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IMMUNOPATHOLOGY OF MANSONS'S SCHISTOSOMIASIS

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SUMMARY

1 — *Reactions of the immediate type*, involving circulating antibodies, are important in granulomatous formation around the *S. mansoni* ova in the tissues. They appear while the miracidium is still alive and exuding antigenic material and become stronger after its death and disintegration. Immature eggs and heated mature eggs (dead miracidia) provoke little or no tissue reaction. Circulating antibodies are active also against the other evolutive stages of the parasite, but since no antigenic material is actively eliminated, antigen-antibody complexes can only be formed after desintegration of adult worms or schistosomules.

General antigenic stimulation can be observed in the splenic struture of infected mice. There is proliferation of reticular cells, plasma cell differentiation and γ-globulin synthesis (antibodies). Changes due to portal hypertension may appear later.

2 — *Reactions of the delayed type* are probably represented by the chronic portal hepatitis: the presence of "immunologically competent cells" around numerous small venules, forming focal accumulations; the tendency to fibrosis; the signs of activity of the inflammatory process and the lack of correlation between the intensity of the reaction and the number of parasitic elements.

3 — *Resistance* can develop not only by limiting the parasitic factors (less worms, less eggs) but also by forming minimal and more effective inflammatory reactions to the parasitic stimuli. Such a situation can be observed in animals with prolonged infections.

I — INTRODUCTION

Immunopathology deals with the study of morphological changes resulting from immunological processes. Lately, this branch of pathology has experienced great progress not only as a result of the progress of immunology as a whole, but also due to the immunological knowledge gained in the study of organ transplantation and auto-immune diseases, and to new morphological techniques, specially the Coon's florescent antibody method. However the subject is a broad and complex one and many gaps and obscurities still exist. On the other hand, the data on the immunology of schistosomiasis is still fragmentary, although excellent reviews (11, 21, 26) have been made.

A study on the immunopathology of schistosomiasis at the moment cannot do more than to present a correlation of some morphological and immunological data as an initial effort of systematization of the subject, which, however, may prove worthwhile for future studies. Such is the aim of the present paper.

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II — REACTIONS OF THE IMMEDIATE TYPE

a) *Generalities:*

..These reactions are dependent on circulating antibodies. Man and animals infected with *S. mansoni* or injected with its products, develop circulating antibodies that are active against the several evolutive stages of the parasite. Demonstration of these antibodies has served as a basis for many indirect processes of diagnosis of schistosomiaisis, which have recently being reviewed by KAGAN & PELLEGRINO [12]. These antibodies are present in the gamma-globulin fraction of the serum and due to their relatively high molecular weight, cannot get through the external surface of living parasites in the body and thus cannot produce reactions. Their protective function, causing immobilization of schistosomules and destruction or stunting of worms, has not yet been documented, although adult worms put in contact with immune serum "in vitro" die within 24-36 hours and undergo phagocytosis, which does not happen with control serum (22).

Immunity or resistance does not seem to depend on circulating antibodies (25), since it cannot be obtained by passive transfer of serum, while it can occur in unisexual infection with no elevation of the gamma-globulin level in the plasma.

b) *Reactions to the ova*

BIOCHEMICAL [27] and histochemical [3] studies have indicated that the main component of the *S. mansoni* ovum has a complex mucopolyssacharide nature, which may be highly antigenic. Furthermore, the miracidium contains a large spectrum of hydrolytic enzymes, such as acid phosphatase, alkaline phosphatase, 5-nucleotidase, ATPase and at least one proteolytic enzyme, amino-peptidase [3].

