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HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS ON
PERITONEAL LESIONS AFTER ENDOABDOMINAL INOCULATION
WITH A SINGLE DOSE OF POLYVINYL CHLORIDE (PVC) IN
THE RAT

V. Costa^{bb}

and N. Frongia^{bb}

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HISTOLOGICAL AND ULTRASTRUCTURAL OBSERVATIONS ON THE PERITO-
NEAL CHANGES INDUCED BY THE ENDOABDOMINAL INOCULATION OF A SIN-
GLE DOSE OF POLYVINYL CHLORIDE POWDER (PVC) IN RATS.

SUMMARY — *The inoculation into the abdominal cavity of rats of a single dose of PVC powder causes a granulomatous non phlogistic reaction of the peritoneum that histologically and ultrastructurally appears pretty typical and perhaps exclusive for this substance.*

Furthermore it is pointed out that after seven months from the inoculation no changes even ultrastructural indicative of a possible neoplastic evolution were observed in the examined rats.

INTRODUCTION

As part of a broad programme of research into the possible toxicity of polyvinyl chloride which has already been in progress for some time at our Institute, and whose results have been published in part (N. Frongia, A. Spinazzola, A. Bucarelli, 1972 and 1974; E. Dessy, A. Bucarelli, A. Spinazzola, 1976), we now report morphological studies using optical and electron microscopy in rats inoculated intraperitoneally with a single dose of polyvinyl chloride powder and sacrificed seven months later.

^{bb} Second Chair of Anatomy and Pathological Histology, Cagliari University

^{bb} First Chair of Anatomy and Pathological Histology, Cagliari University

In spite of the by now well known conclusions on the oncogenic power of the monomer (P.L. Viola, A. Bigotti, A. Caputo, 1971; C. Maltoni, G. Lefemine, 1975; L.B. Thomas, H. Popper, 1975; etc) - on which no doubt seems now to persist, so much so that in the working environment its concentration has been reduced to extremely low levels, deemed harmless to workers - there is no corresponding agreement, even today, on the absolute harmlessness of polyvinyl chloride, as would seem to be demonstrated - after a study by B. Szende et al (1970) on human "pneumoconiosis" following inhalation of polyvinyl chloride dust - by the results obtained from experimental studies up to now using various administration routes (B.S. Oppenheimer et al, 1955; F.J.C. Roe et al, 1967; J. Popow, 1969, etc) and, very recently, by the report of a case of diffuse carcinoma in the mouth of a subject of just 22 years who used to "chew" polyvinyl chloride plastic material (C.L. Casterline & colleagues, 1977).

The intraperitoneal route we used in this study, which is being used for the first time in this field, seems perhaps to offer a more convenient way to evaluate local tissue reaction, and above all, given the very large absorption area, to assess possible effects of vinyl polymer away from the inoculation point.

MATERIALS AND METHODS

The experiments were carried out on 30 male Wistar white rats, with initial weights 180-200g, which were given a single intraperitoneal injection of 0.5g of pure PVC powder, diluted in 2cc of sterile physiological solution. This powder was examined by electron microscope in order to establish its exact granular composition, using the following method: small drops of a suspension, in distilled water, of polyvinyl chloride powder in decreasing concentrations were placed on gratings coated with a thin film of Formvar, and, after drying, shadowed and examined in the electron microscope. The powder was found to consist mainly of more or less spheroidal granules between $0.1 - 0.9 \mu$ in diameter.

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In addition, numerous portions were taken from the liver and from some nodules or plaques found on the epiploic, hepatic and splenic surfaces, some of which were used for electron microscopy. These latter were reduced to a thickness not greater than 1 mm, fixed in Karnovsky liquid for 1 hour and, after washing with cold 0.1 M Millionig buffer, post-fixed for 1½ hours in cold 1.5% OsO₄, buffered with Millionig, then dried and treated for setting in Epon.

Sections obtained using the Porter - Blum ultramicrotome, were treated with saturated aqueous uranyl acetate solution and by the Reynolds lead citrate method, then examined on the Phillips 300 electron microscope at our University's electron microscopy centre.

Semifine sections, 500-600 Å in thickness, were stained with Giemsa liquid or 1% toluidine blue and others were treated with Sudan III, then observed on the optical microscope.

