THE DETERMINATION OF BETA-SUBSTITUTED GLUTARIMIDES IN BLOOD: TIME-CONCENTRATION CURVES AFTER INTRAVENOUS ADMINISTRATION OF TWO BARBITURATE ANTAGONISTS

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A simple and rapid spectrophotometric procedure has been developed which is capable of estimating 2 mg. per cent of β-substituted glutarimides in blood with an accuracy of 90 per cent. Within 10 minutes of intravenous administration in guinea pigs 90 per cent of two barbiturate antagonists, β-methyl-β-ethyl glutarimide and *β-spirocyclo*pentane glutarimide were removed from blood. Some discussion follows on whether the compounds were rapidly metabolised in blood or distributed evenly throughout the body tissues. The phase of rapid removal is followed by a period of moderately slow removal, apparently due to excretion.

The barbiturate-antagonistic activity of the drug β-methyl-β-ethyl glutarimide (bemegride) has recently received considerable attention in the clinical literature. Its ability to shorten pentobarbitone anaesthesia is still detectable in rats up to 48 hours after a single oral dose of 80 mg./kg.[1], but apart from one reported metabolite[2], nothing is known of its distribution or metabolic fate. A method for the quantitative estimation of the chemically related sedative, α-ethyl-α-phenyl glutarimide (glutethimide) in blood and urine has been published[3], and is applicable to other substituted glutarimides such as bemegride. However, it did not give good recoveries of added glutarimide from blood in this laboratory. A simple spectrophotometric procedure has enabled us to obtain information on the concentrations of two barbiturate antagonists, bemegride and *β-spirocyclo*pentane glutarimide (N.P.122)[4], in the blood of guinea pigs at intervals after intravenous administration.

Materials and Methods

In the undissociated form in aqueous solution bemegride exhibited an ultra-violet absorption peak at 205 mμ. This was too close to the lower spectral limit of our spectrophotometer (Hilger-Uvispek) to be reproducible quantitatively and led us to investigate the dissociated form. By determining the absorption spectra of bemegride in various buffers it was seen that dissociation occurred largely between pH 10 and 11·75 (pKa approx. 11·3). Above pH 12 the compound was apparently fully dissociated and showed a strong absorption peak at 230 mμ. Furthermore the optical density at 230 mμ was almost exactly double the optical density at 220 and 240 mμ. Although this characteristic pattern of absorption was not always fully recognisable in the glutarimide recovered from blood, there was sufficient approximation, as will be shown later, to justify using the data as an aid to identification of the absorbing

substance. Bemegride was unstable in solution at pH 12 but losses could be minimised by working at low temperature. In all that has so far been said the behaviour of N.P.122 was identical to bemegride, though each had its specific extinction coefficient at 230 mμ. The same analytical procedure was therefore, after suitable calibration, applicable to either compound.

Two to 5 ml. aliquots of heparinised blood containing at least 100 μg. of glutarimide were extracted with 30 ml. of chloroform for 30 minutes with mechanical shaking and the two layers separated by centrifuging. Ten ml. aliquots of the chloroform layer were pipetted into stoppered cylinders (25 ml.) and chilled to 0°. These were extracted with five successive 10 ml. volumes of cold 0·04N NaOH in a room at 0°. The aqueous layers were removed by aspiration. The combined alkaline extracts were centrifuged under refrigeration to remove suspended chloroform and the absorption of the clear aqueous layer immediately determined at 230, 220 and 240 mμ against a blank similarly prepared from glutarimide-free blood. Concentrations of the drug were ascertained from a calibration curve showing the absorption at 230 mμ of varying concentrations of the pure compound in cold 0·04N NaOH. When estimating very low blood levels (less than 4 mg. per cent) it was desirable to concentrate the chloroform extract. This was conveniently achieved by blowing a stream of warm air on to the extract until a 20 to 25 ml. aliquot was reduced to 10 ml. This procedure occasionally led to the formation of emulsions during the subsequent alkaline extraction but these were readily broken in the refrigerated centrifuge.

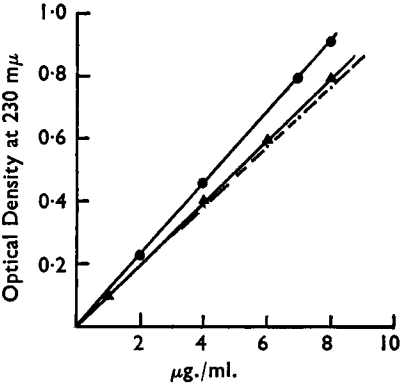


FIG. 1. Calibration curves for the glutarimides in cold 0·04N NaOH. ● Bemegride; ▲ *β-spiro-cyclo*pentane glutarimide; —— bemegride after standing for one hour at room temperature.

Information on the levels in blood after intravenous administration of the compounds was obtained using female guinea pigs (700 to 1100 g.) which had been fasted overnight. It was necessary to expose the jugular vein for administration and, since barbiturates interfere in the assay, anaesthesia for this purpose was obtained with intraperitoneal urethane assisted if necessary with a little ether. Bemegride was given at a dose of 30 mg./kg. in saline. N.P.122 is less soluble than bemegride and is a powerful, long-acting convulsant. It was given at a level of 15 mg./kg. in saline. The animals were killed by decapitation at intervals up to 4 hours after the injection and blood was collected into a beaker containing heparin. Suitable aliquots were assayed by the above procedure and optical densities of the final solutions were determined at 230, 220 and 240 mμ.

