Clin. exp. Immunol. (1978) 33, 232–238.

The immune-dependence of schistosomicidal chemotherapy: relative lack of efficacy of an antimonial in *Schistosoma mansoni*-infected mice deprived of their T-cells and the demonstration of drug-antiserum synergy

M. J. DOENHOFF & JEAN BAIN *Department of Medical Helminthology, London School of Hygiene and Tropical Medicine, Winches Farm Field Station, 395 Hatfield Road, St. Albans, AL4 0XQ*

(*Received* 14 *March* 1978)

SUMMARY

When T-cell deprived CBA mice, infected with *Schistosoma mansoni*, were treated orally with potassium antimony tartrate, the reduction in size of their worm burdens was less than in similarly treated, immunologically-intact animals. The defect in deprived mice could be restored by the administration of serum obtained from *S. mansoni*-infected normal mice simultaneously with the drug, but by a different route. A serum component, probably immunoglobulin, obtained from rabbits which had been injected with an extract from *S. mansoni* adult worms was also found to act synergystically with the antimonial in the chemotherapeutic eradication of *S. mansoni* worms from immunologically intact mice.

INTRODUCTION

During screening of schistosomicidal drugs for any immune-dependence of their action in mice it was observed that potassium antimony tartrate was less effective at curing *Schistosoma mansoni* infections in T-cell deprived animals than in immunologically intact controls. Preliminary results on this phenomenon are presented here together with evidence to suggest that the defect in the immunologically depressed mice is the inability to synthesize anti-parasite antibody. The possibility that this model system might serve as a prelude to the development of chemotherapeutic agents which are more specifically targeted towards the parasite by conjugating the drugs with anti-parasite antibody is discussed.

MATERIALS AND METHODS

Mice. Male CBA/Lac mice were thymectomized by the method of Law, Bradley & Rose (1963), and received four injections each of 0·25 ml rabbit anti-mouse thymocyte serum (ATS) on alternate days within 10 days of the operation. ATS was prepared according to the method of Levey & Medawar (1966), the thymocytes being obtained from random bred T.O. strain mice. Immunologically intact control mice were always of the same age as the deprived animals, experiments with the latter being initiated 30 to 50 days after treatment with ATS.

Evidence that thymectomy and administration of ATS is an effective means of inducing a state of relatively permanent immunosuppression in mice may be summarized as follows: (1) thymectomized, ATS-treated mice retained H-2 similar AKR mouse skin grafts for a median time of 46 days compared with a median rejection time of 18 days on intact mice. Four deprived mice out of a group of nine retained their skin grafts for < 365 days. (2) The mean number of indirect (rabbit anti-mouse IgG-developed) haemolytic plaque-forming cells (Cunningham & Szenberg, 1968) detected per million nucleated cells in the spleens of ATS-deprived mice, 7 days after intraperitoneal injection of 5×10^8 sheep erythrocytes, was twelve compared with a mean of 1740 found in the spleens of similarly erythrocyte-challenged control mice. (3) Fewer than 10%

Correspondence: Dr M. J. Doenhoff, Winches Farm Field Station, 395 Hatfield Road, St. Albans, AL4 0XQ, England.

0099-9104/78/0800-0232$02.00

of the normal number of PHA-responsive lymphocytes were detected in the blood of deprived mice as determined by the method described by Doenhoff, Janossy & Kerbel (1976).

Parasite. A Puerto Rican strain of *Schistosoma mansoni* was maintained by laboratory passage in *Biomphalaria glabrata* snails and random bred T.O. strain mice (Taylor, Amin & Nelson, 1969). Infected snails were phototropically induced to shed cercariae into dechlorinated water, and the larvae used for infection within 3 hr of emergence (Olivier, 1966).

Method of infecting and perfusing mice. Percutaneous infections and portal system perfusions were performed according to the method of Smithers & Terry (1965).

Schistosomicidal chemotherapy. Potassium antimony tartrate (PAT) was purchased from British Drug Houses Ltd. (Poole, Dorset) and administered orally in aqueous solution.

Acute infection serum. CBA mice were infected with 200 *S. mansoni* cercariae, and were serially bled from the retro-orbital sinus twice-weekly from approximately day 46 after infection until the survivors were killed and exsanguinated on approximately day 65. The serum was stored at −20°C, and on the first day of use all serum batches from the same infected mouse donors were pooled, and that which remained after injection was aliquoted and refrozen for use on subsequent days if necessary.

