Autoradiography of End-plates with Carbon-14 – Calabash – Curarine I and Carbon-14 – Decamethonium

A HUNDRED years have passed since Claude Bernard's publication of his investigations on the action of curarine in "Leçons sur les effets des substances toxiques et médicamenteuses"¹. He was able by indirect experiments to localize the curare-block in the end-plates of skeletal muscle. Some time ago when we investigated the metabolism of carbon-14 – curarine I in the cat², we tried to obtain autoradiographs of muscle, intending to show the fixation of this alkaloid directly at the neuro-muscular junctions.

For this purpose we had to synthesize carbon-14 – curarine of the highest possible radioactivity by methylation of 2.0 mgm. nor-curarine with an excess of 6.4 mgm. $^{14}\text{CH}_3\text{I}$, having the activity of 1 mc. After recrystallization we obtained 0.25 mgm. pure carbon-14 – curarine – iodide (biquaternary) with an activity of 24.2 $\mu\text{c./mgm.}$ The minimal lethal dose of the water solution was injected into the veins of the tails of mice, which died of asphyxia within 40–120 sec. The diaphragms of these animals were stretched over steel rings of the same diameter (22 mm.) and dried in air. They were then brought into contact with X-ray film (Ilford X-ray PM_1) or mounted on microscope slides and covered by stripping film (Kodak). After developing the films the end-plates were stained by the Koelle method, in which acetylthiocholine is split by the cholinesterase, brown copper sulphide finally resulting³.

After 60 days the resulting pictures on X-ray film showed a slight blackening in the region of the end-plates, which lie in a band of 260 μ thickness around the centrum tendineum. In addition, there was blackening over the centrally situated vessels due to radioactivity in the blood. On microscopic examination of the stripping film a diffuse formation of silver grains over the end-plates was seen; but the crossfire over this region had been so heavy that only twice the grain density existed over each end-plate as compared to its immediate surroundings. The whole region had a 4–6 times higher density of grains com-

Fig. 2. Stained end-plates (Koelle method, $\times c. 700$) and silver grains in stripping film by carbon-14-decamethonium

pared with a corresponding region of muscle without end-plates.

In order to obtain a much higher specific activity in a curarizing molecule we then methylated decamethylendiamine with $^{14}\text{CH}_3\text{I}$ and obtained 0.44 mgm. decamethonium with six carbon-14 atoms. The specific radioactivity was approximately five times larger than that of carbon-14 – curarine. After five days the resulting autoradiographs again showed clearly the localization over the end-plates, especially when compared with the stained diaphragms (Fig. 1). Over this region we counted four times more silver grains in the unit area of stripping film than in the muscle layer farther out (Fig. 2).

These pictures thus prove the fixation of 'curarizing' and 'depolarizing' drugs in the end-plate. They make possible the calculation of the number of molecules reacting with the receptor surface. We found 8×10^6 curarine molecules bound to one end-plate and 1.7×10^{12} molecules/cm.² of apparent area of end-plate membrane, thus neglecting the possibility of a large increase in surface due to the palisade structure described by Couteaux⁴. The order of magnitude of apparent coverage of the end-plate surface is 5 per cent.

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¹ Bernard, C., 238–353 (Paris, 1857).

² Waser, P., Schmid, H., and Schmid, K., *Archives Internat. de Pharmacodynamie et de Thérapie*, **96**, 386 (1954).

³ Koelle, G. B., and Friedenwald, J. S., *Proc. Soc. Exp. Biol. and Med.*, **70**, 617 (1949).

⁴ Couteaux, R., Thèse de l'université de Paris: "Contribution à l'étude de la synapse myoneurale" (Montréal, 1947).

Fig. 1. Autoradiography of mouse diaphragm with carbon-14-decamethonium in comparison with staining of end-plates in the same diaphragm by the Koelle method ($\times c. 4$)

Globulins in the Chicken Egg-White

LONGSWORTH *et al.*¹ demonstrated, by electrophoresis in the Tiselius apparatus, three globulins in the white of an egg. These were labelled G_1 , G_2 , G_3 . (G_1 was identified later with the lysozyme.)

The antigenic properties of egg-globulins were demonstrated first by Hektoen and Cole² and later by me³.

In my studies on egg-white and its constituents^{3–5}, I have employed the methods which permit the analysis of complex mixtures, such as electrophoresis