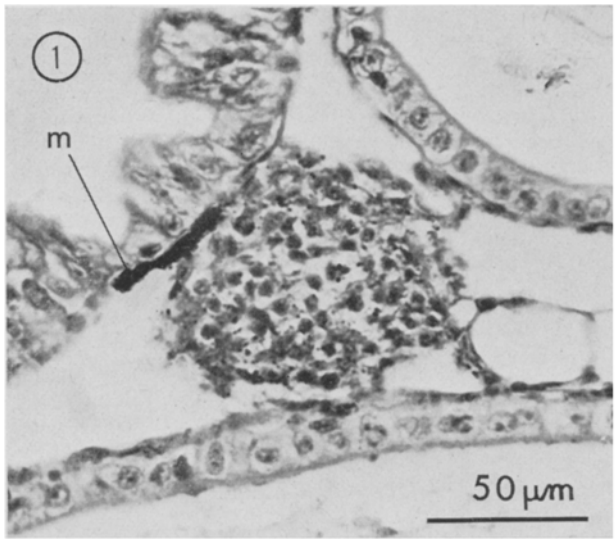

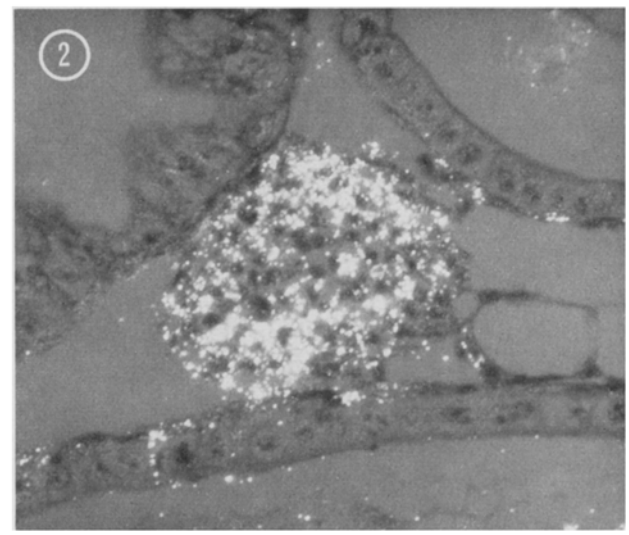

Figs. 1 and 2 show the spleen of a young *Xenopus* tadpole (Stage 48 of NIEUWKOOP and FABER[5]), injected 24 h previously with carbon via the subcutaneous route.
In Fig. 1 the organ is seen using standard light microscopy. At this immature stage the spleen is very small and lacks the follicular organization and small lymphocyte population seen in older animals. Some carbon particles can be seen, but their distribution is difficult to make out. (m) melanin granules. Staining: H and E. Photomicrography: Ilford R20 plate (ASA 50).

Fig. 2 shows the same field, viewed with incident light. Carbon particles reflect the light and show up clearly as bright specks on the dark background, whereas melanin granules and cell nuclei do not. It is clear from this figure that considerable amounts of injected carbon have been taken up by the immature spleen; there is, however, little sign of carbon aggregation or of selective localization of particles in specific areas of the organ. Photomicrography: Kodak Panchro Royal cut film (ASA 400).

In our initial examination of animals injected with carbon[1], the presence of pigment and to a lesser extent the intense staining of the lymphoid tissues frequently necessitated examination under oil immersion for positive identification and localization of carbon when a transmitted light microscope was used. It was subsequently found, however, that use of an incident light system (Leitz 'Ultropak') in conjunction with a standard light microscope (Leitz 'Ortholux') enabled carbon particles to be readily distinguished, even under low power, and their distribution patterns to be easily worked out: the incident light is reflected as bright specks by carbon particles but not by cells or pigment granules; these remain as black outlines on a dark background (Figures 1 and 2). Once the carbon has been localized in this way, examination of the area can then be made using transmitted light, without needing to move the slide or change any parts of the apparatus. The present finding thus extends the use of the 'Ultropak' apparatus described in detail by ROGERS[4], whereby light-reflecting silver grains in autoradiographs can be distinguished from dark material in the section.

For both autoradiographic work and carbon uptake studies we have found the most efficient 'Ultropak' objectives to be $\times 22$ and $\times 60$ (oil immersion). These give a good light signal on a dark background, whilst the high power dry objective ($\times 50$) gives too little contrast in light intensity between the granules and background to be useful. The low power ($\times 6.5$) objective gives good results provided a dipping cone is used[4].

Hopefully the system described will facilitate the examination of reticulo-endothelial tissues in other species, particularly where phagocytosis or trapping of injected particles is obscured by pigmentation or dark staining of tissues.

Riassunto. Il riconoscimento e la localizzazione di cellule del reticolo-endotelio, che fagocitano particelle di carbone, può talora essere difficile impiegando la convenzionale microscopia a luce trasmessa su sezioni istologiche colorante o in presenza di pigmento. D'altro canto, le particelle carboniose sono facilmente distinguibili impiegando la luce incidente, che e'riflessa da queste, ma non dalle cellule o da granuli di pigmento.

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[4] A. W. ROGERS, *Techniques of Autoradiography* (Elsevier Publishing Co., Amsterdam 1967).
[5] P. D. NIEUWKOOP and J. FABER, *Normal Table of Xenopus laevis* (Daudin) (North-Holland Publishing Co., Amsterdam 1967).
[6] This work was carried out during tenure of a Science Research Council studentship at the Zoology Department, University of Hull, England. The author is indebted to Dr. M. J. MANNING for her helpful advice and criticism.