Granulomas around the ova of *S. mansoni* are formed in the tissues of susceptible host while the miracidium is still alive. PRATA [25] observed that all the granulomas formed by the 50th day of infection in the mouse had live miracidia. The miracidium exdudes an antigenic material, which may react with schistosomal antibodies "in vitro" and cause the circumoval reaction [23]. In the tissue this antigenic material also reacts with circulating antibodies, as can be demonstrated by the Coon's technique [4]. The antigenic material can be seen free in the tissue or phagocytised by the granulomatous cells (Fig. 1). Following disintegration of the miracidium a large amount of antigenic material is suddenly liberated in the tissue and an area of hyaline necrosis can appear in the center of a well developed granuloma. This area is later invaded by cells, giving rise to purulent necrosis. Antigen as well as gamma-globulin, which can be eluted by a 2 hour treatment in pH 3,2 phosphate buffer, can be demonstrated in the necrotic area, immunocytochemically [4].

The immature egg, which has a basophillic content [25], evokes little or no tissue reaction. SOROUR [28] has demonstrated that the mature eggs lose their capacity of producing granulomas if previously heated at 55°C for 10 minutes, a treatment that kills the miracidia.

In the granulomas around the *S. mansoni* eggs there are many eosinophils. Sometimes they form dense infiltrations in the area of periovular necrosis. Although the presence of such cells has been ascribed to reactions of an allergic nature, the real role of these cells in schistosomal granulomas has not been clarified. The facts listed above indicate that antigen-antibody reactions play a fundamental role during granulomatous formation around *S. mansoni* ova in the host tissue.

c) *Reactions to adult worms*

The immunocytochemical technique shows that circulating antibodies also bind to adult worms present in the liver of infected animals, specially at the cuticula [4]. As the adult worms do not seem to excrete an antigenic material such as the egg with a mature miracidium, there is no antigen-antibody reaction as long as the parasite remains alive. After disintegration of the worm such a reaction then occurs and at least part of the necrotic-exudative lesion formed around dead worms is due to the formation of antigen-antibody complexes, since the toxic nature of the latter has already been demonstrated [7].

d) *Reactions to schistosomules*

Cercariae penetrate through the skin by way of their active movements and by the secretion of their penetrating glands. These

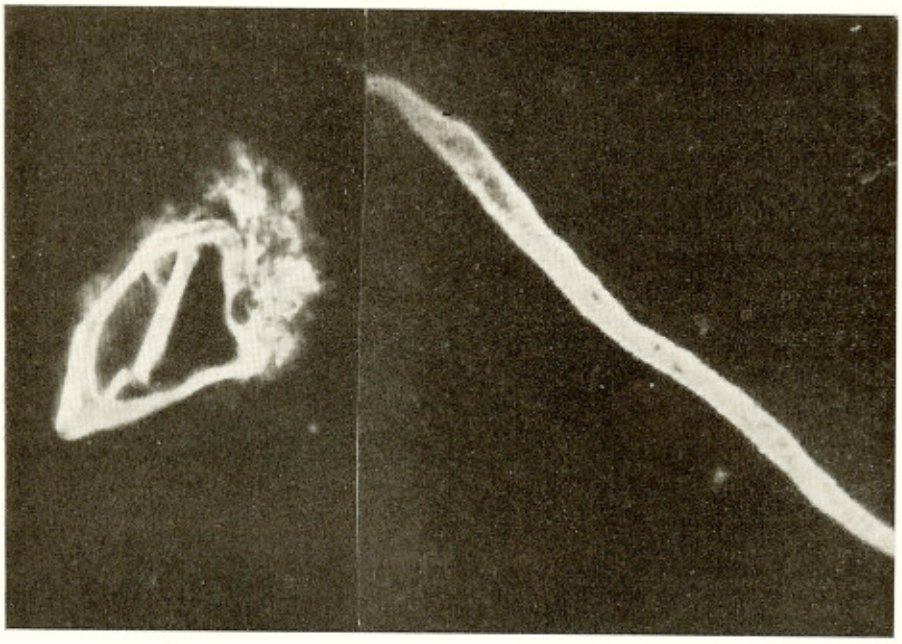

Fig. 1 — Antibodies against *S. mansoni* (gamma-globulin from the serum of an infected patient tagged to fluorescein) bind to material contained in the ovum (left) and in adult worm (right). Material from the ovum is in part outside the egg shell and some may be into the cytoplasm of macrophages. Crysostat section of mouse liver. Direct Coons'technique. 200×.

