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The relation between primidone and phenobarbitone blood levels

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A dose of primidone or one fifth of the same weight of phenobarbitone produced equal levels of phenobarbitone in the blood.

IN 1965, Bogan, Rentoul & Smith described the findings in a fatal poisoning by primidone. It appeared from this case and from the others summarized in that paper that most, if not all, of the effects of primidone are due to its conversion in the body to phenobarbitone. This paper describes work designed to clarify this point.

Experimental and results

ULTRAVIOLET SPECTROMETRY

The quantitative estimation of barbiturates is based on the method of Broughton (1956) as modified by Bogan & Smith (1967).

GAS-LIQUID CHROMATOGRAPHY

The method for the estimation of primidone and phenobarbitone by gas-liquid chromatography is summarized below.

A sample of 5 ml of blood or urine (acidified to pH 3) is shaken with chloroform (50 ml). The chloroform layer is filtered, washed with phosphate buffer (5 ml, pH 7·4) and extracted with N sodium hydroxide

TABLE 1. PHENOBARBITONE IN BLOOD

Subject	Body weight (kg)	Dose (mg)	Dose/kg (mg)	Phenobarbitone in blood (mg/100 ml)	*Corrected phenobarbitone in blood (mg/100 ml)
1	61·0	195	3·2	1·7	0·51
2	60·5	133	2·2	1·9	0·86
3	57·5	98	1·7	0·8	0·47
4	65·2	98	1·5	0·7	0·47
5	65·0	195	3·0	1·4	0·47
6	54·5	98	1·8	0·9	0·50
7	56·0	185	3·3	2·1	0·64
8	98·0	88	0·9	0·4	0·44
9	68·0	88	1·3	0·5	0.38
10	55·0	88	1·6	1·1	0·69
11	50·0	185	3·7	0·9	0·24
Average	62·8	132	2·2	1·1	0·52

* Corrected to a dose of 1 mg phenobarbitone/kg body weight.

solution (5 ml). The aqueous layer is adjusted to pH 3 with hydrochloric acid and re-extracted with chloroform (50 ml). The chloroform is evaporated to a suitable small volume and an aliquot used for gas-liquid chromatography.

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Column: stainless steel, 6 ft × $\frac{1}{8}$ in O.D. packed with 5% SE30 on aeropak 30 (100–120 mesh), maintained at 180°; injection port, 225°, flame ionization detector at 250°. Nitrogen carrier gas and hydrogen supply for the flame flow rate 60 ml/min. Under these conditions the retention times of phenobarbitone and primidone are 1·6 and 3·4 min respectively. A disc integrator is used to measure peak areas. Primidone concentrations are calculated by comparison with the phenobarbitone peak obtained spectrophotometrically. The relationship of phenobarbitone and primidone peak areas is found in the usual manner using internal standards.

THIN-LAYER CHROMATOGRAPHY

The method used is that reported by Bogan, Rentoul & Smith (1964).

SAMPLES

Arrangements were made to collect blood from patients on long term treatment with phenobarbitone, primidone or a mixture of both. Samples were taken 3 hr after a dose. The samples were extracted without delay, and the phenobarbitone levels measured quantitatively spectrophotometrically. As the patients all received differing weights of drugs, the values obtained were adjusted for dose and body weight so that a valid comparison could be made. The values for phenobarbitone are given in Table 1 and for primidone in Table 2. Table 3 gives the blood levels of phenobarbitone and primidone in patients treated with a mixture of these drugs.

TABLE 2. PRIMIDONE-PRODUCED PHENOBARBITONE IN BLOOD

Subject	Body weight (kg)	Dose (mg)	Dose/kg (mg)	Phenobarbitone in blood (mg/100 ml)	* Corrected phenobarbitone in blood (mg/100 ml)
12	61·0	750	12·3	1·40	0·114
13	73·0	750	10·3	0·55	0·053
14	65·5	1,500	23·0	2·85	0·124
15	63·8	750	11·8	1·60	0·135
16	91·0	2,000	22·0	3·10	0·141
17	71·0	1,500	21·0	2·90	0·138
18	58·0	1,500	26·0	1·75	0·067
19	80·0	2,000	25·0	2·20	0·088
20	52·0	750	14·5	2·00	0·138
Average	68·4	1,280	18·4	2·04	0·111

* Corrected to a dose of 1 mg primidone/kg body weight.

RESULTS

If the average blood phenobarbitone level [mg/mg (dose)/kg (body weight)] is calculated, the values found for administered phenobarbitone and primidone are 0·520 and 0·111 respectively. When the value of 0·520 is used to calculate the contribution of the administered phenobarbitone to the phenobarbitone blood level resulting from mixed primidone-phenobarbitone treatment the resulting average contribution by the primidone works out to 0·118 mg/mg (primidone administered)/kg (body

weight). Comparison of this value with that of 0·111 found in pure primidone therapy shows surprisingly good agreement considering the small number of samples.

