longer fixation (up to 24 h), even when it was followed by an extended washing period. The deleterious effects of long fixation were much more evident in pancreas, which is rich in autolytic enzymes, than in duodenum, where such enzymes are less active. This observation suggests that, after the initial rapid fixation reaction of CDI, further exposure leads to a breakdown of the zymogen granules, with a release of active proteolytic enzymes. Hence it would appear that the action of CDI on tissues is biphasic, the second phase being responsible for disruption of the zymogen granules, nuclei and other constituents. The chemical or physical basis of this biphasic effect remains a matter for speculation since the known side-reactions of CDI [1] could scarcely be responsible for any significant disruption of structure. In the first phase it is considered that cross-links form between tissue carboxyl and amino groups which are in close proximity and that these stabilize and fix the structure. In the second phase it is conceivable that, under the influence of the CDI, condensations occur between tissue groups which necessitate spatial rearrangement. The latter then brings about a degradation of structural integrity.

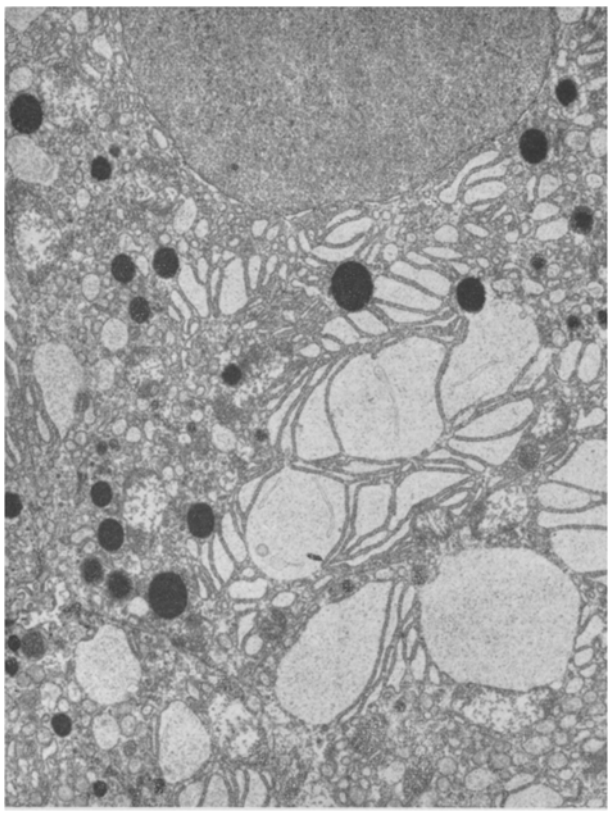

Fig. 5. Exocrine pancreas (mouse) fixed in freshly prepared formaldehyde for 1 h, washed for 21 h. Nuclei, zymogen granules and cytoplasmic membranes are well preserved but there is considerable distortion of the endoplasmic reticulum. ×5.250.

Zusammenfassung. In mit wasserlöslichen Carbodiimiden unter bestimmten Bedingungen fixiertem Gewebe können Polypeptidhormone immunfluoreszenzoptisch-nachgewiesen werden. Unter optimalen Bedingungen fixiertes Material ist auch zur Herstellung elektronenmikroskopischer Präparate vorzüglich geeignet. Carbodiimide werden deshalb als Fixierungsmittel in elektronenoptischen, immunzytochemischen Studien vorgeschlagen.

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[6] We wish to thank Dr. S. BLOOM for preparing the anti-porcine secretin used in this study. This work was supported by the Wellcome Trust and the Cancer Research Campaign.

The Use of Incident Light for Carbon Localization in Studies of the Reticulo-Endothelial System

Cells of the reticulo-endothelial system (RES) are morphologically heterogeneous and as such must be identified by the functional criterion of phagocytic activity. Colloidal carbon is usually used to identify these cells since the particles persist for a considerable period of time after injection, are of a convenient size and are easily recognized in some histological material. However, the use of certain staining techniques and the heavy deposition of pigment in some tissues may make carbon localization tedious when a conventional transmitted light microscope is used, and may demand a more efficient means of visualizing the injected particles.

Such problems were encountered in our laboratory during an investigation of the development and extent of the RES in an amphibian species, *Xenopus laevis* [1]. Animals of several stages were injected with colloidal carbon ('Dag 554', Acheson Colloids, Plymouth, England; or 'Pelikan C11/1431a', Günther Wagner, Hannover, Germany) then killed and fixed whole in Bouin's fluid at various time intervals thereafter. Paraffin-embedded sections of carbon-injected and normal animals were cut at 6 μm, stained routinely in haematoxylin and eosin and examined for carbon uptake. Haematoxylin and eosin was used since it gives excellent histological definition, but it obscures carbon uptake where darkly staining, closely packed cells are located, as in lymphocytic foci. Furthermore, pigment in the form of brown-black melanin granules was found widely distributed in tissues from both larval and post-metamorphic *Xenopus*: this pigment was present not only in the epidermis but also over all coelomic membranes, most blood vessels and in numerous glands, including the thymus, liver, spleen and kidney. The melanin granules are not restricted to melanophores, where they can be readily identified, but may also be found in extracellular locations or in phagocytes [2, 3].

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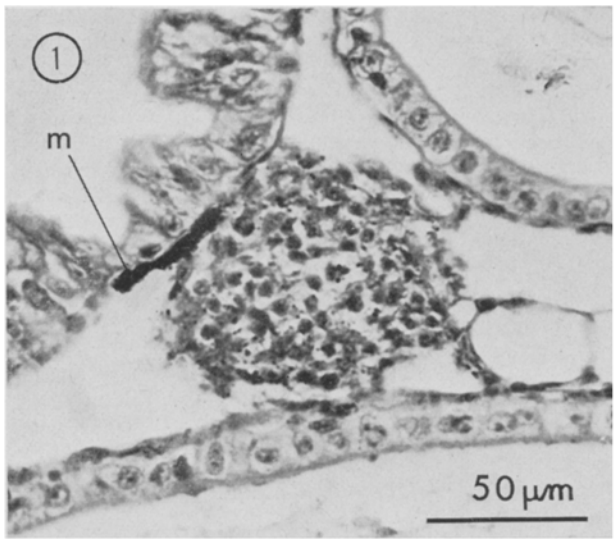
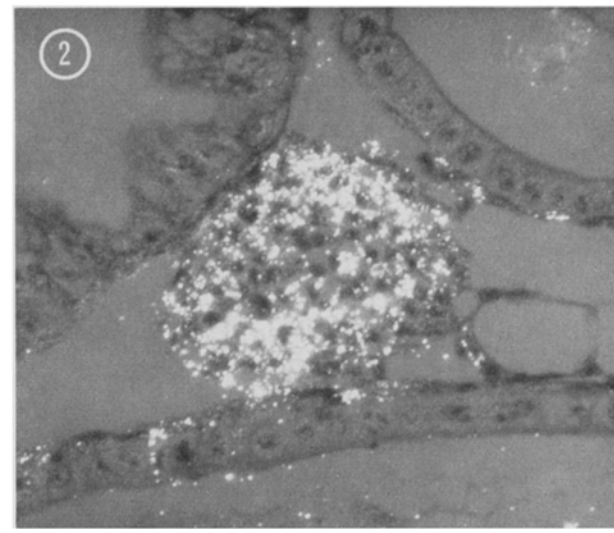
[3] L. KORDYLEWSKI, Bull. Acad. Pol. Sci. Cl. II Sér Sci. biol. *17*, 347 (1969).





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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[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