Rabbit anti-S. mansoni adult worm immunoglobulin. Random-bred ATS-deprived T.O. mice were infected with 300 *S. mansoni* cercariae and were perfused 46 days later. The adult parasites collected from twenty mice (a total of approximately 2000 worms of both sexes) were rinsed in excess isotonic saline until visibly free of mouse erythrocyte contamination and then resuspended in 2 ml tissue culture medium 199 (Burroughs Wellcome) in a 5 ml plastic culture tube. The tube was continuously rotated (Rolamix RM/54; Luckham, Burgess Hill, Sussex, England) at room temperature for 5 hr. The supernatant was then removed and 0·2 ml injected (without adjuvant) intramuscularly into the thigh of albino rabbits. Each rabbit

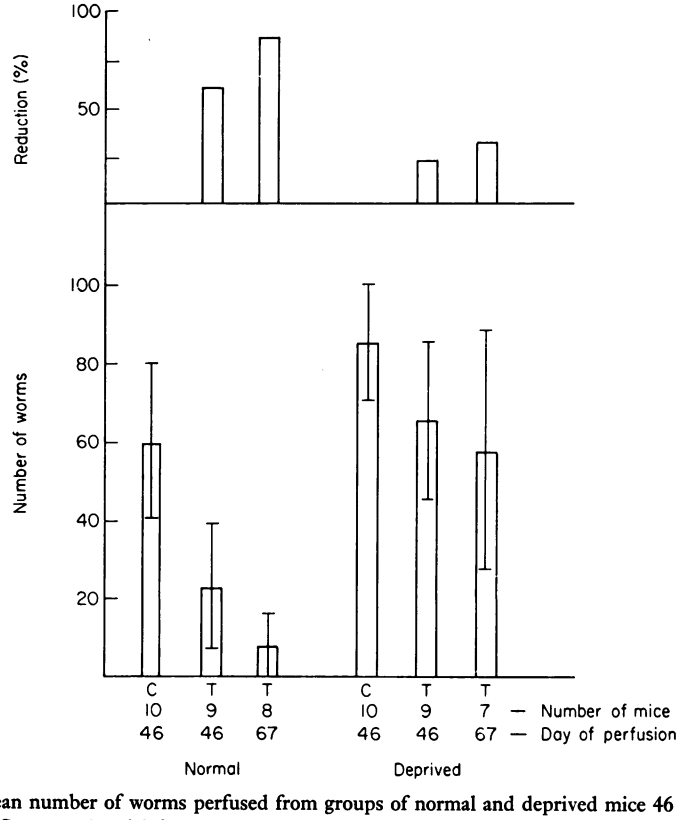

FIG. 1. The mean number of worms perfused from groups of normal and deprived mice 46 or 67 days after infection with *S. mansoni* and left untreated (C) or treated with PAT (T). Upper histogram indicates the degree to which the worm burden has been reduced in the drug-treated groups, calculated according to the formula:

$$\text{percentage reduction} = 100 - \frac{\text{mean number of worms in T}}{\text{mean number of worms in C}} \times 100.$$

P values for the number of perfused worms: normal C day 46 compared with normal T day 46, $<0{\cdot}001$; normal T day 46 compared with normal T day 67, $<0{\cdot}05$; deprived C day 46 ccmpared with deprived T day 46, $<0{\cdot}05$; deprived T day 46 compared with deprived T day 67, n.s.

received 10–12 injections spaced 1–2 weeks apart before being exsanguinated and the serum separated. A crude immunoglobulin fraction of the serum was obtained by slowly adding half the serum volume of saturated ammonium sulphate solution and washing the resulting precipitate twice with 40% saturated ammonium sulphate solution. The precipitate was redissolved in isotonic saline to a total of half the original serum volume, and dialysed for 48 hr in excess saline solution passed through a 0·2 μ filter (Millipore) and stored in suitable volumes at −20°C until use.

Statistics. The significance of the difference in the mean number of worms perfused from groups of animals has been analyzed by the Student's *t*-test. Values of $P < 0{\cdot}05$ have been considered not significant (n.s.). Results have been illustrated in histogram form with variability about the mean indicated in terms of ±1 s.d.

