forded the same protection against a standardized electrical convulsive stimulus. At a time when activity for both compounds was the same, at 2 and 3 hr, the brain levels of phenobarbital were the same. Whole blood and total-body phenobarbital levels paralleled those in the brain. The equivalency of the brain phenobarbital levels correlated well with the fact that the ratio of the ED_{50} for both compounds was the same as the ratio of their molecular weights (31/42 mg/kg = 254/320 = 0.93).

If some of the activity after a single oral dose was due to DMMP or a metabolite or metabolites other than phenobarbital at a time when the brain contained the same amount of phenobarbital, then a greater degree of activity should be seen following DMMP administration. An alternate view would be that if active metabolites are present, then less phenobarbital should be present in the brain following DMMP administration than after giving an equivalent amount of phenobarbital. The alternate viewpoint is seen when either 120 or 200 mg/kg of DMMP was given in combination with SKF-525A. The brain levels of phenobarbital were less than one-half those found when the ED_{50}, 42 mg/kg, of DMMP was given alone. Although the brain phenobarbital levels decreased, protection against MES seizures was observed, 58% at 120 mg/kg. *N*-Methoxymethylphenobarbital (MMP) was found in the brains of mice when this dose of DMMP was given along with the SKF-525A, indicating that the intermediate probably has activity. MMP was also found when large amounts of DMMP were given alone.

The importance of MMP as an anticonvulsant has yet to be fully determined. Its activity must be evaluated at a time when no phenobarbital has been produced through metabolism. The rate of conversion of DMMP to MMP as well as the metabolism of MMP to phenobarbital must also be established in man as well as in animals. Only then can the interrelationships of the various compounds to activity be determined. The MMP brain levels appear to increase with increasing amounts of administered DMMP, whereas DMMP levels remain less than 1 μg/g, the lower limit of the glc analysis. These facts indicate that the metabolism of DMMP to MMP is faster than the metabolism of MMP to phenobarbital. Butler[5] found that the metabolism of 1,3-dimethylbarbital to monomethylbarbital was several times faster than the latter's conversion to barbital.

In conclusion, these data indicate that in mouse the activity of DMMP following the oral administration of an ED_{50} dose of the drug is due to its metabolism to phenobarbital. However, other metabolite(s) may play an important role when the drug is given chronically.

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Anticonvulsants. 3. Phenobarbital and Mephobarbital Derivatives

