The irradiation of arachin and conarachin II with γ-rays

Arachin, prepared according to the method of JOHNSON AND NAISMITH[1], was dissolved in phosphate buffer (ionic strength $I = 0.2$, pH = 7.8), dialysed against the same buffer and irradiated with γ-rays from a cobalt source of 103 curies intensity for 24 hours, the dosage rate being 1700 Rontgens/minute/10 ml. The solution and control were then examined in the Spinco ultracentrifuge Model E at 50,740 r.p.m., (190,000 g), the sedimentation diagrams being shown in Figs. 1(a) and (b) respectively. It is clear from the presence of the more rapidly sedimenting components in Fig. 1(a) that association of the $s_{13.5}$ component has occurred as a result of the irradiation. A solution of arachin at $I = 0.025$, pH = 8 containing an appreciable amount of the dissociation product (s_9 component) and irradiated for the same time as above gave the sedimentation diagram shown in Fig. 2(a). Fig. 2(b) gives the diagram for the control solution. It will be observed that some association of the s_9 component has occurred as a result of the irradiation although no association products of the $s_{13.5}$ component are visible. The usual arachin dissociation reactions ($s_{13.5} \rightleftharpoons 2\ s_9$) occurred with both the irradiated solutions by appropriate adjustments of the ionic strength (*c.f.* JOHNSON AND SHOOTER[2]). The association products of the $s_{13.5}$ component were not affected by lowering the ionic strength to 0.025. At $I = 0.5$, pH = 7.8, irradiation of an arachin solution resulted in the precipitation of most of the protein.

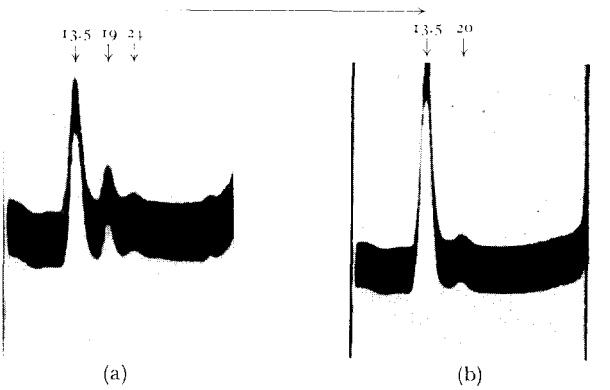

Fig. 1. Sedimentation diagrams of arachin in phosphate buffer, ionic strength $I = 0.2$, pH = 7.8 (a) after and (b) before irradiation with γ-rays. Protein conc. = 1 g/100 ml. Numbers above peaks in this and subsequent diagrams refer to rounded sedimentation constants in Svedberg units.

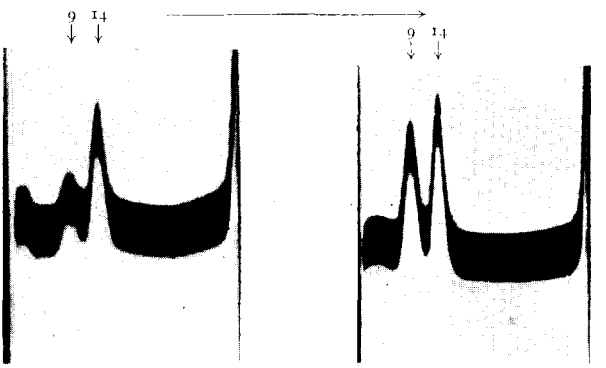

Fig. 2. Sedimentation diagrams of arachin in phosphate buffer, $I = 0.025$, pH = 8.0, (a) after and (b) before irradiation with γ-rays. Protein conc. = 1 g/100 ml.

