non-specific reactions of the phenolic group with ferric ion(1), the Folin-Ciocalteu reagent(3,6) and nitrates(4) or development of fluorescence in alkaline solution(5). Separation of certain derivatives has been achieved by extraction with organic solvents(1,2,5). All of these methods require several reagents and involve a number of steps. The ultraviolet method either by itself or in combination with a colorimetric method achieves a simple, direct separate determination of the various salicyl compounds that are likely to be present simultaneously in biological fluids.

Summary. A method is described for determination of salicylate blood levels, based on the absorption spectrum of salicylic acid in the ultraviolet range. The method is suitable both for routine clinical use and for research work.

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Effect of Epinephrine, Glucose and Certain Steroids on Fatal Convulsive Seizures in Mice.* (19518)

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This is one of a series of experiments in an attempt to define the cause of immediate death following convulsive seizures. In previous studies one of us reported that the incidence of susceptibility to sound-induced convulsive seizures was associated with age(1-3). This was established from a survey of various inbred mouse strains; the age level of maximum susceptibility varied with the strain. The experiments reported here represent attempts to locate, by preventive measures, the cause of lethal convulsion and its onset in the dilute brown, DBA, mouse strain. This closely inbred strain is characterized by its high seizure susceptibility (97% incidence) and high lethal seizures (80% incidence) at the age level of 28-34 days. The pattern of the seizure is similar in appearance to the grand mal epileptic seizure of children.

Since the blood sugar level is low in convulsive human subjects, and glucose is often administered for treatment, this substance was given to a group of lethal-seizure susceptible mice. Behavior disorder may well be dependent upon abnormalities of blood composition and the sympathoadrenal system may be associated with the blood composition, on this assumption injections of glucose and of epinephrine were tested.

Material and procedures. A group of highly "lethal-seizure-susceptible" mice of the dilute brown, inbred strain, DBA, was employed. Both sexes in equal numbers were used and the ages at the first injection was 28-32 days. A description of the physical characters of the strain and the method used for inducing seizures by the sound of an electric bell were given in a former paper(3). The intensity of the sound was approximately 91 decibels with a variation of about ± 2 decibels in different parts of the apparatus. The animals were individually tested in a galvanized iron tub with the bell hanging inside its wall. In each group there were from 40-50 individuals. Controls genetically related to the experimental

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TABLE I. Protective Effects of Epinephrine and Glucose on Fatal Convulsive Seizures in DBA Mice.

Groups and No. of animals	Size of each dose	No. of inj.	% of mice showing seizures 4 hr after last inj.* 0	Non-fatal	Fatal	Total
Epinephrine						
A (49)	10 μg	1	90	0	10	10
B (39)	50 "	1	100†	0	0	0
Epinephrine-glucose (10%)						
C (46)	10 μg-1 cc	1	94†	4	2	6
D (43)	"	5‡	90†	0	10	10
Glucose alone						
E (50)	1 cc-10%	1	50§	25	25	50
F (50)	"	2‡	54§	15	31	46
G (50)	1 cc-5%	1	55	17	28	45
Controls						
Direct, genetically related (76)			4	17	79	96
Standard, for strain (1316)			3	17	80	97

* Optimum duration of protection occurred between 1-4 hr.
† Continued protection from seizures to 24 hr.
‡ Inj. once a day on successive days.
§ Continued protection to 10 hr.

TABLE II. Protective Effects of Certain Steroids on Fatal Convulsive Seizures in DBA Mice.

Groups and No. of animals		Size of each dose	No. of inj.	% of mice showing seizures 8 hr after last inj.§ 0	Non-fatal	Fatal	Total
Pregnenolone	(50)	50 μg	10†	66	0	34	34
Testosterone	(50)	50	6*	85	8	7	15
Estradiol	(50)	25	10*	50	0	50	50
	(48)	50	6*	71	14	15	29
Cortisone	(10)	50	1	25‖	37	38	75
Cortin‡	(40)	.05 cc	6*	12	28	60	88
	(20)	.5	1	30	30	40	70
Controls							
Direct, genetically related (64)				4	17	79	96
Standard, for strain (1316)				3	17	80	97

* Inj. once a day on successive days.
† Inj. twice (10 hr apart) a day on successive days.
‡ 50 dog units per cc.
§ All gave some protection to 24 hr except cortisone which lost its effect after 6 hr.
‖ 4 hr after inj.

groups were used, also the established standard performance of this strain reported(3) previously was used for comparison. Experimental and control animals were all treated alike as to food and environment. The epinephrine doses were made by dilution of Parke Davis 1:1000 adrenalin chloride with physiological salt solution. The glucose was given in 5% and 10% strength. The size of each dose and the number of injections for each group are given in Table I. The animals were tested for convulsive seizures 1, 4 and 8 hours after the last injection. In cases where the protection was still effective the animals were tested up to 20 hours and longer. Previous experiments(4,5) with 6n-propyl thiouracil, administered in the food of DBA mice showed that this drug was a protective agent against the onset of seizures. This suggested a possible endocrine relationship. On this basis a group of hormonal compounds were tested: 1) pregnenolone acetate (Natolone-National

Drug Co.), 2) testosterone (Ciba-Methandren), 3) estradiol (Ciba ethinyl estradiol), 4) cortisone-Kendall's compound E, 5) cortin, (Parke Davis, Eschatin). From these compounds specific dosage strengths were made and were administered by injection. Two-hundred and twenty mice of the DBA strain were employed in this hormonal series. The ages of mice tested were 28-32 days except for the second estradiol (6 injections) group which were 36-38 days of age. The amount of each dose and the number of injections for each group are given in Table II, together with the percent of the individuals in each category which showed: 1) non-seizures, 2) non-fatal seizures, 3) fatal-seizures, and 4) total seizure incidence.

