glyceride could be added without significantly raising the normal levels of surface activity in such systems.

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The Anticonvulsant Activity and Toxicity of Methylparafynol (Dormison®) and Some Other Alcohols[1]

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Although a number of effective drugs are currently available for the control of the human convulsant state (epilepsy), the occurrence of toxic manifestations attributable to these agents stimulates the search for new compounds with low order of toxicity. A good anticonvulsant drug should suppress seizures without hypnosis and, if possible, without untoward effect upon other physiological processes such as hematopoiesis or liver function. Effective use of the barbiturates, hydantoins, or oxazolidines may often be limited by inordinate somnolence, gastrointestinal irritation, personality change, skin eruptions, bone-marrow depression, liver damage, gum hypertrophy, or photophobia.

In October 1951, Margolin, Perlman, Villani, and McGavack (*1*) reported the hypnotic activity of the unsaturated tertiary carbinol, 3-methyl-pentyne-ol-3, which has been used clinically as a soporific under the generic name methylparafynol (Dormison®). This compound, which was shown to have a hypnotic activity in man of about 40% relative to phenobarbital and pentobarbital, was reported to have remarkably low toxicity. Clinical studies revealed no liver, kidney, or bone-marrow damage. No respiratory depression or addiction was observed. Since a number of alcohols, notably isopropyl, have in recent years been studied in our laboratory (*2*), the possible anticonvulsant action of methylparafynol seemed apparent to us. In a preliminary, unpublished study, using the method of Merritt and Putnam (*3*), we found that this pentyne alcohol elevates the cortical threshold for electrically induced seizures in rats. With a dose of 250 mg/kg, the cortical threshold is raised an average of 70.6%. In May 1952, we reported (*4*) the ability of methylparafynol to alter the convulsion pattern of supramaximal stimulation in rats. We found that the duration of anticonvulsant action is in excess of 8 hr, that the action of phenobarbital is potentiated, and that the drug is capable of controlling seizures in human epileptics.

Clinical trials of methyparafynol by the staff of the Stanford Neuropsychiatry Department, however, were suspended after 6 weeks when two of six epileptics under treatment developed strongly positive (3 and 4 plus) cephalin flocculation tests. One week after discontinuation of the alcohol, these tests were negative. A seventh patient, a 13-year-old girl under the care of a private pediatrician, developed a 2 plus cephalin flocculation test during the first 2 months of treatment; but this test reverted to negative under continuing methylparafynol therapy, which was not abandoned since this was the only agent capable of preventing her grand mal seizures. Since, however, clinical laboratory evidence of hepatotoxicity had become apparent in the adult patients, chronic feeding experiments were performed in rats. Five control rats and 10 rats given 0.175% methylparafynol in the drinking water were studied for 4 months. No significant difference was found in growth rate, or in food and water consumption. At the end of each month, a control and a treated animal were sacrificed by decapitation, and the livers were examined histologically by Lelland Rather, of the Stanford Pathology Department. There was no apparent difference in nuclear volume; there was a slight but definite diminution of cytoplasmic basophilia in the hepatic cells of the methylparafynol-fed rats. Loss of cytoplasmic basophilia may under some, but not all, circumstances indicate diminution of ribonucleic acid (*5, 6*). The metabolic and clinical significance of such a change remains to be determined. It might conceivably be associated with changes in the protein production of the liver cell. In order to determine whether the presence of the pentyne alcohol in the blood might produce a "false-positive" cephalin flocculation, this test was performed after the addition of methylparafynol to blood samples from four normal human subjects. Even with excessive concentrations (400 mg/100 ml) of the alcohol, these sera all yielded negative cephalin flocculation tests. It would appear, therefore, that methylparafynol is possibly capable of producing alteration of liver function.