METABOLISM STUDIES

When examining the above samples using thin-layer chromatography, both phenobarbitone and hydroxyphenobarbitone were produced during primidone metabolism. The metabolism was investigated further by the *in vitro* metabolism method of Smith, Waddell & Butler (1963) using rat liver microsomes and soluble fraction. The method uses a solution of nutrients made up as follows: (mg) nicotinamide, 123; nicotinamide adenine dinucleotide phosphate, 11·7; glucose 6-phosphoric acid, 37·2; semicarbazide, 75·6; magnesium chloride, 100; disodium hydrogen phosphate, 492; sodium dihydrogen phosphate, 54·2; water to 25 ml. Metabolism was in 25 ml flasks, flushed with oxygen in which 2 mg primidone (or phenobarbitone) was placed together with 2·5 ml of a nutrient solution

TABLE 3. PHENOBARBITONE IN BLOOD FROM MIXED PHENOBARBITONE AND PRIMIDONE

Subject	Body weight (kg)	Drug	Dose (mg)	Dose/kg (mg)	Phenobarbitone in blood (mg/100 ml)	Calculated contribution of phenobarbitone (mg/100 ml)	Phenobarbitone due to primidone (mg/100 ml)	*Corrected primidone contribution (mg/100 ml)
21	64·7	Primidone	1,500	23·2	4·20	1·57	2·63	0·118
		Phenobarbitone	195	3·02				
22	56·4	Primidone	750	13·3	2·90	1·76	1·14	0·082
		Phenobarbitone	195	3·45				
23	75·5	Primidone	1,000	13·2	2·10	0·89	1·21	0·092
		Phenobarbitone	130	1·72				
24	65·5	Primidone	1,500	23·0	4·40	1·03	3·37	0·146
		Phenobarbitone	150	1·99				
25	57·5	Primidone	750	13·0	2·25	0·26	1·99	0·154
		Phenobarbitone	30	0·52				

* Corrected to a dose of 1 mg primidone/kg body weight.

and 2·5 ml of rat liver homogenate. [This was prepared by homogenizing liver in cold 0·2N potassium phosphate buffer of pH 7·4 (1 g liver/4 ml buffer). The homogenate was centrifuged at 9000 *g* for 30 min in a refrigerated centrifuge. The supernatant containing microsomes and the soluble fraction was refrigerated and used within a few hr.] The flasks were again flushed with oxygen and the resulting mixture shaken for 3 hr at 37°. Protein was then precipitated by adding 0·1 ml sodium hydroxide solution (10% w/v), 1·0 ml sodium tungstate solution (10% w/v) and 0·35 ml of sulphuric acid (10% v/v) to the flask which was then shaken and heated on a boiling water bath for 10 min. The precipitated protein was filtered off and the filtrate shaken directly with 100 ml of chloroform to extract the barbiturate. The chloroform layer was filtered, dried with anhydrous sodium sulphate and evaporated to dryness. The residue was ready for thin-layer chromatography.

RESULTS

Analysis of the solutions from metabolism studies containing primidone showed the presence of phenobarbitone, hydroxyphenobarbitone and primidone, while those containing phenobarbitone showed phenobarbitone and hydroxyphenobarbitone. Though the thin-layer chromatographic method allowed only semiquantitative measurement of barbiturates in blood, it appeared that the concentrations of phenobarbitone and hydroxyphenobarbitone were almost equal in the system starting from primidone but phenobarbitone was in excess in the system starting from phenobarbitone. This may indicate either the direct production of hydroxyphenobarbitone from primidone or that the phenobarbitone is being metabolized almost as rapidly as it is produced. This would certainly be in keeping with the fact that much larger amounts of primidone are required to give the same effect as phenobarbitone.

Discussion

From the Tables it can be seen that for primidone an average dose of 18·4 mg/kg body weight produces an average blood level of phenobarbitone of 2·04 mg/100 ml. For phenobarbitone the corresponding values are 2·2 mg/kg and 1·1 mg/100 ml, i.e. for an equivalent blood level of phenobarbitone the average dose of primidone should be 10 mg/kg. Therefore, if the total effect of primidone is due to its conversion to phenobarbitone we would expect that about five times the dose of phenobarbitone would be required. It has been reported by Gruber, Moisier & Grant (1957) that the dose of primidone required to replace phenobarbitone for similar clinical effects is five times the phenobarbitone dose.

This fivefold increase may be due to the fact, shown by the comparison of blood and urine levels of primidone and phenobarbitone, that there is a large discrimination by the kidneys against primidone leading to rapid excretion. It would appear that the action of primidone is due to its metabolite phenobarbitone.

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