latter contain protease (collagenase type), as well as lipase and muco-polyssacharidase. It has beeen proved that the cercariae can get through the skin of a normal mouse with a minimum of tissue reaction, while in a previously infected animal intense inflammatory changes take place around the schistosomules [18]. This led to the conclusion that the mechanism of resistance to *S. mansoni* is, at least in part, localized in the skin [29]. The participation of circulating antibodies in these reactions has not yet been clarified. Penetration of cercariae through the skin of infected individuals is followed by an erythematous and papulous histamine-type reaction similar to the one obtained in the same individuals with the intradermal skin test with *S. mansoni* antigen.

A bronchiolar pneumonia was frequently observed in immunized animals at the time the schistosomules from a second infection passed through the lungs [17]. This seeemed to be a true hypersensitivity phenomenum, since it was observed regularly in mice infected nine weeks after first exposure, occasionally when the interval was only of two weekss, but never in animals infected for the first time.

e) *Reactions in the lympho-reticular tissue*

Reactions in the lympho-reticular tissue charactherize the general response to antigenic stimulation by the *S. mansoni* and its products. These reactions can better be studied in the spleen of infected animals. They are responsible, in part, for the splenomegaly of schistosomiais. Recently, a combination of histological, histochemical and immunocytochemical techniques was applied to the study of splenic changes in experimental schistosomiasis [1]. It was found that, in the initial phase of infection (first weeek following egg production), there was intense proliferation of reticulo-endothelial cells in the red pulp of the spleen and less so in the white pulp. This was soon followed by plasma cell differentiation, the reticular cells showing cytoplasmic basophilia, secretion of PAS positive material and gamma-globulin synthesis as steps of differentiation. These changes occurred in the absence of parasitic elements in the spleen. They were similar to the so-called primary and secondary responses of the lympho-reticular tissue following antigenic stimulation [13]. The immediate consequence of

these changes is the production of circulating antibodies. Splenic changes are later complicated by the appearance of alterations due to portal hypertension, such as sinus engorgement, hypertrophy and hyperplasia of reticulum fibrils and focal hemorrhages.

III — REACTIONS OF THE DELAYED TYPE

The existence of a delayed type hypersensitivity in schistosomiasis has been little explored. The morphological method, although not being the most adequate, can indeed contribute with some valuable data to this matter. The morphology of the delayed type hypersensitivity is not pathognomonic, but much can be learned by observing the microscopic changes in the tuberculin reaction, during rejection of organ transplant, in auto allergic thyroiditis, in lupoid hepatitis and so on. The common denominator of theses conditions is an infiltration of "immunologically competent cells", which include lymphocytes, plasma and plasmocytoid cells. This infiltration is diffuse with areas of focal accumulation, signs of activity, a destructive character and a progression to fibrosis. As WAKSMAN [30] suggested, they occur more frequently in areas having a large number of small veins.

In schistosomiasis, reaction histologically similar to those listed above, are found in the liver in the form of a chronic portal inflamation. This process has been described by several authors, but no pathogenetic importance has been ascribed to it. FAIRLEY [9], many years ago, called attention to the lack of correlation between this portal inflammation and the presence of parasitic elements in the liver. He even suggested the presence of a toxin to explain it, but such a toxin has never been demonstrated. MELLENEY et al. [19] said that the periportal inflammation appeared in unisexual infection, in bisexual infection before egg production and that it was intensified following egg production. We must bear in mind that any irritation may cause periportal infiltration in the liver. However, the schistosomal portal hepatitis, specially observed in human patients with the hepato-splenic form of the disease [2], is more than a mere round cell infiltration of the portal area (Fig. 2). Besides the diffuse and focal infil-