Suspecting that the triple bond in methylparafynol might be responsible for the possible hepatotoxicity, we have undertaken the investigation of a number of saturated secondary and tertiary alcohols. These are listed in Table 1, where their effects are compared with those of methylparafynol, ethanol, and the established

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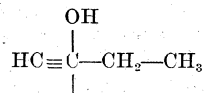
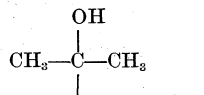
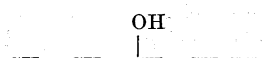
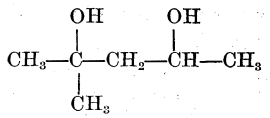
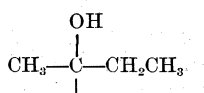
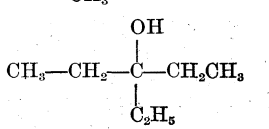
TABLE 1

Compound	Formula	ED_{50}* (mg/kg) oral	TD_{50}† (mg/kg) oral	LD_{50}‡ (gm/kg) oral	PI§	$\frac{LD_{50}}{ED_{50}}$	Duration of action (hr)
Phenobarbital	$C_{12}H_{11}N_2O_3Na$	3.7	28.0	.66	7.6	178	—
3-Methyl-pentyneol-3 (methylparafynol)		50.0	108.0	0.3–0.9 (*1*)	2.2	12	> 8
2-Methyl-2-propanol (tertiary butanol)		59.0	530.0	3.5	9.0	59	> 8
3-Pentanol (diethylcarbinol)		100.0	820.0	2.8	8.2	28	6
2-Methyl-2,4-pentanediol		155.0	650.0	4.7 (*9*)	4.2	30	> 8
2-Methyl-2-butanol (tertiary amyl alcohol)		50.0	160.0	1.0	3.2	20	7
3-Ethyl-3-pentanol (triethyl carbinol)		36.0	72.5	—	2.0	—	—
Ethanol	CH_3CH_2OH	2850.0	6500.0	13.5	2.3	4.7	—

* ED_{50} = Dose which reduces duration of tonic-extensor phase of supramaximal seizures by 50% in rats.
† TD_{50} = Dose which produces ataxia in 50% of rats.
‡ LD_{50} = Dose which is fatal for 50% of rats.
§ PI = TD_{50}/ED_{50}.

anticonvulsant, phenobarbital. The protective index (PI) was calculated by a modification of the "supramaximal" electroshock method of Toman, Swinyard, and Goodman (*7*). Using the apparatus devised by Tainter and associates (*8*), supramaximal convulsions were elicited by the passage of 50 ma (4 times the average threshold) for 1.0 sec through electrodes clipped to the ears of white rats. The ensuing seizures were characterized by a pattern of latent period, tonic-flexor phase, tonic-extensor phase, clonic phase, and stupor. According to Toman, Swinyard, and Goodman, abolition of the tonic-extensor phase represents an anticonvulsant effect. After obtaining control values in seconds for each phase, the animals were starved overnight, given the alcohols by stomach tube, and after careful scrutiny for evidence of ataxia, restimulated 1 hr later. Ten to 20 rats were tested at each dosage level. Plotting on probit paper the percentage decrease in duration of the tonic-extensor phase against the dosage of alcohol results in a straight-line graph, from which can be found the dosage which will reduce the duration of the tonic-extensor phase 50% (ED_{50}). In the same fashion the dose producing ataxia in 50% of the rats (TD_{50}) may be determined. The protective index (PI) is obtained by dividing the TD_{50} by the ED_{50}. When not elsewhere reported, LD_{50} was determined. To determine duration of anticonvulsant action, groups of 5 rats each were tested periodically for reduction of the tonic-extensor phase after medication with the lowest dose affording complete abolition of that component of the supramaximal seizure. In order to avoid aberrant results from restimulation on the same day, a different group of animals was tested at each time interval.

