Rev. Inst. Med. trop. São Paulo
25(5):215-222, *setembro-outubro*, 1983

UDC 616.995.122

THE ASSOCIATION OF SCHISTOSOMA MANSONI INFECTION WITH DEFICIENCY OF VITAMIN A IN MICE

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SUMMARY

The somatic development of **Schistosoma mansoni** was delayed in vitamin A deficient mice when compared with that obtained in controls. The difference was more pronounced on the 12th day. The proportion of worms at the first, second, and third stages of development were 66.5, 31.0, and 2.5%, respectively, for the vitamin deficient mice, and 26.4, 37.4, and 36.2%, respectively, for the controls. On the 18th day after cercarial exposure, the proportion of worms at the first, second, third, fourth, and fifth stages were, respectively, 4.4, 0.0, 46.45, 36.44, and 12.66% for the vitamin A deficient mice, and 0.0, 0.0, 17.54, 54.84, and 27.62%, respectively, for the controls. The oviposition in control and in vitamin A depleted mice was nearly the same. Carotene and vitamin A were not detected in worms derived from vitamin A deficient animals. The levels of carotene and vitamin A were 1020 and 0.086 μg/g of worm, respectively, in schistosomes recovered from control mice. Some mice developed a neuropathy that resulted in spontaneous amputation of one of the forelimbs. In infected control and vitamin deficient mice the incidence of this neuropathy was 4.9 and 23.1%, respectively.

INTRODUCTION

Very little work has been done on the evolution of **Schistosoma mansoni** in experimentally infected hosts submitted to nutritional deficiencies. Research in this field is important since in endemic areas malnutritional and infection often coexist. In schistosomiasis the host diet has an important role in the host-parasite relationship due to nutritional and biochemical alterations that occur in the worm habitat as well as in the efficiency of the host defense mechanisms. Deficient diets intensify the pathological consequences of the infection (DREIZEN [8]). The effect of vitamin A deficiency in the host diet on immunological mechanisms has been reported by KRAKOWER et al. [13].

In the present paper, the consequences of vitamin A deficiency on the host, on the parasite, and on the relationship between them are reported.

MATERIALS AND METHODS

Swiss albino mice were used. After mating, the females were put in individual cages and fed on commercial diet. On the 14th day after

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birth, half of the mothers with their litters were fed on a diet deficient in vitamin A in order to establish a depletion state. This diet contained (in g/kg): ANRC reference casein (Sheffield Chemical Co., Norwich NJ), 200; corn starch, 550; sucrose, 100; peanut oil, 80; salt mixture (AOAC, 1975), 50; cellulose, 10; and vitamin mixture (AOAC, 1975) without vitamin A, 10. The remaining females with their litters were fed on the same diet to which 20.000 units/kg of vitamin A were added. The mice were weaned on the 20th day and separated by sex. They were fed on the same diets as their mothers. The diets were given "ad libitum" during all the experiment. On the 20th and 36th days of age, a total of 48 animals were sacrificed by cervical fracture for determination of the vitamin A level in the liver. The livers were removed and perfused with heparinized 0.9% NaCl at 4°C in order to remove all blood in liver vessels. Perfusion was carried out with a model 40 Brewer type machine, according to BARBOSA et al.[3]. Lipids were extracted (BAYFIELD[4]) and vitamin A was determined in this extract by the method of trifluoroacetic acid (NEELD & PEARSON[17]). The levels of vitamin A in adult **S. mansoni** were assessed by the same method.

On the 37th day, both male and female mice were divided in four groups: (1) control group, (2) vitamin A deficient group, (3) control group infected with **S. mansoni**, and (4) vitamin A deficient group infected with **S. mansoni.** At this time, transcutaneous infection was performed with cercariae (LE strain) maintained in **Biomphalaria glabrata** in the laboratory of the "Grupo Interdepartamental de Estudos sobre Esquistossomose (GIDE)", according to BARBOSA et al.[3]. Each mouse was exposed to 60-70 cercariae on the lower abdomen. The mice were numbered, kept in plastic cages in groups of 10 per cage and weighed weekly.