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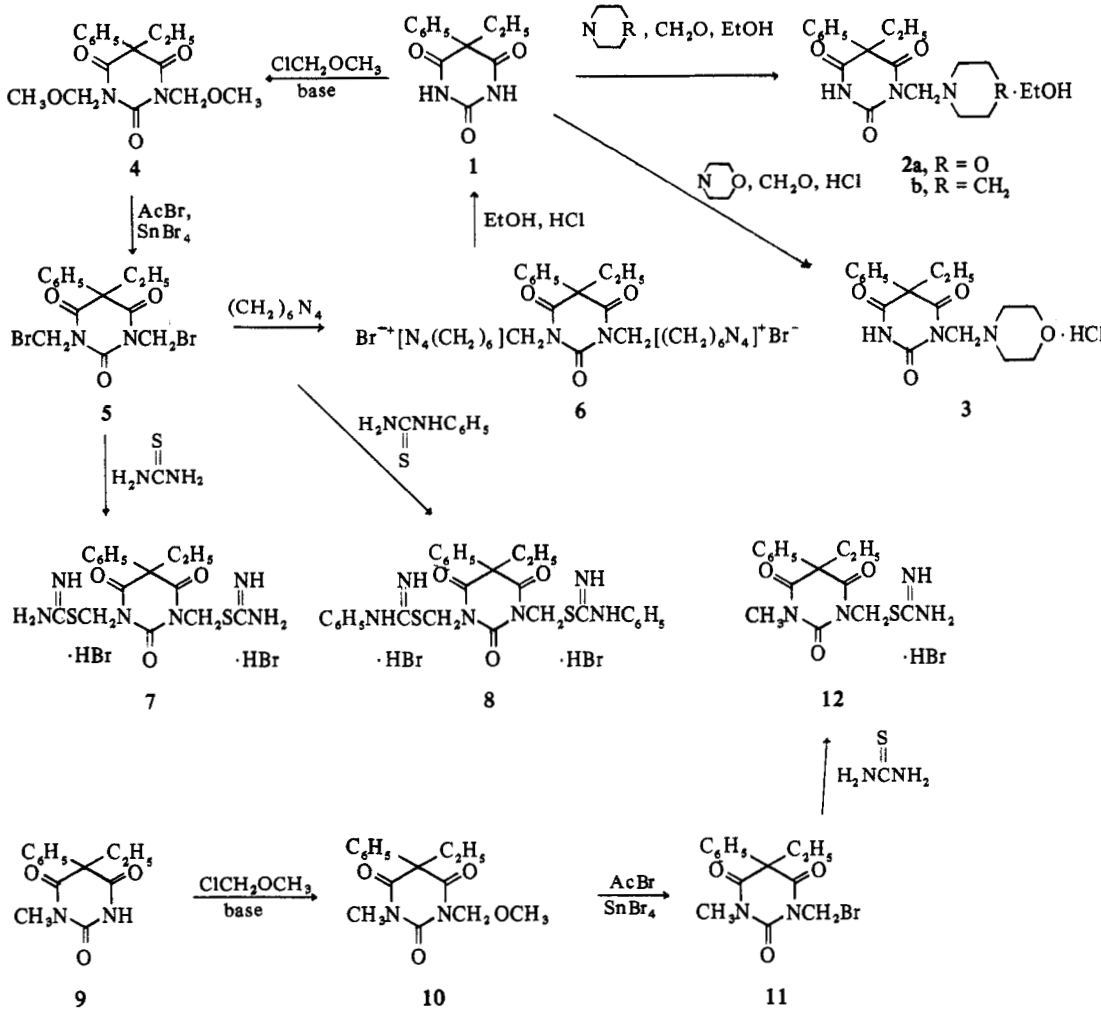
Several phenobarbital and mephobarbital derivatives were found to possess potent anticonvulsant activity and yet were either devoid of the marked hypnotic effects associated with the parent compounds or displayed very weak hypnotic activity. Particularly active compounds were the Mannich-type derivatives **2a,b** and **3**, the bis(hexamethylenetetramine salt) of 1,3-bis(bromomethyl)phenobarbital (**6**), and 1-methyl-3-methoxymethylphenobarbital (**10**).

We reported previously[1] that alkoxymethyl derivatives of phenobarbital possess marked anticonvulsant activity against both maximal electroshock and pentylenetetrazole-induced seizures and yet are devoid of the hypnotic effects associated with the parent compound. We also reported[2] that the bis(acyloxymethyl) derivatives of phenobarbital are active against maximal electroshock seizures as well as pentylenetetrazole, while the bis(halomethyl) derivatives of phenobarbital are effective anticonvulsants against pentylenetetrazole only. At the same time these compounds, unlike phenobarbital, are completely devoid of hypnotic activity. We became interested in finding out whether the salts and other derivatives of phenobarbital and mephobarbital described below would display anticonvulsant properties.

Chemistry. The compounds described herein were synthesized as indicated on Scheme I. The Mannich derivatives were isolated as alcoholates **2a,b** or as the hydrochloride salt **3**.

The bis(hexamethylenetetramine salt) of 1,3-bis(bromomethyl)phenobarbital (**6**) was obtained from the previously described 1,3-bis(methoxymethyl)phenobarbital[1] (**4**) *via* the intermediate 1,3-bis(bromomethyl)phenobarbital[2] (**5**).

Scheme I



It is noteworthy that phenobarbital (**1**) was obtained when compound **6** was treated with ethanolic HCl.

