

dye concentration in the slice to that in the medium. Ability of the slice to concentrate PSP from the medium is not markedly influenced by alterations of tissue weight between 200 and 400 mg.

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Effect of 6n-prophylthiouracil on Lethal Seizures in Mice.* (19203)

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The antithyroid activity of thiouracil has been often demonstrated and is generally accepted. On the theory that functional inhibition of the thyroid gland would produce decreased spontaneous activity, thiouracil was used to produce a reduction in susceptibility of an inbred strain of mice to sound-induced lethal convulsive seizures.

The author previously found that age played an important role in the susceptibility to sound-induced lethal convulsive seizures in tests made on several different inbred strains of mice (1-3). Since the endocrine and physiological levels vary throughout the life span of an individual it was believed that age and metabolism might influence the susceptibility to stress. This was found to be true and, in particular, was demonstrated in the DBA, or inbred dilute brown strain of mice where the highest susceptibility to lethal convulsive seizures at the sound of an electric bell was at the adolescent ages 25-39 days (4). Beyond 80 days of age there were no cases of convulsive individuals. Both sexes were affected, the males slightly more so. The form of convulsive seizures which occur in the DBA strain of mice simulates the grand mal epileptic seizures in humans and it is interesting to note that medical men as Lennox (5) have

found the incidence of epilepsy to be greater among children than adults and that the sexes are equally affected. The mouse seizures are of clonic-tonic form and are usually fatal at the peak of susceptibility in the DBA strain.

Methods.[†] The strain of mice used for these experiments is the dilute brown, DBA/2 Jax, maintained by brother to sister mating. The animals came from the author's colony and are derived from over 200 generations of inbreeding. Two hundred and one animals were used in this experiment: 151 as test or experimental animals and 50 sibs as direct controls. In addition the established performance of the strain as a whole (4) could be used for comparison. Both sexes were used in equal numbers. Seizure susceptibility tests were made at 30 days of age at which age mice are not sexually mature. Thiouracil treatment was started at 21 days of age when the mice weighed about 12 g each. The drug was administered in the food for 9 days previous to the test for seizures, since administering the drug through the drinking water did not prove practicable. The 6n-prophylthiouracil used was obtained from the Overbrook Chemical Co. (Philadelphia). This compound is one of the most highly active antithyroid agents as shown by Astwood (6). Purina Laboratory

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TABLE I. Effect of 6n-prophylthiouracil on Sound-Induced Lethal Convulsions in DBA/2 Mice at 30 Days.

	No. of animals	% of mice showing convulsions—			% of fatal convulsions
		Standing spasms	Clonic-tonic	Total	
Normal animals*	1316	.0	90	90	73†
Sibs of treated animals	50	.0	94.1	94.1	78
Thiouracil treated	151	31.5	.5	32	.0

* From Vicari, 1951.

† Based upon No. of convulsing subjects.

Chow nuggets were ground very fine and 100 mg of the compound was evenly distributed and mixed in 100 g of the ground chow food. From this mixture nuggets were made and stained with red food coloring to distinguish them from the ordinary nuggets given the controls. Also the shape of the drugged nuggets was made different from the ordinary ones. Both sets of animals were fed in the same way. Their food hopper was filled with their respective nuggets and water was available *ad lib*. It was estimated roughly that the daily food intake was approximately 3 g of food and 5 mg of thiouracil per mouse. The procedure of testing and inducing the convulsions by sound was that described previously by Vicari(4). The intensity of the sound used to induce seizures was approximately 90-93 decibels. One stress test was given each animal.

Results. The mice tested after 9 days feeding with 6n-prophylthiouracil gave no lethal seizures and 68% of the individuals had no convulsive seizures of any degree. The 32% of the individuals which had some form of convulsion did not show the severe clonic-tonic convulsive seizure exhibited by the controls. The per cent of mice showing the different patterns of convulsions in the controls and treated animals are given in Table I.