RESULTS

Comparative schistosomicidal efficacy of PAT in normal and deprived mice

Three groups of normal and three groups of deprived mice were infected with 200 *S. mansoni* cercariae. Two groups of each type of animal were given five consecutive daily oral doses of PAT (100 mg/kg/day) commencing on the thirty-fifth day after infection. The two untreated groups and two of the treated groups were perfused on the forty-seventh day of infection and the remaining two treated groups were perfused on day 67.

The mean number of worms perfused from the six groups of mice are illustrated in histogram form in the lower part of Fig. 1. It is apparent that the degree to which the worm burden was reduced in the drug-treated groups relative to the worm burdens perfused from untreated mice (Fig. 1, upper half), was greater in the intact mice (61% on day 46; 87% on day 67) than in deprived mice (24% on day 46; 36% on day 67). During the interval between the two perfusion days the worm burden remaining in the drug

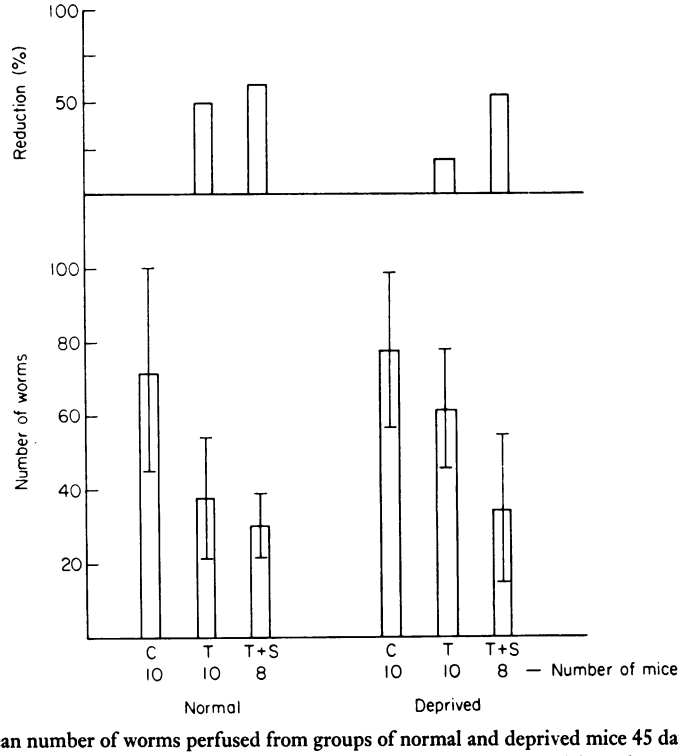

FIG. 2. The mean number of worms perfused from groups of normal and deprived mice 45 days after infection with *S. mansoni* and left untreated (C) or treated with PAT (T) or PAT and homologous acute infection serum (T + S). Upper histogram indicates percentage reduction in worm burden in the treated groups relative to the control group calculated according to the formula given in Fig. 1. *P* values for the number of perfused worms: normal C compared with normal T, $< 0{\cdot}01$; normal C compared with normal T + S, $< 0{\cdot}001$; normal T compared with normal T + S, n.s.; deprived C compared with deprived T, n.s.; deprived C compared with deprived T + S, $< 0{\cdot}001$; deprived T compared with deprived T + S, $< 0{\cdot}01$.

treated, intact mice had been further reduced by about half, whereas in the deprived mice during the same interval the mean worm burden was reduced only from sixty-five adults to fifty-seven.

Restoration of the activity of PAT in S. mansoni-infected deprived mice with homologous acute infection serum

Three groups of normal and three groups of deprived mice were infected with 200 *S. mansoni* cercariae. Commencing 31 days after infection one group of each type of mouse received five consecutive daily oral doses of PAT (100 mg/kg/day). A further group of normal mice and one of deprived mice received daily intravenous injections of 0·5 ml homologous acute infection serum in addition to the same regimen of treatment with PAT. All treated and the two untreated control groups of mice were perfused 45 days after infection.

The worm burdens perfused from the mice in this experiment are depicted in Fig. 2. The results shown in Fig. 1 have been reproduced in as much as treatment with PAT alone was more effective in the immunologically intact mice than in the deprived group. Simultaneous treatment of the deprived mice with the chemical and the serum, albeit via different routes, resulted in a substantial reduction in the worm burden relative to the untreated control group or the deprived group given the drug alone. Even in the normal animals given both the serum and the drug the mean number of worms was marginally lower than in the mice given only PAT.