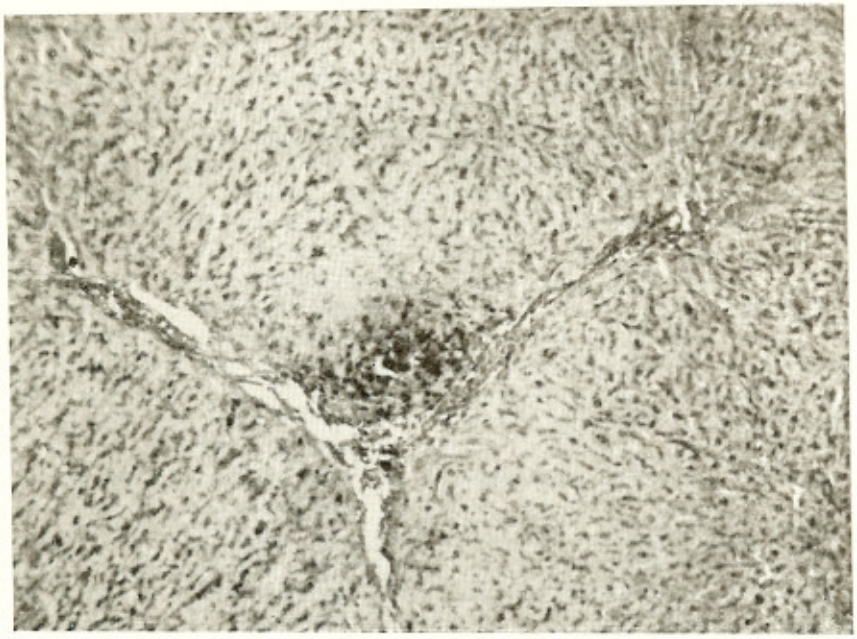

Fig. 2 — Schistosomal granuloma in the portal space. The acid phosphatase staining shows many positive cells (reticulo-endothelial cells) in the granuloma and in the liver sinusoids (Kupffer cells). Mobilization of the latter cells is part of the generalized response to *S. mansoni* antigenic stimulation. Human liver. Barka's azo-dye method for acid phosphatase. Incubation time 15'. 160×.

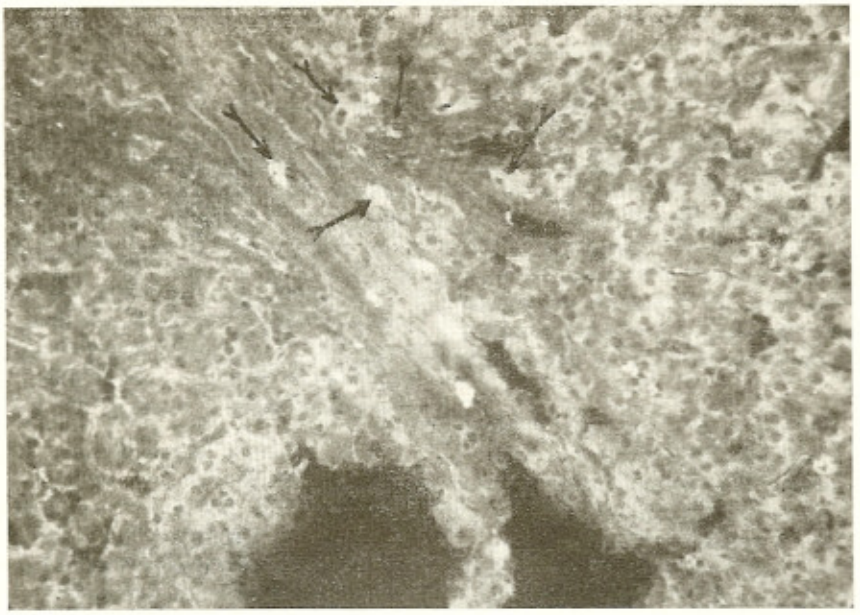

Fig. 3 — Schistosomal portal hepatitis in man. Gamma-globulin producing cells are present in the portal infiltrate. Cryostat liver section treated with fluoresceinated anti-human gamma-globulin. 430×.

