by Skou[2,6]. These results were also obtained in similarly prepared rat, guinea-pig and rabbit-heart "microsomal" fractions[5]. Storage at −5° effects a destruction of the Mg^{2+}-stimulated enzyme thereby revealing the stimulation due to $Na^+ + K^+$, which stimulation can be inhibited by ouabain.

The data presented suggest that the fractions described as having ATPase activity which could be stimulated by the addition of $Na^+ + K^+$ and inhibited by ouabain, consist essentially of endoplasmic reticulum. The possibility of small fragments of cell membrane being associated with the microsomal fraction in these preparations cannot be excluded. The contribution, however, of this "contaminant" to the total ATPase observed in the present study would be very low.

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Effect of oxygen and *N*-ethylmaleimide on the inactivation of ribonuclease by γ-radiation

N-ethylmaleimide is a member of the group of thiol reagents (including iodoacetic acid and phenylmercuric acetate) which are able to sensitize bacteria to the lethal action of ionizing radiation when present during irradiation[1–3]. It was suggested[2,3] that it might react with –SH groups (or possibly –S· free radicals) which result from the radiation-induced breakage of –S–S– bonds, necessary for the functional state of some proteins. Combination of *N*-ethylmaleimide with either of the sulphur

atoms could prevent the -S- -S- bond reforming and result in the loss of the biological integrity of the molecule.

If the breaking and reforming of -S--S- bonds can be caused by the indirect action of radiation (and the work of CAVALLINI and his colleagues[4] suggests that this is the case) then it should be possible to demonstrate sensitization *in vitro* using a dilute aqueous solution of a suitable enzyme. The present paper records some experiments with ribonuclease (EC 2.7.7.16) which was chosen because it has no -SH groups which might interfere with the interpretation of results, and it has four -S—S- bonds, some of which are certainly necessary for enzymic activity[5].

Similarities in the effect of *N*-ethylmaleimide and oxygen on bacterial radiosensitivity have been pointed out[3] and so the effect of oxygen was also tried on the RNAase system, although previous workers[6,7] had failed to demonstrate any effect of oxygen.

An 0.01% aqueous solution of pancreatic RNAase (4 × crystallized, 40 Kunitz units/mg, L. Light and Co. Ltd.) was irradiated at room temperature with gamma rays from a ^{60}Co source (dose rate about $2.6 \cdot 10^5$ rad/h). Unless otherwise stated air, oxygen-free nitrogen or various mixtures of these gases, were bubbled rapidly into the solution through an open-ended tube for 6 min before and throughout irradiation. In some runs *N*-ethylmaleimide (Aldrich Chemical Co.) was present at various concentrations. Samples were withdrawn at zero time and after various doses of irradiation. Irradiated solutions and non-irradiated controls were diluted 1 in 10 into acetate buffer (pH 5) and assayed by the method of ANFINSEN *et al.*[8] in which is measured the optical absorption at 260 mμ of the depolymerized RNA which cannot be precipitated by uranium acetate in $HClO_4$ after the nucleic acid has been treated by the enzyme.

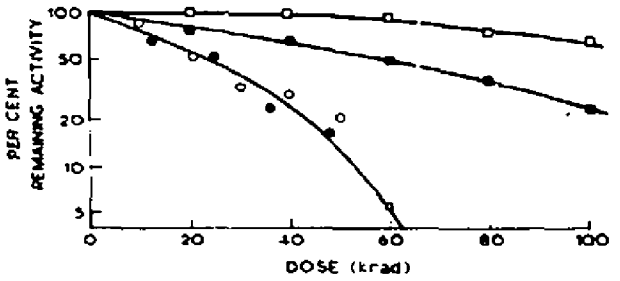

Fig. 1. Inactivation of RNAase by γ-radiation. ●, anoxic (bubbled); □, anoxic (bubbled) with 0.5 mM *N*-ethylmaleimide; ■, aerated (bubbled); ○, aerobic (unbubbled).

From the data in Fig. 1 it can be seen that *N*-ethylmaleimide ($5 \cdot 10^{-4}$ M) showed a considerable protective effect under anoxic conditions (*N*-ethylmaleimide in the absence of radiation did not affect the activity of the enzyme). A similar curve was observed with 0.5 mM iodoacetic acid. Oxygen (when air was bubbled through) also exerted a marked protective affect but this was not observed with unbubbled aerobic solutions. Fig. 2 shows the variation in protective effect with concentration of *N*-ethylmaleimide and bubbled oxygen. Bubbling alone did not produce any significant inactivation of the enzyme.

