TABLE II.
Effect of Dietary Protein Concentration upon Hypertension and Nephrosclerosis Produced by Desoxycorticosterone Acetate Overdosage.
(Averages and standard errors.)

Groups	No. rats at end of exper.	Diets*	Final body wt	Nephrosclerosis		Hypertensive mean pressure		
				Incid. %	Severity %	Avg hypert.† 20 days	Avg hypert.† 40 days	% hypert. rats. 40 days %
1	16	15% casein	130 ± 5.2	100	79	154 ± 2.7 (19)	161 ± 3.8 (15)	100
2	13	30% "	135 ± 7.0	100	79	160 ± 3.0 (25)	157 ± 5.9 (11)	82
3	8	30% " + 10 X choline chl.	144 ± 7.3	100	79	—	163 ± 9.9 (8)	87

* Diets I and II.
† Figures in parentheses refer to number of determinations.

protein diets, the otherwise similar lesions induced by desoxycorticosterone overdosage are largely independent of the dietary protein intake.

We are grateful to the Schering Corporation of New Jersey for desoxycorticosterone acetate and to Misses L. Derome and T. Hansen for technical assistance.

16120

Effects of Dithiobiuret on the Central Nervous System.*†

SOL ALTSCHUL. (Introduced by W. S. McCulloch.)
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Astwood *et al.*[1] reported that 0.002% solution of dithiobiuret, substituted for drinking water, paralyzed and killed rats in 7 days although each took less than .5 mg per diem. Peripheral nerve, myoneural junction and muscle were normal in function. Muscle, peripheral nerve, brain, spinal cord, root and ganglion were microscopically normal. On return to drinking water, the animals promptly recovered, but while paralyzed, strychnine failed to induce convulsions. Effects of dithiobiuret on cortex and convulsant threshold is reported here.

Method. Dithiobiuret, prepared by the American Cyanamid Co., is sparingly soluble in water and as it goes into solution smells of hydrogen sulfide. Saturated solutions in saline or distilled water were used for local applications, intramuscular and intravenous injection, and 0.001 and 0.002% in tap water orally and intraperitoneally.

Two dogs, 3 cats and a fourth that had received 5 cc saturated solution intramuscularly for 8 days were used to determine effects of dithiobiuret when applied to cortex and injected intramuscularly or intravenously in animals under Dial.‡ Electroencephalograms of the fourth cat and one rat that had received 0.002% dithiobiuret for 9 days were

* The author is indebted to Dr. R. O. Roblin of the American Cyanamid Co., for placing dithiobiuret at his disposal.

† The work presented in this paper was done in partial fulfillment of the requirements for the degree of Master of Science in the Graduate School of the University of Illinois.

1 Astwood, E. B., Hughes, A. M., Lubin, M., Vanderlaa, W. P., and Adams, R. D., *Science*, 1945, **102**, 196.

‡ The author wishes to thank Ciba Pharmaceutical Products who kindly supplied this drug.

TABLE I.

Rat	Dosage and route, g/100 cc	Toxic change seen in rate	Threshold* prior to drug, volts	Threshold* prior to toxic changes volts	Threshold* during toxic changes, volts
1	.002 oral	severe	27.9†	50†	70-75†
4	" "	" (died)	45	50-55	70
5	" "	slight	45	50	55
7	" "	severe (pregnant)	40-45	55	70-75
14	" "	" (died)	—	—	—
15	" "	" "	—	—	—
16	" "	slight "	—	—	—
20	" "	died before results obtained	—	—	—
21	" "	severe	—	—	—
22	" "	"	—	—	—
8	.002 I.P.	slight (died after inj.)	—	—	—
9	" "	severe	—	—	90
10	" "	"	—	—	65
2	.001 oral	not toxic	40	50	—
3	" "	severe	46	45	65
6	" "	slight	45	50	75
17	" "	not toxic	—	—	—
18	" "	" "	—	—	—
19	" "	" "	—	—	—
11	.001 I.P.	severe	—	—	80-85
12	" "	"	—	—	70
13	" "	"	—	—	80

* Cortical threshold to electrically induced convu lsions.
† A copper electrode was placed into the mouth; in all other rats a brass electrode was used.
I.P. Intraperitoneal.

taken to determine effects of prolonged administration. Normal animals were used as controls. Electrical activity of cortex was recorded by a 6-channel Grass electroencephalograph.

