been shown to cause liberation of mepacrine into the medium[1], whereas in Tyrode their majority remains intact and retains the drug. This indicates that the described flashing phenomenon is probably connected with quenching of the fluorescence of mepacrine accumulated in the storage organelles. Irradiation of the platelets probably induces liberation of mepacrine from these organelles and subsequent dilution of the drug (e.g. in the cytoplasm). As a consequence, the quenching is diminished, and the fluorescence increases in intensity followed by a decrease due to fading and/or leaking of the mepacrine out of the platelets.

Acridine orange and daunomycin behaved like mepacrine. In fact, platelets loaded with the former drugs showed highly fluorescent (yellow-red) granular structures similar in number and size to the fluorescent granules seen in platelets stained with mepacrine. In addition, the flashing phenomenon was to be seen on exposure of the platelets to violet-blue light.

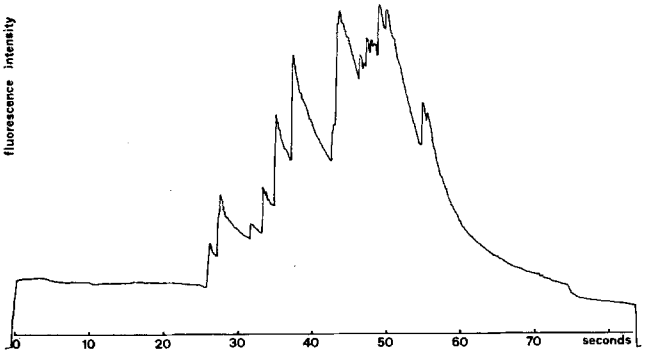


Fig. 2. Microfluorimetric measurement of the fluorescence intensity of a single rabbit blood platelet incubated with mepacrine 5×10^{-5} *M*. After violet-blue irradiation for 25 sec the platelet started to emit flashes.

The mechanism by which irradiation acts on 5HT storage organelles is not known. The alteration of the organelles which leads to the flashing phenomenon seems to be due to the activating (violet-blue) and not to the emitted (green-yellow) light. Thus, mepacrine-loaded platelets, irradiated for 45 min with intense green (546 nm) light, still showed fluorescent granular structures which were flashing on subsequent exposure to violet-blue light. However, the flashing was not bound to live cells; it even took place in mepacrine-loaded platelets fixed for 2 h with 2.5% glutaraldehyde. Therefore, the involvement of contractile proteins in the flashing phenomenon is unlikely.

Both the visualization of the 5HT storage organelles by fluorescent drugs and the flashing phenomenon may be of interest for studying these organelles in situ under various conditions, e.g. during the release reaction. Such observations will probably not be limited to blood platelets since, according to preliminary experiments, flashing also occurs in other cells which contain subcellular particles (e.g. lysosomes in leucocytes and mast cell granules) storing mepacrine. Furthermore, flashing is also observed in isolated chromaffin granules of bovine adrenal medulla loaded in vitro with the drug.

Zusammenfassung. Nach Behandlung von Blutplättchen mit Mepacrin, Acridin-Orange und Daunomycin, die sich selektiv in den 5-Hydroxytryptamin- (5HT)-Speicherorganellen anreichern, werden letztere im Fluoreszenzmikroskop sichtbar. Unter violett-blauer Bestrahlung zeigen die einzelnen Plättchen mehrere sukzessive, blitzartige Zunahmen der Fluoreszenz-Intensität, wobei sie während einiger Sekunden vollständig ausgeleuchtet erscheinen. Die Blitze sind wahrscheinlich durch Freisetzung der fluoreszierenden Stoffe aus je einer 5HT-Speicherorganelle bedingt.

H. P. Lorez, M. da Prada and A. Pletscher[8]

Research Department, F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel (Switzerland), 3 March 1975.

[7] H. Steffen, personal communication.

[8] Acknowledgment. We thank Drs H. R. Baumgartner and H. Steffen, Research Department, F. Hoffmann-La Roche & Co. Ltd, Basel, for advice in performing and interpreting the experiments.

Lung Inflammation in Immunity to *Schistosoma mansoni*

Schistosoma mansoni, the causal agent of a widespread tropical disease, is a parasitic worm which lives in the hepatic portal system of man. Infection occurs when cercariae emerge from a water snail intermediate host, penetrate exposed human skin and migrate via the lungs to the portal system. As the young schistosomes, called schistosomula, are migrating through the lung capillaries they can be recovered in substantial numbers by a simple technique which forms the basis of an assay for measuring immunity to reinfection in rats[1] and mice[2] immunized by a primary infection with *S. mansoni*.

