ratio was elevated by 57% in the deficient group. Brain DNA and protein concentration remained unaltered. But activities of malic enzyme, citrate cleavage enzyme and glucose-6-phosphate hydrogenase were reduced by 9, 39 and 21% respectively.

Discussion :

Post-weaning protein deficiency and also caloric restriction have been associated with significant deficits in body weight and brain weight (15, 16). In the present studies, elevation observed in brain weight to body weight ratio in the deficient rats suggests relative sparing of brain tissue over that of other tissues in general (16).

Post-weaning protein deficiency has not altered brain DNA and protein concentrations. However, brain protein content will alter as per alteration in the brain weight of the deficient animals mentioned above. This observation is in

agreement with that of Winick and Noble (16). Similar observations on brain cholesterol (17) and lipids (18) have been made in the case of rats subjected to either protein deficiency or caloric restriction during post-weaning periods.

Significant deficit of 21 per cent in brain glucose-6-phosphate dehydrogenase of the deficient animals indicates a possible alteration in the pentose phosphate pathway with a concomitant reduction in reducing equivalent NADPH⁺ in extra-mitochondrial space (19). Moreover, reduced activity of malic enzyme in the deficient rat brain is likely to contribute further to the deficits in NADPH⁺ concentration (19). The observed deficit in brain citrate cleavage enzyme can be associated with decrease in acetyl Co A levels in cytosol (19). The enzymes which generate acetyl-Co A and NADPH⁺ particularly for fatty acid synthesis, and those which utilize them change in unison by nutritional stress (20, 21). However post-weaning protein deficiency or caloric restriction have not been associated with alterations in lipid composition (17, 18). This could be attributed to the well known fact that brain lipids mature by 21 days in rats and there after they remain invulnerable to nutritional stress (22). However, it is also known that after 21 days of age, slow but continuous deposition of lipids takes place in brain throughout the life span of rats as judged by the proportion of lipids in grey and white matter (23). Detailed studies on the compartmentation of acetyl-Co A and NADPH⁺ in grey and white matter will throw more light on these aspects.

Effects of Postweaning Protein Deficiency on Rat Brain Enzymes.¹

	Dietary Regimen			
	Control		Low Protein	
Body Wts. (g)	247	± 30	146	± 15*
Brain Wts. (g)	1.736	± 0.039	1.521	± 0.041*
Brain Wt./Body Wt.	0.007	± 0.001	0.011	± 0.001*
BRAIN :				
DNA (mg/g)	1.87	± 0.09	1.87	± 0.08
Protein (g %)	12.49	± 0.19	12.62	± 0.23
Unit Per G Brain				
Malic Enzyme	315	± 5.7	287	± 5.0*
Citrate Cleavage Enzyme	78	± 2.2	47	± 3.7*
Glucose-6-Phosphate Dehydrogenase	395	± 12.0	310	± 20.0*

1 Weanling rats were reared on 20 per cent protein (Control) or 5 per cent protein (Low Protein) diets for a period of 63 weeks. Each group consisted of four rats.

* Values significantly different from the control, $P < .05$.

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Synthesis and Study of 2-Substituted-4-Methyl-5, 6-Dihydrofuro (2, 3-d) Pyrimidines as possible Antimalarial Agents-III

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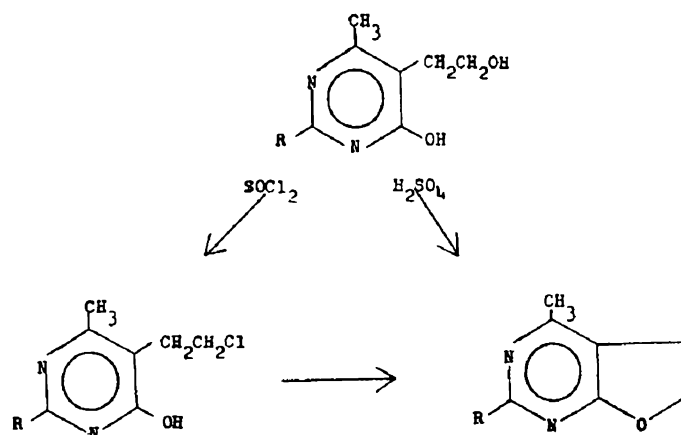
A series of 2-substituted-4-methyl-5,6-dihydrofuro (2,3-d) pyrimidines have been prepared by cyclising 2-substituted-4-methyl-5-(β hydroxyethyl)-6-hydroxypyrimidines and the changes in antimalarial activity caused by this variation were studied.

