mine, a concomitant rise in plasma histamine, and increased peripheral vascular responsiveness to histamine. These findings support the view that histamine performs a crucial role in progressive development of hypotension following administration of endotoxin.

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Circling Syndrome Produced in Mice by Dimethylaminohexose Reductone. (25844)

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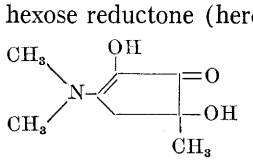
That certain amino-sugar reductones produce circling phenomena in rats, the movements suggesting those seen in waltzing mice, will shortly be reported by Ambrose, Robbins and DeEds of the U. S. Dept. of Agriculture who made the initial observations. After continued feeding the rats circled rapidly lasting many minutes, often set off by such stimuli as a bright light. Pulmonary hemorrhages and thyroid hyperplasia were observed but no primary central nervous or labyrinthine lesion was found. Lack of body fat, presumably from excessive exercise, was characteristic. In this paper reactions produced in mice by one of these reductones, dimethylaminohexose reductone (hereafter called reductone)



are reported.

Results. 1. *Induction of circling.* The circling syndrome was easily produced in white Webster mice by reductone. Table I shows effect of route and dosage on circling. Administration in drinking water or by injection (subcutaneous or intraperitoneal in distilled water or saline) was effective. Maximal effect was obtained after a single injection of 5 mg/20 g mouse, or 4 daily injections of 2.5 mg. At higher dosages early deaths occurred in some animals, and at lower dosages the syndrome was absent or incompletely developed.

2. *Circling syndrome.* Reductone symptoms were noticeable 3 to 4 hours after administration. The first, and most characteristic sign was head tossing, an upward jerk or shake repeated rapidly, accompanied by obvious difficulty in coordination and balance.

TABLE I. Effect of Dimethylaminohexose Reductone Administration to Mice.

No. of mice	Daily dose	No. days admin.	Route	Results
10	.1%	60	In drinking water	Whirling in 24 hr; 4 deaths in 2 mo
6	.6 mg	1	Intraper.	No effect
6	1.25	1	"	*Idem*
10	2.5	1	"	Some head tossing; poor circling
10	5.0	1	"	Rapid head tossing; maximal circling
100	5.0	1	Subcut.	*Idem*
10	7.5	1	Intraper.	"
10	10.0	1	"	" , but 2 deaths in 2 days
6	.6	4	"	Slight head tossing if mouse excited
6	1.25	4	"	Moderate head tossing
6	2.5	4	"	Rapid head tossing; good to maximal circling
6	5.0	4	"	Rapid head tossing; maximal circling

At almost the same time periodic circling began. This consisted of running in tight circles, in either direction, for periods of seconds to several minutes. Periodic maximal episodes of circling occurred within a few hours and continued, though with a gradual lessening of accompanying incoordination, for the lifetime of the mouse, even after a single injection. Head tossing and circling periods could often be induced by noise, handling, bright light, or new surroundings. When not so stimulated the mice tended to be rather quiet, huddling in a group. Several less striking characteristics were also noted. The mice seemed generally more active than controls. They did not build hills or nests in the sawdust on floor of cage but kept it flat, apparently from circling and running. A pregnant reductone mouse, however, if put alone, usually built a good nest. The backs of male animals became scarred, and in places hairless, from backbiting. When tested for swimming ability, these mice still circled, some swimming in circles on the surface, while others consistently dived under the water to circle on a vertical plane. These latter would drown if allowed to do so.

3. *Weight changes.* During first 5 days after administration of reductone, the mice lost as much as 25% of body weight. Although they were seen to eat, their head jerking appeared to interfere when they raised their heads to drink from overhead tubes and water consumption was greatly reduced. By 7 days they seemingly learned to compensate for the head bobbing and had begun to regain weight, and by 2 weeks had reached their original weight. Striking thinness, as described for rats was not characteristic, and an occasional mouse became somewhat obese. A group of 10 mice, 6 months after induction of reductone syndrome, weighed between 26 and 34 g, a range similar to that for normals of same age. Although food consumption was not measured, the impression was that they ate more food than normal mice.

4. *Effect on body temperature.* Rectal temperatures of 26 mice were taken 6 months after administration of reductone, using thermocouple previously described(1). Average temperature, 40.5°C, did not vary significantly from that of similar number of normal controls, 41.0°C. The periodic increased activity did not produce sustained increase in temperature.

5. *Oxygen consumption.* Oxygen consumption was measured by a simple method(1) in mice 6 months after reductone administration. Measurements were made during quiet periods when mice were not circling or actively tossing their heads. Average time for consumption of 4 cc of oxygen was 3.6 ($\pm$ 0.75 SD) and 3.5 ($\pm$ 0.76) seconds/g body weight respectively for 12 reductone mice and 11 control mice of similar weight. It is apparent that resting reductone mice do not have a higher oxygen consumption than normals.