Adult golden hamsters (*Mesocricetus auratus*) were immunized with a small primary infection of 50 *S. mansoni* cercariae and challenged at intervals with 250 cercariae. The number of schistosomula recovered from the lungs of immune hamsters on day 5 after challenge was greatly depressed compared with recoveries from normal controls given only the challenge infection. Immunity to reinfection develops rapidly and reaches a plateau representig 70–80% protection at about 6 weeks after the primary infection.

Many of the immune hamsters suffer from respiratory distress during the period, 4–7 days after challenge when the invading schistosomula are migrating through the lungs, but this is not observed in normal hamsters given the same challenge. Histological examination of the lungs of immune hamsters during this period reveals that the schistosomula stimulate an acute inflammatory reaction. There is a massive infiltration of polymorphonuclear neutrophils into all the lung tissue which persists as more schistosomula enter the lungs. By day 5, when maximum numbers of organisms are present, many macrophages can be seen, fluid exudates containing neutrophils occur in many alveoli and there are extensive haemorrhages which are also visible macroscopically on the lung surface. The histology of control lungs remains essentially

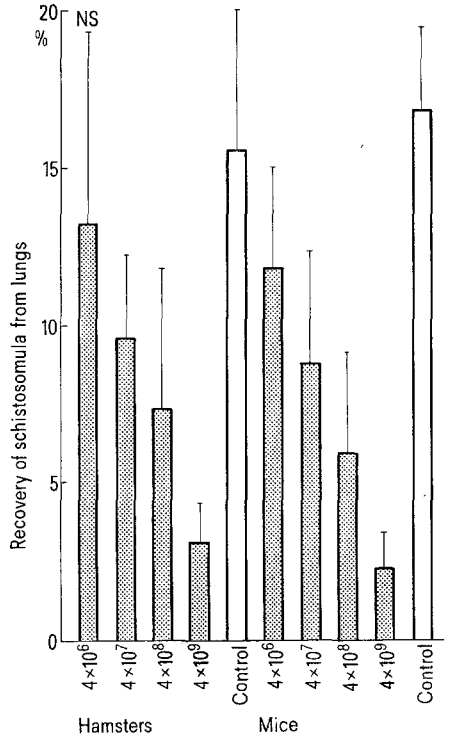
[1] H. Perez, J. A. Clegg and S. R. Smithers, Parasitology *69*, 349 (1974).

[2] F. A. Sher, P. E. Mackenzie and S. R. Smithers, J. infect. Dis. *130*, 626 (1974).

Protection of hamsters against *S. mansoni* infection with human serum albumin (HSA) and *Maia squinado* hemocyanin (MSH)

Substance injected on day 3	Schistosomula recovered from lungs on day 5 (%)
Control	26.5 ± 2.2
1 mg MSH + anti-MSH	11.5 ± 4.6
Soluble MSH	
1 mg	11.3 ± 3.5
5 mg	11.3 ± 4.3
Control	17.1 ± 4.5
Heat-pptd HSA 1 mg	10.0 ± 6.1
Soluble HSA	
1 mg	9.3 ± 3.6
5 mg	8.3 ± 1.6

Each treatment was given to 10 adult normal male hamsters in 2 experiments. 0.1 ml of each substance, suspended or dissolved in Hanks' saline was given intracardiac on day 3 after infection with 250 cercariae and lung recoveries were made on day 5. HSA was heat precipitated at 90 °C for 15 sec. 1 mg MSH was reacted with 1 ml rabbit anti-MSH for 30 min at 37 °C and the precipitate washed twice with Hanks' saline. The differences between the mean for each treatment and the appropriate control mean were statistically significant at the 1% level (Student's *t*-test).



Protection of hamsters and CBA mice against *S. mansoni* infection with different numbers of dead *E. coli*. 3 days after infection with 250 cercariae groups of 5 hamsters or mice were given an intracardiac injection of 0.1 ml Hanks' saline containing 4×10^6 — 4×10^9 formalin-killed *E. coli*. 5 days after infection schistosomula were recovered from the lungs. Each bar represents the mean recovery of schistosomula from a total of 10 animals in 2 experiments with the standard deviation. The differences between the means and the appropriate control were all statistically significant at the 1% level (Students' *t*-test) except for treatment of hamsters with 4×10^6 *E. coli*.

normal during migration of schistosomula, although there are some minute haemorrhages due to mechanical damage to the capillaries. Inflammatory reactions around invading schistosomula have also been observed in the lungs of immune mice[3] and rhesus monkeys[4].