Inspection of the results summarized in Table 1 reveals that two of these alcohols, 2-methyl-2-propanol (PI, 9.0) and 3-pentanol (PI, 8.2), have protective indices greater than that of phenobarbital (PI, 7.6); and two other alcohols, 2-methyl-2,4-pentanediol (PI, 4.2) and 2-methyl-2-butanol (PI, 3.2) are experimentally superior to methylparafynol (PI, 2.2). This, of course, refers to the margin between the effective anticonvulsant dose and the dose producing locomotor incoordination. Consideration of the ratio, LD_{50}/ED_{50}, indicates that the widest margin of safety is present with phenobarbital, followed in decreasing order by 2-methyl-2 propanol, 2-methyl-2,4-pentanediol, 3-pentanol, 2-methyl-2-butanol, and methylparafynol.

Currently the more promising of these alcohols are being subjected to chronic toxicity studies in rats consuming the compounds in their drinking water. The results of this investigation, including blood counts and autopsy, will be reported at a later date. It should be recorded that two long-chain alcohols tested for anticonvulsant activity are not included in the table. Both 2-ethyl-2-hexanol and 3,9-diethyltridecanol were found to be feeble and erratic anticonvulsants as measured by the supramaximal stimulation test.

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Relationship between Inorganic Phosphorus and Vitamin A in the Rat and Sheep[1]

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Ross and Gallup (*1*) have reported an inverse relationship between blood plasma inorganic phosphorus and plasma carotene in beef cattle fed phosphorus-deficient rations. Kleiber, Goss, and Guilbert (*2*) found a decreased utilization of energy in beef heifers fed rations low in this mineral.

Six experiments have been conducted with rats to study a possible relationship between inorganic phosphorus and carotene and/or vitamin A utilization. Weanling rats of the Sprague-Dawley strain were used in these studies. The rats were first depleted of vitamin A on a ration of cerelose, 68 parts; casein, 15 parts; corn oil, 5 parts; USP salt mixture No. 2, 4 parts; and irradiated dry yeast, 8 parts, by weight.

When the rats became depleted of vitamin A, as characterized by weight loss and eye symptoms, they were divided into uniform groups and placed on the experimental rations. The experimental rations were the same as the depletion ration except that different salt mixtures were used and the amount of irradiated yeast was decreased to 1 part, with a corresponding increase in the amount of cerelose. Carotene in cottonseed oil was added in equal amounts to both the high and low phosphorus rations.

In Expts I, II, and III the salt mixture used in the low phosphorus rations was that of Hubbell, Mendel, and Wakeman (*3*), without the inclusion of potassium phosphate. A corresponding increase was made in the potassium chloride content of this salt mixture. The salt mixture used in the high phosphorus ration in Expt I was USP salt mixture No. 2. In Expt II, potassium phosphate was added to the low phosphorus ration and in Expt III di-calcium phosphate was added to produce the high phosphorus rations. Thus in Expts II and III the total salt content of the high phosphorus ration was greater than that of the low phosphorus ration.

The phosphorus and carotene content of the rations fed, feed consumption, and the liver analyses obtained in the first three experiments are presented in Table 1. The rats were fed the experimental rations *ad lib* for 10 days during Expts I and II and for 15 days during Expt III.

TABLE 1

FEED CONSUMPTION AND LIVER ANALYSES OF RATS FED VARIOUS LEVELS OF PHOSPHORUS AND CAROTENE

	Experiment I		Experiment II		Experiment III	
	Low phosphorus	High phosphorus	Low phosphorus	High phosphorus	Low phosphorus	High phosphorus
Ration composition						
Phosphorus (%)	.15	.53	.18	1.07	.15	.85
Carotene (ppm)	9	9	50	50	6	6
Number rats	12	11	10	13	12	13
Feed consumed per rat						
Total (g)	79.3	72.4	88.5	41.1	58.2	40.0
Carotene (mg)	.71	.65	4.42	2.06	.35	.24
Liver analyses						
Av wt (g)	3.33	3.18	4.31	2.63	3.99	2.41
Vitamin A (IU/g)	26.4	19.3	76.2	59.4	4.3	3.5
Total IU/liver	86.6	60.2	293.1	159.6	16.4	8.3

[1] Contribution from the North Dakota Agricultural College Experiment Station. Published with the approval of the director of the station.

It will be noted in Table 1 that the rats fed the low phosphorus rations ate more total feed, had larger