Lungs

General histotectonics quite easily recognisable, even though generally modified by marked thickening of the peri- and intra-lobular septa by abundant lymphomonocytic exudation and concomitant moderate proliferation of histial elements, many of them more or less activated.

Another interesting observation concerns the peri- and para-bronchial lymphatic formations, quite voluminous and developed, especially at the level of the large bronchi, where considerable quantities of elements are observed, mainly lymphocytic, which, beside forming broad sheaths covering the whole bronchial wall, protrude into the lumen in the form of large nodules, raising, detaching and sometimes enveloping the epithelial lining.

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Vascular neoformation is rare; there were no exudative cells, nor foci of necrosis.

In the semifine sections the structure of these cells is better discernible and two aspects appear which could not be seen in the histological sections; the first relates to the fact that the granulosity and the vacuolisation of the cytoplasm in the above cells is due to the presence of minute spherules which seem to fill the cytoplasm almost completely; the second is the fact that numerous narrow vessels of capillary type are readily discernible (fig 3).

Electron microscopy allows full elucidation of the nature of the spherules which occupy the greater part of the histial cell cytoplasm : they are aggregates or small groups of PVC granules, whose profile is modified, compared with that of the powder granules inoculated, appearing mainly ovoid or spherical in shape, doubtless through modifications induced by the procedures used in setting the specimen in Epon. These granules are few in the context of the cytoplasm, or packed in large aggregates or small groups, or even isolated; and sometimes, especially in the last case, seem to relate to the nuclear membrane (figs 4 & 5). The granules are almost always surrounded by ribosomes and often have about them hyperosmiophile areas of radial conformation, which, at optimum resolving power, are seen to consist of microfilaments and microtubules; where the granule is absolutely adjacent to the nucleus, the concentrations of the fundamental cytoplasmic substance give the impression that bridges or fusions with the nucleus itself are forming : in reality, on close inspection, it can be seen that the nuclear membrane is intact. (fig 6).

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The number of mitochondria in the different cells is hard to assess, since the majority of the cytoplasmic structures are swamped by the enormous number of PVC granules enclosed within the cell itself : the mitochondria present appear varied in form, generally swollen, with partial or total disappearance of the cristae, sometimes with interruptions in the external membrane (fig 7).

No particular changes are observed in the nucleus, whose chromatinic inheritance seems also to remain normal.

Liver

Lobular histotectonics substantially unchanged; the hepatocytes are rather voluminous, especially because of a certain abundance of cytoplasm, which appears uniformly swollen, finely granular, and often vacuolised around the nucleus; binucleate hepatocytes are common, with abundant cytoplasm, affected by the same modifications observed in the other hepatic cells.

In the sections coming from samples taken in the perihepatic region and, more precisely, from the plaques or nodules firmly adhering to the surface of the organ, a neoform tissue is seen, apparently of mesenchymal nature, situated on the fibrous covering of the liver, detached at some points, and which, depending on the septa of the fibrous capsule itself, penetrates the structure of the organ (fig 8).

This tissue is composed of cells of histial type, randomly aggregated, voluminous, round or globular, with relatively small nuclei, round or ovoid, nucleated, centrally and peripherally located, and with very abundant, slightly acidophilic, finely granular cytoplasm. Vascular neoformation is rare. In this tissue, which is traversed by fine bundles of collagen fibres, neither elements of cellular exudation nor foci of necrosis are found. The hepatic

parachyma adjacent to this neoform tissue shows no histotectonic alterations, and the hepatocytes show structural modifications quite analogous to those observed in more distant parts of the rest of the parenchyma.

In the semifine sections the structure of the neoform tissue is even better distinguished : its cells have cytoplasm full of minute spherules; the vascular neoformation seems more apparent compared with what could be seen in the traditional histological sections; the histological findings are in substance perfectly superimposable on those already described in relation to the neoform tissue found on the perisplenium (fig 9).

Semifine sections of the hepatic parenchyma show the swelling of the hepatocytes even better, and provide the exact size and conformation of the apparent areas of perinuclear rarefaction; in those treated with Sudan III a structural modification of considerable interest is found, namely the presence of fat droplets situated at the periphery of the cell, sometimes in considerable number, without any particular distribution in the context of the hepatic lobule, occurring both in centro-medio-lobular and peripheral locations.