The salt, 1,3-bis(2-isothioureidomethyl)phenobarbital dihydrobromide (**7**), was obtained from compound **5** and thiourea, while compound **8**, 1,3-bis(*N*-phenyl-2-isothioureidomethyl)phenobarbital dihydrobromide, was obtained from compound **5** and phenylthiourea. Compound **10**, 1-methyl-3-methoxymethylphenobarbital, was obtained from mephobarbital (**9**) and $ClCH_2OCH_3$ in the presence of base. In turn, compound **10** could be converted into compound **11**, 1-methyl-3-bromomethylphenobarbital, with acetyl bromide in the presence of stannic bromide. Compound **12**, 1-methyl-3-(2-isothioureidomethyl)phenobarbital hydrobromide, was obtained from compound **11** and thiourea.

Pharmacology Results. Various Mannich bases of 5,5-disubstituted derivatives of barbituric acid have been reported in the literature[3,4] without indicating any specific therapeutic uses for these compounds. We found that the alcoholates of 1-morpholinomethylphenobarbital (**2a**) and 1-piperidinomethylphenobarbital (**3**) possessed significant anticonvulsant potency compared to that of phenobarbital (**1**), whereas they were weaker than phenobarbital as hypnotics.

Results summarized in Table I indicate marked activity against MES following administration of compounds **2a,b** and **3** (in addition to the previously reported activity of compound **4**), suggesting potential therapeutic effectiveness in grand mal epilepsy. The time of peak activity was shown to be approximately 1 hr after the administration of the drug. Compounds **2a,b** and **3** also displayed good activity against pentylenetetrazole, suggesting potential therapeutic effectiveness in petit mal epilepsy. Compounds **2a,b** and **3** in mice produced a progressive depression of the CNS, death resulting from respiratory paralysis. Sleep, however, did not appear except at lethal doses In addition, compound **2a** is a potent analgesic.

All three disubstituted phenobarbital derivatives, compounds **6–8**, were devoid of hypnotic activity. However, only one of these compounds, the bis(hexamethylenetetraamine salt) of 1,3-bis(bromomethyl)phenobarbital (**6**), was a potent anticonvulsant against both maximal electroshock and pentylenetetrazole-induced seizures, suggesting potential therapeutic effectiveness in both grand mal and petit mal epilepsy. Administration of compound **6** also resulted in skeletal muscle flaccidity without death, suggesting effectiveness as a centrally acting muscle relaxant.

Compound **7**, 3-bis(2-isothioureidomethyl)phenobarbital dihydrobromide, was devoid of activity against maximal electroshock and pentylenetetrazole-induced seizures up to 1000 mg/kg dose levels. When compound **7** was tested against a lethal dose of strychnine sulfate, the ED_{50} was found to be 600 mg/kg indicating CNS depressant activity. Also, when administered to 15 mice, a dosage of 100 mg/kg caused ptosis of the eyelids. The animals remained undisturbed after the administration of compound **7** but showed exaggerated motor activity when touched.

Compound **8**, 1,3-bis(*N*-phenyl-2-isothioureidomethyl)-phenobarbital dihydrobromide, exhibited definite, albeit weak anticonvulsant activity. Compound **8** afforded no pro-

Table I. Pharmacologic Activity of Phenobarbital and Mephobarbital Derivatives

Compd no.	MES-ED_{50}, mg/kg	Met-ED_{50}, mg/kg	HD_{50}, mg/kg	LD_{50}, mg/kg	Other
1	20.0 (13.8-29.0)	9.8 (6.7-14.2)	100 (72.5-138.0)	270 (216-337.5)	
2a	12.5	~50	250	>250 < 500	Analgesic ED_{50}, 3.125 mg/kg
2b	<12.5	<3.125	>250 < 500	>250 < 500	
3	~25	<3.125	>250 < 500	~500	
4	13.5 (8-22.7)	47.0 (29.4-75.2)	None	470 (376-588)	
5	None	~27	None	>1000	
6	42 (28.4-62.2)	~50	None	>1000	Muscle relaxation
7	None	None	>1000	>500 < 1000	Antistrychnine ED_{50}, 600 mg/kg, ptosis of eyelids at 100 mg/kg, exaggerated motor activity when touched
8	>500	330	None	>500	Inactive against strychnine, analgesic ED_{50} ~500 mg/kg
9	16 (11.4-22.4)	24 (16.9-34.1)	180 (148.7-217.8)	~300	
10	~50	16	>500 < 1000	>500 < 1000	
11	>500	500	None	>1000	Ptosis of eyelids, piloerection at 100 mg/kg
12	>100 < 250	>100	None	>500 < 1000	Potentiation of subhypnotic dose of hexobarbital, ED_{50} ~250 mg/kg