Standardisation of Biological Ink for the Study of Vascular Injury in Inflammation

In certain strains of mouse, guinea-pig and rat, the occurrence of toxic effects with circulating Pelikan Ink may be sufficiently likely to make it desirable to attempt purification. Simple dialysis against water removes the toxicity, but the resulting ink is unreliable for labelling damaged blood vessels, especially in the delayed phase of injury. Furthermore the large volumes of ink required for adequate carbon deposition may cause cardiovascular

collapse and, if given immediately before an injury, supplementary labelling of vessels in the surrounding tissue. Effective preparations of a dialyzed but concentrated ink can however be made from Pelikan Ink No. C11/1431a (Günther Wagner, Hannover) which contains 0.1% carbon, fish glue, and 1.0% phenol as a preservative.

The carbon content may be determined gravimetrically on small samples by drying them overnight at 60 °C followed by acid hydrolysis of the other ingredients; the batch result is then reproducible to ± 5.0%. Estimation by specific gravity of diluted material by hydrometer is easier but the error is greater. Figure 1 shows the relation between carbon content and specific gravity (by 60 °F hydrometer) of ink samples diluted 1 part to 2.5 parts of distilled water. The hydrometric determination required 18.0 ml of ink, in a large boiling tube, held at 30 °C.

The carbon content of 25 batches of untreated material ranged from 80 to 150 mg/ml, with mean 110.5 ± 3.62 S.E. Dialysis reduces this by up to 24.0 mg/ml but centrifugation or filtration has no appreciable effect on it.

Pressure dialysis at 700 mm Hg (Figure 2) is done through a 'visking' membrane (C) supported on a perspex cylinder (A) with walls 0.7 mm thick and diameter of 20 mm, perforated with numerous 3.0 mm holes. The membrane is protected from rupture by an intermediate nylon sheath (B) but, as it bulges into the holes in the perspex it is sufficiently distorted to permit the passage of lower molecular weight components in the suspending glue. Continuous circulation of the ink by peristaltic pump prevents any deposition of sludge. After 48 h dialysis, the carbon suspension (now 250 to 300 mg/ml) is ready for filtration[1] or centrifugation[2], as required. A convenient preservative is para-chlor-meta-xylenol (4-chloro-3, 5-xylenol) 30 to 50 mg/100 ml ink.

The carbon suspension was given i.v. in doses ranging from 60 to 270 mg/kg body weight. Immediate vascular injury to guinea-pigs and rats was induced at randomly placed sites in the dorsal skin by 0.1 ml of saline containing up to 0.2 µg histamine, in the normal guinea-pig, or Locke's solution and 0.25 µg 5-hydroxytryptamine in normal rats. Thermal injury was applied 2 h before i.v. carbon from a disc heated to 54 °C[3] for 10 to 40 sec. Immunological injury was induced 20 h before carbon, by 1.0 unit of old tuin berculin in appropriately sensitized guinea-pigs.

After deposition of circulating carbon in the blood vessels, the carbon labelling in the lesions was estimated in cleared sections 100 µm thick; or by micro-photometry of the whole skin after fixation as the mean difference between readings through the centre of the lesion and that the adjacent normal skin.

With all these types of injury in the guinea-pig, labelling of both venules and capillaries was poor or absent with carbon doses of 105 mg/kg or less. It was always good with 125 to 170 mg/kg but larger doses did not improve it consistently. In rats the minimally effective dose was also 125 mg/kg but selective venular labelling occurred with 90 mg/kg.

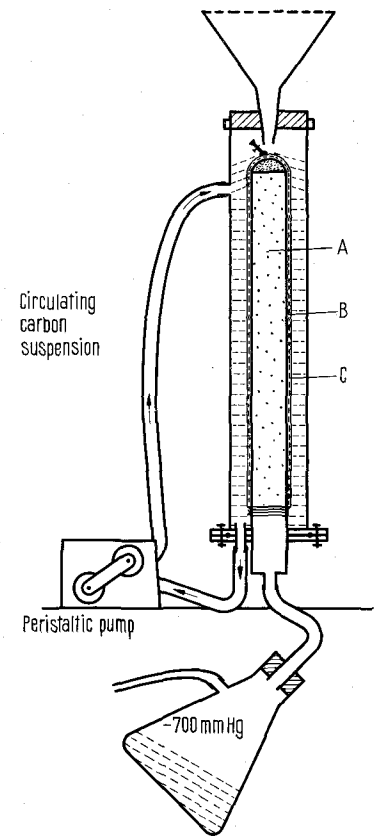

Fig. 2. Pressure dialysis apparatus. (Not to scale – for explanation see text).

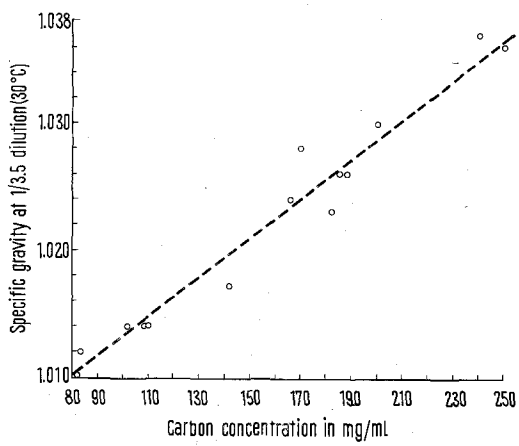

Fig. 1. Pelikan black samples. Density of material prepared for injection, diluted 1/3.5 in distilled water. Determined in 100 ml boiling tubes at 30 °C using a 60 °F urinometer.

Zusammenfassung. Es wird eine Methode zur Markierung geschädigter Blutkapillaren mit standardisierter und entgifteter Tusche entwickelt. Die Konzentrierung der Tusche durch Druckdialyse ergibt ein nichttoxisches Produkt mit einer wirksamen Mindestdosis Ruß von 120 mg/kg.

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