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Istochimica. — *Comparison of the histochemical properties of tissues embedded in Araldite and in paraffin* (*). Nota di ERMANNO BONUCCI, ENZO MORETTI e MARIANO SALVATORE PERGOLA, presentata (**) dal Corrisp. A. ASCENZI.

RIASSUNTO. — Ad eccezione delle metodiche basate sull'uso del nitrato d'argento, tutte le tecniche istochimiche controllate nella presente ricerca sono risultate negativamente influenzate dall'inclusione in Araldite. La rimozione della resina mediante metossido di sodio consente il ripristino di tutte le colorazioni, senza che le proprietà istochimiche dei tessuti ne risultino modificate.

In recent years, with the wide-spread use of the electron microscopy, the necessity has arisen to perform comparative histological and ultrastructural investigations on serial semi-thin and ultra-thin sections of the same tissue. For this purpose, many staining methods have been proposed for staining semi-thin sections obtained from plastic embedded tissues [1, 8, 9, 12–14, 17, 19, 21, 24–27, 29, 30]. Moreover, many histochemical techniques usually applied to sections from paraffin-embedded tissues have been adapted for staining sections from plastic blocks [4, 5, 10, 12, 13, 15, 17, 20, 22, 24, 26, 28]. Many of these methods give relatively satisfactory results if the embedding plastic is permeable, as in the case of methachrylates and Vestopal W [6, 7, 11, 27]. They are less suitable when applied to sections from Araldite and Epon embedded tissues because of the reduced permeability of these resins [1]. This hinderance can be overcome by removal of the embedding plastic before staining [1, 12, 18]. However, to our knowledge and as far as histochemical techniques are concerned, no investigations have been made to ascertain if the results of different staining methods differ in the presence of the embedding medium or in its absence.

The aim of the present investigation is to verify if the embedding plastic, and its removal, can change the results of histochemical staining techniques. It has been carried out by comparing sections from paraffin embedded tissues with corresponding sections from specimens embedded in Araldite.

MATERIAL AND METHODS

Small specimens from kidney, ileum and epiphyseal cartilage of 1-month-old mice were fixed in 4 % formalin buffered at pH 7.2 with phosphate buffer. Each specimen was divided into two parts; one was embedded in paraffin, and the other in Araldite.

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Two groups of semi-thin sections (0.5–2.0 μ) were obtained from Araldite embedded specimens. One group was stained without removal of the plastic; in the other group, staining followed removal of the plastic by treatment of sections with sodium methoxide [18]. Similarly, two groups of sections were collected from paraffin embedded specimens. Both groups were deparaffinized; one was stained directly, the second was treated with sodium methoxide to compare its staining properties with those of Araldite-free semi-thin sections.

The four groups of sections were simultaneously stained with the following methods: *a*) periodic acid–Schiff (PAS); *b*) periodic acid-silver nitrate-methenamine [17]; *c*) silver nitrate-methenamine [16]; *d*) Alcian blue, pH 1.8; *e*) colloidal iron, pH 1,8 [23]; *f*) hyaluronidase digestion (Sigma Chemical Company, 1 mg/1 ml in 0.9 NaCl), followed by staining with colloidal iron; *g*) amylase digestion (Merck's Lab., 1 mg/1 ml in 0.2 M phosphate buffer, pH 5.5), followed by PAS staining.

Results and Discussion

The staining properties of sections obtained from paraffin embedded tissues do not change in consequence of treatment with sodium methoxide. This clearly shows that, as far as the staining reactions tested in this investigation are concerned, sodium methoxide can be used for the removal of Araldite without changing the histochemical reactivity of the tissues. Following treatment with sodium methoxide, sections do not adhere to the slides as well as untreated sections.

No differences have been observed in the localization and distribution of PAS-positive structures in sections from Araldite blocks when compared with sections from paraffin embedded tissues. However, the basal membranes are only faintly stained when the plastic is present (Tav. I, fig. 1) and their PAS-positivity is evident only after removal of the Araldite (Tav. I, fig. 2). Amylase digestion is ineffective in plastic embedded sections, while glycogen is completely digested in Araldite-free semi-thin sections.