tection against a lethal dose of strychnine sulfate; however, analgesic activity was demonstrated according to the Eddy hot-plate technique, the ED_{50} being less than 500 mg/kg. Although a marked sedative effect was exhibited by compound **8**, it did not cause sleep.

Mephobarbital **(9)** is an anticonvulsant drug with less hypnotic activity than phenobarbital **(1)**. Mephobarbital is almost completely metabolized in the body[5] and the primary pathway for the metabolism of mephobarbital is N-demethylation, yielding phenobarbital.[6] Nevertheless, mephobarbital is claimed to be more effective than phenobarbital in petit mal[7] and less effective than phenobarbital in the management of grand mal epilepsy. We were interested, therefore, to learn whether the activity of methoxymethyl, bromomethyl, and 2-isothioureidomethyl derivatives of mephobarbital resembled that of the parent compound, mephobarbital, or the respective 1,3-bis(methoxymethyl), 1,3-bis(bromomethyl), or 1,3-bis(2-isothioureidomethyl) derivatives of phenobarbital.

We found that 1-methyl-3-methoxymethylphenobarbital **(10)** was more effective against pentylenetetrazole than either mephobarbital or compound **4**, indicating potential therapeutic usefulness in petit mal epilepsy. On the other hand, compound **10** was less active against maximal electroshock seizures than either mephobarbital or compound **4**. Unlike mephobarbital, compound **10** was devoid of hypnotic activity. In this respect, compound **10** resembled compound **4** since it induced sleep only at lethal doses. Death resulted from respiratory paralysis following progressive CNS depression.

Unlike mephobarbital and compound **5**, both potent drugs against pentylenetetrazole, 1-methyl-3-bromomethylphenobarbital **(11)** showed weak activity against pentylenetetrazole. Peak time was at least 2 hr, indicating delayed onset of activity. Like compound **5**, and unlike mephobarbital, compound **11** was devoid of hypnotic activity. We also found that compound **11** resembled compound **5** rather than mephobarbital itself in another way since both compounds **11** and **5** were devoid of anticonvulsant activity against maximal electroshock. There were no immediate or delayed deaths following a dose of 1000 mg/kg of compound **11**. The pattern of toxicity included piloerection and ptosis, suggesting autonomic effects.

It was also of interest to discover that compound **12** had anticonvulsant activity against both electrically and chemically induced seizures, although such activity was weaker than that of mephobarbital. In contrast, the structurally similar compound **7** was devoid of anticonvulsant activity. Compound **12** did not induce sleep either, but mild sedative activity was disclosed by its conversion of a subhypnotic dose of hexobarbital into a hypnotic dose.[8]

Experimental Section

Pharmacology. All compounds were administered orally in 10% aqueous acacia suspension. Adult male albino mice (18-30 g, Charles River) were used throughout this study. Protection against maximal electroshock (MES) and pentylenetetrazole (Met) and toxicity (LD_{50}) was determined according to Swinyard, *et al.*[9] Central nervous system depressant activity was measured by the ability of the test compound to protect against the lethal effect of a toxic dose of strychnine sulfate. Central nervous system depressant activity was also determined by observation (visual and touch) of muscle flaccidity in the animals tested, without interference with spontaneous respiration after administration of the test compound. Where indicated, analgesic activity was determined by the hot-plate method.[10]

Peak Time. Time of peak anticonvulsant activity (maximal electroshock seizures) was determined according to Swinyard, *et al.*,[9] except that different groups of five mice were tested at the approximate ED_{50} at intervals of 0.5, 1, 2, and 3 hr (or longer) following drug administration. In our experience different groups were necessary since repeated shocking of animals leads to false positive results. The peak time was taken to be the time at which maximum protection occurred.

