THE EFFECT OF α-RADIATION ON PROTEIN MONOLAYERS*

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Although an α-particle source is commonly used as an electrode for measuring the surface potentials of monomolecular films spread on water, the possibility that errors may occur due to the effect of the ionizing radiation on the film does not seem to have received attention in the literature. However, during measurements on films of complex lipo-proteins derived from oat coleoptiles, a hint that such an effect may exist was given by a series of very erratic results due, apparently, to a marked lowering of the surface potential of any part of the film which was directly under the electrode for any length of time. This note gives details of a brief investigation of the phenomenon using the more easily specified bovine serum albumin.

The particular electrode used was type PDD1 from the Radiochemical Centre, Amersham, England. It consisted of 1 mc of polonium 210 electrodeposited as a flat disk 7 mm diameter on platinum, and protected by thin mica. This quite large source area was undoubtedly the reason that the effect was so prominent. In an alternative source, PDD2, the polonium is deposited on the end of a platinum wire 0·9 mm diameter, and the surface potential of the irradiated film becomes averaged out with that of the surrounding area to a much greater extent than in the first case. The potential between the active electrode and a calomel reference electrode was measured on a Keithley 610A electrometer with an input resistance above 10^{14} Ω. A typical Langmuir–Adam surface balance was used with a Pyrex glass trough, all enclosed by an earthed sheet-metal and wire-gauze case. The substrate was acetate buffer of pH 4·3 and ionic strength 0·005.

Bovine serum albumin (Cohn fraction VC, Commonwealth Serum Laboratories, Melbourne) was made up as an 0·03% solution in an aqueous solvent of 60% isopropanol with 0·05M sodium acetate (Cumper and Alexander 1950). It was spread onto the water surface using an "Agla" micrometer all-glass syringe with two different spreading techniques: firstly, using the conventional dropwise method, where each drop was touched upon a different part of the trough; secondly, by Trurnit's (1960) method, where the solution flowed from the syringe down a 7 mm diameter glass rod projecting 6 cm above the water surface. In each case the film was allowed to spend 5 min at maximum area before compression.

The pressure–area curves of the protein spread by Trurnit's method on the very dilute buffer showed the larger area, compared with the dropwise spread film, as noted by him. In the present case typical areas at 10 dynes/cm were 0·73 and 0·46 m^2/mg respectively, i.e. the area of the Trurnit spread film was 59% greater than that of the dropwise spread film. It was found, however, that the α-source had a similar effect of lowering the surface potential in each case, and so the extent of surface denaturation can be eliminated as a major factor.

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The precise nature of the effect was investigated as follows. After thoroughly cleaning the water surface the electrode was set 2 to 3 mm above the surface and gradually moved across the width of the trough. The clean potential was recorded in five positions along this sweep (see the upper part of Fig. 1). The protein film was then applied and left in an expanded state for 5 min before being compressed to 15 dynes/cm pressure. Observations of the potential of this fresh film were now made as quickly as possible so that the electrode did not remain over any of the five positions for more than 5 or 10 sec. Under these circumstances it was found that the potential of the film slowly decreased for an hour or more, finally settling down

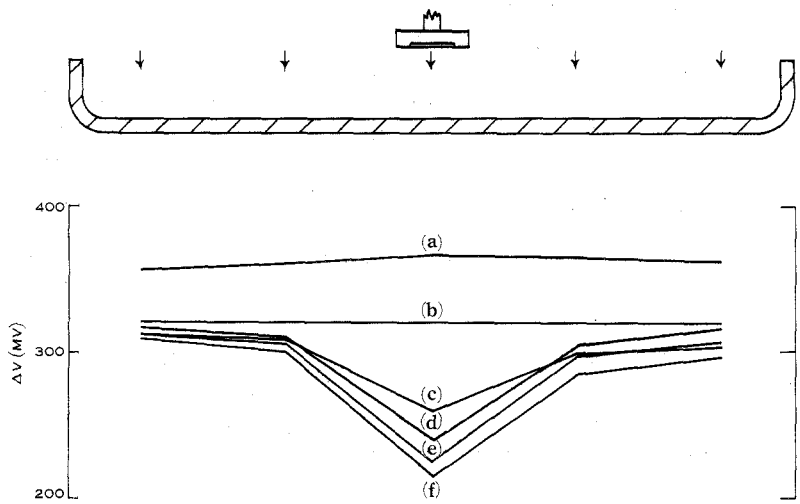

Fig. 1.—*Top*: Cross section of trough and electrode with five recording positions. *Bottom*: Surface potentials of protein film (*a*) freshly compressed; (*b*) after standing for 60 and 120 min; (*c*) after electrode had been left in place for 15 min; (*d*), (*e*), (*f*), after further 15 min periods of irradiation.

some 20–30 mV lower than that of the fresh film. The area needed to maintain the film pressure constant at 15 dynes/cm needed minor adjustments over this period, but subsequently was not affected by the presence of the electrode. At this stage the electrode (still 2–3 mm above the surface) was left for 15 min above the centre of the trough. A quick recording of the potential over the five positions was made and the electrode was returned to the centre position for a further 15 min. This procedure was repeated three or four times. The results of a typical run are shown in the lower part of Figure 1, as a plot of $\Delta V = V$ (film) $- V$ (clean) against position on the surface. The freshly compressed film gave ΔV values along (*a*) and after standing for 1 hr with the electrode well away from the film ΔV dropped to line (*b*). A further 1 hr standing gave no change in ΔV. However on leaving the polonium electrode over the central position for 15 min, ΔV at this spot dropped by 60 mV, and further irradiation gave additional but smaller decreases. A similar run at a constant pressure of 5 dynes/cm gave essentially the same type of behaviour.

The exposure of protein films to these radioactive electrodes can accordingly lead to serious errors in surface potential data, especially if the electrode is kept in one place during gradual compression and measurement of the film.

The actual change in the protein molecules due to radiation has been suggested, in work with γ-rays by Kumpta and Tappel (1961), for example, as being both aggregation and scission, with the sulphur-containing amino acids as the most susceptible points of attack. This attack is probably carried out mainly by H and OH radicals arising in the aqueous medium (Maxwell and Peterson 1959), with oxygen and/or hydrogen peroxide formed from the radicals also reacting with the protein (Swallow and Velandia 1962). Irradiation by γ-rays has been shown also to cause unfolding of the secondary structure of ovalbumin powder (Fricke *et al.* 1959), but the extent of this process in a protein molecule which is already surface denatured is likely to be insignificant. In studying the effects of 2350–2400 Å light on protein monolayers, Mitchell and Rideal (1938) showed that the net result was an increase in ΔV and liquefaction of the gelled structure. However, the sites of action of this radiation were the aromatic chromophores, leading to oxidation and fragmentation, whereas the free-radical attack is probably more widespread, allowing polymerization as well. Rideal and Mitchell (1937), using stearic anilide as an analogue of the above protein chromophores, found that as with proteins, u.v. light caused splitting of the molecule and an increase in ΔV. The polonium electrode procedure has been applied to a film of stearic anilide on 5N sulphuric acid, with no apparent effects on the surface potential. This suggests that the peptide linkage is not the site of primary attack by the α-particles and their products in aqueous systems, a conclusion consistent with the prominent part given to sulphur-containing amino acids in the irradiation of proteins.

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