Results similar to those obtained with the PAS method are observed after periodic acid oxidation and silver nitrate-methenamine staining (Tav. I, figs. 3 and 4). However, with the latter method the positive structures are more discernable than with the former method, possibly because of their higher contrast. Basal membranes are clearly visible even without removal of the plastic (Tav. I, fig. 3). It seems, therefore, that periodic acid oxidation and staining with silver nitrate-methenamine is a very suitable technique for staining glycoproteins in Araldite sections with, or without, previous treatment with sodium methoxide. However, a slight staining of nuclei seems to be indicative of some degree of unspecificity.

The results of silver nitrate-methenamine without periodic acid oxidation are similar in all tissues examined. The removal of the plastic is without effect on the staining reaction.

The results of Alcian blue staining, on the contrary, are highly influenced by the presence of Araldite. A part from the mucous cells of the ileum, all other structures containing acid proteoglycans, including the cartilage matrix, are very faintly stained. Araldite removal only slightly improves the intensity of the reaction. It is interesting that, on the contrary, the calcified areas of the cartilage are rather deeply stained. This could be explained in several ways. It could be due to the acidity of the staining solution which might cause decalcification, thus allowing better penetration of Alcian blue molecules in the previously calcified matrix; or it could be explained by assuming that the inorganic substance in some way protects the acid proteoglycans of the calcified matrix during fixation, dehydration and embedding, while they are partially lost or damaged in the uncalcified areas [2, 3].

The results of the colloidal iron method are similar to those obtained with Alcian blue. In this case, too, tissue stainability is greatly reduced and in some cases almost completely abolished when Araldite is present. Only the calcified areas of the epiphyseal cartilage are stained, a result similar to that obtained with Alcian blue. After Araldite removal, colloidal iron stains all of the structures containing acid proteoglycans. The intensity of the reaction is greater than that obtained with Alcian blue, but lower than that expected on the basis of the high stainability of proteoglycans with colloidal iron in paraffin sections.

Independent from the removal of the plastic, hyaluronidase digestion is ineffective in reducing the colloidal iron stainability of intestinal goblet cells. On the contrary, the stainability of the cartilage matrix is greatly reduced, providing Araldite is removed previously.

The results of the present investigation show that the histochemical reactivity of tissues embedded in Araldite is not the same as that of tissues embedded in paraffin. The presence of Araldite decreases the stainability of glycoproteins with the PAS method and almost completely abolishes the stainability of acid proteoglycans with Alcian blue and colloidal iron. Moreover, the possibility of digesting the tissues with enzymes seems almost completely lost. On the contrary, the methods based on the use of silver nitrate as a stain, are not affected.

All of the staining reactions are restored or at least improved by the removal of the plastic. Moreover, the use of sodium methoxide does not change the histochemical properties of the tissues, at least those tested in the present investigation. In particular, after Araldite removal, Alcian blue and colloidal iron give the same results as those obtained in sections from paraffin embedded tissues, although the intensity of the staining reaction remains rather faint.

It can be concluded that the histochemical reactions tested in this investigation can be performed on semi-thin sections from Araldite embedded blocks if the resin is previously removed.