HD_{50}. Compounds were administered to groups of ten animals generally at five dosage levels. Hypnotic activity (sleep) was determined by loss of the righting reflex without regard to the duration of effect, *i.e.*, the interval between the loss and the return of the righting reflex. The number of mice sleeping was recorded for each dose, and the dose required to induce sleep in 50% of the animals (HD_{50} with 95% fiducial limits) was determined graphically according to Litchfield and Wilcoxon.[11] LD_{50}'s were also determined graphically, death being the end point. In this study a compound was considered as having hypnotic activity only if the LD_{50} and HD_{50} values differed significantly.

Analyses. Microanalyses were within ±0.3% of the theoretical values as performed by Galbraith Laboratories, Knoxville, Tenn. Melting points were obtained on a Fisher-Johns hot stage and are corrected. Ir spectra were recorded on a Perkin-Elmer 337 grating infrared spectrophotometer. Uv spectra were recorded on a Bausch & Lomb Spectronic 505 spectrophotometer. Nmr spectra were run on a Varian Associates A-60A spectrometer in $(CD_3)_2SO$ using

Me_4Si as internal reference. Mass spectra were determined on a Hitachi RMU-6D double-focusing spectrometer at 70 eV. Merck HF-254 and 366 silica gel, according to Stahl, was used for tlc development with PhH–EtOAc mixtures. Ir, nmr, uv, mass spectra, and tlc were all appropriate.

1-Morpholinomethyl-5-ethyl-5-phenylbarbituric Acid Alcoholate (2a). To a 37% aqueous HCHO solution (8.0 ml, 0.1 mol) and morpholine (8.7 ml, 0.1 mol) in absolute EtOH (50 ml) 5-ethyl-5-phenylbarbituric acid (23.2 g, 0.1 mol) was added and the solution was heated at reflux for 30 min and then allowed to cool. Upon standing, crystals appeared which were filtered. Recrystallization from EtOH gave compound **2a** (32 g, 85% yield), mp 73°. *Anal.* ($C_{19}H_{27}O_5N_3$) C, H, N.

1-Piperidinomethyl-5-ethyl-5-phenylbarbituric Acid Alcoholate (2b). Compound **2b** was obtained by using piperidine (10 ml, 0.1 mol) in place of morpholine in the same procedure as described for the preparation of **2a** (33 g, 88% yield), mp 67–68°. *Anal.* ($C_{20}H_{29}O_4N_3$) C, H, N.

1-Morpholinomethyl-5-ethyl-5-phenylbarbituric Acid Hydrochloride (3). To a solution of morpholine (17.4 ml, 0.2 mol), 37% aqueous HCHO solution (16 ml, 0.2 mol), and 38% aqueous HCl solution (20 ml) in absolute EtOH (100 ml) 5-ethyl-5-phenylbarbituric acid (23.2 g, 0.1 mol) was added and the solution was kept at reflux for 45 min. Upon standing crystals appeared which were filtered. Recrystallization from EtOH gave compound **3** (15.6 g, 41.5% yield), mp 158°. *Anal.* ($C_{17}H_{22}O_4N_3Cl$) C, H, N, Cl

Bis(hexamethylenetetramine salt) of 1,3-Bis(bromomethyl)-5-ethyl-5-phenylbarbituric Acid (6). Hexamethylenetetramine (1.5 g, 0.0107 mol) was added to a solution of 1,3-bis(bromomethyl)-5-ethyl-5-phenylbarbituric acid (**5**) (2.1 g, 0.005 mol) in $ClCH_2CH_2Cl$ (75 ml). The mixture was kept at 50° overnight; then the product was filtered and washed with $ClCH_2CH_2Cl$. Obtained was compound **6** (3.4 g, 97% yield), mp 137–140°. *Anal.* ($C_{26}H_{38}O_5N_{10}Br_2$) C, H, N.

