the maximum effect on skin being seen at 3 $\mu\text{g/g}$ per day.

Animals that lived beyond the 5-day dosing period were killed on the fifteenth day, and showed an increased thickness of keratin, although the granular layer was only slightly thicker than that of the controls. However, the dermis was thicker and also there was an apparent increase in dermal fat.

EGF antiserum did not always prevent the action of EGF. The epidermis was frequently thicker than with EGF alone.

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Cross-Resistance between Radiation and Cytotoxic Drugs in a Mammalian Cell Line *in vitro*

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When murine lymphoma cells, strain L5178Y, are grown continuously in tritiated water, mutants arise that are considerably more resistant to X-radiation than the parent strain (Courtenay, 1969). A radioresistant mutant able to survive continuous irradiation at 24rd/h was found to tolerate certain drugs (e.g. methotrexate, vinblastine, dimethylmyleran) to a greater degree than the parent strain. This mutant, strain I, has been found to be 20-fold more resistant to methotrexate.

The uptake rate of ^3H -labelled methotrexate into this strain was found to be about one-quarter of the rate into the parent strain. The dihydrofolate reductase (EC 1.5.1.3) activity was identical with that of the parent strain, namely 250–300nmol of substrate/h per mg of soluble protein.

Mutants resistant to methotrexate can be produced by continuous culturing of the parent strain in concentrations of methotrexate (Fischer, 1961, 1962). Two such mutants were examined with respect to uptake rate of methotrexate and folate reductase activity. Strain II developed in cultures containing 0.2 μg of drug/ml, i.e. 100-fold increase in the concentration tolerated by the parent strain, had a drug uptake rate one-quarter that of the

parent strain, and an identical folate reductase activity. Strain III, developed in cultures containing 100 μg of drug/ml, had a drug uptake rate again one-quarter that of the parent strain, but a 30-fold increase in reductase activity. The response of strain II towards acute X-irradiation was no different from that of the parent strain.

Strains II and III resemble those developed by Fischer (1961, 1962) and suggest that at least two mechanisms are concerned in this drug resistance. Strain I is of considerable interest since the radioresistance resulting from a mutation is associated with a resistance to methotrexate. However, the reverse is not necessarily the case, as evidenced by strain II, where methotrexate resistance is not associated with cross-resistance to radiation.

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The Entry and Oxidation of Succinate by Mouse Fibroblast Cells

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At physiological pH the oxidation of exogenous succinate by strain L fibroblasts in Krebs–Ringer phosphate buffer is limited by its rate of entry. The evidence for this is as follows. (1) The respiration rate increases linearly with external succinate to 40 mM, but even at this high concentration it is only 25% greater than the endogenous rate. (2) The uncoupler 2,4-dinitrophenol, which markedly stimulates the oxidation of glucose and pyruvate, increases respiration in various succinate concentrations only by the small increment it induces in endogenous rates. (3) Damage to the membrane by freezing and thawing increases the Q_{O_2} in succinate, and the maximum is induced by concentrations as low as 5 mM. (4) Isolated mitochondria readily oxidize exogenous succinate at very low concentrations.

Lowering of the pH facilitates succinate entry, and at pH 6.3 2,4-dinitrophenol stimulates respiration by increments directly related to succinate concentration, and much greater than those at pH 7.4. With pyruvate, which easily permeates the membrane, a maximum Q_{O_2} (50 $\mu\text{l/h}$ per mg of protein) was induced at pH 6.3 and 7.4 by 0.03 mM- and 0.1 mM-2,4-dinitrophenol respectively, confirming that the uncoupler activity increases with decreasing pH (Simon, 1953). At the lower pH succinate (40 mM) oxidation in 0.03 mM oxidation reached rates similar to that of pyruvate, but at pH 7.4 no comparable stimulation could be obtained by up to 0.5 mM-uncoupler, again indicating that at

physiological pH succinate oxidation is limited by its entry.

Short exposure to pH 6.3 with succinate and uncoupler present did not significantly increase membrane permeability to a sulphonated azo-dye. Lowering the pH to 6.3 changes the quantities of ionic species $\text{H}_2\text{succinate}:\text{Hsuccinate}^{1-}:\text{succinate}^{2-}$: from 0.1mM:2.5mM:37.4mM to 0.4mM:6.2mM:33.4mM and the faster succinate entry is probably due to the increased proportion of uncharged molecules, which can permeate the relatively hydrophobic osmotic barrier by a diffusion pathway. It appears that the membranes of these cells, grown in suspension in modified Waymouth medium (Sinclair, Reid & Mitchell, 1963), are virtually impermeable to succinate²⁻, which predominates at physiological pH. This is in contrast with the apparent ability of succinate²⁻ to permeate the mitochondrial membrane by a carrier mechanism (Chappell & Haahrhoff, 1967).

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Indirect Evidence that Light-Induction of Adenosine Triphosphate Hydrolysis by Chloroplasts Depends on Electron-Transfer Reactions

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The dark hydrolysis of ATP by spinach or lettuce chloroplasts, after activation by light in the presence of cysteine (80mM), is stimulated by ammonium chloride (5mM) added after illumination; the ATP hydrolysis then becomes progressively slower, thus confirming earlier observations with dithiothreitol (Carmeli & Avron, 1969; Carmeli, 1969; cf. also Marchant, 1969). A distinct difference is found between the use of *N*-methylphenazonium methyl sulphate on the one hand and FMN on the other as added redox substance. If the reaction mixture is not stirred during the illumination period (5min) when *N*-methylphenazonium methyl sulphate was used the initial stimulated rate is high and falls off relatively slowly, whereas with FMN the initial rate is lower and falls off rapidly. If on the other hand the mixture is continuously stirred during the light phase, the initial high rate with *N*-methylphenazonium methyl sulphate now falls off more rapidly, whereas with FMN the initial rate is now considerably higher and no longer falls off so rapidly. This higher rate with FMN is even

observed if a few seconds' agitation is given during the latter part of the illumination period. Neither stirring nor agitation are necessary for these higher rates with FMN if the illumination period is shortened to 2min.

The effects with FMN can be attributed to the need for either oxygen or oxidized FMN for electron transfer, the FMN having become reduced during early illumination and not then available in the oxidized form unless the mixture is stirred. With *N*-methylphenazonium methyl sulphate a possible explanation is that stirring in from the surface layers of the oxidized cofactor itself, or a derivative of it, is responsible for the decreased response to ammonium chloride subsequently, assuming that maintenance of efficient cyclic electron transfer throughout illumination depends on the nature and redox state of this cofactor (cf. Zweig & Avron, 1965). Both of these explanations would imply that a maintained stimulation by ammonium chloride depends on prior electron transport.

Further support for the involvement of electron transfer in the light-induction is given by the absence of inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (0.1mM), with or without ammonium chloride, when *N*-methylphenazonium methyl sulphate is used (cf. Carmeli & Avron, 1966), but very clear inhibition with FMN, as is found for photophosphorylation.

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Preparation of Human Pathological γ -Macroglobulins by Rate Zonal Centrifugation in a BXV Ti Rotor

By K. B. COOKE and MARGARET E. APSEY. (*Department of Chemical Pathology, Westminster Hospital Medical School, London S.W.1, U.K.*)

Zonal rotors (Anderson, 1966) have mostly been used for the preparation of cell particles, but both Fisher & Canning (1966) and Anderson *et al.* (1967) used the separation of a macroglobulin peak from rat plasma as a test of resolution for zonal rotors. Their results suggested that the technique could be