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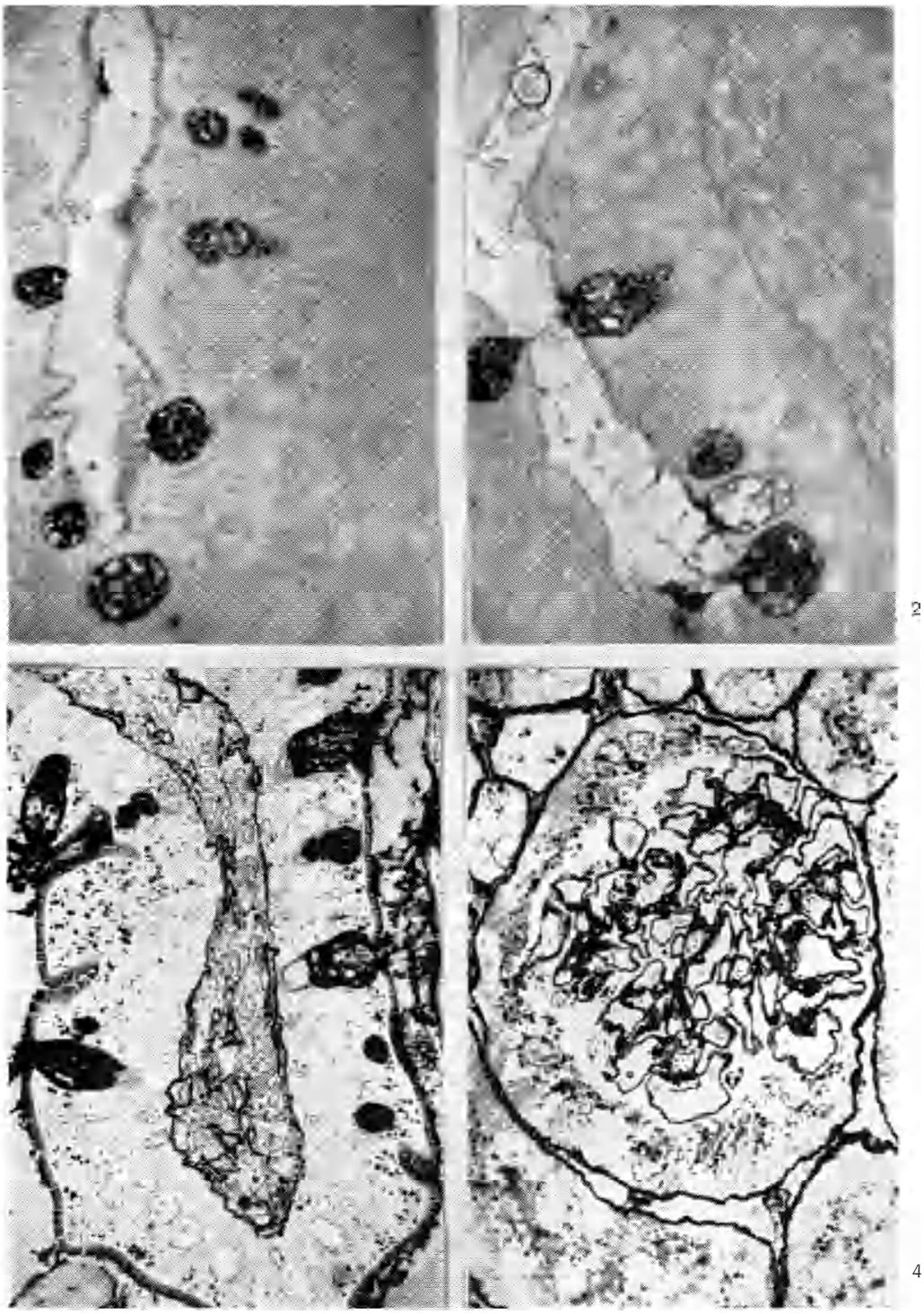
EXPLANATION OF PLATE I

Fig. 1. – Semi-thin section of ileum from a specimen embedded in Araldite. PAS staining without removal of the plastic. The goblet cells and the mucus coating the microvilli of the intestinal cells are stained; the basal membranes are not visible. ×1100.

Fig. 2. – Semi-thin section of ileum from a specimen embedded in Araldite. PAS staining after removal of the plastic. The basal membranes are evident. ×1100.

Fig. 3. – Semi-thin section of ileum oxidized with periodic acid and stained with silver nitrate-methenamine without Araldite removal. The goblet cells, the mucus coating the microvilli of the intestinal cells and the basal membranes are deeply stained. ×700.

Fig. 4. – Semi-thin section of a renal glomerulus oxidized with periodic acid and stained with silver nitrate-methenamine after removal of the plastic. The basal membranes are deeply stained. ×700.



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