Summary. A single injection of 0.5 ml of phenolized tularemia vaccine elicited detectable agglutinating antibodies in 67 of 162 (41.4%) patients with hematologic disorders as compared to 45 of 48 (93.7%) in normal controls. Particularly significant was the lack of response in 23 of 25 chronic lymphatic leukemia and in 37 of 57 lymphoma patients. In the latter group only 5 of 27 females as compared to 15 of 30 males exhibited antibodies.

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Received January 3, 1961. P.S.E.B.M., 1961, v106.

Acute and Subacute Toxicity of Amino-Hexose-Reductones. (26433)

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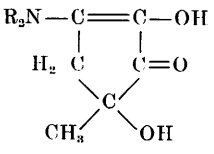
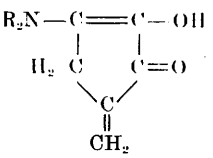
The well-known Maillard reaction, responsible for browning discoloration in many foods, involves combination of reducing sugars with amino groups of amino acids, peptides, and proteins, with subsequent enolization and dehydration of the sugar radical(1, 2). In studies on the sugar rearrangement and dehydration reactions, Hodge and collaborators(3,4) isolated new "amino-hexose-reductones," $R_2N \cdot C_6H_7O_3$ (I), and "anhydro-amino-hexose-reductones," $R_2N \cdot C_6H_5O_2$ (II), from reactions of hexoses with secondary amine salts and determined their structure(4). Because these reductones showed excellent antioxidant properties in fats and oils(5,6), toxicity studies were undertaken to determine their suitability for food use.



(I)



(II)

The present report presents data on the acute and subacute toxicity of 5 reductones, namely, dimethylamino - hexose - reductone (DMA) and its anhydro derivative (ADMA), piperidino-hexose-reductone (PIP) and its anhydro derivative (APIP), and morpholino-hexose-reductone (MORPH).

Methods. Aqueous propylene glycol was used as solvent for DMA and MORPH in acute toxicity studies employing oral and intraperitoneal administration. Because of lack of a suitable solvent of low toxicity for PIP, APIP, and ADMA toxicity determination of these reductones was limited to single oral administration of a suspension in 20% gum acacia. The limited amount of material available for determination of acute toxicity necessitated the use of mice (weighing 15 to 20 g each) except in the case of DMA for which an approximate determination of the toxicity was made by single intraperitoneal injection in rats (weighing 125 to 150 g) as well. In addition 2 groups of 5 mice each were given daily oral doses of 25

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and 50 mg/kg of DMA respectively for a period of 3 weeks, Saturdays and Sundays excluded, for detection of cumulative effects. All dosages refer to mg/kg of body weight.

A more severe test for detection of cumulative effects of each of the 5 reductones was a 100-day feeding experiment. Each reductone was studied by placing 5 young rats of each sex on dietary levels of 0.0, 0.06, 0.12, 0.25, 0.5, and 1.0% reductone in a basal diet having the following percentage composition: corn meal 73, casein 10, linseed oil cake meal 10, alfalfa 2, cod-liver oil 3, bone ash 1.5, and sodium chloride 0.5. The rats ranged in weight from 32 to 47 g and were distributed to give groups of 5 rats of each sex with approximately the same average weight. Each group was housed with shavings as bedding, and with free access to food and water.

Results. DMA. Fifty-six mice injected intraperitoneally with DMA dissolved in aqueous propylene glycol received doses ranging from 25 to 1000 mg. Propylene glycol concentration varied from 5 to 50% to permit injection of approximately the same volume of solution. These concentrations of glycol produced no reactions in 4 control mice. All mice given 200 mg or less of DMA survived. All those given 400 mg or more died. Four of 7 mice receiving 300 mg died on the second day, indicating an LD_{50} of approximately 300 mg DMA injected intraperitoneally. Death occurred in 2 to 3 hours after doses of 400 and 500 mg, and in about one hour after doses of 600, 800, and 1000 mg. In all instances where death occurred depression developed shortly after injection of DMA, followed by coma.

