Interaction of Dimethysulfoxide and Ascorbic Acid on Fibroblasts of Rats. (32443)

J. M. Archer, K. B. Shilkin, J. M. Papadimitriou and M. N-I. Walters.
(Introduced by N. F. Stanley)
Department of Pathology, University of Western Australia, Perth

Despite the current wide employment of dimethylsulfoxide (DMSO) in many scientific disciplines, its basic mechanism of action at the cellular level is ill-understood. It has been shown that DMSO in equivalent concentrations to those used in human therapy damages striated muscle cells(1) and hepatocytes(2) of rats. The endoplasmic reticulum of these cells dilates to form vacuoles; agents which are believed to stabilize cell membranes and so prevent toxic cellular damage are ineffective in the case of DMSO(2). Similar ultrastructural changes in the endoplasmic reticulum have also been observed in fibroblasts in the wounds of scorbutic guinea pigs(3,4). A study was therefore undertaken to assess the relationship of DMSO and ascorbic acid in the production and prevention of ultrastructural abnormalities in the fibroblasts of healing wounds.

Materials and methods. Two parallel linear incisions 2.5 cm in length were made on the shaved abdomen of 16 ether-anaesthetised Wistar rats (200-250 g) through the skin and subcutaneous tissue. The animals were divided equally into 4 groups; all groups had skin biopsies taken after 5 days. Group 1 received an application to the wound site of 90% DMSO 1 hour before biopsy. Groups 2 and 3 received intraperitoneal doses of 100 mg ascorbic acid after 48 hours and again at 7½ hours and 1½ hours before biopsy. In addition DMSO was applied in Group 2 as described above for Group 1. Group 4, which served as controls, was given neither ascorbic acid nor had DMSO applied. A total of 16 healing wounds was examined. Small blocks from these wounds were rapidly immersed in cold osmium tetroxide buffered in S-collodine. After dehydration in graded solutions of ethanol, the tissue was embedded in araldite. Sections were cut on a Huxley ultramicrotome, stained with lead, and examined on a JEM T6 electron microscope at an accelerating voltage of 60 KV. In addition, fibroblasts from DMSO-treated cremaster muscles of Wistar rats were examined as previously described(1).

Results. Fibroblasts from rats receiving neither DMSO nor ascorbic acid (Group 4) or only ascorbic acid (Group 3) appeared normal. Rats receiving DMSO only (Group 1): All fibroblasts from wounds of animals to which DMSO had been applied exhibited some ultrastructural anomaly. The cytoplasm of these cells was swollen and electron lucent. The cisternae of the endoplasmic reticulum were dilated and contained fine fibrillar material (Fig. 1). The cisternal membranes sometimes showed loss of ribosomal material. Mitochondria were also swollen and had fewer and less prominent cristae. The clusters of cytoplasmic ribosomes were diminished. No obvious abnormality of collagen fibrils was seen.

Striated muscle fibres were also swollen and displayed loss of the myofibrillar elements. Mitochondria were diminished in number and those present were swollen. Remnants of the sarcotubular system exhibited gross dilatation producing large electron lucent vacuoles measuring 3-8 μ in diameter (Fig. 2).

In rats receiving both DMSO and ascorbic acid (Group 2) cytoplasmic swelling and dilatation of cisternae of the endoplasmic reticulum were again seen. However, these were much less severe (Fig. 3) than in the group receiving DMSO alone. Myocytic vacuoles were not present although swelling of striated muscle fibres, myofibrillar loss and mitochondrial swelling were present.

Discussion. The ultrastructural changes produced in rat fibroblasts by DMSO in these experiments occur mainly in the endoplasmic reticulum. The cisternae of this organelle dilate and assume a clear vacuolated appearance. There are fewer clusters of ribosomes scattered throughout the cytoplasm, which is