In the 68% of the treated animals not exhibiting any convulsions, the pattern of behavior was a "sit-and-walk" form in 54% of the individuals, and the "run-and-stop" form in 14% of the individuals. The typical behavior of a treated animal at the sound of the bell was as follows, in succession: the startle reaction, at 8 seconds the mouse circled around, at 16 seconds it stopped, and then sat, at 33 seconds it started running fast, at 45 seconds it began to tremble, quivering all over the

body, then at 58 seconds it jumped high, circled, tried wall jumping. At 80 seconds it stopped and sat breathing fast and quivered or sat and groomed. If no convulsion occurred within two minutes the observation was terminated.

In the 32% of the individuals convulsing, the duration of the convulsive spasm was lessened and the preconvulsive period was lengthened as compared with the controls. About one half of the mice, undergoing some form of convulsion, showed a peculiar behavior following the spasm. The animal went into a sort of cataleptic posture and remained so for from 2 to 3 minutes. The other half of the convulsing individuals showed a slightly different behavior. The animal would start to go into a clonic spasm, would *not* fall over on its side but would show a stiffness in the front legs as if paralyzed. This would give its movements a "hopping" gait for several minutes before the normal gait was resumed. Both forms of behavior were observed in both sexes.

A smaller group of 30 individuals which were fed the 6n-prophylthiouracil for 5 days previous to testing at 30 days showed similar protection from lethal seizures as the 9 days treated group.

Discussion. The slight variations in the manifestations of the thiouracil-fed animals may possibly be explained by the fact that the amount of the food ingested by each individual could not be accurately controlled. However, the results *do* show a reduced activity of the animal under sound stress and a very high reduction of susceptibility to clonic-tonic convulsions with total elimination of fatal seizures. That thiouracil has influenced the susceptibility to seizures shows that the thyroid gland has some role in the expression

of seizures and that it may be a contributory factor in its relation to the chief or primary cause.

Thiouracil has been used in morphological experiments but not, as far as can be ascertained in the literature, has it been used for the direct purpose of protecting an animal from convulsive seizures.

Summary. The effects of feeding 0.1% 6n-prophylthiouracil in the food for 9 days to an inbred strain of mice, DBA/2, recognized for its high susceptibility to sound-induced convulsive lethal seizures, were observed. One hundred and fifty-one mice were started the drug feeding at 21 days of age and 30 animals at 25 days. The animals were tested for sound-induced seizures at 30 days when the susceptibility was normally highest. Practically 100% protection from lethal seizures was produced by the use of the drug. Approximately 68% of the individuals showed no form of convulsion. The 32% which had some form of convulsive spasm did not show

the severe seizures exhibited by the controls. In this convulsive group the influence of the drug was manifested by a temporary paralysis of the front legs causing hopping movements or the behavior took the form of a trance-cataleptic posture which lasted for 2 to 3 minutes. Also the spasmodic convulsion was shortened in duration and the preconvulsive period was lengthened. It may be that the thyroid gland has some important role, even if secondary, in the onset or prevention of convulsive seizures as elicited by sound in the mouse.

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Effect of Bacteriophage on Virulence of *Corynebacterium diphtheriae*. (19204)

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For many years the question as to whether or not virulent (toxigenic) *Corynebacterium diphtheriae* can become wholly avirulent (non-toxigenic) has vexed the public health official and the microbiologist alike. A view widely held for years was that, in persons convalescent from diphtheria, virulent diphtheria bacilli revert to an avirulent state with passage of time. No sound evidence has ever supported this belief. On the contrary, extensive studies(1-7) showed that if the change from the virulent to avirulent state occurs in nature, it must do so very rarely. Until very recently, attempts in the laboratory to alter the virulence of *C. diphtheriae* have failed completely, with the possible exception of the instance cited by Morton(8). No evidence

has yet been offered that virulent strains become avirulent in nature.

On the other hand, all views on the stability of *avirulence* of *C. diphtheriae* must now be revised, and all epidemiological considerations concerning diphtheria must be altered in view of the demonstration by Freeman(9) in 1951 that certain bacteriophages readily induce certain strains of avirulent diphtheria bacilli to become definitely toxigenic and virulent for animals. We regard this discovery as one of the most fundamental in the field of diphtheriology since Loeffler's work. Dr. Freeman kindly sent us his avirulent strains and his bacteriophages. Two phages, A₆ and B₆, and five cultures, 324, 325, 326, 334, and 337B, were received. It is to be noted, as a pecu-