Synergy between PAT and heterologous anti-S. mansoni immunoglobulin in immunogolically-intact mice

In each of two experiments four groups of normal mice were infected with 200 *S. mansoni* cercariae and

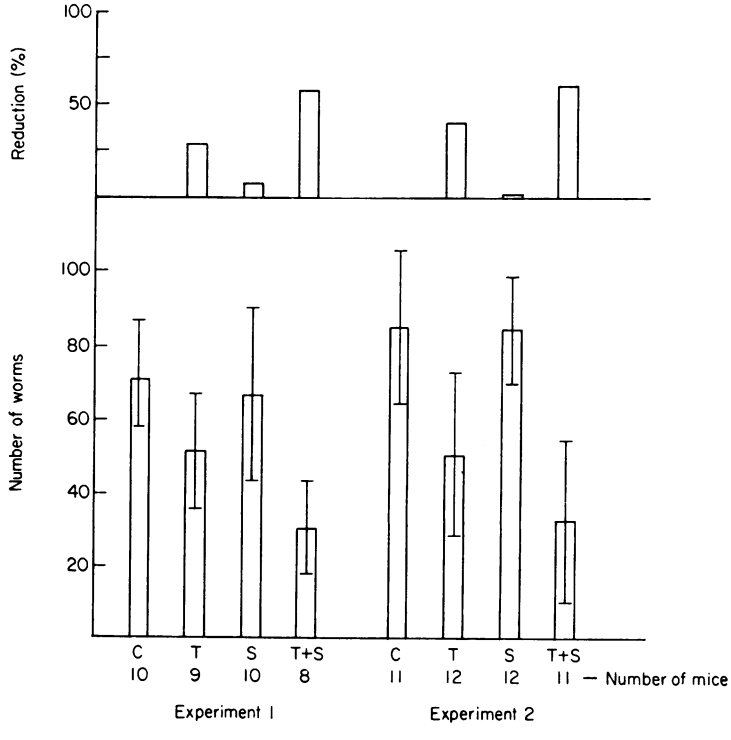

FIG. 3. The mean number of worms perfused from four groups of immunologically intact mice infected with 200 *S. mansoni* cercariae and left untreated (C), or treated with PAT alone (T), rabbit anti-S. *mansoni* adult worm immunoglobulin solution alone (S), or simultaneously with PAT and rabbit immunoglobulin solution (T + S). Upper histogram indicates percentage reduction in worm burden in the treated groups relative to the untreated control group calculated according to the formula given in Fig. 1. *P* values for the number of perfused worms, Expt. 1: C compared with T, $<0{\cdot}01$; C compared with S, n.s.; C compared with T + S, $<0{\cdot}001$; T- compared with T + S $<0{\cdot}01$; S compared with T + S, $<0{\cdot}001$; *P* values for Expt. 2 were the same as Expt. 1, except that T compared with T + S, n.s.

from 33 days (Exp. 1) or 31 days (Exp. 2) after infection, three of the groups of mice were respectively given 5 daily oral doses of PAT (100 mg/kg/day; T), 5 daily intravenous injections of rabbit anti-*S. mansoni* adult worm immunoglobulin solution (0·2 ml/mouse/day; S), or both PAT orally and immunoglobulin solution intravenously (T + S). All four groups of mice were perfused 48 days (Expt. 1) or 45 days (Expt. 2) after infection. Two different rabbits injected with batches of *S. mansoni* worm extract prepared on different occasions, were used as the serum immunoglobulin donors for the two experiments.

Results of the two experiments are depicted in histogram form in Fig. 3, and in both instances treatment with the drug and the immunoglobulin solution has had a more marked effect than either the drug or the serum alone in terms of the percentage reduction in the worm number. The drug alone reduced the mean worm burdens by only 30–40% and serum alone had no effect, whereas PAT and serum administered together reduced the worm burden sizes by approximately 60% in both experiments. It was further observed in the first experiment, but not in the second, that the worms from the drug and serum treated groups were visibly stunted relative to the worms from the other three groups. The second rabbit antiserum appears to have been less effective in this system in as much as the difference in size of the worm burdens of the drug alone-treated and drug + serum-treated groups was not significant.

