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.

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

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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.

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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

bubblers, one aliquot of each sample was subjected to 40 parts per million (ppm) ozone in room air from a Mast ozone generator; the second aliquot to room air only; and, a third portion was allowed to stand without bubbling. All procedures were carried out at room temperature and terminated at 2 hours. Osmotic fragility was measured in phosphate buffered saline pH 7.4; lipid peroxidation was assessed by the 2-thiobarbituric acid (TBA) method(7) with minor modifications. This measures a breakdown product of lipid peroxidation, malonaldehyde, and probably underestimates total lipid peroxidation by a factor of 20(8). The TBA procedure was performed on each erythrocyte suspension after completion of the exposure period. In order to ascertain whether the TBA reactants were intimately associated with the erythrocyte or were released into the supernatant, following some of the exposures an aliquot of the erythrocyte suspension was centrifuged, the supernatant removed, and the red cells washed three times in phosphate buffered saline. TBA reactants were then measured in the original suspensions, the erythrocyte free supernatants and the washed cells.

Results. In this *in vitro* erythrocyte system, exposure to ozone was associated with an increase in lipid peroxide formation ($p < .001$) (Table I) which could be detected within 10

TABLE I. Lipid Peroxidation in Erythrocyte Suspensions. mμmoles malonaldehyde per gram hemoglobin: average of 16 studies.

Non-exposed control	Room air × 2 hr	40 ppm ozone × 2 hr
12 2	11.8	51.1

TABLE II. Lipid Peroxidation in Washed Cells. mμmoles malonaldehyde per gram hemoglobin: average of 9 studies.

Non-exposed control	Room air × 2 hr	40 ppm ozone × 2 hr
13.2	11.7	20.4

minutes. Neither depletion of substrate by prior incubation of the erythrocytes in buffer at 37°C for 2 hours, nor addition of glucose before ozone exposure resulted in a marked difference in the amount of lipid peroxidation after 2 hours of ozone exposure. The majority

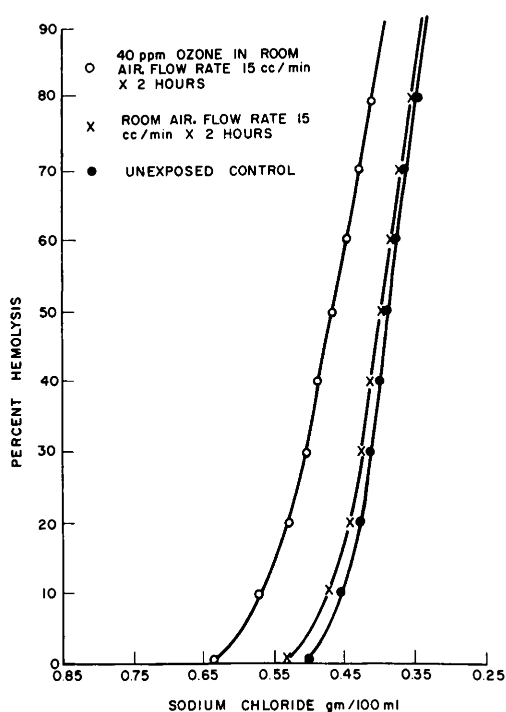

FIG. 1. Osmotic fragility average of 16 studies.

of TBA reactants were found in the supernatant. However, a significantly ($p < .01$) higher level of TBA reactants was discovered in cells washed after ozone exposure as compared to the washed unexposed controls suggesting that some of the TBA reacting substance remains intimately associated with the intact erythrocytes.

Erythrocytes exposed to 40 ppm ozone in room air have an increased osmotic fragility compared to those exposed in a similar manner to room air or allowed to stand (Fig. 1). Statistical analysis of the 50% hemolysis level reveals that the difference is significant at $p < .005$.

Discussion. Increased osmotic fragility in congenital spherocytosis or in erythrocytes exposed to sulfhydryl inhibitors is thought to be due to partial loss of membrane through the release of small membrane fragments followed by reapproximation of the remaining membrane producing an intact erythrocyte with decreased surface area. This eventually results in a change in red cell shape from that of the normal biconcave disc to the relatively fragile sphere(9,10,11). Stained

preparations of the red cells exposed to ozone confirm the formation of spherocytes. Erythrocyte fragmentation may be due to direct membrane damage, short of overt hemolysis, for which the normal reparative cell processes cannot compensate; or, to interference with the intracellular metabolic processes necessary for membrane integrity. The increase in TBA reacting substances suggests that the former mechanism is operative with peroxidation of unsaturated fatty acids contained in the cell membrane and the formation of free radicals perhaps causing damage to neighboring protein. However, interference with biochemical pathways necessary for the maintenance of the cell membrane cannot be excluded.

It is interesting to speculate that the hypothesized involvement of lipid peroxidation in the aging process(12) is related to the frequent observation of generalized aging in animals chronically exposed to ozone(4). A further speculation is that if emphysema be considered a normal aging change, the increase in chronic respiratory disease in areas of urban air pollution found in epidemiologic studies is due to the acceleration of aging in the lung by air pollutant-induced lipid peroxidation.

Summary. In vitro exposure of erythrocytes to ozone resulted in an increased osmotic fragility associated with the formation of TBA reactants. This suggests that lipid peroxidation may be involved in the mechanism of ozone toxicity.

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Caseinolytic and Fibrinolytic Activities of Human Plasma in Starch Block Electrophoresis. (32445)

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Depending upon the circumstances of purification profibrinolysin (plasminogen) has been reported to appear in a number of different plasma fractions. For example, plasma fractionated on DEAE-cellulose columns yields the bulk of the profibrinolysin near the β-globulin fraction but traces appear also in the γ-globulin fraction(1). Profibrinolysin, purified by the method of Kline(2), has been found by several investigators to move toward the cathode in starch gel electrophoresis(3,4, 5).

Slotta and Gonzalez(6), using starch gel electrophoresis in the presence of epsilon-aminocaproic acid (E-ACA), separated purified profibrinolysin into 6 different bands of active protein. Cohly and Shulman have reported that human euglobulin migrates within the β- and γ-globulin region on cellulose acetate membrane in veronal buffer at pH 8.6(7). The heterogenous character has also been observed when profibrinolysin is subjected to chromatography on various cellulose preparations(8). It is apparent that the properties of purified profibrinolysin relate to the methods by which it was ob-

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