enzyme may be available for the dehydroxylation of the 5α-(allo) bile acids; (*d*) neomycin acts by preventing the re-absorption of allodeoxycholic acid from the intestinal tract.

E. H. Mosbach
V. Bokkenheuser
D. P. Chattopadhyay

Public Health Research Institute,
New York.

M. Schmidt
R. L. Hirsch

Columbia University Research Service,
Goldwater Memorial Hospital,
New York.

A. F. Hofmann

Rockefeller Institute,
New York.

[1] Bevans, M., and Mosbach, E. H., *Amer. Med. Assoc. Arch. Path.*, **62**, 112 (1956).
[2] Hofmann, A. F., and Mosbach, E. H., *J. Biol. Chem.*, **239**, 2813 (1964).
[3] *Standard Methods for the Examination of Water and Waste Water*, eleventh ed. (Amer. Public Health Assoc., 1960).
[4] Chattopadhyay, D. P., and Mosbach, E. H., *Anal. Biochem.*, **10**, 435 (1965).
[5] Mosbach, E. H., Bevans, M., Kaplan, R., and Halpern, E., *Arch. Path.*, **66**, 72 (1958).
[6] Borgström, S., Fajers, C. M., and Van der Linden, W., *Acta Chir. Scandinav.*, **126**, 329 (1963).

RADIOBIOLOGY

In vitro Effect of Gamma-radiation on Different Cholinesterase Preparations

The interaction of high-energy radiation with proteins has received a great deal of attention and in the past few years much has been published on this subject.

Although the interactions with proteins undoubtedly play a vital part, the biochemical effects are frequently ambiguous, and this is the case in the results described for the variation of the cholinesterase activity after γ-irradiation.

Experiments were designed to investigate the *in vitro* effect of γ-rays on cholinesterase from various sources and of differing grades of purity. Irradiations were performed at 18° C in a thermostatically controlled cylindrical 2,000-c. cobalt-60 source. The dose-rate used was $2{\cdot}5 \times 10^5$ r./h. Ten samples were simultaneously irradiated in sealed glass ampoules with a head space of 6 ml., containing air. Controls were handled in the same fashion, but they were not placed within the cobalt source. Dosimetry for experiments was performed by the method of Weiss[1].

The preparations exposed to γ-radiation were: (1) bovine red blood cell cholinesterase (Sigma Chemical Co.); (2) common fly-brain cholinesterase preparations at different stages of purification. These stages were: whole heads; homogenate; and a purified fraction. Heads were collected by the Moorefield method[2] and the homogenate had 60 mg of fresh tissue per ml. distilled water.

The purified fraction was obtained according to the following method: a homogenate was adjusted to *p*H 8·5 with 0·1 N sodium hydroxide and allowed to stand 20 h at 5° C, after which time it was centrifuged in the cold at 100,000*g* for 30 min. The supernatant was brought to 41 per cent saturation with solid ammonium sulphate and centrifuged again at 15,000*g* for 15 min. The supernatant was brought to 78 per cent saturation. Centrifugation at 15,000*g* yields a purified cholinesterase preparation which was used as a suspension in distilled water[3].

The enzymatic activities of the samples were controlled within 1 h of irradiation. Cholinesterase activity was measured potentiometrically using as substrate a solution of 0·5 M sodium chloride–0·04 M magnesium chloride–

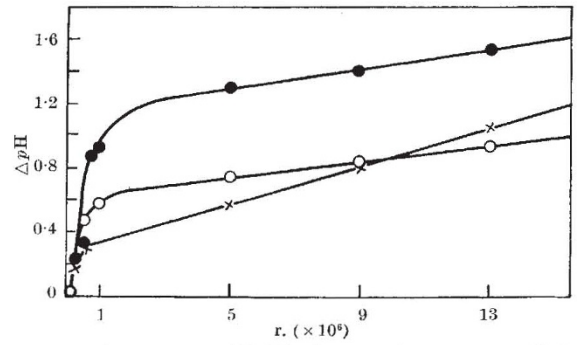

Fig. 1. *p*H decrease (Δ*p*H) following several doses of γ-radiation. ×, Whole heads; ●, purified samples of common fly brain cholinesterase; ○, red cell cholinesterase

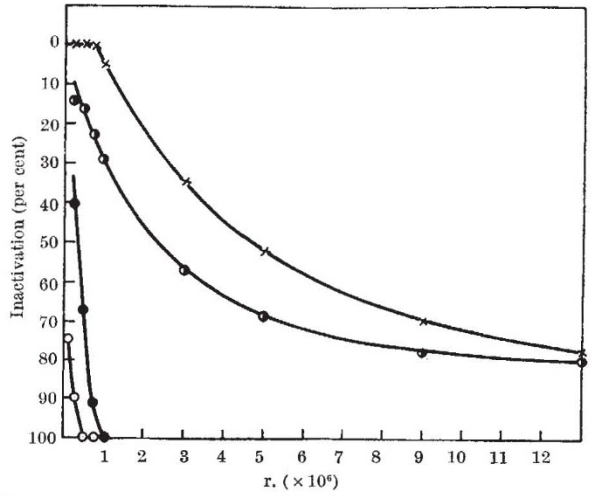

Fig. 2. Effect of irradiation on the activity of cholinesterase preparations. ×, Whole heads; ◑, homogenate; ● purified preparation; ○, red cells

0·002 M acetylcholine bromide in 0·5 M phosphate buffer *p*H 7 and 38° C. The acetic acid liberated was automatically determined by titration with 0·005 N sodium hydroxide. Enzymatic activity was expressed as ml. consumed alkali per min.

Fig. 1 gives the extent to which the *p*H of the samples was affected by the γ-irradiation.

Experimental results were evaluated by plotting the percentage of the remaining enzymatic activity as a function of irradiation dose (Fig. 2).

From these results it is clear that the more purified the samples were, the more was the inactivating effect of the irradiation. In the cholinesterase preparations from common fly, the order of inactivation was: purified sample > homogenate > whole heads. Assays carried out with red blood cells and cholinesterase from the electric eel (Sigma Chemical Co.) showed a high grade of inactivation (100 per cent of inactivation in a 50 u/ml. solution receiving a 10^6 r. dose). Some data on the nature of this radio-resistance were obtained. The homogenate completely lost its cholinesterase activity by heating it at 100° C for 2 min; when the filtrate was added to the purified sample, a clear protection could be shown ranging from 80 to 40 per cent. Nevertheless, the supernatant from the homogenate precipitated by addition of trichloroacetic acid did not show any protective properties.

R. E. Catalan
A. M. Municio

Institute of Chemistry,
Madrid.

[1] Weiss, J., *Nucleonics*, **10**, 7 (1952).
[2] Moorefield, H., *Contrib. Boyce Thompson Inst.*, **18**, 413 (1957).
[3] Barberan, C., Ph.D. thesis (Madrid, 1964).