DISCUSSION

Ehrlich (1909, quoted by Taliaferro, 1948) was probably the first to envisage the possibility that drugs act synergistically with the immune response. However, for the most part investigations on the chemotherapy and the immunology of parasitic infections have been pursued independently and discussions such as that by Taliaferro (1948), in which a deliberate effort was made to discover any interrelationships between the immune response and the action of quinine on malaria, are rare. In contrast, synergistic activity between antibody and chemotherapeutic agents has already been demonstrated in cancer research (Davies, 1974; Newman *et al.*, 1977), and Carter *et al.*, (1973) have shown that asparaginase-treatment of an allogeneic murine lymphoma was considerably less effective in T-cell deprived mice than in immunologically intact controls. The present work was initiated on the basis of the interpretation which was given to the latter observation.

With respect to schistosomes, Standen (1955) suggested that immune mechanisms, and in particular phagocytosis, may play some part in the final destruction of worms that had been initially weakened by chemotherapy. However, Newsome (1964) failed to enhance the schistosomicidal activity of the antimonial Stibophen by "vaccinating" *S. mansoni*-infected baboons with homologous worm homogenate, even though serum from animals immunized with this homogenate had been found to enhance the attachment of "leucocytes" to worms *in vitro* (Newsome, 1962).

The use of a relatively well-defined rodent model has here made it evident that the schistosomicidal activity of potassium antimony tartrate is indeed immune-dependent. The nature of the immunodeficiency in deprived mice which is responsible for the lack of efficacy of PAT has by no means been unequivocally determined, but the result showing that the schistosomicidal action of PAT can be reconstituted in these animals by administration of homologous acute infection serum (Fig. 2) suggests that antibody may be the immune effector mechanism which is lacking. The marginal effect that the same serum had in drug-treated, immunologically intact animals may be due to the serum donors having been infected for somewhat longer than the recipients. The fact that crude immunoglobulin preparation derived from immunized rabbits was also synergistically active with the drug in immunologically intact mice substantiates the idea that antibody may be important in the successful treatment of schistosome infections with antimony. Attempts are currently being made to standardize the rabbit serum preparations and render them more potent in this system by injecting the worm extract with adjuvant. The manner in which PAT and antibody putatively act together is not clear. It has been shown that antimonials inhibit the enzyme phosphofructokinase (Bueding, 1969), and it is possible that as a result of a disruption of energy metabolism by the metal, the worm is unable to protect itself from the ravages of immune attack. From this hypothesis it follows that during a primary infection of *S. mansoni*, immunological effector mechanisms are generated against which the worms are successfully able to

protect themselves and survive under normal circumstances. Protective measures normally used by the worms could include the rapid replacement of surface membrane components (Wilson & Barnes, 1974; 1977; Kusel & Mackenzie, 1975) and in fact the rate of replacement may depend on the immune status of the host (Perez & Terry, 1973). Schistosomes may also protect themselves from immune attack by incorporation of host erythrocyte antigens (Smithers, Terry & Hockley, 1969). In spite of protective devices such as these, host immunoglobulins have been detected on the surface of worms after perfusion, and perfused worms have been shown to bind antibody from chronic infection serum *in vitro* (Goldring *et al.*, 1977). Therefore PAT, rather than being itself lethal to the worm, might upset the balance which exists between the self-defence mechanisms of the parasite and the immune effector mechanisms of the host; for example, by inhibiting either membrane turnover or the incorporation of sufficient host antigen by the parasite, or by allowing greater attachment of host antibody, each to an extent which allows the immune mechanisms to destroy the worms.

An alternative explanation may be that therapy with PAT results in the release of a substantial quantity of antigen which rapidly boosts the level of anti-parasite antibody. The worms, already in a weakened state as a result of the drug treatment, succumb to the effects of the newly synthesized antibody, but are able to survive better in deprived mice which are less capable of producing the antibody.

If the component in serum which acts synergistically with PAT in killing schistosomes proves to be antibody, it may be possible to conjugate it with the drug and use it for 'targetting' the latter more specifically towards the parasite, thereby making the drug more effective against the parasite and less toxic to the host. Although this idea has been recurrent in cancer research for some time, more rapid progress may be made with parasites than with tumours because of the more readily demonstrated antigenic disparity between parasites and their hosts. The choice of a drug which depends on its metal ion content for its activity, as here, may be appropriate because of the unlikelihood of there being any intermediate metabolites between the injected and the schistosomicidally active form. However, whether the species of antibody which putatively acts synergistically with PAT in killing schistosomes could also be used for 'targetting' purposes, and whether the antibody would still show synergistic schistosomicidal activity with the drug to which it was conjugated, are questions which remain to be answered.

