BIOPHYSICS

Mechanical Damage of Keratin Proteins observed by Electron Spin Resonance

It has been shown by electron spin resonance that mechanical damage to biological material causes the formation of free radicals and changes of chemical valence. The change of haemoglobin to methaemoglobin and the appearance of an unidentified free radical have been observed, and were due to mechanical damage of whole blood by freeze drying¹. Mechanically excited electron spin resonance spectra of synthetic organic polymers were studied by Bresler *et al.*².

In the experiments with mechanically damaged keratin carried out in this laboratory, intense electron spin resonance spectra were obtained. Most experiments were performed with hoof keratin, but other keratinous substances, such as finger nails, feather, etc., have similar spectra. The material was filed with a rough file, in atmospheres of both air and nitrogen with 7 per cent oxygen.

The powder was inserted into the cavity of a standard spectrometer operating at 9 kMc/s. and with 100 kc/s. magnetic modulation, in open or closed sample tubes, at room temperature. Fig. 1 shows the spectra obtained by filing the hoof in air and their change with time; Fig. 2 is a recording of the spectrum from the material treated in nitrogen.

The spectra are very similar to those of X-irradiated keratin proteins³⁻⁵, but show greater intensity and are better resolved. Just as with X-irradiated keratin, they can be assigned to the presence of amino-acids containing sulphur.

Fig. 1. Electron spin resonance spectra of hoof proteins excited by filing in air. (a) Fresh powder; (b) after 4 min.; (c) after 10 min.

Fig. 2. Electron spin resonance spectra of hoof proteins excited by filing and measuring the samples in nitrogen. (a) Fresh powder; (b) after 80 min.; (c) after 3 days

The spectra obtained in air (Fig. 1) closely resemble those of cysteine and cystine³⁻⁵, and the spectra observed in nitrogen are similar to those of glutathione, observed by Henriksen and Pihl⁶. It may be noted that McCormick and Gordy⁴ found no distinct difference between the spectra of cystine, cysteine and reduced glutathione, while oxidized glutathione was not clearly resolved.

Due to the ease with which mechanical excitation of the electron spin resonance spectra is brought about, it is probable that many effects in the spectra of biological objects, which have been thought previously to be of metabolic origin, are artefacts caused by mechanical damage during the treatment of the samples. This is true especially in the case of substances containing electron sinks⁷.

The effects observed may be of importance in connexion with the processes of wounding and healing, for the initiation of cancer by mechanical stimulation etc.

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¹ Ingram, D. J. E., *Free Radicals as Studied by Electron Spin Resonance*, 241 (Butterworths Sci. Publ., 1958).

² Bresler, S. E., Žurkov, S. N., Kazbekov, E. N., Saminskij, E. M., and Tomaševskij, E. E., *J. Tech. Phys. U.S.S.R.*, **29**, 358 (1959).

³ Gordy, W., Ard, W. B., and Shields, H., *Proc. U.S. Nat. Acad. Sci.*, **41**, 983 (1955).

⁴ McCormick, G., and Gordy, W., *J. Amer. Chem. Soc.*, **82**, 789 (1958).

⁵ Gordy, W., and Shields, H., *Rad. Res.*, **9**, 611 (1958).

⁶ Henriksen, T., and Pihl, A., *Nature*, **185**, 307 (1960).

⁷ Gordy, W., and Miyagawa Ichiro, *Rad. Res.*, **12**, 211 (1960).

Generation Time of Epithelial Cells in the Human Colon

THE duration of the mitotic cycle in intestinal epithelial cells of rodents has been measured by radioautography after tritiated thymidine^{1,2}. Proliferating jejunal and colonic epithelial cells in rodents have total generation times of about 18 hr., DNA synthesis (*S* phase) times of 5-8 hr., and pre-mitosis (*G*₂) and mitosis times of 1.5-2 hr. In man, investigations of mitotic indices and autoradiographic studies with tritiated thymidine have suggested turnover times of intestinal epithelial cells of several days³.

This investigation gives measurements of the component parts of the mitotic cycle of human intestinal epithelial cells after tritiated thymidine and the effect of 5-fluorouracil on the proliferative cycle. Two patients having a colostomy opening with normal mucosa of the transverse colon attached to the anterior abdominal wall after resection of carcinoma of the rectum were examined; both had liver metastases. The first was given intravenously 0.057 μ Ci/gm. of tritiated thymidine, the second 0.26 μ Ci/gm. (specific activity 6.59 curies/mM dissolved in 10 ml. of water). Three months later the second patient was treated with 5-fluorouracil, total dose 105 mgm./kgm., for 16 days. At the onset of toxicity, the drug was discontinued and a second tritiated thymidine injection given. After each injection, serial biopsies of colonic mucosa were taken from the colostomy opening from the surface to a distance of 2 in. below. Tissue