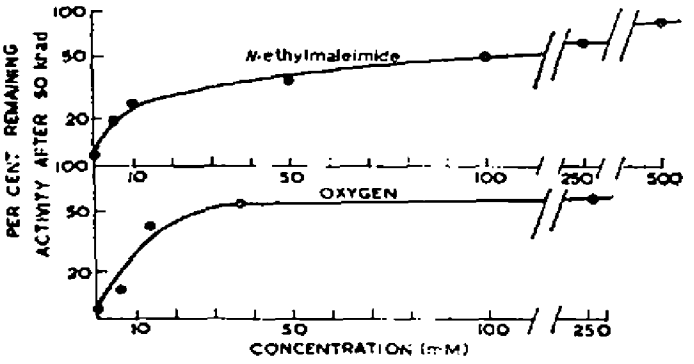

Fig. 2. Effect of concentration of oxygen (bubbled) and *N*-ethylmaleimide on their protective effect.

The protection observed with *N*-ethylmaleimide may be due to its acting as a radical scavenger. If so, it is a remarkably efficient scavenger. *N*-Ethylmaleamic acid, which did not sensitize bacteria[3], had a similar protective effect on RNAase.

It was considered possible that *N*-ethylmaleimide might only sensitize the direct effect of radiation. Some experiments were therefore carried out with RNAase solutions in which an attempt was made to minimise the effect of aqueous free radicals by irradiating in the presence of 2% alanine; under these conditions the slope of the nitrogen curve was reduced by a factor of eight. Protection was again observed with $5 \cdot 10^{-4}$ M *N*-ethylmaleimide and with air bubbling but to a lesser extent.

The failure to demonstrate sensitization in this model system casts considerable doubt on the hypothesis that *N*-ethylmaleimide sensitizes bacteria by combining with –SH or –S· arising from ruptured –S—S–bonds. An alternative hypothesis might be that *N*-ethylmaleimide reacts with protein –SH groups which are normally masked but which are temporarily exposed during irradiation by unfolding of the protein molecules. As there were no –SH groups (masked or unmasked) in the RNAase used here, this type of sensitization would not be shown. Aldolase, with 28 –SH groups, many of which are masked, would be more likely to demonstrate such sensitization. Experiments have been carried out elsewhere to test this possibility[9].

The apparent protective effect of oxygen in aqueous solution is interesting but not unique. Examples have been noted with trypsin at low pH, (ref. 10), with phage[11,12], and with lysozyme[13]. The fact that neither HOLMES[6] nor BRIGHENTI AND FALASCHI[7] observed any effect with oxygen using RNAase might have been due to the inadequacy of their techniques for removing oxygen; neither group used a bubbling technique. It is, however, generally true that enzymes in dilute aqueous solution do not show an oxygen affect (see *e.g.* HUTCHINSON[14]).

Although previous examples of oxygen protection have been interpreted in terms of production of peroxides and free radicals[10,11], there is a possibility that some other mechanism might be responsible in some cases. SHALEK *et al.*[13] only observed protection of lysozyme by oxygen when there was vigorous agitation of the solution. The present results are in agreement with their findings. It could be that

physical agitation of the solutions in the presence of air causes some change in the secondary structure of the molecules resulting in higher radiation resistance.

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Mechanism of D-glutamyltransferase repression in mammalian cells

This investigation was undertaken to obtain information about the mechanism involved in an adaptive enzyme system in cultured mammalian cells. Repression of D-glutamyltransferase ,which is almost certainly identical with glutamine synthetase (L-glutamate : ammonia ligase (ADP), EC 6.3.1.2)[1] by glutamine was described by DEMARS[2] in human cervical carcinoma cells, strain HeLa[3]. We have observed a similar phenomenon in mouse subcutaneous (fibroblasts) strain L[4]. The cells were grown in WAYMOUTH's medium[5], modified as described below, to which 5% dialysed calf serum was added. They were harvested by treatment with trypsin and extracts were prepared by repeated freezing and thawing in 0.8% NaCl, followed by centrifugation at 12 000 × *g* for 45 min. D-glutamyltransferase activity in the supernatant was determined by measuring the capacity to form γ-glutamylhydroxamic acid[6] in conditions slightly modified from the original to obtain greater sensitivity[7].

When L cells were propagated in a glutamine-free medium containing 20 mM glutamic acid an 8-fold increase in glutamyltransferase activity occurred within 48 h. On the addition of glutamine at a final concentration of 2.4 mM D-glutamyltransferase activity rapidly decreased and reached the initial level within 16–24 h (Fig. 1). The questions posed by this observation are: Is active protein synthesis involved in