Conarachin II[3], which has been shown to undergo dissociation-association reactions with variation in ionic strength[1,3], was irradiated at $I = 0.5$, pH = 7.8 (dissociated state) and at $I = 0.1$, pH = 8 (associated state). Figs. 3 and 4, which give the sedimentation diagrams of the irradiated solutions and their controls at $I = 0.5$ and $I = 0.1$ respectively, indicate that association has again occurred in each case although the association products did not give discrete peaks as in the case of arachin. The solution irradiated at $I = 0.5$ was dialysed to $I = 0.1$, pH = 8 when further association occurred (Fig. 5) as would be expected with conarachin II. The solution irradiated at $I = 0.1$, pH = 8 was dialysed to $I = 0.5$, pH = 7.8 when dissociation would be expected to take place giving a sedimentation diagram similar to Fig. 3(b). No dissociation occurred.

Thus γ-ray irradiation causes association of both arachin and conarachin. Whereas the irradiation does not hinder the subsequent dissociation of the arachin ($s_{13.5}$) component at low ionic strengths, the dissociation of conarachin II at high ionic strengths is prevented.

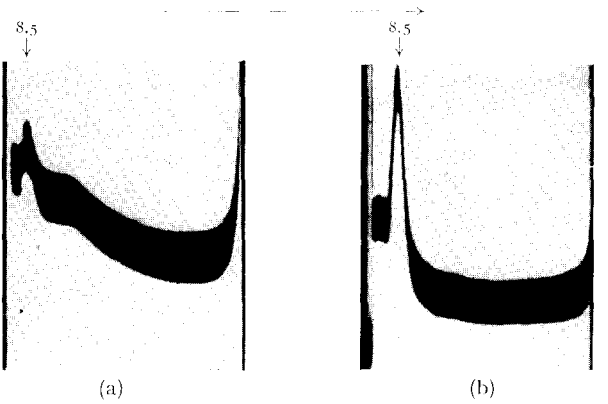

Fig. 3. Sedimentation diagrams of conarachin II in phosphate buffer, $I = 0.5$, pH = 7.8, (a) after and (b) before irradiation with γ-rays. Protein conc. = 1 g/100 ml.

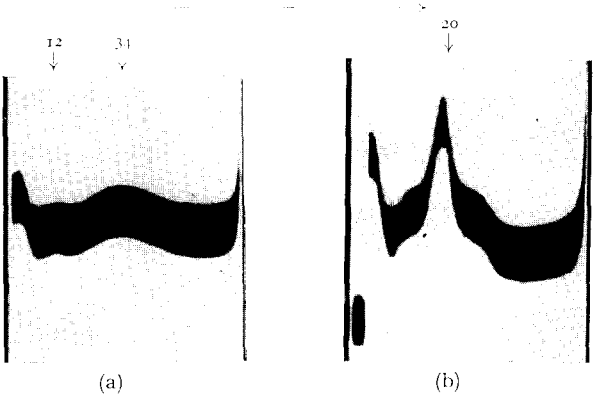

Fig. 4. Sedimentation diagrams of conarachin II in phosphate buffer, $I = 0.1$, pH = 8.0, (a) after and (b) before irradiation with γ-rays. Protein conc. = 1 g/100 ml.

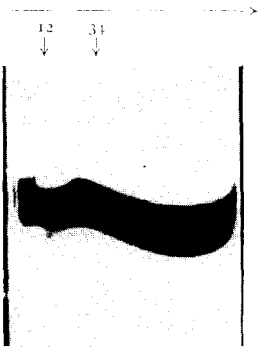

Fig. 5. Sedimentation diagram of conarachin II irradiated with γ-rays at $I = 0.5$, pH = 7.8 and then dialysed to $I = 0.1$, pH = 8.0. Protein conc. = 1 g/100 ml.

Grateful acknowledgement is made to Dr. P. TIMMONS of King's College, University of Durham Newcastle-on-Tyne for carrying out the γ-ray irradiations.

W. E. F. NAISMITH

Imperial Chemical Industries Limited, Nobel Division, "Ardil" Fibre Factory, Dumfries (Scotland)

[1] P. JOHNSON AND W. E. F. NAISMITH, *Discussions Faraday Soc.*, 13 (1953) 98.
[2] P. JOHNSON AND E. M. SHOOTER, *Biochim. Biophys. Acta*, 5 (1950) 361.
[3] P. JOHNSON AND W. E. F. NAISMITH, *Biochim. Biophys. Acta*, 15 (1954) 377.

Received May 10th, 1955