Levels of Serotonin during Convulsion and the Effect of Reserpine

IN view of the contradictory reports¹⁻³ with respect to changes in the 5-hydroxytryptamine (5-HT) levels of the mammalian central nervous system during convulsion, this problem was re-examined in rats in which convulsion was induced by intraperitoneal injection of fluoroacetate or 'Metrazol'. The former convulsant was particularly preferred because, unlike the convulsive agents generally employed, its action can be related to specific biochemical events, that is, blocking of the citric acid cycle⁴ with concomitant changes in certain amino-acid⁵ and ammonia⁶ levels of the central nervous system.

The serotonin content of brain, small intestine and spleen of rats treated with convulsants, with or without simultaneous administration of reserpine, was compared with control animals. The solution of reserpine for intraperitoneal injection was prepared by dissolving reserpine crystals (100 mgm.) in 0.1 ml. of concentrated formic acid, 2.5 ml. propylene glycol, 2.5 ml. absolute alcohol and water to make the volume 20 ml. (Formate was used instead of glacial acetic acid to avoid the competition of acetate with fluoroacetate.) The animals which did not receive reserpine received only intraperitoneal injection of reserpine solvent. The animals were killed at 60 min., with the onset of convulsion at 30 min. The amount of fluoroacetate injected was greater than the LD₅₀, 5 mgm./kgm. for rats, in order to ensure the severity and the recurrence of seizures between onset and time of killing.

Statistical analysis of the values in Table 1 indicates that fluoroacetate-produced convulsion does not cause an increase of serotonin-level in the brain or in the other organs analysed. Administration of reserpine did not produce any delay in the onset of convulsions or in the incidence of seizures. However, it depleted serotonin within 1 hr. by 78 per cent from the brain of convulsing animals, but not from the spleen or the intestine, a pattern similar to that produced by reserpine in non-convulsing rats⁸. Administration of reserpine did not alter the fluoroacetate-produced citrate accumulation in the brain to any degree of statistical significance. In another series of experiments 'Metrazol' (60 mgm./kgm.) was administered intraperitoneally alone and with reserpine. 'Metrazol', like fluoroacetate, had no effect on the 5-HT values of the brain of convulsing rats killed 1 hr. after administration of the drug. Again,

convulsions produced by 'Metrazol' had no effect on the release of 5-HT by reserpine from the brain.

It can safely be stated that there is no statistically proved evidence to demonstrate that fluoroacetate, and, under the conditions of our experiments, 'Metrazol', have any effect on the 5-HT levels of rat brain.

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Enzymic Release of Sialic Acid and Inactivation of Human Menopausal Gonadotropin

It has been shown that the biological activity of chorionic and pituitary gonadotropins is lost when the hormone materials are treated with influenza virus or with receptor-destroying enzyme¹⁻³. This enzyme has been recognized as neuraminidase⁴, and the loss of biological activity of gonadotropins on treatment with the above catalytic agents was accompanied by the release of sialic acid. It seemed desirable to investigate the effect of neuraminidase on the chemical composition and biological activity of a purified preparation of human menopausal gonadotropin^{5,6}.

The relative potency of the preparation used, in terms of human menopausal gonadotropin-24, was 100 when tested by the augmentation of uterus weight in 3-week-old female mice. It contained 7.8 per cent of sialic acid, determined by the diphenylamine reaction⁷ and expressed as N-acetylneuraminic acid. Heating at 100° C., pH 2, for 1 hr. destroys the activity and quantitatively removes sialic acid, but it is possible that other bonds (sugar, amino-acid) were broken.

10 mgm. of human menopausal gonadotropin preparation, dissolved in 10 ml. M/15 phosphate buffer, pH 6.3, was incubated with about 30,000 units of neuraminidase (receptor-destroying enzyme, Behringwerke) at 35° C. After 24 hr. the solution showed a loss of gonadotropic activity of 80 per cent, as compared with a control kept under the same conditions but without the receptor-destroying enzyme. Reaction mixture was then dialysed twice against 10 vol. of water at 0° for 48 hr. About 60 per cent of initial sialic acid was found in the dialysate. After concentration, the dialysate was applied to a column of 'Dowex 2' 200-400 mesh (20 × 0.8 cm.), equilibrated in 0.1 M formic acid⁸. The effluent was collected in 3-ml. fractions which were analysed for their sialic acid content. Elution with 0.3 M formic acid yields two peaks (fractions 13-19 and 24-29).

Table 1. EFFECT OF CONVULSION ON 5-HYDROXYTRYPTAMINE CONTENT OF SOME RAT TISSUES AND THE INFLUENCE OF RESERPINE. Sprague-Dawley male rats (350-400 gm.) received intraperitoneally sodium fluoroacetate (8 mgm./kgm.) and reserpine (6 mgm./kgm.) as indicated. 5-Hydroxytryptamine was determined by the spectrofluorometric method (ref. 7) and the citrate, as described elsewhere (ref. 4). Numbers of animals indicated in brackets

Group	5-Hydroxytryptamine (μ gm./gm. wet tissue)			Citrate (μ gm./gm. wet tissue) Brain
	Brain	Spleen	Small intestine	
Control (10)	0.41 $\pm$ 0.03*	2.02 $\pm$ 0.22	2.08 $\pm$ 0.30	19 $\pm$ 1.3
Fluoroacetate (10)	0.48 $\pm$ 0.03	1.78 $\pm$ 0.27	2.11 $\pm$ 0.36	151 $\pm$ 10
Fluoroacetate + reserpine (10)	0.11 $\pm$ 0.05	1.90 $\pm$ 0.28	2.30 $\pm$ 0.30	119 $\pm$ 13
Reserpine (5)	0.12 $\pm$ 0.05	2.34 $\pm$ 0.67	3.00 $\pm$ 0.39	
'Metrazol' (6)	0.40 $\pm$ 0.02	2.17 $\pm$ 0.18	3.43 $\pm$ 0.31	
'Metrazol' + reserpine (6)	0.25 $\pm$ 0.05	2.40 $\pm$ 0.25	3.16 $\pm$ 0.26	

* S.E.