Introduction

Pyrimethamine and trimethoprim owe their antimalarial activity to selective inhibition of dihydrofolate reductase which converts dihydrofolic acid to tetrahydrofolic acid in the parasite. This inhibition interferes with plasmodial biosynthetic pathways leading to the formation of purine and pyrimidine bases and eventually of DNA. Although, these agents have no selective action on the enzyme of the parasite, they bind to

dihydrofolate reductase of plasmodia much more tightly than to the same enzyme of the host. Furthermore, the host is not dangerously affected by the blockade of this reaction, because the folic acid required is supplied in the food (1).

In recent years, a number of fused pyrimidines such as purines, pyrazolo (3,4-d) pyrimidines, pyrrolo (2,3-d)-pyrimidines, etc. have shown interesting



where,

$\text{R} = \text{ClC}_6\text{H}_4$; $4\text{-BrC}_6\text{H}_4$; $4\text{-OC}_8\text{H}_7$ C_6H_4 ; $3,4\text{-(OC}_2\text{H}_5)_2$ C_6H_8 , etc.
4-pyridyl, 3-pyridyl, 2-aryloxy-4-pyridyl.

antimetabolite and antitumor activity. Further, Roth (2) reported the antiparasitic (such as anthelmintic and antiprotozoal) properties, of 2,4-diamino thieno (2,3-d) pyrimidines.

In view of these facts, it was thought interesting to study systematically the chemistry and biological importance of the fused furano pyrimidines in the present work. The furan ring was fused at 5 and 6 position of pyrimidine ring to get 2-substituted-4-methyl-5,6-dihydrofuro (2,3-d) pyrimidines (II) and changes in the antimalarial activity caused by this variation were studied.

Chemistry

These furopyrimidines (II) were obtained by the cyclization of 2-substituted-4-methyl-5-(β -hydroxyethyl)-6-hydroxypyrimidines (I) (3) by reaction of thionyl chloride followed by the treatment with base like sodium carbonate or potassium carbonate (4). Alternatively, compounds (I) were also dehydrated using concentrated sulphuric acid to get 2-substituted-4-methyl-5,6-dihydrofuro (2,3-d) pyrimidines (II) (5) (Table-I).

Experimental

(a) 2-(4'-Chlorophenyl)-4-methyl-5,6-dihydrofuro-(2,3-d) pyrimidines

2-(4'-Chlorophenyl)-4-methyl-5-(β -hydroxyethyl)-6-hydroxypyrimidine (2.65 g; 0.01 mole) was treated with 5 ml of conc. sulphuric acid. The reaction mixture was warmed on a steam bath for 8 hours. It was then poured on crushed ice and made gradually alkaline with sodium hydroxide solution (40%) below 15°C, when the title compound was precipitated as white solid. The product was filtered, washed with water and recrystal-

lised from 2:1 mixture of hexane and benzene.

Yield : 1.05 g (41.0%); m.p. 166-67°C.
 Anals. calcd. for $C_{13}H_{11}ClN_2O$:

Calcd. : C, 63.28; H, 4.46; N, 11.36%.

Found : C, 62.89; H, 4.13; N, 11.63%.

(b) 2-(3',4',5'-Trimethoxyphenyl)-4-methyl,5,6-dihydrofuro (2,3-d) pyrimidine :

2-(3',4',5'-Trimethoxyphenyl)-4-methyl-5-(β -hydroxyethyl)-6-hydroxypyrimidine (1.4 g; 0.05 mole) and 20 ml chloroform was boiled under stirring. The contents were cooled and saturated with dry hydrogen chloride gas. Then 2 ml of thionyl chloride in 10 ml of chloroform was added dropwise. The reaction mixture was stirred for an additional one hour at room temperature and left overnight. Next day, it was gently refluxed under stirring for 2 hours. The chloroform was evaporated under vacuum, the residual thionyl chloride being supplemented with benzene. The residue thus obtained was suspended in 10 ml of ethanol and refluxed with 1 g of sodium carbonate for five hours. The insoluble product filtered off and the filtrate concentrated to get the title compound. It was recrystallised from 2:1 mixture of hexane and benzene in the form of white needles.

