again in the human adult only pathologically in the liver-principle deficiency anemias (pernicious anemia, tropical sprue, bothriocephalus anemia, pernicious anemia of pregnancy), the leukemias and agranulocytosis (one case in Dr. Hal Downey's collection).

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A Micro-Bioassay Method for Acetylcholine.*

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One of the most specific and sensitive tests for acetylcholine in body fluids is the leech muscle suspended in a saline bath containing eserine (Fuehner, Minz[1,2]). When only small quantities of blood are available they must be diluted with sufficient saline to fill the bath. This procedure naturally decreases the sensitiveness of the test. Moreover, in smaller animals, the repeated drawing of several cc. of blood is often detrimental to the carrying out of a series of tests. The method presented here eliminates these disadvantages by suspending the leech in foam from small quantities of blood.

The leech is attached to a hook at the bottom of a glass bath (2x6 cm.), the other end leading to a writing lever. A narrow opening at the funnel-shaped bottom of the bath is connected with an air tank by means of rubber tubing, so that air is allowed to enter at the desired rate. A wire loop serves as a guide to prevent the blood soaked thread from adhering to the wall of the tube. The leech is prepared in the usual manner and is suspended in eserinized saline until it is relaxed. The saline is then drained completely and the blood which is to be tested is placed in the bottom of the tube. The air passing through the blood creates a foam, which passes over the muscle as it is carried upward. If there is acetylcholine present in the blood, the contraction of the muscle then starts immediately. (See Fig. 1.) In order to obtain uniform results, it is necessary to keep the amount of air entering the bath constant. After the foam has exerted its effect on the leech, the bath is washed with saline,

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1 Fuehner, H., *Biochem. Z.*, 1918, **92**, 347.

2 Minz, B., *Arch. f. exp. Path., u. Pharmakol.*, 1932, **167**, 85; **168**, 292.

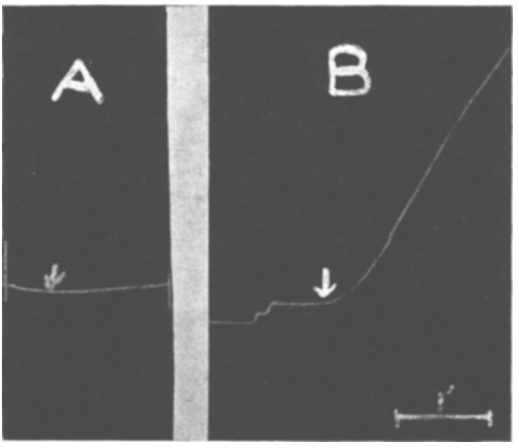

FIG. 1.
Leech Muscle, 1:7 Amplification.
A. Action of foam from 0.25 cc. blood from femoral vein of dog, containing Chlorazol Fast Pink and eserine 1:200,000.
B. Foam from 0.25 cc. blood from femoral vein of dog, containing Chlorazol Fast Pink, eserine 1:200,000 and acetylcholine chloride, 1:125 million.

with which it is left filled, until the muscle is relaxed and ready for another test. In order to prevent coagulation of blood and foam, a small quantity of an anti-coagulating substance must be added. This is done by rinsing the syringe with an 8% solution of "Chlorazol Fast Pink" before drawing the blood, the amount adhering to the syringe walls being sufficient to prevent coagulation. This solution may contain eserine in order to prevent the rapid destruction of acetylcholine. In experiments in which a known quantity of acetylcholine chloride (Hoffmann-LaRoche) was added to the eserinized blood, the resulting curve of the contraction of the leech caused by the blood foam could be matched by filling the bath with saline containing the same concentration of acetylcholine, provided the volume of the blood used was not less than 0.5 cc. If only 0.3 to 0.4 cc. of blood are available, their effects must be matched against foam from an equal quantity of old serum containing the known acetylcholine concentration. During a period of 2 or 3 minutes, foam from old serum (without acetylcholine) has no effect on the leech.

With this method we have been able to determine a given concentration of acetylcholine in as little as 0.3 cc. of blood or serum with a margin of error of about ±20%. With less than 0.3 cc. of blood containing acetylcholine, the leech still contracts (See Fig. 1) but the margin of error becomes greater when the effect is matched with known concentrations.

We believe that the principle of this method can be successfully adapted to other bio-assays on isolated organs.