On the 12th day of infection the perfusion and schistogram were performed in 10 mice of the control group infected with **S. mansoni,** and 10 of the vitamin A deficient group infected with **S. mansoni**, according to BARBOSA et al.[3].

On the 18th day after infection the same procedure was repeated in 16 animals of each infected group.

The method used to assess the development of **S. mansoni,** the schistogram was based on morphological criteria proposed by FAUST et al.[9]. Due to technical facilities the gastric caecum shape was chosen: stage 1. — schistosomula presenting only a light stain which stands for the beginning of the caecum; stage 2. — a darker stain now bifurcates but does not bypass the acetabulum; stage 3. — the dark stain bypasses the acetabulum and its branches link themselves later on; stage 4. — the dark bifurcated stain after reconnection grows to the parasite end, but not longer than the bifurcated caecum; stage 5. — the final linked caecum grows longer than its bifurcated section, but shorter than three times of its lenght; stage 6. — their linked caecum grows three times longer than the bifurcated caecum (mature adults).

On the 50th day after infection, the perfusion to recover mature worms was conducted according to the method of BARBOSA et al.[3], and the quantitative oogram (PELLEGRINO & FARIA[18]) of 10 mice in each infected group was done.

Autopsy was performed in six animals of each group. Fragments of liver lungs, small intestine, heart and kidneys were fixed in 10% neutral formaldehyde solution for histological examination.

Fragments of spinal cord, peripheral nerves, and the lesioned foot were also examined histologically in 11 animals with paw lesions. The dissection of the medullary canal and its nerve roots was performed in four animals.

Staining with hematoxilin-eosin and Gomori's trichromium were routinely used. Weil's method (GRIDLEY[12]) was used to stain both spinal cord and peripheral nerve sections.

A total of 300 animals was used in the following way: 48 for the establishment of vitamin A deficiency; 120 for determination of the effects of vitamin A deficiency on **S. mansoni**; 35 for histological studies and 92 for the evaluation of weight gain on host. Five vitamin A deficient animals infected with **S. mansoni** died during the experiments.

RESULTS

The levels of vitamin A in the liver of mice at different ages are shown in Table I. In 36

day old mice the levels were very low. In the animals fed on vitamin A containing diet these levels increased during all the experimental period, whereas in the deficient animals the stores of vitamin A were depleted.

The weight gain of the animals from the 20th to the 89th day of age is recorded in Table II. Weight gain in infected animals was always smaller than in non infected animals. There was no effect of vitamin A deficiency on weight gain during the period of experimentation.

Figure 1 shows the somatic development of **S. mansoni** evaluated by schistogram, 12 days

TABLE I

Levels of vitamin A, in μg per whole liver, of control and vitamin A deficient mice, infected or not with **Schistosoma mansoni** *

Age (Days)	Days after infection	Control	Vitamin A Deficient	Infected Control	Infected Vitamin A Deficient
20	—	2.19 ± 1.16(12)	1.00 ± 0.49(11)	—	—
36	—	12.20 ± 5.58(12)	1.08 ± 1.37(12)**	—	—
49	12	16.17 ± 4.87(6)	0.00 ± 0.00(6)	21.32 ± 7.73(10)	1.73 ± 1.63(10)**
45	18	—	—	27.02 ± 8.30(16)	0.00 ± 0.00(16)
87	50	42.37 ± 17.21(6)	0.00 ± 0.00(6)	50.29 ± 12.15(10)	0.00 ± 0.00(10)

* Average ± standard deviation. Number of animals in parenthesis.
** 50% of the animals with total absence of vitamin A.

TABLE II

Weight gain, in grams, of control and vitamin A deficient mice infected or not with **Schistosoma mansoni** from the 20th to the 89th day of age. Infection was carried out on the 37th day of age *

Sex	Control	Vitamin A Deficient	Infected Control	Infected Vitamin A Deficient
Males	29.17 ± 3.46(3)[a]	26.33 ± 4.73(10)[a]	15.75 ± 4.84(15)[b]	13.82 ± 5.55(10)[b]
Females	25.43 ± 1.17(3)[a]	20.38 ± 3.34(10)[a]	14.47 ± 3.80(21)[b]	13.25 ± 4.09(20)[b]

* Average ± standard deviation. Number of animals in parenthesis. Statistical difference ($P < 0.01$) in Tukey's test for each sex was indicated by different letters.