tration by lymphocytes, plasma and plasmocytoid cells, macrophages, there is frequent invasion of the parenchymal border by the inflammatory cells and focal ductular cell proliferation, indicating activity of the inflammatory process. Furthermore, fibrosis and vascular involvement are conspicuous features [2]. Recently local formation of gamma-globulin by the cells of the portal infiltration has been demonstrated (ANDRADE, unpublished observations) (Fig. 3). PARONETTO et al. [24] correlated the local formation of gamma-globulin by the cells invading the liver parenchymal border ("piece meal necrosis") with the presence of a self perpetuating hepatic condition (auto-immune liver destruction). Further studies are necessary to determine the real significance of portal hepatitis in schistosomiasis, its role in the production of portal fibrosis, and its relationship to a delayed hypersensitivity reaction to *S. mansoni*, to liver cell breakdown products or to both.

IV — RESISTANCE

Acquired resistance to *S. mansoni* infection has mainly been studied by observing the ability of an infected animal to deal with a challenging infection. There is also indirect evidence that man can acquire resistance to a second infection, since the disease is more frequent and severe in children in endemic areas and severe disease has been observed in adults infected for the first time [26]. Animals with previous infection [15, 17, 20, 29] or injected with *S. mansoni* antigen [10, 31] show resistance to a challenging infection, later showing less worms, stunted worms, and prolonged survival when compared to controls. Also, much more severe inflammatory reactions can be observed around schistosomules in immunized animals [18].

ANDRADE & WARREN [6] showed that hepatic granulomas formed around mature eggs in the liver of mice with prolonged infection are small, well delimited and composed of a few histiocytes and fibroblasts. Such granulomas are in sharp contrast with those found earlier in the liver of the same animals. These latter granulomas, formed in the first weeks following egg deposition were characterized by marked necrotic exudative reactions with many eosinophils. So, a host that initially may have a severe hypersensitivity reaction can develop later a proliferative, encapsulating, small, immune type reaction to the same pa-

thogenic element, the mature egg of *S. mansoni*. It can be said now that a host can acquire protection not only by limiting the parasitic elements (less worms, stunted worms, less egg prodution) but also by developing a more economical and more effective tissue reaction to the parasitic stimuli. It is worthwhile to recall that such a situation is similar to the one occuring in *S. mansoni* infection in animals with natural resistance to schistosomiasis [16]. Also, the reactions around penetrating schistosomules in abnormal host is reminiscent of those occuring in immunized animals [8, 16].

By injecting eggs of *S. mansoni* intravenously in a normal mouse, LICHTENBERG [14] observed that the granulomas formed in the lungs did not reach the size or severity of those formed in animals with cercarian infection. LICHTENBETG's data can now help us to catalogue the three classical types of reaction as occuring around *S. mansoni* eggs in the tissues: a *normergic type*, observed after intravenous injection of eggs in non-infected animals and having an intermediate morphology between the following types; a *hyperergic type* observed in susceptible hosts in the initial period of infection, and presenting a large necrotic exudative reaction around the eggs; and finally an *immune type* observed in animals with prolonged infection that forms small, round, discrete, proliferative reaction around mature eggs.

The factors responsible for the development of immunity or resistance to schistosomiasis are incompletly known at present. So far we know that both cellular and humoral factors appear to be important.

In conclusion, investigation of the immunopathology of schistosomiasis, in spite of paucity of data and lack of knowledge in some areas, can be fruitful for understanding the pathogenesis of the disease and for opening up new avenues for future studies.

SUMARIO

Imunopatologia da esquisitossomose mansônica

1 — *Reações de tipo imediato*, são importantes na formação dos granulomas em tôrno dos ovos. Elas ocorrem enquanto o miracídio está vivo e eliminando material antigênico e se acentuam após a desintegração ovular. Os ovos imaturos e os ovos com miracídio prèviamente aquecido (mortos) provocam pouca ou nenhuma reação tecidual. Os anticorpos circulantes são também ativos contra os vários estágios evolutivos do *S. mansoni*, mas não havendo eliminação de material antigênico, as reações antígeno-anticorpo só se processam após a desintegração dos mesmos.

