Antihypertensive Action of Neotetrazolium. (22602)

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Tetrazolium salts have been used to measure various aspects of the dehydrogenase activity in living tissue systems. Most experiments have dealt with excised structures(1,2) or with tissue homogenates(3) incubated *in vitro*. The reaction between the tetrazoles and living enzyme systems involves reduction of the colorless salt to an insoluble purple to black formazan, with the greatest accumulation in areas of growth, multiplication and high metabolic activity(1). It has also been shown that pp'-diphenyl bis 2-(3,5 diphenyl) tetrazolium chloride (Neotetrazolium), when injected intraperitoneally or subcutaneously in the monkey, rat or rabbit, lodges in considerable amount in the cerebrospinal and sympathetic ganglia of the nervous system where it is reduced to the insoluble formazan particularly in the satellite cells, but also in the nerve cells. The darkly colored ganglia stand out prominently in contrast to the remainder of the nervous system which remains uncolored(2,4).

Since it has been shown that blood pressure can be reduced in hypertensive conditions by interfering either by surgical or pharmacological means with nerve transmission through the sympathetic ganglia, the possibility was entertained that the intracellular deposition of tetrazolium salts might produce ganglionic blockade. Accordingly, the effect of these salts on the blood pressure of hypertensive rats was investigated.

Methods. Neotetrazolium (NT) was the agent used, hypertensive rats receiving daily subcutaneous injections of 0.5 ml or 0.25 ml of a 0.5% solution in saline. The studies were carried out on a series of 12 rats made hypertensive either by a figure-of-eight loop about both kidneys, or unilateral nephrectomy and constriction of the remaining renal artery. The hypertension was allowed to develop for periods of from 2-6 months, before placing the rat on the tetrazolium regime. Blood pressures were recorded by the Friedman microphonic method(5) on the tail of lightly etherized rats.

Experimental. In most instances the NT was administered for test periods of from 5-10 days. After 3 days of treatment, the blood pressure began to fall and remained at hypotensive levels as long as the NT was administered. On the average, the blood pressure was reduced by 90-100 mm Hg, reaching levels as low as 60-70 mm Hg. The lowest level was reached within 3-5 days after the initial NT injections and the pressure remained in this range for an additional 2-3 days after terminating treatment. Thereafter, the blood pressure rose and within a week had returned to its previous high level. In 2 of the rats, the blood pressure did not return to its original hypertensive levels for at least a month.

Fig. 1 is a representative protocol which shows the rapid decline in mean blood pressure after daily administration of 0.5 ml of the NT solution to a 4-month hypertensive rat. As indicated, a second series of 4 injections with 0.25 ml of the NT solution likewise had a dramatic antihypertensive action.

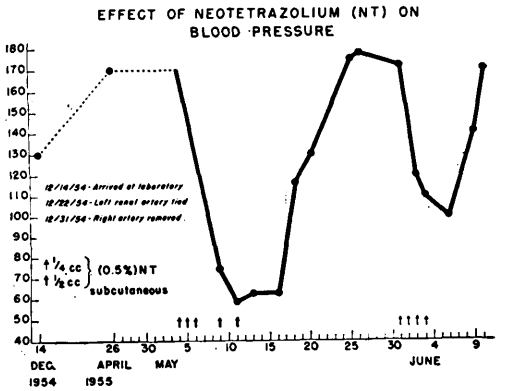

FIG. 1. Representative protocol of blood pressure measurements (indirect) of renal hypertensive rat (partial ligation of renal artery). Each point on B.P. curve represents avg of 4-5 readings at indicated interval. (C. F. Wistar strain, female, wt at initial operation 140 g, at final reading 210 g.)

Although injection of NT, in the amounts used, resulted in a moderate loss of weight (20-30 g), the animals appeared in good health. In addition to the formazan present in the ganglia, various other tissues showed evidence in coloration. The adrenals were lightly colored, with the formazan chiefly in the innermost reticularis. The kidney and liver had a faint purplish hue, which on histological examination showed formazan deposits in the parenchymal cells. In the brain proper, no reduced NT was evident in section including the hypothalamus and vasomotor centers.

A small series of normotensive controls (4 rats) were placed on a comparable NT regime and blood pressure measurements taken. The larger dose, 0.5 ml of a 0.5% NT solution, dropped the pressure from control levels of 110-120 mm Hg to 60-65 mm Hg. The smaller dose 0.25 ml of 0.5% NT produced only a transient and fleeting drop of 20-30 mm in blood pressure for 2-3 days when administered for 6 consecutive days.

Discussion. The mechanism whereby NT reduces the blood pressure of hypertensive rats is not clear. The fact that NT is irreversibly reduced to formazan in the nervous system ganglia suggests a chemical blocking action equivalent to temporary ganglionectomy. It is unlikely that the physical presence of formazan *per se* is responsible for the fall in blood pressure since the deposits remain in the ganglia almost indefinitely, while the blood pressure returns to hypertensive levels in about 5 days. The possibility therefore remains that with the irreversible reduction of NT, enzyme activity is impaired(3,6). Black, Zweifach and Speer(7) have shown that sodium azide, a metabolic inhibitor, produces a lowering of blood pressure in rat and man with hypertension.

Inasmuch as the tetrazolium salts are also deposited in the adrenal cortex, particularly in the reticularis, following subcutaneous injection(1,2), the role of an adrenal pathway is likewise to be considered. In view of the minor *in vivo* deposition of NT in the kidneys, the possible interference with the elaboration of a renal factor appears unlikely, although not ruled out.

Summary. When the tetrazolium salt pp'-diphenyl bis 2-3 (3, 5 diphenyl) tetrazolium chloride (Neotetrazolium) is injected subcutaneously, it lodges in considerable amount in the sympathetic ganglia of the nervous system where it is reduced to the insoluble formazan. The satellite cells are heavily colored in contrast to the nerve cells. A series of renal hypertensive rats were given daily subcutaneous injections of from 0.25-0.5 ml of a 0.5% solution of neotetrazolium (NT) for periods of from 5-10 days and the blood pressure recorded by an indirect microphonic method on the tail. The NT lowered the blood pressure within 2 days after the treatment was initiated to levels as low as 60-70 mm Hg. Within 1 week after treatment, the pressure had returned to control levels. Subsequent injections had a comparable hypotensive action. It is suggested that NT may reduce the blood pressure by impairing the metabolic activity of the ganglionic cells, although an indirect effect on other organs such as the adrenal has not been ruled out.

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