Results. The records and data of the epinephrine and glucose treatments are given in Table I. Among the combinations tried, the best treatment, in preventing lethal seizures was a combination of epinephrine with glucose. Fatal seizures were reduced to 2% of the individuals as compared with the 80% in untreated controls. The percentage of the individuals *not* exhibiting the onset of seizures was 90-94% as compared with the 3-4% of the control groups. Although the dose of 50 μg of epinephrine alone proved 100% effective, the treatment seemed to leave the animals somewhat lethargic.

The results in testing the few hormonal compounds are shown in Table II. Of the 5 compounds tested, testosterone was most effective with estradiol and pregnenolone close seconds. Cortisone and cortin were least effective.

Discussion. The wide range of effectiveness in the treatment of convulsive seizures indicates that the *mechanism* involved in the causes of such behavior disorder, as seizure susceptibility, is a complicated one. The results of our tests have been interpreted tentatively as indicating that susceptibility to sound-induced lethal convulsive seizures, in the DBA inbred strain of mice, is associated with the blood sugar level and also with the endocrine mechanism. Epinephrine alone and with glucose was very beneficial and the combination of the two appeared to be the best sustained protective measure against, not only the incidence of lethal convulsive seizures, but also against the incidence of the onset of seizures.

The hormonal compounds all steroids showed beneficial effects in various degrees on the incidence of, not only fatal seizures, but also the incidence of non-fatal seizures. Testosterone reduced the fatal seizure incidence from the control 80% to 7%, estradiol reduced it to 15% and pregnenolone to 34%. Moreover the incidence of seizures as a whole was also reduced from the control 97% to 15% by testosterone, 29% by estradiol, 34% by pregnenolone, 70% by cortin and 75% by cortisone. Since the availability of cortisone, at the time, was very limited it may be that the optimum dosage was not hit upon. It appears from our epinephrine tests that the medulla of the adrenal gland has more direct effect on the incidence of seizures than its cortex. The fact that all these hormonal compounds have some protective effect against the onset of sound-induced seizures in susceptible animals suggests that the mechanism involved is nonspecific in its physiology.

In his experiments relating to adrenalin injections to seizure-susceptibility in rats O. L. Lacey(6) found that a dose of 0.025 mg of adrenalin (¼ cc of 1:10,000 adrenalin chloride) administered to rats of 200 g body weight at 2-day interval over a period of 16 days, did not appreciably affect seizure-susceptibility. The number of individuals used by Lacey were 10 experimental (6 albinos and 4 gray rats) and 10 controls. The ages of his animals were "one year minus 3-8 days at the beginning of the experiments". Lacey claims no definite conclusion from his data as to the effect of the adrenalin injections. However he found the adrenalin injected rats averaged a 60% susceptibility incidence while the controls had a higher incidence or 70%. Also the average time to onset of the seizures of the adrenalin injected albino rats was 22.5 seconds while the control's average was 13.3 seconds. Larger doses of adrenalin might have given more definite results. His work(7) on the dependence of behavior disorder in the rat upon blood composition indicated that seizure-susceptible animals show significantly higher serum protein and significantly greater group

variability in blood sugar than do the non-susceptible animals.

Ginsburg, B., *et al.*(8) in their experiments with glutamic acid on DBA mice, used for their placebo injections a 5% dextrose solution (0.1 cc per 10 g of body weight) and claimed not to have found any effect of the dextrose injections. The dosages employed in our experiments were from 8 to 16 times greater than those of Ginsburg, and it will be noted that the larger dose gave better protection.

It has been shown that the susceptibility to seizures of the DBA mouse is hereditary(9-13), and although its precise mode of inheritance is not absolutely clear, there is evidence that multiple factors are involved. Our results strongly suggest that the manifestation or expression or release of the interaction of the genes involved may be controlled by a series of physiological factors, such as metabolism, and blood sugar level along with an optimum balance of hormonal secretions. All this supports the idea that the endocrine glands do play a definite role in the expression of convulsive seizures.

Summary. In an attempt to locate the cause which brings on convulsive seizures, agents to prevent lethal seizures in the DBA mouse strain, such as glucose, epinephrine and several hormonal compounds, steroids, were used. The DBA inbred mouse strain is characterized by its extremely high incidence of "lethal-convulsive-seizures" during the adolescent period. Testing mice in this period with various compounds showed that the DBA mouse responds to injections of all of them in various degrees of protectiveness against, not only fatal seizures, but also on the total seizure incidence. The untreated related animals showed 96% seizure incidence, while the epinephrine-glucose treated showed 6% incidence. As to "lethal-seizures", epinephrine with glucose reduced the fatality to 2%, while the untreated related controls had a 79% fatality. The hormonal compounds, especially testosterone, pregnenolone, and estradiol were effective in reducing the total seizure incidence from 96% to 15% and the fatal seizure incidence from 79% to 0-2%. The findings of our investigation support the conclusion that the supposedly genetic mechanism controlling the susceptibility to seizures, responds to treatments of glucose and various hormonal compounds. This experimental control of its expression suggests a mechanism which is sensitively responsive to endocrine secretions, and their interrelationships.

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