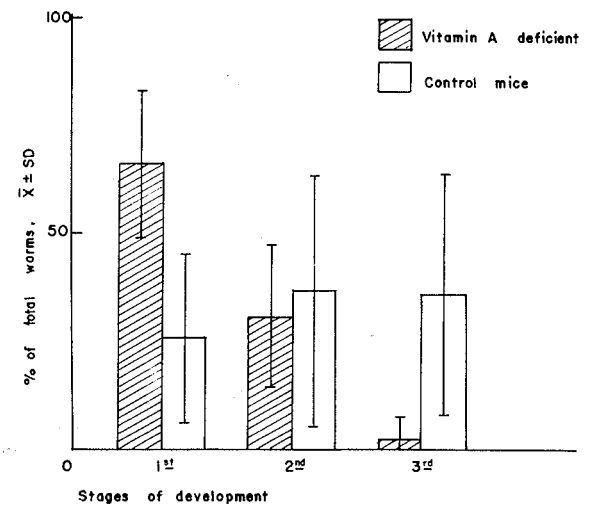

Fig. 1 — Pattern of somatic development of **Schistosoma mansoni** (schistogram) in control and in vitamin A deficient mice, 12 days after infection.

after infection. Schistosoma harbored by vitamin A deficient mice showed a slower rate of development. In the deficient animals there was a predominance of worms at lower stages of development. This picture was not so evident on the 18th day after cercarial exposure (Fig. 2), even though a tendency for the predominance of lower stages of development of S. **mansoni** was still evident in vitamin A deficient mice.

There were no significant differences between the oograms of controls and vitamin A deficient mice. The average numbers of viable eggs, per female, per gram of tissue were 4059 ± 1896 and 4228 ± 2140 for vitamin A deficient and control mice, respectively. The distribution of these eggs among the first stage and maturity was 724 ± 405 and 1027 ± 536, respectively, for the vitamin A deficient animals and 699 ± 436 and 1336 ± 681 for the controls. The mean number of dead (non viable) eggs

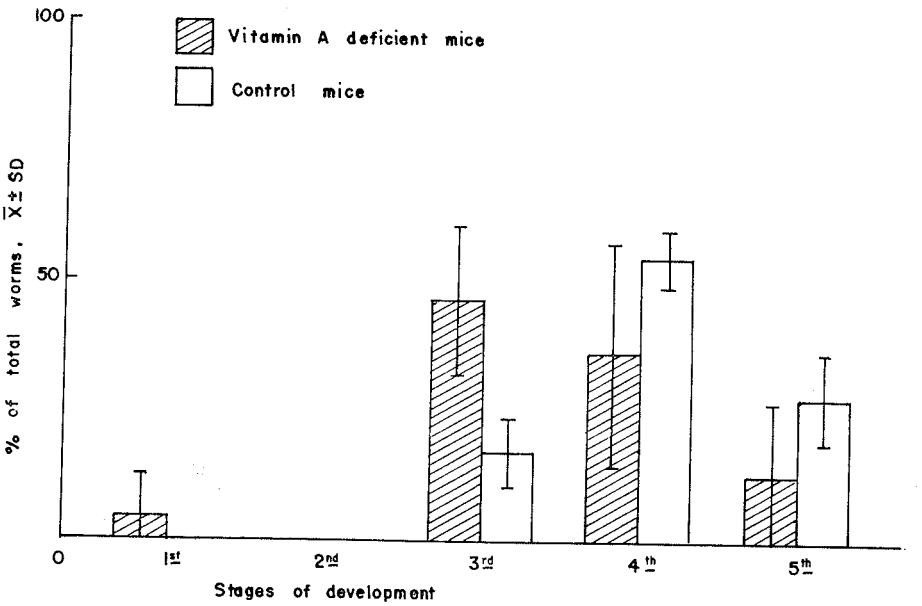

Fig. 2 — Stages of development of **Schistosoma mansoni** (schistogram) in control and in vitamin A deficient mice, 18 days after infection.

per female, per gram of tissue, was 535 ± 402 and 869 ± 724 for control and vitamin A deficient mice, respectively.