O estímulo geral causado pelo *S. mansoni* e seus produtos se refletem bem na estrutura esplênica. Em camundongos infectados há proliferação de células reticulares, diferenciação plasmocitária e secreção de γ-globulina.

2 — Talvez a hepatite crônica portal esquistossomótica seja uma expressão morfológica de *reação imunitária de tipo retardado*. São dados sugestivos: a presença de "células imunològicamente competentes "em tôrno de numerosas vênulas, os adensamentos focais do infiltrado e seus sinais de atividade, além da falta de correlação entre a intensidade da reação e a presença de elementos parasitários.

3 — Um hospedeiro pode desenvolver resistência à esquistossomose não só limitando os fatôres parasitários (menor número de vermes e ovos), maior sobrevida, mas também desenvolvendo reações inflamatórias mínimas e todavia mais eficientes aos elementos parasitários. Tal situação pode ser bem observada em animais com infecção prolongada.

REFERENCES

1. ANDRADE, Z. A. — Aspectos experimentais da esplenomegalia da esquistossomose. *Rev. Inst. Med.Trop. São Paulo* **4**:249-255, 1962.

2. ANDRADE, Z. A. — A hepatite crônica esquistossomótica. *"O Hospital"* **62**:171-184, 1962.

3. ANDRADE, Z. A. & BARKA, T. — Histochemical observations on experimental schistosomiaisis of mouse. *Am. J. Trop. Med. & Hyg.* **11**:12-16, 1963.

4. ANDRADE, Z. A. — PARONETTO, F. & POPPER, H. — Immunocytochemical studies in schistosomiasis. *Am. J. Path.* **39**:589-598, 1961.

5. ANDRADE, Z. A.; SANTANA FILHO, S. & REBOUÇAS, G. — Patologia da esquistossomose hepática avançada. *Rev. Inst. Med. Trop. São Paulo* **4**:170-179, 1962.

6. ANDRADE, Z. A. & WARREN, K. S. — Mild prolonged schistosomiasis in mice. Al-

terations in host responses with time and the development of portal fibrosis (To appear in Trans. Roy. Soc. Trop. Med. & Hyg. London, 1964).

7. COCHRANE, C. G. & WEIGLE, W. C. — The cutaneous reaction to soluble antigen-antibody complexes. A comparison with the Arthus phenomenon. *J. Exper. Med* **108**:591-604, 1958.

8. COUTINHO-ABATH, E. & JAMPOLSKY, R. — Comportamento das cercárias de *Schistosoma mansoni* na infestação experimental de animais refractários. I — Histopatologia das reações cutâneas observadas no pombo doméstico *(Columba livia domestica). An. Soc. Biol. Pernamb.* **15**:93-125, 1957.

9. FAIRLEY, N. H. — A comparative study of experimental bilharziasis in monkeys contrastated with the hitherto described lesions in man. *J. Path. & Bact.* **23**:289-314, 1920.

10. HUNTER, G. W.; CRANDALL, R. B.; ZICKAFOOSE, D. E. & PURVIS, Q. B. — Studies on schistosomiasis. XVIII. Some factors affecting resistance to *Schistosoma mansoni* infections in albino mice. *Am. J. Trop. Med. & Hyg.* **11**:17-24, 1962.

11. KAGAN, I. G. — Contributions to the immunology of schistosomiasis. *Rice Inst. Pamphlet* **45**:151-183, 1958.

12. KAGAN, I. G. & PELLEGRINO, J. E. — A critical review of immunological methods for the diagnosis of bilharziasis. *Bull Wld. Hlth. Org.* **25**:611-674, 1961.

13. LEDUC, E. H.; COONS, A. H. & CONNOLLY, J. M. — Studies on antibody production. II — The primary and secondary responses in the popliteal lymph mode of rabbit. *J. Exper. Med.* **102**:61-72, 1955.