With the object of determining whether the acute inflammation is the cause of the greatly reduced recovery of schistosomula from the lungs of immune hamsters we artificially stimulated inflammation in the lungs of normal hamsters following an infection. 3 days after infection with 250 cercariae normal hamsters were given an intracardiac injection of 4×10^9 formalin-killed *Escherichia coli* (type 0111). Lung recoveries at day 5 showed a marked depression equivalent to 81% protection (Figure). Smaller numbers of dead *E. coli* had progressively less effect. Intraperitoneal injection of 4×10^9 dead *E. coli* gave no protection against infection. Similar results were obtained with CBA mice (Figure). Conventional assay of protection by recovery of adult worms by perfusion 6 weeks after infection confirmed that hamsters given a single intracardiac injection of dead *E. coli* on day 3 are able to destroy most of an infection.

Intravascular administration of gram-negative microorganisms has long been known to stimulate sequestration of neutrophils in the lungs[5] and there is recent evidence that this effect may involve activation of the complement cascade[6]. Histology of normal hamster lungs fixed at intervals following intracardiac injection of 4×10^9 dead *E. coli* showed that the organisms alone stimulate an inflammation involving both neutrophils and macrophages which reaches a maximum at 48 h and is greatly exacerbated if migrating schistosomula enter the lungs.

The stimulation of protection against infection with *S. mansoni* in normal hamsters is not specific to *E. coli*. Intracardiac injection of heat-aggregated human serum albumin (HSA) or *Maia squinado* (Spider Crab) hemocyanin (MSH) complexed with rabbit anti-MSH antibody stimulated inflammation of the lungs and gave a degree of protection (Table). Antigen-antibody precipitates and other aggregated antigens are known to cause lung inflammation when given i.v.[7]. In our experiments both MSH and HSA in soluble form also stimulated lung inflammation and protection against infection (Table).

Although i.v. administration of 3 widely different foreign antigens causes inflammation of the lungs in normal hamsters, schistosomula themselves migrating through the capillaries of normal lungs do not provoke detectable cellular infiltration. However, circulating antibody, raised in hamsters by vaccination with a particulate fraction of adult schistosomes mediates sequestration of inflammatory cells in the lungs in response to invading schistosomula. Hamsters were each given an i.m. injection of pellet material in complete Freund's adjuvant, obtained by centrifugation at 10,000 × *g* of 100 adult schistosomes homogenized in saline. A booster dose of the same size in incomplete Freund's adjuvant was given 2 weeks later followed after 7 days by an infection with 250 cercariae. The vaccination itself did not cause any inflammation of the lungs but migrating schistosomula stimulated sequestration of neutrophils and macrophages

[3] A. MAGALHAES-FILHO, Am. J. trop. Med. Hyg. *8*, 527 (1959).
[4] F. VON LITCHENBERG and L. S. RITCHIE, Am. J. trop. Med. Hyg. *10*, 859 (1961).
[5] F. W. ANDREWES, Lancet *2*, 8 (1910).
[6] C. E. MCCALL, L. E. DE CHATELET, D. BROWN and P. LACHMANN, Nature, Lond. *249*, 841 (1974).
[7] H. Z. MOVAT, T. URIUHARA, N. S. TAICHMAN, H. C. ROWSELL and J. F. MUSTARD, Immunology *14*, 637 (1968).

in the lungs of vaccinated hamsters. Passive transfer of serum from vaccinated into normal hamsters (1 ml i.p.) on the day of infection demonstrated that humoral factors are responsible for this response. Although a challenge infection of vaccinated hamsters caused infiltration of inflammatory cells the lung recovery assay showed clearly that destruction of schistosomula had not occurred.

Our interpretation of this evidence is that antibody raised by vaccination of hamsters with crude schistosome material can mediate sequestration of inflammatory cells in the lungs in response to migrating schistosomula but the cells are unable to destroy the schistosomes. Intravascular administration of several foreign antigens however, stimulates sequestration of inflammatory cells in the lungs and apparently also causes some kind of non-specific activation of the cells enabling them to damage schistosomula. This effect could possibly be due to the phagocytosis of antigen which is known to cause enhanced metabolic activity and enzyme synthesis in neutrophils[8] and macrophages[9], the cell types detected in the inflamed lungs.

Zusammenfassung. Bei durch Primärinfektion mit *Schistosoma mansoni* immunisierten Goldhamstern wird die Zerstörung einer Sekundärinfektion nachgewiesen. Intravenöse Injektion diverser Fremdantigene führt bei normalen Hamstern 3 Tage nach der Infektion zu einer unspezifischen Lungenentzündung und verursacht die Vernichtung der meisten Jung-Schistosomen.

M. A. Smith[10,11], J. A. Clegg, J. R. Kusel and G. Webbe

London School of Hygiene and Tropical Medicine, Winches Farm Field Station, St. Albans (Hertshire, England), and National Institute for Medical Research, Mill Hill, London NW7 1AA (England), 9 December 1974.

[8] P. C. Wilkinson, *Chemotaxis and Inflammation* (Churchill, Livingstone 1974), p. 150.