No statistically significant differences were detected between the total worm recovery for control (28 ± 5,n=10) and deficient mice (23 ± 7,n=10) using Student's test.

Neither carotene nor vitamin A could be detected in worms derived from vitamin A deficient animals. The levels of carotene and of vitamin A in schistosomes recovered from control mice were 1020 and 0.086 μg/g of worm, respectively.

Histological findings in vitamin A depleted animals showed signs of cellular injury without necrosis mainly in the kidney tubules and in the liver. Schistosomiasis was found to be similar in both control and deficient animals. The general picture of the disease was consistent with that described by DE WITT & WARREN [6].

During the experiment a very peculiar lesion was observed in one of the front paws of 11 infected mice fed on either control or on deficient diet. Macroscopically the lesion was characterized successively by hipotrophy, retraction, and edema of the paw, followed by a necrotic ulcerative process and, then, gangrene. Frequently, there was a spontaneous amputation of the paw (Fig. 3). This condition, once manifested, was irreversible. On microscopic

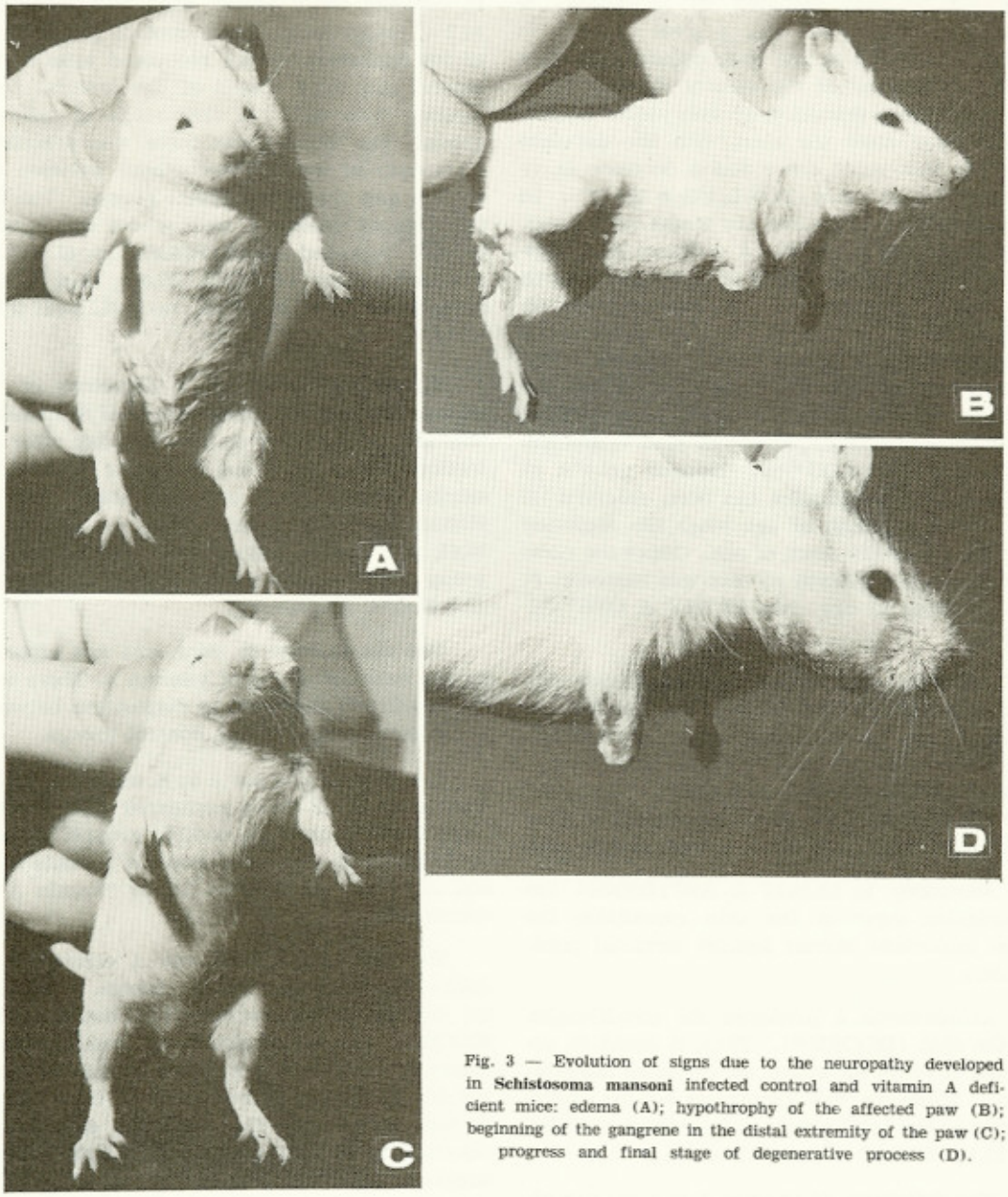

Fig. 3 — Evolution of signs due to the neuropathy developed in **Schistosoma mansoni** infected control and vitamin A deficient mice: edema (A); hypothrophy of the affected paw (B); beginning of the gangrene in the distal extremity of the paw (C); progress and final stage of degenerative process (D).

examination a peripheric demyelinating neuropathy was detected. There was no histologic evidence for other type of medular or nerve root pathology. The incidence of these signs occurred only in **Schistosoma** infected mice. In infected control and in vitamin A deficient mice the incidence of this neuropathy was of 4.9 and 23.1%, respectively ($P < 0.018$).

DISCUSSION

The increasing levels of vitamin A in livers of control and of infected mice suggest that **Schistosoma mansoni** does not interfere with the storage of this vitamin.

The chronology of vitamin A deficiency in mice, described in the present work was com-