This work was supported by Edna McConnell Clark Foundation Grant No. 274–0095. Assistance, advice and encouragement from Prof G. S. Nelson, Dr G. Webbe, Dr M. Taylor, Dr E. Long, Mr Q. Bickle and Mr A. Dobinson, and secretarial expertise from Miss H. Falconer and Mrs D. Mulholland are gratefully acknowledged.

REFERENCES

BUEDING, E. (1969) Some biochemical effects of anthelminthic drugs. *Biochem. Pharmacol.* **18**, 1541.

CARTER, R.L., CONNORS, T.A., WESTON, B.J. & DAVIES, A.J.S. (1973) Treatment of a mouse lymphoma by L-asparaginase: Success depends on the host's immune response. *Int. J. Cancer*, **11**, 345.

CUNNINGHAM, A.J. & SZENBERG, A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology*, **14**, 599.

DAVIES, D.A.L. (1974) The combined effect of drugs and tumor-specific antibody in protection against a mouse lymphoma. *Cancer Res.* **34**, 3040.

DOENHOFF, M.J., JANOSSY, G. & KERBEL, R.S. (1976) Enumeration of polyclonal mitogen-responsive cells in different lymphoid tissues of the mouse. *Immunology*, **30**, 367.

EHRLICH (1909) Chemotherapie von Intektionskrankheiten. *Zeitschr. Arztl. Fortbildung*, **6**, 721.

GOLDRING, O.L., SHER, A., SMITHERS, S.R. & MCLAREN, D.J. (1977) Host antigens and parasite antigens of murine *Schistosoma mansoni*. *Trans. Roy. Soc. Trop. Med. Hyg.* **71**, 144.

KUSEL, J.R. & MACKENZIE, P.E. (1975) The measurement of relative turnover rates of proteins of the surface membranes and other fractions of *Schistosoma mansoni* in culture. *Parasitology*, **71**, 261.

LAW, L.W., BRADLEY, T.R. & ROSE, S. (1963) Reversal of the thymus dependent influence in radiation leukemagenesis of C57B1 mice. *J. Nat. Cancer Inst.* **31**, 1461.

LEVEY, R.H. & MEDAWAR, P.B. (1966) Some experiments on the action of antilymphoid antisera. *Ann. N.Y. Acad. Sci.* **129**, 164.

NEWMAN, C.E., FORD, C.H.J., DAVIES, D.A.L. & O'NEILL, G.J. (1977) Antibody drug synergism: An assessment of specific passive immunotherapy in bronchial carcinoma. *Lancet*, **ii**, 163.

NEWSOME, J. (1962) Immune opsonins in *Schistosoma* infections. *Nature (Lond.)*, **195**, 1175.

NEWSOME, J. (1964) Investigation of anti-schistosome opsonins *in vivo*. *Trans. Roy. Soc. Trop. Med. Hyg.* **58**, 58.

OLIVIER, L.J. (1966) Infectivity of *Schistosoma mansoni* cercariae. *Amer. J. Trop. Med. Hyg.* **15**, 882.

PEREZ, H. & TERRY, R.J. (1973) The killing of adult *Schistosoma mansoni in vitro* in the presence of antisera to host antigenic determinants and peritoneal cells. *Int. J. Parasitol.* **3**, 499.

SMITHERS, S.R. & TERRY, R.J. (1965) The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology*, **55**, 695.

STANDEN, O.D. (1955) The progress of degenerative changes in schistosomes following the treatment of experimental infections with 1:7-bis (p-dimethylaminophenoxy) heptane. *Trans. Roy. Soc. Trop. Med. Hyg.* **49**, 416.

TALIAFERRO, W.H. (1948) The rôle of the spleen and the lymphoid-macrophage system in the quinine treatment of gallinaceum malaria. *J. infect., Dis.* **83**, 164.

TAYLOR, M.G., AMIN, M.B.A. & NELSON, G.S. (1969) "Parthenogenesis" in *Schistosoma mattheei*. *J. Helm.* **43**, 197.

WILSON, R.A., & BARNES, P.E. (1974) An *in vitro* investigation of the dynamic processes occurring in the schistosome tegument, using compounds known to disrupt secretory processes. *Parasitology*, **68**, 259.

WILSON, R.A. & BARNES, P.E. (1977) The formation and turnover of the membranocalyx on the tegument of *Schistosoma mansoni*. *Parasitology*, **74**, 61.