[9] E. Suter and L. Hulliger, Ann. N.Y. Acad. Sci. *88*, 1237 (1960).

[10] We thank Mrs. N. Robson and Mr. K. Gammage for expert technical assistance. M.A.S. was supported by a grant to G.W. from the Tropical Medicine Research Board through the Overseas Development Administration.

[11] Present address: Department of Zoology, Imperial College, London S.W.7 (England), to whom reprint requests have to be addressed.

Cyclic Activity of the Corpus Allatum Related to Gonotrophic Cycles in Adult Female *Periplaneta americana*

Oocyte maturation is dependent on the secretion of the corpora allata in the majority of insect species studied[1,2], including the cockroach *P. americana*. Extirpation of the corpora allata during the latter nymphal instars results in total failure of ovarian growth after metamorphosis[3], and allatectomy of reproductively active adult females leads to a cessation of ootheca formation[4]. Periodic changes in corpus allatum appearance parallel the cycles of ovarian activity in the viviparous cockroaches *Leucophaea maderae* and *Diploptera punctata*, these species have protracted periods of pregnancy during which the corpora allata are believed to be inactive[5–7]. *P. americana* differs in that it is ovoparous and the ovarian cycles overlap to the extent that vitellogenic oocytes are always present in the ovaries of mature females[8,9]. Would one therefore expect to find differences in the activity of the corpora allata in relation to the gonotrophic cycle in this species?

The corpus allatum is the physiological source of juvenile hormone[1], and the recent identification of the hormone of *P. americana* as methyl-10,11-epoxy-3,7,11-trimethyl-*trans*, *trans*-2,6-dodecadienoate ($C_{16}JH$)[10,11], coupled with the results of short-term, radiochemical, in vitro incubation experiments on locust glands[12,13], suggested that similar techniques might provide direct quantitative measurement of the rates at which *P. americana* corpora allata synthesise and release hormone at different times during the gonotrophic cycle.

Materials and methods. Adult female *P. americana* were maintained, in the presence of males, at 27 °C in dim light and fed ad libitum with a ground mixture of oatmeal: dog chow: peanuts: yeast powder (17:10:4:1). Ovarioles and corpora allata were dissected from unanaesthetised animals under citrate-fortified Ringer solution[12]. The lengths of the T and T-1 oocytes were measured; and the patency of the follicular epithelium, an indication of active vitellogenesis, was tested by the Evan's blue method of Pratt and Davey[14].

The biosynthetic activities of the corpora allata from individual animals were calculated from the incorporation of radio-labelled methionine into $C_{16}JH$ present in the glands plus medium following 3 h incubation in vitro. The procedures for separation, identification and quantification of the incubation products by radio-thin layer chromatography and liquid scintillation counting were as described previously[12,13]. In certain experiments corpora allata from pairs of animals, sacrificed at the same time in relation to the deposition of the previous ootheca, were divided between 2 incubation tubes, such that each tube contained 2 glands, one from each animal (split pairs)[15].

Results and discussion. The animals used in these experiments formed an ootheca every 3–4 days, while dye penetration tests show that each batch of oocytes spent 6–8 days in the state of active vitellogenesis. Shortly after ovulation of the T oocytes the new basal oocytes (previously T-1) are 2.0–2.3 mm in length and already at an advanced stage in vitellogenesis, whereas, the new

[1] V. B. Wigglesworth, *Insect Hormones* (Oliver and Boyd, Edinburgh 1970).

[2] F. Engelmann, *The Physiology of Insect Reproduction* (Pergamon Press, London 1970).

[3] A. Girardie, J. Insect Physiol. *8*, 199 (1962).

[4] D. H. Chen, W. E. Robbins and R. E. Monroe, Experientia *18*, 577 (1962).

[5] F. Engelmann, J. Insect Physiol. *1*, 257 (1957).

[6] B. Scharrer and M. Von Harnack, Biol. Bull. *115*, 508 (1958).

[7] F. Engelmann, Biol. Bull. *116*, 406 (1959).

[8] G. E. Pratt, Nature, Lond. *214*, 1034 (1967).

[9] W. J. Bell, Biol. Bull. *137*, 239 (1969).

[10] G. E. Pratt and R. J. Weaver, J. Endocr., in press.

[11] P. J. Müller, P. Masner, K. H. Trautmann and M. Suchý, Life Sci. *15*, 915 (1974).

[12] G. E. Pratt and S. S. Tobe, Life Sci. *14*, 575 (1974).

[13] S. S. Tobe and G. E. Pratt, Biochem. J. *144*, 107 (1974).

[14] G. E. Pratt and K. G. Davey, J. exp. Biol. *56*, 201 (1972).