parable to that obtained by DOWLING & WALD [7], who showed that a great decrease in vitamin A levels in the liver occurred 17 days after withdrawal of the dietary vitamin. However, complete exaustion of liver stores occurred 24 days after the start with the deficient diet: at this point, there was a decrease in vitamin A blood levels (DOWLING & WALD [7]). In rats, growth is impaired six weeks after starting feeding on a vitamin A deficient diet, whereas in mice the deficiency did not affect growth up to the 11th week of age. Mice are very resistant to vitamin A deficiency. The internal and external signs of the deficiency are not manifested even though the hepatic stores are virtually exhausted (POMERENE & BEARD [19], WOLFE & SALTER [20], FENTON et al. [10], McCARTHY & CERECEDO [14,15]). Normal growth of vitamin A depleted mice has been observed up to the sixth month of age when the depletion started at the 11th week of age. Under the same conditions the growth of rats was hastened at the 7th week of age (McCARTHY & CERECEDO [14]).

Table II suggests that schistosomiasis, and not vitamin A deficiency, was responsible for growth impairment. There was no statistically significant differences between the controls and the vitamin deficient counterparts. However, growth was impaired in either control or vitamin A deficient mice infected with **S. mansoni.**

According to CLEGG & SMITHERS [5], the Malpighian layer of the skin constitutes the most important barrier against cercarial penetration.

Avitaminosis A produces the cornification of the skin (MOORE [16]). Thus, it could be expected to have an effect on cercarial penetration. However, this hypothesis can be rejected due to the total number of parasites recovered in the control and experimental groups.

The deficiency of vitamin A in mice retarded the somatic development of **S. mansoni** on the 12th day after infection (Fig. 1). This effect was less pronounced but still significant on the 18th day of infection (Fig. 2).

On the 12th day after infection the proportion of worms at the first, second, and third stages of development were 66.5, 31.0, and 2.5%, respectively, in the vitamin A deficient mice; in controls these values were 26.4, 37.4, and 36.2%, respectively. In the control group 73.6% of the immature worms recovered were at the second and third stages of development, contrasting with 66.5% of first stage in deficient group. On the 12th day there was a striking difference in worm development between the control and the experimental groups. The distribution of immature worms was comparable to that reported by BARBOSA et al. [3], who obtained values of 14.8%, 37.0%, and 48.1%, respectively, for the first, second, and third stages.