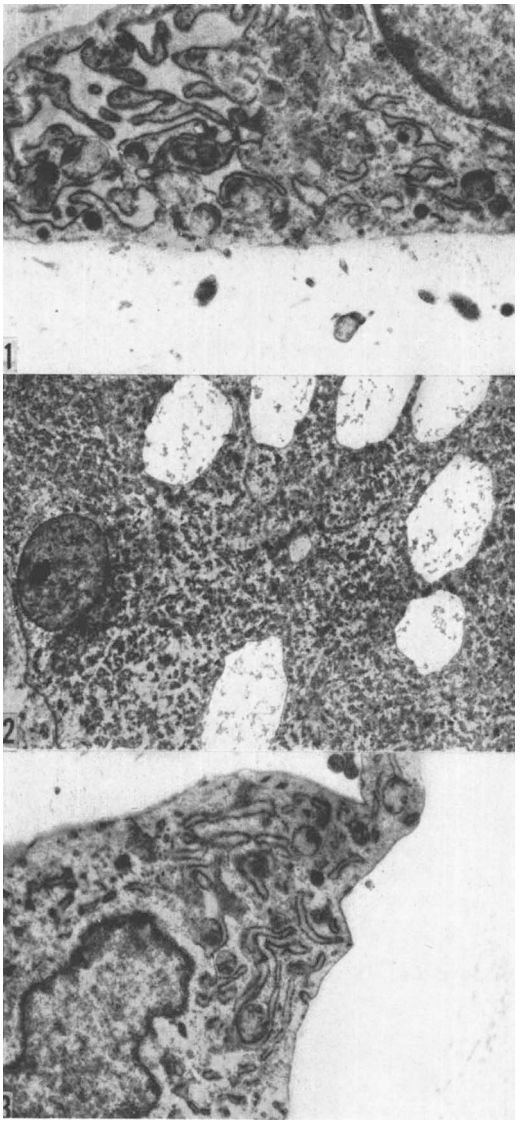

FIG. 1. Fibroblast from wound treated with DMSO. The cytoplasm is swollen and electron lucent, mitochondria are enlarged, the cisternae of endoplasmic reticulum are dilated and free ribosomes are diminished in number. Magnification 8695.

FIG. 2. Striated muscle fibre from rat after DMSO application. The cell is swollen and there is myofibrillar disarray. The elements of the sarcotubular system are grossly dilated. Magnification 8418.

FIG. 3. Fibroblast from animal receiving both DMSO and ascorbic acid. Although the cytoplasm is swollen and mitochondria enlarged the cisternae of ergastoplasm are only slightly dilated. Magnification 2579.

most likely swollen by the accumulation of water. The endoplasmic reticulum is similarly deranged in fibroblasts in wounds of scorbutic guinea pigs. Here the ribosomes lose their orientation to the cisternal membrane and become scattered(3,4). Collagen production is also impaired in this deficiency disease.

Insofar as the ribosome—endoplasmic reticular system is the morphologic counterpart of the protein synthetic pathway, the disturbance of these structures in scorbutus explains the cytologic basis for the lack of manufacture of adequate collagen. Furthermore, after treatment with ascorbic acid there is a reversion to the normal organellar appearance concomitant with the synthesis of new collagen(4). In our experiments, treatment with ascorbic acid also reduces the endoplasmic reticular abnormalities produced by DMSO. The nature of DMSO-induced changes in relation to collagen synthesis has not been assessed in this situation. Collateral evidence raises the possibility that such DMSO-damaged fibroblasts would have impaired protein synthetic ability. Using analyses of collagen formation in rats with experimental granulomata as an index it has been shown that application of DMSO results in lesions containing less of the components of collagen than controls(5). Likewise, the amount of collagen determined by hydroxyproline content, in human radiation scar tissue was less in areas treated by DMSO than in similar untreated areas. Collagen fibrils that had formed before application of DMSO showed no ultrastructural derangement. Although the possibility exists that the decreased content of collagen could be the result of degradation caused by DMSO, the evidence for this is lacking(6). Other human fibroproliferative disorders have benefited from treatment with DMSO(7,8,9). It may well be that in these conditions the improvement results from damage to fibroblasts which cannot then synthesise collagen; the balance of synthesis and degradation is altered to favour the latter.

