13193

Effect of Neural Stimulation on Choline-Esterase Activity of the Blood.

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Croxatto, Huidobro, Croxatto, and Salvestrini[1] published a paper showing an increase of almost 100% in the choline-esterase activity of the renal blood of the cat, during neural stimulation of the leg muscles. In an attempt to verify these findings, similar experiments were performed on the frog as well as on the cat with modified technics.

For the frog experiments, a perfusion technic was used. The cannulae were introduced into the dorsal aorta and the ventral abdominal vein as described by Fenn, *et al.*[2] The perfusion fluid was a mixture of gum acacia, Ringer's solution, and beef red blood cells.[3] Five experiments were done, analyzing only the supernatant fluid after the red cells had been removed by centrifugation, and one experiment in which the whole perfusion fluid was analyzed. In all cases, analyses were made of arterial samples and of venous samples taken, (a) in the resting state, (b) during about 10 minutes of electrical stimulation of the leg muscles through the sciatic nerve, and, (c) after stimulation had ceased. The stimulation was accomplished by means of a condenser discharge type stimulator. The frequency and intensity of the shocks were adjusted to produce maximal contraction and were not constant. The samples were analyzed for choline-esterase with the differential volumeter apparatus of Fenn[4] according to the principle used by Stedman and Stedman.[5] One cc of bicarbonate buffer (.65% NaCl and .4% $NaHCO_3$), and one-half cc of 1% acetylcholine solution in saline were placed in the bottom of the flask, and 1/32 to ¼ cc of the test solution, depending on the activity, was placed in the side arm. In cases where there was appreciable hemolysis of the red blood cells, the degree of hemolysis

1 Croxatto, H., Huidobro, F., Croxatto, R., and Salvestrini, H., *Comptes Rendus Soc. Biol.*, 1939, **130**, 236.

2 Fenn, W. O., Koenemann, R. H., and Sheridan, E. T., *J. Cell. and Comp. Physiol.*, 1940, **16**, 255.

3 Saslow, G., *Am. J. Physiol.*, 1938, **124**, 360.

4 Fenn, W. O., "The Differential Volumeter for the Measurement of Cell Respiration and Other Processes." *The Science Press*, 1935.

5 Stedman, E., and Stedman, E., *Bioch. J.*, 1935, **29**[2], 2107.

was determined by measuring the hemoglobin content of the supernatant fluid by dilution and color comparison. In the experiment using the whole perfusion fluid, the amount of dilution was calculated from a measure of the hemoglobin content by the acid hematin method.[6]

Two experiments were done on the cat. The animals were anesthetized with Dial and injected with chlorazol fast pink (1 cc of 5% solution/kg) to prevent blood clotting. Blood samples were taken by cannula from the saphenous vein[7] before stimulation, during 2 minutes of stimulation of the leg muscles through electrical excitation of the severed sciatic nerve in the sciatic notch, and 10 minutes after stimulation had ceased. Other samples were taken from the carotid artery at the beginning and end of the experiments. Both whole blood and plasma of each sample were analyzed for cholinesterase by the same method as in the frog experiments. In both experiments, records of the tension developed by the gastrocnemius group of one leg were made and found to show maximal response. On each of these blood samples, plasma potassium analyses had been made by Mr. J. R. Jordan in connection with another experiment and these determinations showed a normal rise of potassium on stimulation, indicating normal functioning of both muscle and nerve.

The results of the frog experiments were negative. When there was no hemolysis of the red cells, there was little or no cholinesterase activity in the plasma and the amount bore no relation to stimulation. When the red cells had hemolysed, the activity was proportional to the degree of hemolysis, *i. e.*, plotting the activity against the percent of hemolysis gave a straight line. In the experiment in which the whole perfusate was analyzed, the data showed no correlation between stimulation and high choline-esterase activity of the perfusate.

In the cat experiments, the results were quite different from those of Croxatto *et al.*[1] In these experiments there was a rather wide scattering of the values found but again there was no correlation with stimulation. A summary of the frog data and the data on Cat I are shown on the graph (Fig. 1). The second cat experiment was not as complete as No. 1 (fewer samples were taken) but the cholinesterase value for the one stimulated sample was not statistically different from those of the resting and arterial samples. The average

[6] Hawk, P. B., and Bergeim, O., "Practical Physiological Chemistry," Blakiston, 11th edition.

[7] Fenn, W. O., Wilde, W. S., Boak, R. A., and Koenemann, R. H., *Am. J. Physiol.*, 1939, **128**, 39.

