13837

Action of *Triturus* Toxin on the Heart of the Frog.

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Since Twitty and coworkers[1] demonstrated the existence of a powerful toxin in the eggs and embryos of the amphibian *Triturus,* investigations have been conducted to determine its physical and chemical characteristics and physiological effects.

Horsburgh, Tatum and Hall[2] showed that the toxin produces death in mammals, usually preceded by convulsions, by direct depression of the central nervous system. These workers demonstrated that while paralysis of the respiratory nerves and muscles occurs as a result of depression of the centers, the toxin paralyzes somatic motor nerves and skeletal muscle generally. Vagus and splanchnic nerve responses are abolished, but not, according to the evidence obtained, those of the cervical sympathetic trunk. The experiments gave no evidence of an effect on cardiac or smooth muscle since arterial pressure responses to adrenalin were not affected. The arterial fall in pressure was attributed to vasomotor paralysis.

Fuhrman and Field[3] demonstrated that the toxin has little effect on the oxidative metabolism of nervous tissue in experiments treating rat brain *in vitro* with the toxin.

Although Horsburgh, Tatum and Hall[2] were unable to observe any effects of the toxin in the concentrations used on cardiac tissue, it seemed of interest to determine whether at higher concentrations the toxin might not have some direct effect on the beating heart. It seemed possible that further light might be cast on the action of the toxin by testing its effects on rhythmically contracting mechanisms in which it might be possible to separate effects on the pacemaker, on conduction and on contractility. Accordingly both mechano- and electrocardiograms were made of the heart of *Rana pipiens* under treatment with toxin.

The toxin, kindly provided by Dr. Tatum, was dry and had been computed to be of a potency of approximately 3500 mouse units per g. This unit was arbitrarily chosen and defined by Horsburgh, Tatum and Hall[2] as the amount of toxin that killed a 20 g mouse in 10 min. Test solutions were made by adding the dry toxin to frog Ringer's. Although the weight of the toxin was accurately computed and the approximate potency recorded for each test solution, the meager knowledge of the chemical nature of the toxin necessarily limited this work to a qualitative analysis of its effects on the heart. It was observed that over a space of a few months the effectiveness of the dry toxin, even though it was kept on ice, decreased markedly. It was thus noted that in the earlier tests solutions made up to contain .125 m.u. per cc of perfusate had a marked effect, while in later experiments the concentration had to be doubled to duplicate the former results.

Tests were run on the isolated heart in a perfusion chamber, on hearts *in situ* cannulated in the left auricle and on hearts *in situ* with the postcava cannulated. The latter technique produced the most uniform results and gave more normal electrocardiograms.

The first characteristic reaction to occur under treatment with adequate concentrations of toxin was ventricular stoppage in diastole. Direct electrical stimulation showed that the quiescent ventricle retained its contractility but that on continued treatment the contractility was gradually lost. Continued treatment of the heart in which ventricular stoppage had occurred next brought about

[1] Twitty, V. C., and Elliott, H. A., *J. Exp. Zool.*, 1934, **68**, 247; Twitty, V. C., and Johnson, H. H., *Science*, 1934, **80**, 78; Twitty, V. C., *J. Exp. Zool.*, 1937, **76**, 67.

[2] Horsburgh, D. B., Tatum, E. L., and Hall, V. E., *J. Pharm. and Exp. Therap.*, 1940, **68**, 284.

[3] Fuhrman, F. A., and Field, John, 2d, PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 435.

cessation of sino-auricular activity. This ventricles- auricles-sinus order of stoppage was characteristic. In a number of tests after complete stoppage had been brought about in the heart, washing with Ringer's brought back normal beat, and as was expected, the resumption of sino-auricular activity preceded that of auriculo-ventricular activity.

Electrocardiograms of hearts treated with concentrations inadequate to produce stoppage showed a decrease in rate and a lengthening of the P-R interval. If concentrations great enough to produce stoppage were used, there occurred first a lengthening of the P-R interval and later disappearance of the QRST complex with transient persistence of the P wave. Finally the P wave disappeared. The disappearance of the QRST complex coincided exactly with the observed cessation of ventricular activity. Recovery in Ringer's showed the same reversal of activity as did the mechanocardiograms. It was noted in both mechano- and electrocardiograms that low concentrations brought about a decrease in rate, while with high concentrations no perceptible decrease in rate occurred before cessation of activity.

To conclude, it may be said that *Triturus* embryonic toxin primarily affects the conducting mechanisms in the frog heart and secondarily contractility. Auriculo-ventricular conduction is first impaired, then sino-auricular activity, and lastly the contractility of the heart muscle itself.

13838

Estimation of Tryptophane Content of Various Proteins.

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An extensive study of the tryptophane content of various proteins was made by Jones, Gersdorff and Moeller.[1] They employed the Holm and Greenbank[2] modification of the May and Rose[3] method, in which the protein, together with a solution of paradimethylaminobenzaldehyde in 17.5% hydrochloric acid is incubated at 37° until maximum intensity of color develops. This usually requires from 6 to 8 days. Comparison is made with a standard casein similarly treated.

The desirability of shortening the time required for maximum color development led Bates[4] and Sullivan, Milone and Everitt[5] to propose modifications based on the suggestions of Komm[6] and of Boyd,[7] involving the use of mild oxidizing agents as accelerators. Bates[4] adds sodium nitrate to the mixture of casein and reagent in hydrochloric acid, and obtains maximum intensity of color very speedily at room temperature. Sullivan, Milone, and Everitt[5] digest the mixture of protein and reagent in 17.5% hydrochloric acid at 85° for 15 min, add a 0.3% solution of hydrogen peroxide, and read after cooling to room temperature.

The short procedure of Sullivan *et al.*[5] would permit the rapid estimation of the tryptophane content of a large number of proteins. Accordingly, 20 proteins, obtained, with the exception of egg albumin, from Jones,[1] were analyzed by this method. This permitted the comparison of the results so obtained with those reported by Jones, Gersdorff and Moeller.[1]

1 Jones, D. B., Gersdorff, C. E. F., and Moeller, O., *J. Biol. Chem.*, 1924, **62**, 183.

2 Holm, G. E., and Greenbank, G. R., *J. Am. Chem. Soc.*, 1923, **45**, 1788.

3 May, C. E., and Rose, E. R., *J. Biol. Chem.*, 1922, **54**, 213.

4 Bates, R. W., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, 1937, **119**, vii.

5 Sullivan, M. X., Milone, H. S., and Everitt, E. L., *J. Biol. Chem.*, 1938, **125**, 471.

6 Komm, E., *Z. physiol. Chem.*, 1926, **156**, 35.

7 Boyd, W. J., *Biochem. J.*, 1929, **23**, 78.