6. *Quantification of activity.* The general activity of reductone mice was compared to that of normals on activity table previously described(1). Ten normal mice had average activity table count of 678 recorded move-

TABLE II. Effect of Stress on Normal and Reductone Mice, the Latter Showing Slight Impairment on Turntable and Marked Impairment on Rotating Rod.

Wk since reductone (5 mg/mouse subcut.)	No. of mice	% success in 15 trials	
		Turntable	Rotating rod
Controls	80	96 ± 3*	92 ± 7*
4	10	84 ± 5	13 ±12
12	"	93 ± 5	43 ± 5
16	"	100 ± 0	50 ± 7

* ± S.D.

ments in the standard 4 minute test. Each was then given reductone, 5 mg subcutaneously, and tested 7 and 8 days later, when there was no sustained circling. Average activity counts were 736 and 674, indicating a lack of change in activity (when there was no circling). Minor head tossing was not of magnitude great enough to activate the activity table counter. When animals were tested during active circling, counts rose to high levels. Thus 2 mice that circled almost constantly during the test, produced counts of 3398 and 2879, one that spun intermittently had a count of 1137, but one that spun only once or twice gave a count of 722, *i.e.*, in the average range. Thus the basic (non-circling) activity registered on this apparatus was not changed by reductone administration. Periods of increased ordinary movement were obviously balanced by periods of subnormal activity in a 4 minute period. All-out spinning produced a high activity count during circling.

7. *Response of reductone mice to stress* was studied on the turntable and 3 cm rotating rod(1). Each mouse was tested 15 times. Data are recorded as percent of successful tries ± SD. Results are shown in Table II. Reductone does not interfere greatly with successful riding of the turntable, but interferes seriously, though decreasingly as weeks pass, with performance on the rotating rod.

8. *Gross pathological findings.* Mice were sacrificed daily for 12 days after administration of reductone. Diminution in body fat was noticeable on fourth day, but largely rectified by twelfth day. Lung hemorrhages, prominent in rats, were minor and occurred similarly in controls and reductone mice.

Discussion. The effectiveness of dimethylaminohexose reductone in producing a characteristic activity in mice prompts a comparison with other types of similar activity. The type most studied is undoubtedly that associated with genetic abnormalities and often called "waltzing"(2,3).

The "Japanese waltzer" with a *V waltzing* gene has a characteristic syndrome which consists of head shaking, running in circles, deafness and inability to swim. The pathology underlying this behavior is incompletely understood, but degeneration of inner ear structures within the first few weeks of life has been reported and degeneration of all acoustic tracts in the brain has been noted. Waltzing behavior characterized by choreic head movements, circling and inability to swim in varying degree, has also been found in other genetically independent strains, as follows: a. *Sh-1 shaker-1*—after 2-4 weeks degenerative changes of inner ear and possible atrophy of corpus callosum, deafness, nervous rapid up and down movements of the head; b. *Sh-2 shaker-2*—pathology not studied, deafness and movements indistinguishable from type above; c. *St shaker short*—embryological failure of development of semicircular canals and endolymphatic appendages; cochlea and cortical organs abnormal; deafness, disturbances of equilibrium as in shaker 1; d. *Kreisler*—resembles shaker-short mice; e. *Jerker and pirouette*—pathology not studied, deafness; f. *Fidget*—pathology not known, similar in behavior but do not become deaf. Some of these names may be duplicates as the genetic characteristics are not known in all cases. Some mice not ordinarily considered waltzers show abnormal excitability in youth. This is particularly true of the C-57 Black line; males of the NB strain are aggressive and show characteristic tail rattling. Also somewhat similar are seizures which can be produced by audiogenic stimuli, particularly in the DBA strain.

The effects of drugs on genetic waltzing were studied by Rothlin and Cerletti(4) who reported that lysergic acid, mono- or di-ethyl amide decreased waltzing in congenitally waltzing mice while at the same time increas-

ing general activity. A preparation of dihydroergot alkaloids (Hydergin) decreased circling and showed a generally sedative effect, while ergotamine and dihydroergotamine did not influence activity.

Temporary or permanent waltzing behavior in mice similar to that of genetic waltzers has been produced by a number of drugs. According to Goldin(5), Ehrlich reported that arsacetin was active in this respect. Goldin also reported waltzing from dialkyl and heterocyclic derivatives of β-chloroethylamine. Pathological studies of the mice revealed destruction of cells of the granular layer of the lingula of the cerebellum within 2 to 5 days after injection and also small areas of gliosis in brain stem, medulla and upper cord. Inner ear and auditory nerve appeared undamaged.