Yield : 1.1 g (74.0%); m.p. 200-201°C.
 Anals. Calcd. for $C_{16}H_{18}N_2O_4$:

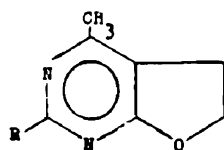
Calcd. : C, 63.57; H, 5.96; N, 9.27%.

Found : C, 63.30; H, 5.64; N, 9.36%.

Biological Results and Discussion

In all, eighteen furopyrimidines were synthesised and six representative members were screened for their *in vivo* antimalarial activity against *P. yoelii*.

Table I
2-Substituted-4-methyl-5, 6-dihydrofuro (2, 3-d)pyrimidines.



No.	R	M.P. °C ^a	Mol. formula ^b
1	C ₆ H ₅	142-44	C ₁₃ H ₁₂ N ₂ O ^c
2	4-ClC ₆ H ₄	166-67	C ₁₃ H ₁₁ ClN ₂ O
3	4-BrC ₆ H ₄	171-72	C ₁₃ H ₁₁ BrN ₂ O
4	4-FC ₆ H ₄	137-38	C ₁₃ H ₁₁ FN ₂ O
5	4-(n)-PrOC ₆ H ₄	164-66	C ₁₆ H ₁₈ N ₂ O ₂
6	3, 4-(EtO) ₂ C ₆ H ₃	162-64	C ₁₇ H ₂₀ N ₂ O ₃
7	3, 4-EtO (MeO)C ₆ H ₃	167-68	C ₁₆ H ₁₈ N ₂ O ₃
8	3, 4-MeO (EtO)C ₆ H ₃	172-73	C ₁₆ H ₁₈ N ₂ O ₃
9	3, 4-MeO (n-PrO)C ₆ H ₃	153-54	C ₁₇ H ₂₀ N ₂ O ₃
10	3, 2-EtO (PhCH ₂ O)C ₆ H ₃	124-25	C ₂₂ H ₂₂ N ₂ O ₃
11	3, 2-EtO (PhCH ₂ O)C ₆ H ₃	183-85	C ₂₁ H ₂₀ N ₂ O ₃
12	3, 4-EtO (2'-ClPhCH ₂ O)C ₆ H ₃	135-36	C ₂₂ H ₂₁ ClN ₂ O ₃
13	3, 4-EtO (2'-ClPhCH ₂ O)C ₆ H ₃	156-57	C ₂₁ H ₁₉ ClN ₂ O ₃
14	3, 4, 5-(MeO) ₃ C ₆ H ₂	200-01	C ₁₆ H ₁₈ N ₂ O ₄
15	3-Pyridyl	133-35	C ₁₂ H ₁₁ N ₃ O
16	4-Pyridyl	144-46	C ₁₈ H ₁₅ N ₃ O ₂
17	2-Phenoxy-4-pyridyl	144-46	C ₁₈ H ₁₅ N ₃ O ₂
18	2-(4'-chlorophenoxy)-4-pyridyl	175-77	C ₁₈ H ₁₄ ClN ₃ O ₂
19	2-(4'-Chloro-3'-methyl-	151-53	C ₁₉ H ₁₆ ClN ₃ O ₂

(a) Melting points are capillary melting points and are uncorrected.

(b) Analysed correctly for C, H and N within — 0.4%.

(c) Reported (7) m.p. 143-44°C.

Rane's test described by Osdene *et al.* (6).

mg/kg. Also compound No. 14, was active at 80 mg/kg.

