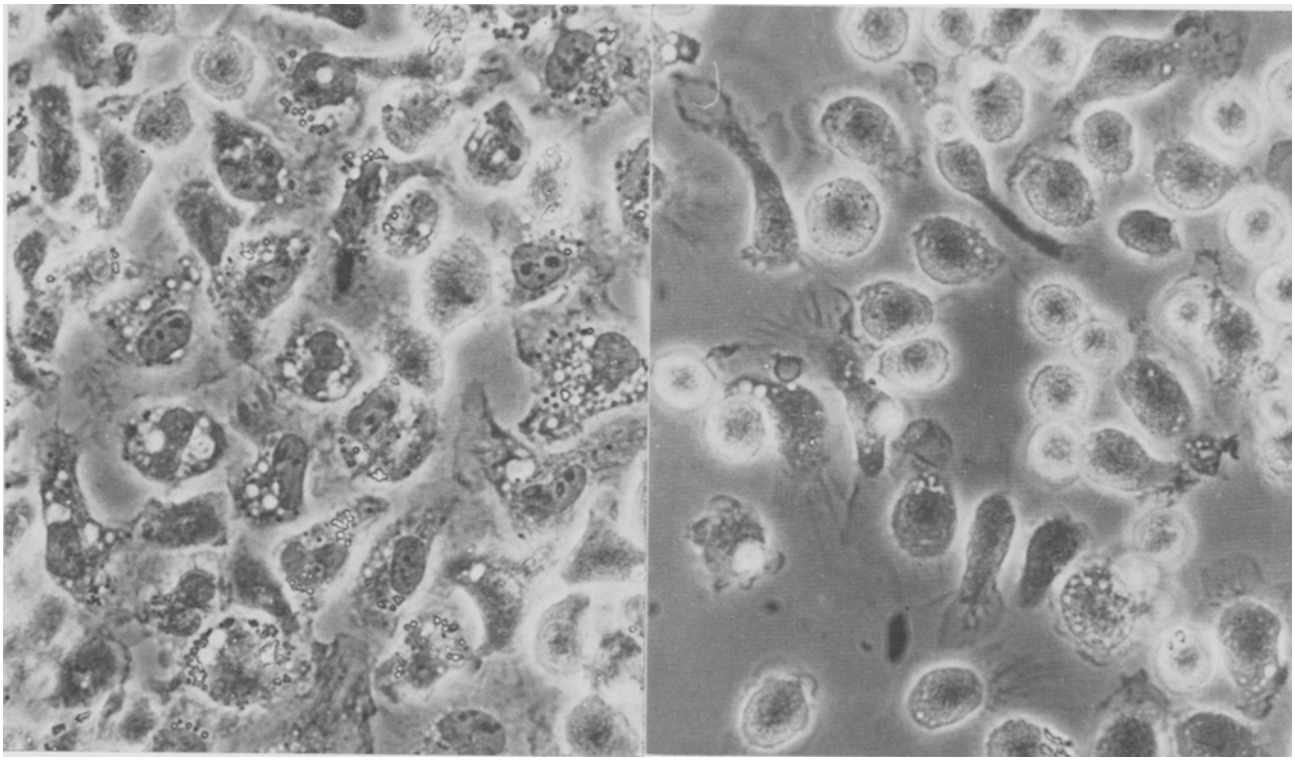

Peritoneal macrophages from nude mice nu/nu (a) and their litter mates nu/+ and +/+ (b) 30 min after plating into plastic dishes at 37 °C. The macrophages of nude mice show extensive spreading and some phase-dense and phase-lucent vesicles. ×400.

This is superficially incompatible with findings of CHEERS[8] which indicate suppression of the growth of i.v. inoculated *Listeria* in the liver and spleen. But the disagreement can be reconciled if it is assumed that macrophages of nude mice are heterogenous with respect to bactericidal activity, and that fixed macrophages in the liver and spleen and perhaps those remaining in the peritoneum under our experimental conditions are much superior to those obtained in peritoneal washings.

Zusammenfassung. Ein gewisser Grad von Makrophagenaktivierung, gemessen an der Fähigkeit der Listerien-Elimination, wird auch in adult thymektomierten, mit Knochenmark rekonstituierten Mäusen sowie in nude/nude-Mäusen beobachtet und daraus der Schluss gezogen, dass die Makrophagenaktivierung nicht unter allen Umständen mit der Anwesenheit von T-Lymphozyten verknüpft sein muss.

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Flashing Phenomenon in Blood Platelets Stained with Fluorescent Basic Drugs

Fluorescent basic drugs such as mepacrine, acridine orange and daunomycin (an antitumor agent) have been shown to selectively accumulate in the 5-hydroxytryptamine (5HT) storage organelles of blood platelets[1,2]. The present work deals with the fluorescence properties of isolated platelets loaded in vitro or in vivo with these compounds.