The effect of vitamin A deficiency of the host on the somatic development of **S. mansoni** was less pronounced on the 18th day after infection. The proportions of worms at the first, second, third, fourth, and fifth stages for the vitamin A deficient mice were 4.4, 0.0, 46.45, 36.44, and 12.66%, respectively. In the control group these values were 0.0, 0.0, 17.54, 54.84, and 27.62%, respectively.

The egg burden in mice was not affected by the host deficiency of vitamin A. There was no significant difference in oviposition between the experimental and the control groups.

The level of vitamin A in **S. mansoni** bodies was insignificant when compared with the hepatic levels in infected control groups. Therefore, it may be concluded that the parasite does not expoliate the host as far as vitamin A is concerned.

The deficiency of vitamin A elicited only inespecific lesions in mouse viscera. Metaplasia and epithelial keratinization, described by MOORE [16] as characteristics of vitamin A deficiency in rats, were not found in mice, probably due to the higher resistance of this species to that deficiency. The similarity of the manifestations of schistosomiasis in both groups suggests that the action of the parasite on the host was not aggravated by the experimental vitamin A deficiency.

The occurrence of peripheral neuropathy in the front paws of mice infected with **S. mansoni** was independent of the vitamin A deficiency. This suggests that the phenomenon is related to the parasitosis itself, although such aspect has never been described as a complication of schistosomiasis. No further neurologic

TURCHETTI-MAIA, R. M. De M.; BAMBIRRA, E. A.; COELHO, P. M. Z.; MORAES E SANTOS, T. & VIEIRA, E. C. — The association of **Schistosoma mansoni** infection with deficiency of vitamin A in mice. **Rev. Inst. Med. trop. São Paulo** 25:215-222, 1983.

lesions were observed. Vitamin A deficiency alone did not elicit the neuropathy. The morphologic picture observed in infected mice does not seem to be related to the ones described in the literature on vitamin A deficient animals secondary to a compressive medular lesion (ABERLE [1]; FLETCHER & RIGDON [11]). In the present work, neither myelopathy nor nerve root lesion were morphologically detected. Dissection of the affected nerves up to their roots in the spinal cord revealed that the demyelination was restricted to the peripheral nerves. No elements suggestive of a mechanism for this demyelination could be developed. It is interesting to note that there was a higher incidence of the neuropathy in vitamin A deficient animals when compared with the infected controls. So far, no explanation for these findings can be advanced. This neuropathy had already been observed in laboratory mice maintained on commercial diet and infected with **S. mansoni.** Further experiments must be carried out to clear up these facts.

RESUMO

Associação de infecção esquistossomótica com deficiência de vitamina A em camundongos

Verificou-se que o desenvolvimento somático do **Schistosoma mansoni** foi retardado em camundongos com deficiência de vitamina A, quando comparado com aquele obtido no grupo controle. A diferença foi mais acentuada no 12.° dia. A proporção de vermes no 1.°, 2.° e 3.° estágios de desenvolvimento foi de 66,5; 31,0 e 2,5%, respectivamente, para os camundongos em avitaminose A, e 26,4; 37,4 e 36,2%, respectivamente, para os controles. No 18.° dia após a exposição cercariana, a proporção de vermes no 1.°, 2.°, 3.°, 4.° e 5.° estágios foi de 4,4; 0,0; 46,45; 36,44 e 12,66% para os camundongos com deficiência de vitamina A, e 0,0; 0,0; 17,54; 54,84 e 27,62%, respectivamente, para os controles.

A oviposição foi praticamente a mesma em ambos os grupos. Não foi possível detectar caroteno ou vitamina A nos vermes obtidos de animais em avitaminose A. Nos esquistossomos recuperados de animais-controles, os níveis de caroteno e vitamina A foram 1020 e 0,086 μg/g de verme, respectivamente.

Alguns animais desenvolveram uma neuropatia, com conseqüente amputação espontânea de uma das patas dianteiras. Nos grupos controle e deficientes de vitamina A infectados, a incidência desta neuropatia foi de 4,9 e 23,1% respectivamente.