All the six compounds (Table II) proved to be non-toxic even at the high dose level of 160 mg/kg. The compounds No. 1,2 and 4 were active at 160 mg/kg dose and No. 3 was active at 40 and 80

Acknowledgement

The authors are greatly indebted to Dr. M. H. Shah, Assistant Director, Chemotherapy Department, for his keen

Table - II

Antimalarial activity against *P. yoelii*

Sr. No. ^a	MST T or C ^b dosages mg/kg		
	40	80	160
1	1.9	—	6.0(A)
2.	3.9	—	8.5(A)
3	9.5(A)	6.2(A)	—
4	3.8	—	8.8(A)
6	1.2	—	2.5
14	—	7.2(A)	—

Mice which live more than 40 days are regarded as cured (C). Drug which double the survival time of mice compared to control are considered active (A). In each run pyrimethamine was included as standard drug (curative dose 4.5 mg/kg).

(—) = not tested.

(a) Numbers refer to those from Table I. Hydrochlorides of these compounds were screened.

(b) MST is the mean survival time (days) of control mice (MSTC). Death prior to MSTC is toxic (T).

interest in present work and to Dr. D. M. Renapurkar, Assistant Director, Zoonosis Department, for making available the anti malarial screening data. Thanks are also due to Mr. M. T. Jaokar for elemental

microanalysis. They are also grateful to Dr. B. B. Gaitonde, Former Director, and Dr. K. D. Sharma, Director, Haffkine Institute, Bombay for providing facilities to carry out the present work.

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BOOK-REVIEW

Title : Membrane Transport in Biology

Ed. : Tosteson D. C.

Vol. II : Transport across single Biological Membrane 439 pages, cloth.

Springer Verlag, Berlin Heidelberg New York ISBN 3-540-08780-X, 148 DM.

The second volume of the series on Membrane Transport in Biology is devoted mainly to the study of transport systems across single cell (or more correctly biological) membranes.

The myriad of biochemical processes, which include, inter alia, chemical transformation and transport of native and ionic forms of physiological agents, bequeath on an organism the properties of life. While transports across a physiological compartments of the organism are essential for a variety of life processes like nutrition, respiration, reproduction etc., in the maintenance of the 'internal milieu' transport across single biological membranes plays an important role.

The first three chapters of the volume are devoted to transports across red cell membranes. By virtue of their biochemical functions the RBC form the most important constituent of the blood. The entire functions of these cells are possible due to their unique limiting membrane. The RBC membrane possesses specific characteristics and transport processes which are responsible for gas, electrolyte and non ionic transport mechanisms which make them the vital link between tissue and the external environment.

Since the last 85 years a large amount of work has been going on, on the trans-

port of water and solutes into the RBC. This work has been aimed towards understanding the characteristics of the membrane. A large variety of techniques using isotopes, high pressure technology, optics etc. have been employed for the same. R. I. Macey has reviewed these in the first chapter of the volume and made a critical evaluation of the interpretation of the data obtained from various experimental designs.

The chloride bicarbonate shift of the RBC is yet another important function by which gas exchange is achieved. The CO_2 from the tissues is carried in the blood in the form of HCO_3^- . An exchange of HCO_3^- between tissue and RBC is affected with the aid of Cl^- . The mechanism of this anion transport has been reviewed in detail by Gunn in the second chapter.

Living cells maintain steady states with concentration gradients across their membranes. The maintenance of these gradients is an energy requiring process, fuelled by metabolic energy, which work to counter balance passive flux of ions (especially cations — Na^+ K^+) towards the equilibrium. The passive fluxes and the active transport systems have been discussed in the 3rd and 4th chapters by Lew and Beauge, and Sarkadi and Tosteson. As the Na^+ K^+ pumps play a vital role in the functioning of excitable cell membranes the importance of these chapters can hardly be overestimated. The RBC provide a simple model for these studies as the passive permeability of the membrane of Na^+ K^+ is so low that it behaves like a pure phospholipid

bilayer, enabling active transport measurements.

The conduction of impulses across the surface of excitable cell membranes is an important outcome of cation fluxes across axonal membranes. In fact it is this transport mechanism which revolutionized the understanding of the mechanics of nerve transmission. L. J. Mullins has exhaustively dealt with axon transport in the fifth chapter. The excellent treatment of the data makes the chapter an important document for those involved in the physiology of neurotransmission and conduction.

To the experimental pharmacologist the chapter on ion movements in skeletal muscle membranes and the sarcoplasmic reticulum are probably the most useful while studies on transport mechanisms of mitochondrial and lysosomal enzymes would find favour with biochemists. The data available on these topics is both huge and confusing. The authors of these chapters have done well to review it in the light of its relevance.