Conversion of Bis(hexamethylenetetramine salt) of 1,3-Bis(bromomethyl)-5-ethyl-5-phenylbarbituric Acid (6) to 5-Ethyl-5-phenylbarbituric Acid (1). To an ice-cold suspension of compound **6** (6.4 g, 0.01 mol) in EtOH (35 ml) a cold solution of 38% aqueous HCl (17 ml) in EtOH (8 ml) was added. The mixture was stirred for 16 hr at 25°. The mixture was poured into a saturated $NaHCO_3$ aqueous solution, the product was extracted into EtOAc, and the dried (Na_2SO_4) EtOAc solution was evaporated. To the oily residue H_2O was added and the solidified product was filtered and then crystallized from aqueous EtOH to give compound **1** (1.8 g, 78% yield).

1,3-Bis(2-isothioureidomethyl)-5-ethyl-5-phenylbarbituric Acid Dihydrobromide (7). To a solution of 1,3-bis(bromomethyl)-5-ethyl-5-phenylbarbituric acid (**5**, 4.2 g, 0.01 mol) in Me_2CO (30 ml) a solution of thiourea (1.5 g, 0.02 mol) in Me_2CO (50 ml) was added. Upon mixing a white solid precipitated. The mixture was kept for 16 hr at 25°; then the product was filtered, washed with Me_2CO, and crystallized from MeCN. Obtained was compound **7** (5.1 g, 89% yield), mp 230–233°. *Anal.* ($C_{16}H_{22}O_3N_6S_2Br_2$) C, H, N, S, Br.

1,3-Bis(*N*-phenyl-2-isothioureidomethyl)-5-ethyl-5-phenylbarbituric Acid Dihydrobromide (8). **8** was prepared from compound **5** (2.1 g, 0.005 mol) and phenylthiourea (1.5 g, 0.01 mol) in the same way as described for the preparation of compound **7**. Obtained was compound **8** (3.2 g, 88% yield), mp 192–198° dec. *Anal.* ($C_{28}H_{30}N_6O_3S_2Br_2$) C, H, N, S, Br.

1-Methyl-3-methoxymethyl-5-ethyl-5-phenylbarbituric Acid (10). LiH (3.97 g, 0.5 mol) was added to an ice-cold, stirred solution of 1-methyl-5-ethyl-5-phenylbarbituric acid (**9**, 123.1 g, 0.5 mol) in DMF (1250 ml). After 90 min, $ClCH_2OCH_3$ (44.2 g, 0.55 mol) was added dropwise over a period of 30 min. The solution was stirred for 2 hr and then poured into 2000 ml of ice H_2O. The solid precipitate was filtered, washed with H_2O, and crystallized from EtOH (600 ml) to give compound **10** (101.6 g, 70% yield), mp 115–116°. *Anal.* ($C_{15}H_{18}O_4N_2$) C, H, N.

1-Methyl-3-bromomethyl-5-ethyl-5-phenylbarbituric Acid (11). To a suspension of compound **10** (60.5 g, 0.21 mol) in AcBr (74 g, 0.6 mol) $SnBr_4$ (16 g) was added and the mixture was kept at 55°, with stirring, for 7 days. The mixture was poured into ice H_2O (1200 ml) and stirred for 4 hr. The product was filtered, washed with H_2O, and dried. Crystallization from hexane gave compound **11** (55.7 g, 79% yield), mp 121–122°. *Anal.* ($C_{14}H_{15}O_3N_2Br$) C, H, N, Br.

1-Methyl-3-(2-isothioureidomethyl)-5-ethyl-5-phenylbarbituric Acid Hydrobromide (12). A solution of compound **11** (25.4 g, 0.075 mol) in MeCN (150 ml) was added to a warm (40°) solution of thiourea (5.7 g, 0.075 mol) in MeCN (300 ml). The solution was kept at reflux for 3 hr, the MeCN evaporated, and the oily residue crystallized from a mixture of EtOH (250 ml)–Et_2O (250 ml) to give compound **12** (27.2 g, 88% yield), mp 214–215°. *Anal.* ($C_{15}H_{19}N_4O_3SBr$) C, H, N, S, Br.

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