Rats were used to determine effects of dithiobiuret upon cortical threshold to electrically induced convulsions. Convulsions were produced by a Goodwin, condenser discharge, stimulator§ with one electrode placed into the mouth; the other on the vertex of the scalp. Voltage was varied from 25 to 90 volts in 5 volt increments, while frequency of stimulation was 120 per sec. and time duration of the falling phase of each stimulus was 0.5 millisec. Duration of stimulation in all cases was 5 sec. and the interval between attempts to induce convulsions was 20 to 30 min. Cortical threshold to electrically induced convulsions was defined as voltage (other factors remaining constant) required to produce toni-clonic activity lasting more than 5 sec. after stimulation. Sixteen rats received unrestricted amounts of dithiobiuret orally and 10 rats received daily 10 cc doses of dithiobiuret intraperitoneally. Four rats given 0.002 and 3 rats 0.001% dithiobiuret orally were used to determine cortical threshold before, during, and after administration of the drug. Two rats receiving 0.002 and 3 rats 0.001% dithiobiuret intraperitoneally were used to determine cortical threshold only after toxic changes were seen. (Table I). After toxic changes in the animals were noted, 10% glucose in 10 cc doses were injected intraperitoneally. One dose was given to each of 6 rats and 2 doses were given to each of 2 rats.

Results. Electroencephalographic tracings of 4 cats and 2 dogs did not change after local application of dithiobiuret or after single intravenous and intramuscular injections of saturated solutions of dithiobiuret in doses ranging up to 100 cc. Prolonged administration of dithiobiuret in one cat and one rat failed to produce any significant difference in electrical activity of cortex from that of normal animals.

The following progressive physical changes were noted in rats 5 to 12 days after the

§ The Goodwin Stimulator used in this study was obtained through the courtesy of Lab-Tronics, Inc.

initial dose of dithiobiuret. First was a decreased ability to use their hind limbs, as indicated by a shuffling gait; the animals would lie still most of the time, unless their tails were pinched. Next, they completely lost the ability to use their hind limbs. Finally, the rats could not move at all and died. After toxic changes were first seen, it took from 2 to 7 days for severe changes and death to occur. A slight toxic reaction was defined as the beginning disturbance of gait, while a severe reaction was defined as an inability to use the hind limbs. Of 10 rats receiving 0.002% orally, 7 showed severe reactions, 2 showed slight reactions while one died before changes were noted. Five rats receiving daily intraperitoneal doses of 10 cc of either 0.001 or 0.002% solutions showed the same severe changes, which appeared however, a few days earlier. A sixth rat on 0.002% intraperitoneally died shortly after an injection, but had already displayed slight effects to previous doses. Of 6 rats on 0.001% orally, only 2 showed toxic effects.

With respect to the cortical threshold‖ to electrically induced convulsions, the following results were noted. Prior to administration of dithiobiuret, 6 rats displayed typical toni-clonic muscular contractions of a grand mal type after stimulation at approximately 45 volts. A seventh rat had a cortical threshold of 28 volts. A copper electrode, however, was placed into its mouth while in all others a brass electrode was used. In these 7 rats stimulated after the drug had been given and before toxic changes were seen, a change in cortical threshold varying from a decrease of one volt to an increase in 20 volts was noted (Table I). The average change was a 6-volt rise in threshold. These same animals when showing toxic effects had a rise in the cortical threshold of 10 to 45 volts, with an average rise of 26 volts. The rat showing only 10 volts change died before severe toxic effects were present. In 5 animals, tested only after toxic changes had been observed, the increase in cortical threshold ranged from 20 to 45 volts, with an average increase of 28 volts over the average cortical threshold of animals tested prior to administration of the drug. The form of the convulsions changed when the animals became intoxicated. Non-intoxicated rats responded to stimulation with typical toni-clonic seizures, while seizures in intoxicated rats were decreased in intensity with some animals showing sustained tonic seizures followed by minimal clonic movements. In these animals the voltage was raised until a grand mal type reaction occurred.

Recovery was not hastened by one or two daily intraperitoneal injections of 10 cc of 10% glucose.