Electron microscopy, finally, removes all histological doubt, showing irrefutably that the more or less broad perinuclear halo, observed under the optical microscope, is due to the presence of glycogen, both in the form of abundant small granules (β particles) and less frequently in larger aggregates (α particles), sometimes forming cluster or rosette shapes. The nucleus is voluminous, fairly homogeneous, and sometimes includes one or more nucleoli, often of reticulated form. The nuclear membrane appears double, with a normal perinuclear space. Mitoses are not observed. The mitochondria, spherical or rodlike in shape, show almost normal structures, and the endoplasmic reticulum is quite well developed.

Ultrastructural study confirms moreover the information obtained from the semifine sections treated with Sudan III : slightly osmiophile vacuoles are observed, variously distributed in the cytoplasm of the hepatocytes; in addition many binuclear cells are observed, which display structures analogous to those of the other hepatocytes described above, with the exception of course, of the presence of two large nuclei; in these cells also abundant glycogen is observed.

Visceral Peritoneum

In the adipose tissue of the epiploon small areas are seen, of varied shape and size, of a neoform tissue very rich in cells of apparent mesenchymal nature, sometimes surrounded by a fine capsule of fibrous connective tissue; the structure is perfectly superimposable on that of the perihepatic and perisplenic neoform tissue (fig 10). In fact this is a tissue made up of voluminous, globular cells, without any particular distribution, having particularly abundant cytoplasm, finely granular, and with a relatively small nucleus. In this location also, the cellular proliferation is accompanied by occasional vascular neoformation, and no elements of cellular exudation or foci of necrosis are observed.

SUMMARY AND CONCLUSIONS

Inoculation of PVC powder into the rat abdomen has produced modifications both at the peritoneal level and at a distance even in extra-abdominal organs. Ultrastructural research has, at least for the moment, been concentrated on the peritoneal lesions, although the hepatic, splenic and pulmonary results even from the histological work, show a pattern of undeniable interest, so that they will be the subject of further work and more detailed precise study.

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In the peritoneum we observed a reactive process of essentially granulomatous type, in the form of granules, nodules or plaques of greyish-white colour, dispersed on the epiploic and hepatosplenic serous membrane and partially affecting also the liver itself: these are agglomerates of histial cells, voluminous, globular and with rather abundant cytoplasm, full of PVC granules, without appreciable inflammatory or necrotic phenomena, but with a certain regular vascular neoformation. Electron microscopy confirms that the spherules of vinyl polymer injected are enveloped in the histial elements, in large aggregates, small groups, or isolated, and appear slightly modified compared with their original form, probably because of the Epon setting technique; when isolated in the cytoplasm, these granules are surrounded by many ribosomes, as if they were contained in dilatations of the reticulum. Moreover around the PVC spherules there is nearly always an almost radial thickening of the basic cytoplasmic substance, in the form of microtubules and microfilaments which, when the tubules are in a perinuclear location, create striking impressions of some kind of link with the nucleus itself; this is an observation which undoubtedly merits more precise study and detailed evaluation, taking account above all of the apparent integrity of the nuclear membrane. The mitochondria often appeared swollen, with a scarcity of cristae, and the external membrane sometimes interrupted.

Concerning the lesions away from the inoculation zone, in the liver an increase in glycogen was observed, together with phenomena of fatty degeneration, as was seen histochemically in semifine sections and in partial studies on the electron microscope, and a considerable numerical increase in binuclear hepatocytes.

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granules on the cytoplasmic structures of the histio-phagocytic cells, and neither the rich vascular neoformation reported by many authors in vinyl chloride poisoning as a prelude to lesions of angiosarcomatous type, nor any cytocariological changes which could in some way be considered indicative of an actual or potential anaplastic process, have been observed.

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Fig. 6 - Granuli di cloruro di polivinile (PVC) in stretta vicinanza della membrana nucleare apparentemente intatta di una cellula istiale; spiccato inspessimento della sostanza fondamentale, in forma di microtubuli e microfilamenti (frecche), attorno ai granuli stessi. N = nucleo. ... 51.000 X.

Fig 6 Polyvinyl chloride (PVC) granules in close proximity to the apparently intact nuclear membranes of a histial cell; pronounced thickening of the base substance, in the form of microtubules and microfilaments (needles), around the granules themselves; N = nucleus

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