ACKNOWLEDGEMENTS

This work was supported by grants from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), and from "Financiadora de Estudos e Projetos" (FINEP). The help of Yehuda Waisberg with the statistical analysis is greatly appreciated.

REFERENCES

1. ABERLE, S. B. D. — Paralysis in young rats kept on a diet deficient in vitamin A. **J. Nutrition 7:** 445-459, 1934.

2. A.O.A.C. (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS) — Official Methods of Analysis. W. Horwitz ed., 12nd ed., 1975.

3. BARBOSA, M. A.; PELLEGRINO, J.; COELHO, P. M. Z. & SAMPAIO, J. B. M. — Quantitative aspects of the migration and evolutive asynchronism of **Schistosoma mansoni** in mice. **Rev. Inst. Med. trop. São Paulo 20:** 121-132, 1978.

4. BAYFIELD, R. F. — Simplified methods for the examination of liver lipids. I — Determination of vitamin A using a direct solvent extraction technique. **Analytic. Biochem. 64:** 403-413, 1975.

5. CLEGG, J. A. & SMITHERS, S. R. — Death of schistosome cercaria during penetration of the skin. II — Penetration of mammalian skin by **Schistosoma mansoni. Parasit. 58:** 111-128, 1968.

6. DE WITT, W. B. & WARREN, K. S. — Hepato splenic schistosomiasis in mice. **Am. J. Trop. Med. Hyg. 8:** 440-446, 1959.

7. DOWLING, J. E. & WALD, G. — Vitamin A deficiency and night blindness. **Proc. Nat. Acad. Sci.** USA 44: 648-661, 1958.

8. DREIZEN, S. — Nutrition and the immune response, a review. **Int. J. Vit. Nutrition Res. 49:** 220-228, 1979.

9. FAUST, E. C.; JONES, C. A. & HOFFMAN, W. A. — Studies on schistosomiasis mansoni in Puerto Rico. III — Biological studies. 2. The mammalian phase of the life cycle. **Puerto Rico J. Publ. Hlth. Trop. Med. 10:** 133-196, 1934.

10. FENTON, P. F.; SHRADER, R. E. & COWGILL, G. R. — Chronic vitamin A deficiency in three inbred strains of mice. **Fed. Proc. 8:** 382, 1949.

11. FLETCHER, D. E. & RIGDON, R. H. — Neurological manifestations associated with vitamin A deficiency in young ducks. **Arch. Neurol. Psych. 61:** 199-209, 1949.

12 GRIDLEY, M. F. — Manual of histologic and special staining technics. **Arm. Forc. Inst. Pathol.**, Washington D.C., pp. 164-167, 1957.

13. KRAKOWER, C.; HOFFMAN, W. A. & AXTMATER, J. H. — The fate of schistosomes **(S. mansoni)** in experimental infection of normal and vitamin A deficient with rats. **Puerto Rico J. Publ. Hlth. Trop. Med. 16:** 269-345, 1940.

14. McCARTHY, P. T. & CERECEDO, L. R. — Vitamin A deficiency in the mouse. **J. Nutrition 46:** 361-376, 1952.

15. McCARTHY, P. T. & CERECEDO, L. R. — The vitamin A requirements of mouse. **J. Nutrition 49:** 357-367, 1953.

16. MOORE, T. — Vitamin A. Amsterdam, **Elsevier Publishing Company**, pp. 139-148 and p. 228, 1957.

17. NEELD Jr., J. B. & PEARSON, W. N. — Macro and micromethods for the determination of serum vitamin A using trifluoracetic acid. **J. Nutrition 79:** 454-462, 1963.

18. PELLEGRINO, J. & FARIA, J. — The oogram method for the screening of drugs in schistosomiasis mansoni. **Amer. J. Trop. Med. Hyg. 14:** 363-369, 1965.

19. POMERENE, E. & BEARD, H. H. — Studies on the nutrition of the white mouse. VI — The experimental production of xerophtalmia in mice. **Amer. J. Physiol. 92:** 282-286, 1930.

20. WOLFE, J. M. & SALTER, H. P. — Vitamin A deficiency in the albino mouse. **J. Nutrition 4:** 185-192, 1931.

Recebido para publicação em 22/11/1982.