The nature of the effect of ascorbic acid in partially inhibiting the damage caused by DMSO is unclear. It has been shown that ascorbic acid is concerned with electron transport and is transferred in microsomes(10). DMSO, a dipolar aprotic chemical which

forms powerful hydrogen bonds(11), may interfere with these intracellular mechanisms of electron transfer and this physico-chemical disorganisation may result in the morphological changes described. Ascorbic acid, on the other hand, may partially reverse this effect and so limit the changes due to DMSO.

The large vacuoles in muscle fibres treated with DMSO resemble those arising from sarcotubular elements in hypokalaemic periodic paralysis(12,13). Impairment of the function of this organelle would interfere with muscle contraction and may account for the relief of pain in musculoskeletal disorders described after the application of DMSO(14).

Summary. DMSO causes ultrastructural changes in rat wound fibroblasts of dilation of the endoplasmic reticulum and diminution of ribosomes. Such features are similar to those described in the wound fibroblast of scorbutic guinea pigs. Ascorbic acid partly prevents DMSO damage. DMSO probably acts by interfering with cell membrane electron transfer systems and this may be the basis of its beneficial effect in clinical situations.

1. Walters, M. N-I., Papadimitriou, J. M., Shilkin, K. B., Exp. Molec. Pathol., 1967, v6, 106.
2. Shilkin, K. B., Papadimitriou, J. M., Walters, M. N-I., Aust. J. Exp. Biol. Med. Sci., 1966, v44, 581.
3. Peach, R., J. Ultrastruct. Res., 1962, v6, 579.
4. Ross, R., Benditt, E. P., J. Cell. Biol., 1964, v22, 365.
5. Gries, G., Bublitz, G., Lander, J., Ann. N. Y. Acad. Sci., 1967, v141, 630.
6. Frommhold, W., Bublitz, G., Gries, G., ibid., 1967, v141, 603.
7. Scherbel, A. L., McCormack, L. J., Poppo, M. J., Cleveland Clinic Quart., 1965, v32, 47.
8. Scherbel, A. L., McCormack, L. J., Layle, J. K., Ann. N. Y. Acad. Sci., 1967, v141, 613.
9. Persky, L., Stewart, B. H., ibid., 1967, v141, 551.
10. Staudinger, Hj., Krisch, K., Leonhauser, S., ibid., 1961, v92, 195.
11. Parker, A. J., Quart. Rev. Chem. Soc., 1962, v2, 163.
12. Shy, G. M., Wanko, T., Rowley, P. T., Engel, A. G., Exp. Neurol., 1961, v3, 53.
13. Howes, E. J., Price, H. M., Pearson, C. H., Blumberg, J. M., Neurology, 1966, v16, 242.
14. Rosenbaum, E. E., Herschler, R. J., Jacob, S. W., J.A.M.A., 1965, v192, 309.

Received July 17, 1967. P.S.E.B.M., 1967, v126.

Effect of Ozone on Lipid Peroxidation in the Red Blood Cell.* (32444)

BERNARD D. GOLDSTEIN AND OSCAR J. BALCHUM
University of Southern California School of Medicine, and Los Angeles County General Hospital, Los Angeles

Brinkman *et al* have shown that breathing ozone potentiates *in vitro* radiation provoked sphering of human erythrocytes(1). This, and other work(2,3), has led to the hypothesis that ozone is radiomimetic and results in free radical formation(4). Lipid peroxidation has also been found to be produced by radiation and is thought to result in the formation of free radicals(5,6). This suggests the possibility that the deleterious biological effects of ozone are mediated by lipid peroxidation caused by the interaction of ozone with the double bonds of unsaturated fatty acids.

To investigate this hypothesis, an *in vitro* system employing human erythrocytes as a target organ was utilized in preference to animal lungs, because of the difficulty in comparing control non-exposed lungs with exposed lungs heavily infiltrated with peripheral leukocytes. Erythrocyte osmotic fragility was measured as an indication of spherocyte formation.

Methods. Heparinized or defibrinated human blood was washed in isotonic phosphate buffered saline pH 7.4, the buffy coat removed, and the cells resuspended in buffer in a 4% concentration. By means of fritted disc

* This work was supported by the National Center of Air Pollution Control, USPHS Contract PH 86-62-162, and in part by the Hastings Foundation, Univ. of Southern California School of Medicine and by National Institutes of Health Grant AP 00606-01.