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Effect of decaborane on the norepinephrine content of rat brain*

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OTHER investigators[1, 2] have found severe depression, bradycardia, hypotension, and rigidity to be features of decaborane ($B_{10}H_{14}$) poisoning. Some of these symptons resemble those resulting from the action of reserpine.[3] In addition, hyperglycemia has been observed in decaborane intoxication[4] and after administration of reserpine.[5] It seemed possible, therefore, that the same mechanism might be responsible for some of the effects seen with both compounds.

It is well known that brain tissues are depleted of catecholamines by reserpine and that decreased concentrations of norepinephrine are related to sedation, at least in some species.[6]

In view of the similar neurological symptoms exhibited by both animals and humans intoxicated with the boron hydrides, this investigation was initiated to determine whether decaborane alters the levels of norepinephrine in the rat brain.

Decaborane was dissolved in Mazola corn oil (Corn Products, New York, N.Y.) and administered i.p. to male albino rats (400–450 g) of the Sprague–Dawley strain. Since decaborane is known to react with a variety of organic compounds, and has been shown to be hydrolyzed by alcohol and water,[7] the decaborane–corn oil solution was used immediately after dissolution. The animals were sacrificed by cervical dislocation and the brains quickly removed and homogenized in ice-cold 0·4 N perchloric acid. The procedure of Anton and Sayre was used for the assay of catecholamines.[8] Recovery of added norepinephrine from rat brain homogenate was unaffected by pretreatment of the animals with decaborane.

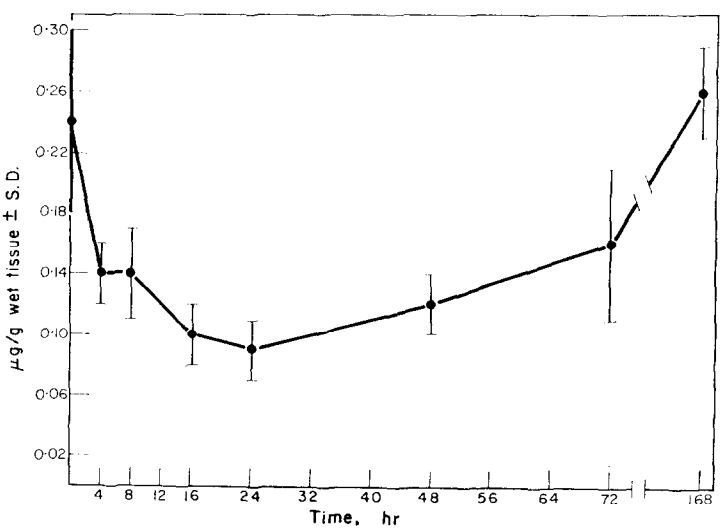

FIG. 1. Rat brain norepinephrine levels vs. time. Control is the mean of ten animals. Other points represent five animals each after i.p. injection of 15 mg decaborane/kg.

The time-response experiment (Fig. 1) used 15 mg decaborane/kg body weight. This dosage is approximately 75% of the 24-hr LD_{50}. Within 15 min after injection the animals became quite sedated and when forced to walk they did so with a peculiar rolling gait. Sedation persisted for more than 48 hr. From 24 to 48 hr the animals were very belligerent when prodded and often began to fight among themselves. The concentration of norepinephrine in the brain declined fairly rapidly

* Experiments were conducted according to the 'Principles of Laboratory Animal Care' of the National Society for Medical Research.

during the first 4 hr, then more slowly to about 37% of control values during the next 20 hr. The norepinephrine concentration remained at this level from 24 to 48 hr, then increased slowly, reaching normal levels in approximately 7 days.

From the dose-response curve (Fig. 2) it can be seen that 10 mg/kg exerts maximal effect in terms of depletion of norepinephrine, i.e. to about 40% of control levels. However, there is a marked

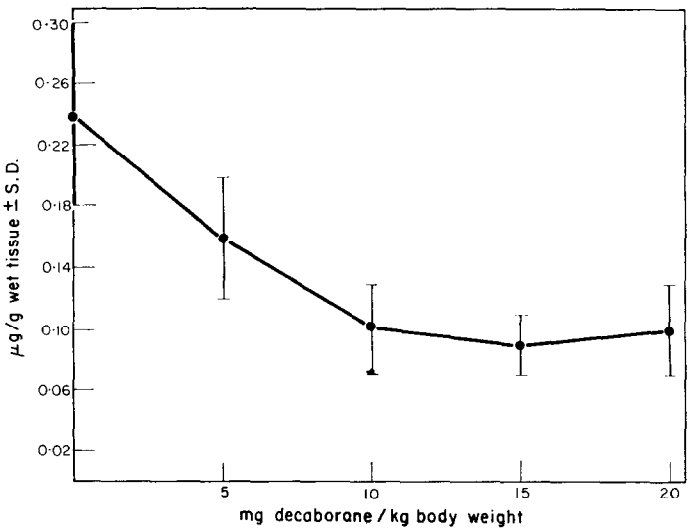

FIG. 2. Rat brain norepinephrine vs. dosage of decaborane. Control is the mean of ten animals. Other points represent the mean of five animals each after i.p. injection of decaborane. All samples collected 24 hr after injection.