Materials and methods. Rabbits, guinea-pigs and rats were exsanguinated using disodiumethylene diaminetetracetate (EDTA, 1/10 vol. 5%) as anticoagulant[3]. Platelet-rich plasma obtained by centrifugation of the blood for 20 min at 300 × *g* was incubated (37 °C, 30 min) with various concentrations (10^{-5}, 5×10^{-5}, *M*) of mepacrine (K & K Labs, USA), acridine orange (DIFCO Labs, U.K.) or daunomycin (Farmitalia, Italy). Platelet-rich plasma was also obtained from rabbits 60 min after i.v. injection of 10 mg/kg of mepacrine, acridine orange or daunomycin. The platelets were then sedimented and washed twice with modified Tyrode buffer[4], and the whole platelets or their 5HT organelles, isolated as previously described[3,4], submitted to fluorescence microscopy.

For qualitative fluorescence microscopy a mercury super pressure lamp HBO 200 W/4 was used. Epi-illumination was performed with a Leitz fluorescence vertical illuminator[5] equipped with an interference dividing plate

[1] M. DA PRADA and A. PLETSCHER, Eur. J. Pharmac., in press.
[2] G. B. PICOTTI and I. GALATULAS, Archo it. Pat. Chim. Tumori *16*, 101 (1973).
[3] M. DA PRADA, K. VON BERLEPSCH and A. PLETSCHER, Naunyn-Schmiedeberg's Arch. Pharmac. *275*, 315 (1972).
[4] M. DA PRADA and A. PLETSCHER, Br. J. Pharmac. *34*, 591 (1968).
[5] J. S. PLOEM, Leitz-Mitt. Wiss. Tech. *4*, 225 (1969).