Mice injected with 25 and 50 mg of DMA differed from controls in exhibiting sensitivity to touch on 6th day after injection. They showed none of the symptoms of surviving mice given 100 mg or more. The latter animals showed increased sensitivity to touch and noise the day after injection. After 24 to 36 hours all survivors periodically held the head to one side, frequently with nodding movements, and in some instances generalized tremors were present. The choreic head movements were frequently followed by circular running. These symptoms persisted during 3 months of observation.

Groups of 4, 4, and 5 mice were given orally 200, 300, and 400 mg respectively of DMA dissolved in 25% aqueous solution of propylene glycol. No deaths occurred, and all animals exhibited the aforementioned symptoms during one month of observation.

None of the mice given daily oral doses of 25 or 50 mg DMA for 3 weeks showed any symptoms until the 8th administration, when some mice on the high dose exhibited circular running movements. At the end of experiment when a total of 16 doses had been given no symptoms had developed on the 25 mg dose, but 2 of the 5 animals receiving 50 mg per dose showed some whirling movements.

Nine rats divided into 3 equal groups were given 100, 200, and 300 mg DMA intraperitoneally. One rat on the highest dose died in 24 hours. All survivors showed the same typical symptoms during 6 months of observation.

ADMA. This reductone, administered orally in 20% gum acacia, was given to 53 mice in doses ranging from 200 to 1500 mg. No deaths and no symptoms occurred in 4 controls given gum acacia, nor in 8 mice receiving 200 mg. Five of 10 mice given 300 mg and 4 of 8 given 400 mg died in 1 to 2 hours. On dosage levels of 500 and 800 mg 25% survival occurred, but mortality was 100% on the higher dosage levels. Convulsions occurred before death, and the heart stopped in systole. No symptoms were noted in any of the survivors.

PIP. Thirty-four mice received this reductone orally as a 20% gum acacia suspension in doses ranging from 400 to 1200 mg. No LD_{50} was established. One mouse receiving 600 mg and 1 receiving 800 mg died after 9 and 6 weeks respectively. Twenty-four hours after administration of all dosage levels spasmodic jerk of the head, generalized tremors and whirling occurred. When observations were discontinued 10 weeks later 25% of the mice given 800 mg or less and 50% of those receiving 1000 or 1200 mg continued to show symptoms.

APIP. A suspension of this reductone in 20% gum acacia was given orally to 46 mice in doses of 200 to 1200 mg. No deaths occurred in controls nor in animals given 200 or 300 mg. Mortality was 25% on doses of 400, 500, and 700 mg; 20% on 800 mg; 75% on 1000 mg; and 100% on 1200 mg. Time of death was unrelated to dosage and varied from 5 to 90 minutes, except on 1200 mg where 2 deaths occurred the following day. No typical symptoms were noted in the survivors.

MORPH. An aqueous 33% propylene glycol solution of MORPH was given intraperitoneally to 61 mice in doses from 100 to 1000 mg and orally to 23 mice in doses from 700 to 1200 mg. No deaths occurred in controls nor in mice injected with 600 mg or less. Mortality was 12.5% after 700 mg, 37.5% after 800 mg, 50% after 850 and 900 mg, and 100% after 1000 mg. No deaths resulted from oral administration of MORPH. Neither intraperitoneal nor oral administration of MORPH produced symptoms characteristic of DMA.

Feeding test-rats—100 days. The two noteworthy effects of feeding the various dietary levels of the 5 reductones were the presence or absence of reductone toxicity symptoms and time of onset, and effects on growth. The typical symptoms of hyperexcitability, elevation and nodding of the head, and whirling were produced only by DMA and PIP.

On 4th day of feeding, rats on a dietary level of 0.06% DMA showed increased activity as compared with controls on the basal diet. At this time daily ingestion of DMA was 55 mg per day. By the 14th day sporadic whirling was seen in some animals and rate of ingestion of reductone was 74 mg. On the 59th day all rats showed whirling. There was no difference in response of male and female rats to the compound. On diet levels of 0.12% and higher typical symptoms were noted on the 4th day and maximum intensity of response to DMA appeared on the 17th to 28th day, whereas maximum response on the 0.06% dietary level occurred about the 59th day. After development of maximum response there was no marked difference between effects on 0.06% and 1.0% dietary levels of DMA.

Increased activity and slight whirling produced by feeding PIP was first noted on the 26th and 3rd day on dietary levels of 0.06% and 0.12% respectively. By the 58th day typical symptoms were developed fully on all dietary levels. Again there was no difference in response of the sexes.