increase in lethality when the dosage is increased from 15 mg/kg to 20 mg/kg. It is recognized that this introduces a bias into the data, since norepinephrine levels were measured only in those animals that survived 24 hr—i.e. 5 out of the 10 given 20 mg/kg. Corn oil given as a control induced no decrease in the concentration of norepinephrine.

In reserpine-treated rabbits, norepinephrine is depleted 90% within 4 hr,[9] and in reserpine-treated rats it is depleted 93% within 6 hr,[10] whereas in decaborane-treated rats a maximum of only 63% norepinephrine depletion occurs after 24 hr. Therefore, the mechanism of depletion of catecholamines by decaborane may not be the same as for reserpine.

Boranes are electron-deficient compounds and are good reducing agents. As a group they react with ammonia, organic amines, unsaturated hydrocarbons, various heterocyclic amines, and a host of other compounds. In a biological system there would be a variety of possible chemical interactions with boranes, e.g. formation of complexes with polyhydroxy molecules such as norepinephrine.

Further research is being conducted into the effects of decaborane on norepinephrine and other tissue amines in order to elucidate its mechanism of action on the biogenic amines of the central nervous system.

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On glucuronide formation in the cat

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IT HAS been reported[1, 2] and is apparently generally accepted[3, 4] that cats do not use glucuronic acid as a means of detoxifying foreign organic compounds, although they do form a glucuronide of bilirubin.[5] Their failure to use this common mammalian detoxication mechanism has been attributed to a lack of glucuronyl transferase, rather than to an inability to form UDP-glucuronic acid.[6] In man and dog, after the administration of iopanoic acid,* the principal biliary iodine-containing compound has been identified (by isolation) as an ester glucuronide.[7] The same is true[8] of tyropanoic acid.† Iopanoic acid-glucuronide is poorly absorbed when given to cats orally but concentrates in the bile more rapidly than the parent compound when given intravenously.[9] It is also known that cats convert iopanoic acid to a highly water-soluble conjugate.[7] If this conjugate is not a glucuronide, a glycinate seems to be the most likely alternative. To establish which type of conjugation actually occurs the following experiments were performed.

Healthy adult cats weighing 2–3 kg were prepared for study as previously described.[7] The radiopaques (100 mg/kg, calculated as the acids) were administered orally as finely divided powders in capsules (iopanoic acid and its glycine conjugate‡ as the acids; tyropanoate and bunamiodyl§ as the sodium salts). Sixteen hr later the cats were sacrificed under pentobarbital anesthesia, the gall bladders were tied off and removed, and the following items were determined on the bile.

A. Total iodine (by the method of Zak and Boyle[10]).

B. Ether-extractable iodine and conjugated glucuronic acid. To 0·1–0·2 ml of bile was added 10 ml of 0·1 N hydrochloric acid and 50 ml of ether. After thorough extraction (3–5 min mechanical shaking in 100-ml g.s. centrifuge tubes) and centrifugation, glucuronic acid was determined[11] on the residue from 1–2 ml aliquots of the ether, and total iodine was determined[10] on the residue from 40-ml ether aliquots. (Note: the ether extraction method as described readily extracts the glucuronide conjugates of iopanoic and tyropanic acids[7, 8] but not necessarily quantitatively).

C. Unchanged radiopaque. To 0·2 ml of bile was added 10 ml of acetate buffer (0·5 M, pH 4·7), and the solution was thoroughly extracted (as described in *B*, above) with 40 ml of a 1:1 mixture (v/v)

* Available from Winthrop Laboratories, New York, N.Y., under the trade name of Telepaque. Iopanoic acid is 3-amino-α-ethyl-2, 4, 6-triiodohydrocinnamic acid.

† Available from Winthrop Laboratories, New York, N.Y., under the trade name of Bilopaque. Tyropanoic acid is 3-butyrylamino-α-ethyl-2, 4, 6-triiodohydrocinnamic acid.

‡ Prepared by Dr. A. A. Larsen; m.p. 197–198°; % iodine = 61·2.

§ Available from E. Fougera Co., Hicksville, N.Y., under the trade name of Orabilex. Bunamiodyl is 3-butyrylamino-α-ethyl-2, 4, 6-triiodocinnamic acid.