MODIFICATION OF THE BIOASSAY OF ACETYLCHOLINE

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Although many different methods like spectrofluorometry (1, 3, 8),
gas chromatography (5, 10), and enzymic assay (9), have been developed
for quantitative determination of acetylcholine, the most commonly em-
ployed and sensitive method is still the bioassay (2, 4, 6, 7).

Fig. 1. Photograph of the apparatus: a, knob controlling the timer; b, indicator; c, scale of the microamperometer; d3, system for stopping the indicator; e, microam-
perometer; k, box containing the photoelement; l, knob for setting the base level measurement (see text); m, plastic tube; n, diaphragm; p, muscle bath; s, double-arm lever.
The apparatus described in this paper is simple and inexpensive. It allows one to obtain more reliable and reproducible results than those from a smoked drum.

The apparatus is shown in Fig. 1. A beam of light falls on a photoelement. A movable diaphragm between the lamp and the photoelement is located. It is connected to the muscle by means of a double-arm lever.

The amount of light which falls on the photoelement depends on the state of the muscle. The diaphragm rises with the contraction of the muscle; when the muscle is only partly contracted less light falls on the photoelement. The reading of the microamperometer (in $\mu$A) is directly proportional to the size of the muscle contraction. The apparatus is suitable for a dorsal muscle of the leech, a frog muscle (rectus abdominis), or a guinea-pig ileum. The bath containing the muscle is the same as that normally used in the smoked drum method. The muscle is connected to one arm of the lever. A free hanging aluminium diaphragm is attached to the second arm. The arm connected to the diaphragm was 17 times longer than that connected to the muscle.

The base level (0% of extinction) may be set with knob 1 (Fig. 1).

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Fig. 2. Construction of the apparatus: k, box containing the photoelement; i, photoelement; s, double-arm lever; p, muscle bath; o, muscle; n, diaphragm; f, light source; m, plastic tube, through which the light flows from its source to the photoelement; u, rod connected to photoelement for setting 100% of extinction (see text); x, connection to microamperometer.
which is used for changing the vertical position of the instrument box (Fig. 2), made up of the photoelement \( k \) and the plastic tube \( m \) (Fig. 1 and 2). The photoelement \( k \) may be moved up and down by means of rod \( u \) (Fig. 2) for setting 100% of extinction. In this case, the muscle bath and the diaphragm remains stationary.

![Diagram of microamperometer](image)

Fig. 3. Sketch showing the basic construction of the modified microamperometer: \( a \), knob controlling timer; \( b \), indicator; \( c \), scale of the microamperometer; \( t \), timer; \( d_1 \), \( d_2 \), \( d_3 \) system which mechanically stops the indicator (see text); \( w \), scale of the timer.

The slightly modified microamperometer is shown in Fig. 3. The indicator \( b \) is stopped mechanically after a preset time interval regulated

![Calibration curve](image)

Fig. 4. Calibration curve for frog muscle.

ng of acetylcholine per volume of the bath
by knob a (Fig. 1 and 2). The system that stops the indicator consists of a frame which is pressing against the indicator $d_3$ (Fig. 1 and 3), a bar connecting the frame to the core of the electromagnet $d_2$ (Fig. 3), and the electromagnet $d_1$ connected to the timer $t$ (Fig. 3). The electromagnet reacts after a set time controlled by means of the knob a (Fig. 3). The indicator is then pressed down and held in place by the frame $d_3$. The contraction time of the muscle may be fixed from 0.5 min (for very sensitive muscles) up to 3 min (for less sensitive muscles).

![Graph](image_url)

Fig. 5. Calibration curve for leech muscle.

The procedure involved here is very much the same as that with standard assays using the smoked drum method. Doses of acetylcholine are given at regular time intervals (1 min) followed by periods of washing the muscle with Ringer solution (2 min); thus, one measurement lasts 3 min. Since the sensitivity of the muscle to acetylcholine changes with time every dose of the unknown sample must be preceded and followed by a matching amount of acetylcholine. As shown in Fig. 4 and Fig. 5, linearity for frog muscle is obtained for the range from 50 ng to 200 ng of acetylcholine per volume of bath (in the described apparatus 2.5 ml), and for the leech muscle for the range from 5 ng to 20 ng, respectively. The results are calculated by comparing the value of $\mu$A obtained by a known amount of standard acetylcholine solution with the value of $\mu$A obtained by the unknown sample.

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