Growth rate was retarded significantly in both male and female rats on all dosage levels of all reductones with the exception of MORPH. Inhibition of growth increased with increasing dietary levels of the reductones. However, only APIP at dietary levels of 0.5 and 1.0% caused a significant decrease in food consumption. In all other instances food intake was equal to or greater than that of the appropriate controls. Increasing toxicity, as judged by effects on growth, places the reductones in the following order: MORPH, DMA, PIP, ADMA, APIP.

Discussion. Cutting and collaborators(7) have described the phenomena produced in mice by dimethylamino-hexose-reductone and compared it with other types of similar activity. The circling movements produced in rats and mice by dimethylamino and piperidino hexose-reductones differ from those produced in rats by magnesium deficiency as described by Kruse, Orent, and McCollum (8). Movement induced by reductones is a rotation in small circles rather than racing in a wide circle, and is not followed by rigidity and death. Symptoms produced by reductones occur in one to 3 days after single doses and persist for long periods of time.

Since long-term feeding tests in rats demonstrated growth inhibition on all dosage levels of all reductones except morpholino, and since anhydrodimethylamino, and anhydro-piperidino-hexose-reductones did not produce whirling under these conditions, it appears that growth inhibition and whirling are unrelated toxic effects.

Summary. The approximate LD_{50} values of dimethylamino-hexose-reductone and morpholino-hexose-reductone injected intraperitoneally in mice were 300 and 850 mg/kg respectively. Oral LD_{50} values of anhydro-

dimethylamino-hexose-reductone, dimethylamino-hexose-reductone, and anhydro-piperidino-hexose-reductone in mice were 300, 400, and 900 mg/kg respectively, while for the morpholino and piperidino-hexose-reductones they were greater than 1200 mg/kg. Single intraperitoneal injections and single oral doses of dimethylamino-hexose-reductone in both mice and rats, as well as long-term feeding of dimethylamino and piperidino-hexose-reductones in rats produced typical symptoms of hyperexcitability, elevation and nodding of the head, and whirling.

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Received January 4, 1961. P.S.E.B.M., 1961, v106.

Effect of Colchicine on Taurine Excretion.*† (26434)

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Many of the characteristic biological effects of colchicine parallel those produced by X-rays. Since Kay and Entenman(1) have demonstrated that X-irradiation markedly increases excretion of taurine by the rat, the effect of colchicine on taurine excretion in the rat was studied.

Materials and methods. Male Wistar rats weighing 200-300 g were used, each rat serving as its own control. During the control period urine was collected for at least 2 successive days of 21 hours each, 3 hours of each full control day being allotted to feeding the animals. Food was withheld during the treatment period but water was given *ad libitum* throughout. After the control period the rats were administered colchicine intraperitoneally in doses ranging from 0.8 to 6.4 mg/kg, and urine was collected for various periods up to 36 hours after colchicine administration. Values for taurine output are expressed as a percentage of each animal's taurine output during the control period. For assay of taurine in urine and tissues a modification of the method of Pentz *et al.*(2) was used. In this method extraction of the chloroform-soluble dinitrophenyl (DNP)-derivatives of Dowex-50-treated samples of the urine or tissues leaves DNP-taurine in the aqueous phase. Taurine content is then determined by comparing the absorption of the sample at 355 mμ in a Bausch & Lomb Spectrophotometer with that produced by standard taurine solutions under the same conditions. Paper chromatograms of the DNP-derivatives from urine and tissues in 3 solvent systems showed one component, the mobility of which was the same as authentic DNP-taurine.

Results and discussion. When urine samples were collected over the periods of 0-3, 3-6, 6-14, and 14-36 hours after colchicine administration, it was found that increased taurine excretion begins in the first 3 hours but does not continue beyond 14 hours after treatment. The data also suggest that the peak rate of excretion occurs sometime between 6 and 14 hours following colchicine.

In the control periods preceding treatment taurine excretion averaged 2 micromoles/hr/

* Supported by Nat. Inst. Health.

† Preliminary report presented at Fed. Am. Soc. Exp. Biol., April 11-15, 1960, Chicago, Ill., (*Fed. Proc.*, 1960, v19, 42).