The volume II of the series is an extremely valuable to the series with carefully selected chapters published in an excellent manner.

R. B. Ghooi
Scientific Secretary

Proceedings of the Critics' Club

(April to August 1981)

Ms. Tharthare of the Immunohaematology Department addressed the members of the Club at its meeting on 24th April 1981. Haptoglobins (alpha-2-glycoproteins) are present in the blood of most mammals and derive their importance from the fact that they complex with haemoglobin. The Immunohaematology Department has been studying haemolytic anaemias since some time, and they are now investigating the relevance of haptoglobin levels in cases of haemolytic anaemias, especially Thalassemia. The data obtained on studies of haptoglobin levels in thalassemic children revealed low levels of haptoglobin which recovered after whole blood transfusion. The relevance of the findings were discussed at the meeting.

On the 25th of June Dr. Laszlo Pesti, Deputy Director, Veterinary Medical Research Institute, Hungarian Academy of Sciences Budapest, addressed the Critics Club. Dr. Pesti has been involved in studies on virulence factors and the immune response to infection. Detailing the work done in his laboratory he discussed the relevance of the heat labile and the heat stable factors from *E. coli* to its virulence and immunogenicity.

There is an increase in the awareness of the incidence of angiostrongyliasis in

India, cases of which have already been reported in South East Asia. Though the infection in rodents was reported as far back in 1966, no human case has so far been reported in our country. Dr. D. M. Renapurkar of the Zoonosis Department in a talk to the Critics' Club on 3rd July revealed that two cases of suspected angiostrongyliasis were admitted to the city hospitals. Investigations by the Haffkine team of scientists revealed that the patients had eaten raw slugs, a known vector of the infection. Studies on slugs revealed the presence of infection which could be transferred to rats. Examination of commensal rodents also revealed the presence of infection in *Rattus norvegicus*. Dr. Renapurkar summed up his talk by discussing the relevance and the implications of his findings.

Shri P. P. Gudadhe of the Department of Chemotherapy delivered before the members of the club on 17th July a talk based on his efforts to synthesize new antimalarial agents. Shri Gudadhe discussed the synthesis and biological activity of substituted Pyrimidine and furo-pyrimidine derivatives.

R. B. Ghooi
Scientific Secretary

DIRECTIONS TO CONTRIBUTORS

Bulletin of Haffkine Institute is a triannual publication. It embodies review and research articles in biomedical sciences, mainly devoted to studies on Communicable Diseases.

Contributions should be precise and concise, highlighting original observations and inferences. The type-script (T.S.) should be sent in duplicate, typed in double space on only one side of the sheet with ample margins.

A brief summary not exceeding 200 words, should accompany the article. The title should be informative and specific, followed by author's name and address.

A short title in not more than forty letters should also be suggested.

The text of the paper should be generally sub-divided into : Introduction, Materials and Methods, or Experimental, Results, Discussion, Acknowledgement and References.

'Short Abstract' of about 100 words suitable for inclusion in 'International Abstracts of Biological Sciences', etc. should be typed on a separate sheet, with author's name, address and title of the paper.

Results should be presented concisely in tables giving only important and relevant data and avoiding incorporation of elaborate observations.

Restraint should be exercised in submitting illustrative matter. Photographs should be well-defined and on glossy paper.

References in the text should be cited by numbers serially as they appear, and to be inserted in parenthesis, e.g., Plasmid-mediated resistances to these heavy metals have also been observed in enteric bacilli, especially in P factor carrying organisms (1), and have recently been studied by several workers (2, 3, 4). References at the end should be arranged numberwise serially as they figure in the text with author's name first and abbreviations according to Index Medicus. The following pattern may be followed :

- (i) Reuben, R. ; Carey, D. E. and Meyers, R. M. (1968) — Japanese encephalitis studies in Vellore, S. India, **Indian J. Med. Res;** 56, 109-18.
- (ii) Cruickshank, R. (1968) — Medical Microbiology, 11th Ed. 180-4, E and S Livingstone Ltd., Edinburgh, p. 28.

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