Athough β-aminoproprionitrile, the poison found in sweet peas which causes lathyrism, did not cause waltzing, the bis form has been reported to produce the waltzing syndrome. Thus Hartman and Stitch(6) showed that after doses of 1.5 to 2.0 g/kg of bis aminoproprionitrile (bis-β-cyanoethylamine; β β′-iminodiproprionitrile) abnormal movements began in about 3 days and lasted many days with characteristic running in circles, moving backwards, twitching and a general resemblance to waltzing mice. Rudberg(7), using somewhat smaller doses, 0.75 to 1.5 g/kg, observed a maximal amount of waltzing in 8 to 15 days which continued for more than 3 months. The pathological changes were described by Bachuber(8) as destruction of Purkinje cells in the cerebellum, and of anterior horn ganglion cells in the spinal cord. The hyperactivity caused by bis-β-aminoproprionitrile was reported by Azima and Grad (9) to be temporarily controllable with reserpine, by Widlocher *et al.*(10) with lysergic acid diethylamide and by Selye(11) to be prevented by thyroxin. (We have not been able to show a similar thyroxin effect on mice given reductone.) Rudberg(7) reported similar effects from chlorpromazine, but noted that hyperactivity was not affected by Hydergin or ergotamine tartrate.

Scholler(12) reported that methylphenyltriazine in doses of 0.5 g/kg intraperitoneally or 1 g/kg orally in mice gave impairment of righting reflexes within 2 hours, followed by depression and death. Half these doses gave head bobbing and inability to swim well, such as characterize waltzing mice.

Another type of abnormal movement, somewhat different from waltzing, was reported by Siegmund, Cadmus and Lu(13), as a writhing syndrome, which followed administration of 2-phenyl-1, 4-benzoquinone in dose of 1 mg/kg intraperitoneally. Various analgesics exerted a suppressive effect. In our laboratory these writhing symptoms appear to be at least in part the result of peritoneal irritation, since vehicles alone such as 0.3% carboxymethylcellulose give similar effects.

The effects produced by reductone resemble those seen in genetically waltzing mice, and those described by the substituted β-chloroethylamines and bis-β-amino-proprionitrile, and to some extent those produced by methyl-phenyltriazine. However, pathological lesions in the central nervous system or in the labyrinthine or auditory portions of the ear have not been observed. The characteristic syndrome after reductone shows all signs associated with waltzing except deafness, although impairment in swimming may be less severe. Although reductone gave similar effects in rats and mice, limited trials in goldfish produced only questionable abnormalities.

Conclusions. 1. A permanent circling syndrome can be produced in mice by a single injection of dimethylaminohexose reductone. 2. The syndrome is characterized by frequent head tossing and running in circles. Body temperature, oxygen consumption and basic activity are not altered but ability to resist stress by staying on a rotating rod is decreased.

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Urinary Sulfate Ion as a Naturally Occurring Inhibitor of DNase II Activity.* (25845)

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It was reported(1) that human urine inhibits its enzymatic activity of desoxyribonuclease II (DNase II) and activates DNase I slightly. The inhibitory material was dialyzable, thermostable and was not retained by anion and cation exchange resins. In the experiments to be described, an attempt was made to establish the chemical identity of the urinary inhibitor of DNase II.

Methods. 1. *Materials.* a. *Desoxyribonuclease II.* DNase II was prepared from calf spleen using the first 3 steps of the preparative procedure of Koszalka *et al.*(2). The sediment obtained in Step 3 was dissolved in a small volume of water and dialyzed overnight against running tap water at room temperature. In those experiments concerned with effect of inorganic ions on enzymatic activity, the preparation was dialyzed against 0.0001% aqueous sodium versenate. b. *Desoxyribonucleic acid.* DNA of salmon sperm was used as purchased from Calif. Fn. for Biochemical Research. c. *Urinary inhibitory material.* 890 ml of freshly voided human urine was dialyzed overnight against 1,080 ml of distilled water. The resulting 960 ml of dialysate was concentrated to 265 ml by lyophilization. In some experiments, the concentrated dialysate was passed subsequently through a column of activated charcoal (80 mesh). d. *Ashing of inhibitory material.* To determine whether the inhibitor removed by the ion-exchange resins was organic or inorganic in nature, the solid residue remaining after evaporation of solvent from the charcoal-treated urinary dialysate was ashed by heating to redness in a porcelain crucible over a Bunsen burner. The white ash so formed was dissolved in 1 N HCl and the resulting solution evaporated to dryness repeatedly, adding water after each evaporation. The residue was then dissolved in a volume of water equal to that of initial concentrated urinary dialysate. 2. *Analytical procedures.* a. *Assay of DNase II activity.* Enzymatic activity was determined by an adaptation of the procedure for DNA determination described by Schneider and Hogeboom(3). Unless stated otherwise, the following test system was employed:

Acetate buffer, 0.2 N, pH 5.2	2.0 ml
DNA, 0.4% solution	.5 "
Distilled water	1.0 "
DNase II	.2 "

This mixture was incubated for one hour at 37°C and then deproteinized with 1 ml of 2.88 M trichloroacetic acid (TCA). Simultaneously, a similar mixture, lacking DNA, was incubated and then deproteinized with TCA. To the latter deproteinized reaction mixture, 0.05 ml of a 0.4% solution of DNA

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