Discussion. The effects of dithiobiuret in raising the cortical threshold to electrically induced convulsions is great only when the rats are almost paralyzed.

Since no change in electrical activity of cortex was detected in 4 cats, 2 dogs, and one rat after administration of dithiobiuret, the action can not be based on a disturbance in the electro-chemical system of the cortex responsible for the electroencephalogram.

Astwood *et al.* demonstrated that strychnine failed to induce convulsions in animals paralyzed by dithiobiuret. This, plus findings of ascending spinal paralysis in animals able to respond to painful stimuli, seems to indicate that dithiobiuret produces a functional change in the efferent system of spinal cord.

Summary. The effects of dithiobiuret on electrical activity of cortex were tested upon 4 cats, 2 dogs, and one rat. Normal animals or the same animals were used as controls. Twenty-two rats were used in chronic administration and 12 of these were used to determine cortical threshold to electrically induced convulsions. The significant findings were: (1) a lack of detectable electroencephalographic change in cortical activity of animals tested after immediate or prolonged administration of dithiobiuret, (2) cortical threshold to electrically induced convulsions of rats was raised by an average of approximately 26 volts and (3) the form of con-

‖ The term "cortical threshold" is used in the accepted manner, but since a non-blocking amplifier was not available for recording local changes, cortical discharges that were blocked at a lower level, might have occurred prior to observed seizures.

vulsion in intoxicated rats was altered.

Conclusion. Dithiobiuret is an effective anti-convulsant in rats only when they are intoxicated. The site of action of dithiobiuret is within the central nervous system; the most probable site is the efferent system of the spinal cord.

16121 P

Specificity of Eczematous Hypersensitivity to P-Aminobenzoic Acid Butyl Ester ("Butesin").

EDWARD L. LADEN AND LOUIS RUBIN, (Introduced by S. Rothman.)

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The present study was carried out on a 15-year-old boy who was originally seen for a contact dermatitis resulting from the application of butesin picrate ointment to a burn on the wrist. Routine patch test with the commercial ointment, which contains 1% butesin picrate, yielded a strongly positive reaction with marked reddening, edema and vesiculation.

Experimental and results. Patch tests were carried out in this patient with a series of related compounds in order to establish the range of specificity of this reaction. The materials studied were dissolved in triethanolamine in 2½% concentrations. The patches were removed after 48 hours and the reactions observed for another 48 hours.

Patch tests with a series of homologous p-aminobenzoic acid alkyl esters including methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, and amyl esters yielded positive reactions. Para-aminobenzoic acid and the alcohols which constitute the side chains of the esters yielded negative results. No reaction was obtained with the methyl ester of meta aminobenzoic acid.

Six commercial anesthetics which are derivatives of p-aminobenzoic acid with a tertiary amine in the side chain were tested with negative results. This series included procaine, larocaine, tutocaine, monocaine, butyn and pontocaine.

Eight local anesthetics, benzoic acid esters with a tertiary amine in the side chain but without an aromatic amino group, were tested.

TABLE I.
Effective Threshold Concentrations of *p*-aminobenzoic Acid Alkyl Esters.

Ester	Effective threshold concentrations
Methyl	$1 : 10^2$
Ethyl ("benzocaine")	$1 : 10^6$
Propyl	$1 : 10^8$
Butyl ("butesin")	$1 : 10^6$
Amyl	$1 : 10^6$
Isopropyl	$1 : 10^6$
Isobutyl	$1 : 10^3$

These were alypin, metycaine, stovaine, nupercaine, diothane, phenacaine, apothesine and intracaine. All gave negative reactions.

The 7 substances which elicited positive reactions were then tested quantitatively to determine threshold concentrations at which a just perceptible reaction occurs. (Table I)

The table shows that the sensitivity to the methyl ester was relatively low. The sensitivity increased with lengthening of the side chain to reach a maximum with the propyl ester. Further lengthening of the side chains resulted in a decreased sensitivity. The iso compounds caused in both cases considerably less reaction than the isomer n-compounds.

Discussion. There are only a few studies dealing with multiple epidermal sensitivity. In one group of the studied cases, contact dermatitis can be provoked in a rather haphazard fashion by a number of not closely related compounds.[1-4] In the other group, however, the sensitivity has a rather narrow