14. LICHTENBERG, F. — Host response to eggs of *S. mansoni*. I — Granuloma formation in the unsensitized mouse. *Am. J. Path.* **41**:711-723, 1963.

15. LICHTENBERG, F. & RITCHIE, L. S. — Cellular resistance against schistosomula of *Schistosoma mansoni* in *Macaca mulatta* monkeys following prolonged infections. *Am. J. Trop. Med. & Hyg.* **10**:859-869, 1961.

16. LICHTENBERG, F. SADUN, E. H. & BRUCE, J. I. — Tissue responses and mechanisms of resistance in schistosomiasis mansoni in abnormal hosts. *Am. J. Trop. Med. & Hyg.* **11**:247-356, 1962.

17. LIN, S. S. & SADUN, E. H. — Studies on the host parasite relationships to *Schistosoma japonicum*. V — Reactions in the skin, lungs and liver of normal and immune animals following infection with *Schistosoma japonicum. J. Parasit.* **45**:549-559, 1959.

18. MAGALHÃES Filho, A. & BARROS COELHO, R. — Estudo histopatológico da penetração de cercárias de *S. mansoni* na pele de camundongos prèviamente infestados. *An. Soc. Biol. Pernamb.* **15**:269-281, 1957.

19. MELLENEY, H. E.; SANDGROUND, J. H.; MOORE, D. V.; MOST, H. & CARNEY, H. H. — The histopathology of experimental schistosomiaisis. II — Bisexual infections with *S. mansoni. S. japonicum* and *S. Hematobium. Am. J. Trop. Med. & Hyg.* **2**:883-913, 1953.

20. NAIMARK, D. H.; BENESON, A. S.; OLIVER-GONZALEZ, J.; McMULLEN, D. B. & RITCHIE, L. S. — Studies of schistosomiasis in primates: observations on acquired resistance (Progress report). *Am. J. Trop. Bed. & Hyg.* **9**:430-435, 1960.

21. NEWSOME, J. — Problems of fluke immunity with special reference to Schistosomiasis. *Trans. Roy. Soc. Trop. Med. & Hyg.* **50**:258-274, 1956.

22. NEWSOME, J. — Phagocytosis of schistosomes in immune serum. *Trans. Roy Soc. Trop. Med. & Hyg.* **56**:10, 1962.

23. OLIVER-GONZALEZ, J. — Anti-egg precipitins in the serum of humans infected with *Schistosoma mansoni. J. Infect. Dis.* **95**:86-91, 1954.

24. PARONETTO, F.; RUBIN, E. & POPPER, H. — Local formation of gamma-globulin in the diseased liver and its relation to hepatic necrosis. *Lab. Invest.* **11**:150-158, 1962.

25. PRATA, A. — *Biópsia retal na esquistossomose. Bases e apliacções no diagnóstico e tratamento.* Salvador, Bahia, 1957. (Tese).

26. SMITHERS, S. R. — Acquired resistance to bilharziasis. *In Bilharziasis,* A CIBA Foundation Symposium, pp 239-258. *J. & A. Churchill Ltd. London,* 1962.

27. SMITHERS, S. R. & WILLIAMSON, J. — Antigenic polyssacharide material in cercariae and eggs of *Schistosoma mansoni. Trans. Roy. Soc. Trop. Med. & Hyg.* **55**:308-309, 1961.

28. SOROUR, M. F. — The pathology and morbid histology of bilharzial lesions in various parts of the body. *C. R. Cong. Internat. Med. Trop. et d'Hyg.* Le Caire. **4**:321-329, 1928.

29. STIREWALT, M. A. — The influence of previous infection of mice with *Schistosoma mansoni* on a challengig infection with the homologous parasite. *Am. J. Trop. Med. & Hyg.* 2S867-882, 1953.

30. WAKSMAN, B. H. — The distribution of experimental auto-allergic lesions. *Am. J. Path.* **37**:673-693, 1960.

31. WATTS, N. P. — Prophylatic use of schistosomal antigen. *J. Immunology* **62**:183-192, 1949.

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