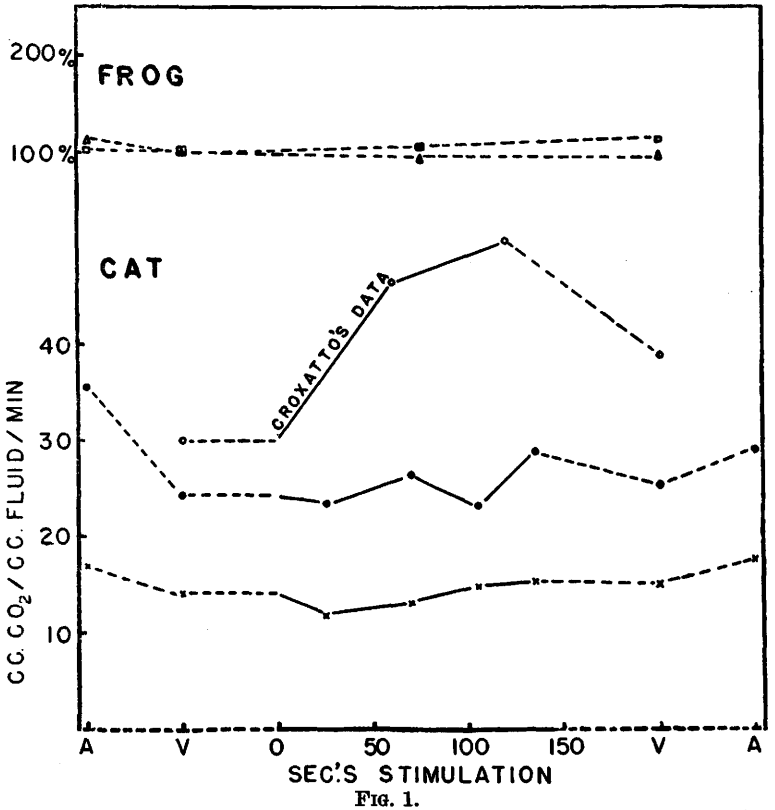

FIG. 1.

For the 5 frog experiments the points are the average choline-esterase activities expressed as percent of the resting venous value. The solid symbols indicate stimulation. □ = whole perfusate; △ = supernatant fluid (the average of four experiments). The lower curves show the results of the first cat experiment. ● = whole blood; × = plasma. The cat plasma values are computed by averaging the results of direct measurement and values obtained by back calculation from the whole blood values and the hematocrit. An average value for choline-esterase content of the red blood cells was computed and subtracted from the whole blood value for each sample in proportion to the hematocrit of that sample. The figures of Croxatto *et al.* are included for comparison. The vertical scale is arbitrary. In all curves, the solid portions of the line are plotted using seconds of stimulation as the abscissa; the dotted portions have no time scale except that the readings were made before and after stimulation as indicated. A is arterial and V is non-stimulated venous.

values were: stimulated venous, 16.5 cc CO_2/cc fluid/min; resting venous, 16.7; arterial, 15.6. In the first cat experiment the values were: stimulated venous, 25.5; resting venous, 24.4; arterial, 32.2.

These experiments indicate that stimulation of the leg muscles in either the frog or the cat does not cause an increase in the choline-esterase activity of the venous blood coming from that limb. The discrepancy between these results and those of other authors may be

due to the fact that, in the work presented here, the blood was taken directly from the limb, the saphenous vein of the cat, while in the previous work, the renal vein was the source of the blood samples. The increase of choline-esterase found under those circumstances may be due to some other factor in some other part of the body rather than to a direct output of choline-esterase by the stimulated muscles.

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13194

Action of Nicotinic Acid on Coagulation of the Blood.

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Calder and Kerby[1] recently reported that the prolonged coagulation time of heparinized blood could be significantly shortened *in vitro* by the addition of 1% nicotinic acid. They stated " . . . . that nicotinic acid does not duplicate the action of, nor can it be substituted for, any of the known factors involved in blood clotting," and furthermore suggested that its effect might be due to chemical neutralization of antithrombin.

The following experiments were performed in order to investigate the mechanism of action of nicotinic acid on the coagulation of the blood.

Methods. The coagulative potency of 1% nicotinic acid in 0.85% NaCl; 1% nicotinic acid amide in 0.85% NaCl; Nicamin,* representing 1% nicotinic acid; 0.001% protamine;** thromboplastin solution;[2] 0.85% NaCl; and distilled water was tested with the blood of 4 healthy human adults in whom the bleeding time, coagu-

† Assisted by grants from Mr. Frank Kennedy and the Pacific Institute of Tropical Medicine.

1 Calder, R. M., and Kerby, G. P., *Am. J. Med. Sci.*, 1940, **200**, 590.

* Monoethynolamine nicotinate, Abbott. Dilutions of the commercial product representing 25 mg of nicotinic acid per cc were made with 0.85% NaCl.

** Kindly supplied by Eli Lilly & Co.

2 Quick, A. J., *J.A.M.A.*, 1938, **110**, 1658.