BIOASSAY OF ACETYLCHOLINE WITH A THIN MUSCLE
STRIP OF THE JAPANESE MEDICAL LEECH:
A PRELIMINARY NOTE ON THE REAPPRAISAL
OF THE AVAILABILITY OF THIS METHOD

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In European countries, the muscle response of the medical leech (*Hirudo medicinalis*) has been found effective in the bioassay of acetylcholine (ACh)^{1-2), 5), 7-11)}; in Japan, however, despite the high sensitivity and specificity of leech dorsal muscle to this amine, this method has been considered to be practically disadvantageous owing to the poor muscle response following induced contraction¹²⁾. On the other hand, it was found that if a strip of the dorsal muscle as thin as 0.2-0.4 mm was prepared from the worm following Szerb's⁹⁾ and Whittaker's procedures¹¹⁾ the specimen contracted with ACh relaxed well within 5 min following rinse. A thinly prepared muscle strip was highly sensitive to ACh. During the winter season, February and March, it contracted in response to ACh over a range of 10^{-8} — 10^{-20} g/ml. We report herein our findings on a promising bioassay procedure for ACh using the Japanese medical leech hitherto regarded as a material of no practical use.

The medical leech, possibly identical with *Hirudo nipponia Whittan* seen in Japan, Korea, and Formosa⁹⁾, was purchased from a local drug house dealing in Chinese medicines. A leech, usually 30-40 mm long, was pinned down on its back through the mouth- and tail-suckers in a suitably extended position, onto a cork board covered with filter paper, mouth towards the operators, and a cut was made with scissors along one lateral line from the mouth nearly to the tail. The ventral and dorsal walls were pinned down, inside up, at either side along the cut line, and the internal organs, such as the whitish intestine and diverticula, were bluntly removed with forceps (Fig. 1a). The dorsal muscle was cut longitudinally about 0.2 mm wide and 20 mm long with double razor blades (Fig. 1b). Two thin threads were attached, about 15 mm apart, at each end of the muscle strip, and the specimen was removed from the leech. During all these procedures, the muscle was often soaked with drops of a diluted mammalian Locke solution described by Szerb⁹⁾, 5 parts Locke : 4 parts water (DML). The removed muscle strip was suspended in a Whittaker's microbath with a capacity of 0.2 ml¹¹⁾, fixing one thread onto the bottom of the bath and attaching the other to a transducer whose minimal sensitivity to displacement force was 1 mg. The transducer was connected to a recorder (AS-3A, Shinko Tsushin Co., Osaka). The specimen was allowed to stand for 1 hr during which time the preparation was washed and sensitized with an eserinated, diluted mammalian Locke (EDML), the DML containing

FIG. 1. (a) The walls of a medical leech, inside up, after the removal of the internal organs. Left half; ventral wall. Right half; dorsal wall. (b) A dorsal muscle strip, about 0.2 mm wide and 20 mm long, cut along the dorsal median line.

eserine sulphate (1 mg/100 ml). The fluid, EDML, came down continuously from a reservoir to the bath, soaked the muscle strip, and then flowed out through an overflow to an aspirator⁽¹⁾. Before adding ACh, the supply of EDML was stopped. The fluid, EDML, remaining in the bath was removed by suction with the tip of the aspirator, and a dose of ACh (ACh chloride), diluted in EDML and 0.2 ml in volume, was put into the bath. The agonist was allowed to act for 0.5 or 1 min depending on the sensitivity of the muscle response, after which time the thin muscle strip was rinsed by EDML. Usually, the muscle relaxed well within 5 min after the starting of the wash. When a low muscle response was observed, an increasing amount of ACh was added in an exponential progression of 2. The sensitivity of the muscle response to ACh varied from preparation to preparation: the muscle strip contracted in response to ACh over a range of 10^{-8} – 10^{-20} g/ml, this range being lower than that reported by previous workers using the Japanese medical leech^{(3-4), (12-13)}. Fig. 2 shows a typical muscle response: 0.5 min contractures with ACh over a range of $1/8 \times 10^{-13}$ – 1×10^{-13} g/ml and relaxation processes within 5 min following rinse. The threshold of the muscle response is low and the relaxation is rapid. The dose-response curve is seen to be quite linear on a semilogarithmic graph paper (Fig. 2; Inset). The muscle response seemed fairly specific to ACh in comparison with the other agonists tested. The muscle which contracted with ACh at 10^{-10} g/ml did not respond to the following substances (g/ml doses in parentheses): choline chloride (10^{-4}), noradrenaline hydrochloride (10^{-7}), dopamine hydrochloride (10^{-2}), histamine hydrochloride (10^{-7}), serotonin creatinine sulphate (10^{-7}), tryptamine hydrochloride (10^{-4}), sodium glutamate (10^{-2}), glutamine (10^{-2}), γ -aminobutyric acid (10^{-2}), and glycine (10^{-2}).

FIG. 2. A record of the muscle response to ACh over a range of $1/8$ - 1×10^{-13} g/ml. The amine was added with 5.5-minute cycle; contraction for 0.5 min followed by washing for 5 min for relaxation. Water temperature; 18°C . Inset; The dose-response curve of the specimen is shown in Fig. 2. Plotted on a semilogarithmic paper.

Four factors in preparation were found critical for muscular relaxation and contraction threshold: 1) A preparation thicker than 0.5 mm would not relax well, as Whittaker had pointed out¹⁰. Usually a thick muscle took a long time to relax, i.e. 15–20 min or even more, as previous workers who had used the Japanese medical leech for ACh bioassay have reported⁴,¹². Moreover, the thick muscle strip was found to be insensitive to ACh. 2) The second factor which affected threshold and relaxation was the temperature of the solution used in soaking the muscle (see ref. 12). The effects of temperature between 13 – 21°C were tested: below 15°C the muscle would neither respond to a high dose of the amine, e.g. 1×10^{-6} g/ml or more, nor relax well. In some cases, the muscle which had responded well to one dose of ACh would not give any contracture on a second try with the same dose. 3) The third point found critical was the time spent in isolating the muscle strip. The more rapid operation gave the better preparation, i.e. the one more satisfactory in both relaxation and contraction. To speed up muscle isolation neither the skin nor the brown fat covering the muscle strip were removed. 4) The fourth point is a selection of the portion to be cut out. Since the dorsal skin and the brown fat are too thin to remove from the muscle without damaging the preparation, an elaborate removal of skin and fat often results in the worst preparations. Care was paid to cut out the muscle strip along the dorsal median line, this portion having the least amount of brown fat.

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ERRATUM

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page 604, line 5:

correct: Moreover, the thick muscle strip was found to be poor in sensitivity to ACh.

incorrect: Moreover, the thick muscle strip was found to be in sensitive to ACh.