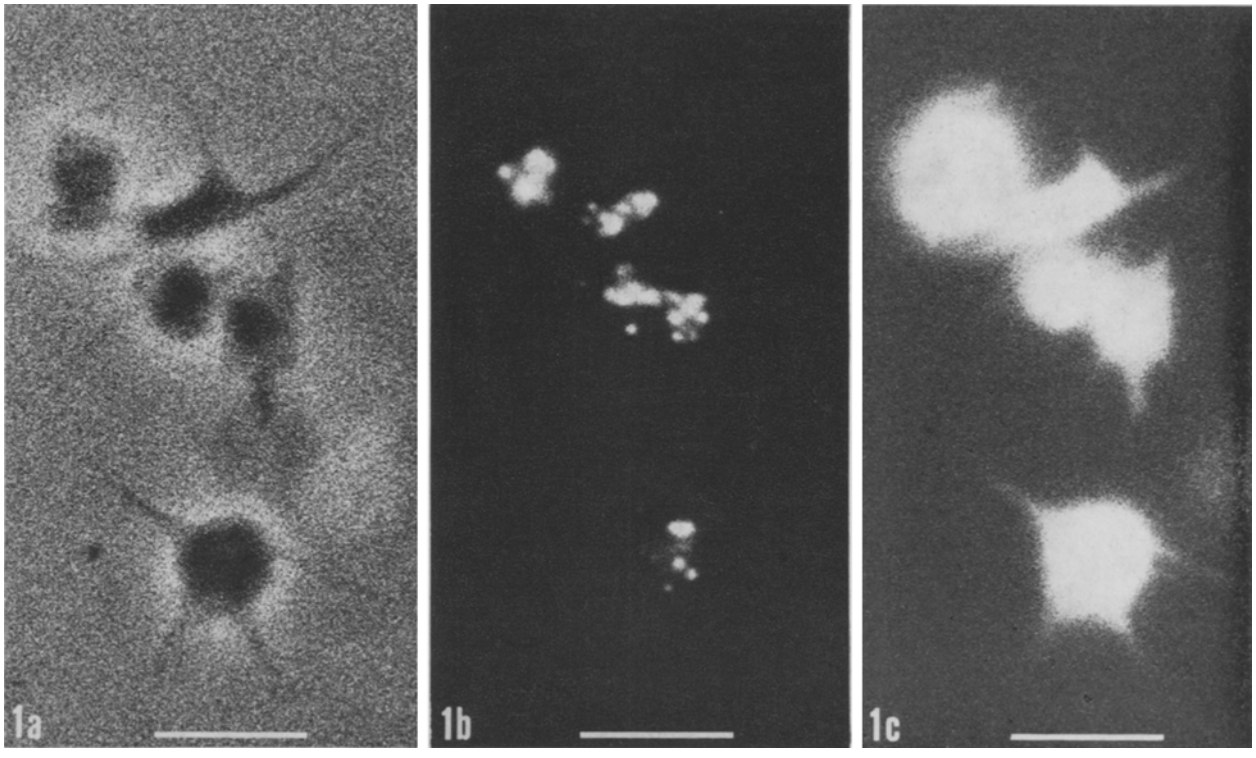

Fig. 1. Rabbit blood platelets incubated with mepacrine 5×10^{-5} *M*. 4000:1 (125×10). 5 μm. a) Phase contrast micrograph. 5 platelets are shown prior to irradiation with violet-blue light. The platelets appear attached to the cover glass, and some pseudopods are visible. b) Fluorescence micrograph of the platelets shown in Figure 1 a. On irradiation with violet-blue light the platelets exhibit green-yellow fluorescent granular structures on a virtually non-fluorescent background. Due to long exposure time some of the granules appear confluent. c) Fluorescence micrograph of the platelets shown in Figures 1 a and b after violet-blue irradiation for 40 sec. Fluorescence has spread out in quick flashes from the granules to illuminate the entire platelets including their pseudopods.

(λH at 495 nm). Violet-blue excitation light for mepacrine, acridine orange and daunomycin was selected with a 3 mm BG 3 coloured glass filter or an interference band filter PAL 437.5 nm (half width 16 nm, peak transmittance 41%) manufactured by SCHOTT et al. Mainz, Federal Republic of Germany. The fluorescence intensity of single platelets was measured with a Leitz-Microscope-Photometer MPV equipped with a mercury super pressure lamp HBO 100 W/2, a 3 mm BG 3 filter, a vertical illuminator for epi-illumination as described above, an oil immersion objective 100× (NA 1.30) and an E.M.I. type 9558 QA photo-multiplier.

Results and discussion. Examination with the fluorescence microscope in violet-blue light of isolated live platelets loaded with mepacrine in vivo or in vitro revealed the presence of fluorescent green-yellow granular structures on a virtually non-fluorescent background (Figure 1, a and b). The diameter of the granules amounted to about 400 nm, their number to approx. 17, 11 and 8 in rabbits, guinea-pigs and rats, respectively. In rabbits, this number was of the same order as that of the 5HT storage organelles estimated earlier by electron microscopy[6]. Furthermore, 5HT storage organelles isolated from rabbit platelets previously loaded with mepacrine could also be visualized by fluorescence microscopy. They had a similar appearance and diameter (about 400 nm) as the 5HT storage organelles seen in whole platelets stained with mepacrine. The subcellular distribution studies of mepacrine[1] together with the microscopic findings indicate that the fluorescent granular elements observed in platelets loaded with mepacrine correspond to the 5HT organelles. Their increased diameter compared to that of 5HT organelles visualized by electron microscopy (150–200 nm) may be connected with scattering of the emitted fluorescent light.

On irradiation with violet-blue light for 10 or more sec (depending on the intensity of the light), isolated platelets loaded with mepacrine started to emit subsequent flashes (peaks in Figure 2). These were characterized by an enhancement of the green-yellow fluorescence which was first observed around the granules and then generally spread out over the entire platelets including their pseudopods (Figure 1, c). The increase was followed by a rapid decrease of the fluorescence intensity. Nearly all the platelets exhibited this phenomenon. According to microfluorimetric registration a single rabbit platelet emitted as many as 10–25 subsequent fluorescence peaks. After cessation of the flashing, the appearance of the platelets (as observed by phase contrast and fluorescence microscopy) was similar to that before the flashing had started, with the exception that the fluorescent granular structures had virtually disappeared. Exposure to violet-blue light of 5HT organelles isolated from platelets preincubated with mepacrine also induced numerous flashes (1 flash per granule) similar to those observed in intact platelets.

Isolated 5HT organelles loaded with mepacrine exhibited (on irradiation with violet-blue light) a much higher fluorescence intensity when lysed in distilled water than after suspension in physiological media such as Tyrode[7]. Lysis of the organelles in distilled water has

[6] J. P. TRANZER, personal communication.

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.

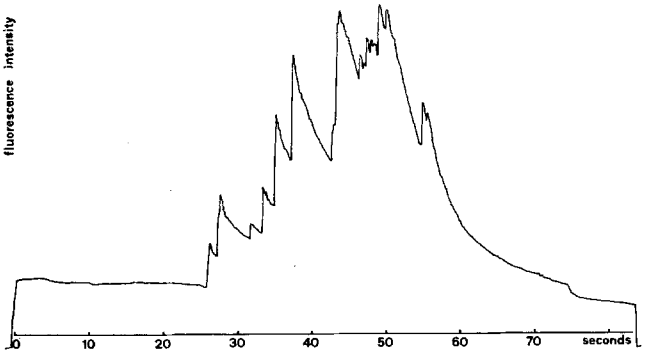

Fig. 2. Microfluorimetric measurement of the fluorescence intensity of a single rabbit blood platelet incubated with mepacrine 5×10^{-5} *M*. After violet-blue irradiation for 25 sec the platelet started to emit flashes.

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.

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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).