This is superficially incompatible with findings of CHEERS 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 heterogeneous 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/nu-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. 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 diaminetetraacetate (EDTA, 1/10 vol. 5%) as anticoagulant. Platelet-rich plasma obtained by centrifugation of the blood for 20 min at 300 × g was incubated (37°C, 30 min) with various concentrations (10⁻⁴, 5 × 10⁻⁴ 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, and the whole platelets or their 5HT organelles, isolated as previously described, 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 equipped with an interference dividing plate.

Fig. 1. Rabbit blood platelets incubated with mepacrine $5 \times 10^{-6}$ M. 4000:1 (125 x 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 1a. 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 1a 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 x (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. 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 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’s. Lysis of the organelles in distilled water has

*J. P. TRANZER, personal communication.*