Results

The calibration curves in Figure 1 are those for bemegride and N.P.122 in cold 0·04N NaOH. The broken line shows the optical densities of the same bemegride solutions after one hour at room temperature. During this period the temperature of the solutions rose from 10° to 18·5°.

The estimated accuracy of the analytical procedure as determined by the recovery of glutarimides added to guinea pig blood *in vitro* in known amounts is shown in Table I.

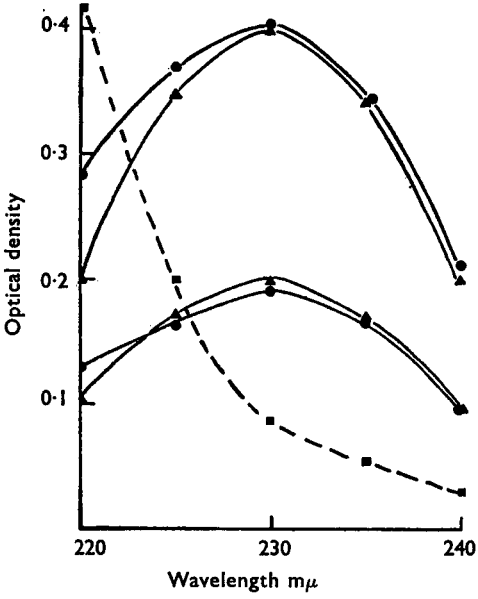


FIG. 2. Absorption curves of the glutarimides in 0·04N NaOH. ▲ Bemegride, 1·75 μg., and 3·5 μg./ml. respectively; ● bemegride recovered from blood; ■ blood blank.

Two of the curves in Figure 2 are those obtained by the extraction of bemegride from blood and for comparison the curves of pure bemegride solutions of 3·5 and 1·75 μg./ml. in 0·04N NaOH are included. The broken line is a typical absorption curve of a blood blank. It can be seen that the ratio of the optical density at 230 mμ to that at 270 mμ ($O.D._{230}/O.D._{220}$) is not constant at a value near 2 after the recovery of bemegride from blood. This is probably due to differences in the blank absorption which is changing rapidly over this wavelength range. The ratio $O.D._{230}/O.D._{240}$ on the other hand is constant at a value of approximately 2. It was a routine step during analysis to check that there was in fact an absorption peak at 230 mμ and that $O.D._{230}$ was approximately twice the value of $O.D._{240}$. Values of the ratio $O.D._{230}/O.D._{240}$ recorded during this investigation are set out in Table II for bemegride and N.P.122 before and after recovery from guinea pig blood.

To reduce the results from the different animals to a common basis the blood concentration of the drug as μg./ml. was multiplied by the animals' blood volume (7·2 ml./100 g.)[5]. The figure so obtained, expressed in milligrams, represented the total glutarimide present in the blood and this was calculated as a percentage of the original dose. Figure 3 shows the percentage remaining at various intervals after administration. Each point on the graph represents the observation from a single animal. Both compounds left the blood at the same rate and only 10 per cent of the initial dose remained in the blood after ten minutes. This amount could still be detected after 30 minutes and in the case of bemegride 5 per cent was present after four hours.

The injections of glutarimide were given over a period of less than one minute and at the conclusion the animals were beginning to convulse.

TABLE I

STANDARD RECOVERIES FROM BLOOD

Amount added mg./100 ml.	Amount recovered per cent	
	Bemegride	N.P.122
125	99·0	94·0
"	94·4	96·4
50	90·0	—
"	89·6	—
25	94·0	92·7
"	93·8	93·1
12·5	91·4	—
"	88·0	—
5·0	90·9	91·3
"	93·1	87·6
2·5	92·2	90·1
"	91·7	90·0
Mean	92·3	91·9
Standardard deviation between duplicates	1·78	1·57

Guinea pigs given bemegride convulsed severely for 5 to 6 minutes and this was followed by a further 5 to 6 minute period of twitching. After this the animals became quiet. There appeared to be a relation between

TABLE II

THE ABSORPTION RATIOS OF THE GLUTARIMIDES

Ratio $O.D._{280}/O.D._{240}$				
Bemegride			N.P.122	
Pure soln.	ex blood *in vitro*	ex blood *in vivo*	Pure soln.	ex blood *in vivo*
1·97	2·08	1·81	2·08	2·03
1·97	2·06	2·13	2·02	1·98
2·06	1·88	1·84	2·02	2·08
2·14	2·22	1·82	2·05	2·00
1·99	1·96	2·12	1·94	2·02
2·03	1·95	1·73	1·95	1·97
2·12	1·90	1·96	1·91	1·88
2·09	1·89	1·93	2·00	—
1·96	1·99	1·80	1·80	—
2·12	2·07	1·99	2·17	—
*Means**				
2·045	2·000	1·913	1·994	1·994

* A difference of 0·1 in the mean of the ratios is significant at the $p < 0·05$ level.

the decrease in blood concentration of bemegride and the decrease in convulsions. Observation of animals given N.P.122 were taken only up to 30 minutes after administration and they convulsed throughout this period.

DISCUSSION

The strong ultra-violet absorption band at 230 mμ appears to depend primarily on an intact glutarimide ring, secondly, ionisation of the –OC–NH–CO– grouping of the ring, and finally the type and position of the ring substituents.

Although not reported here it was shown during the preliminary investigation of this project that several β-substituted glutarimides had identical absorption curves in the 200 to 250 mμ range. Alkaline hydrolysis, however, led to cleavage of the ring at the imide linkage, yielding first the substituted glutaramic acid, which in equivalent concentration showed

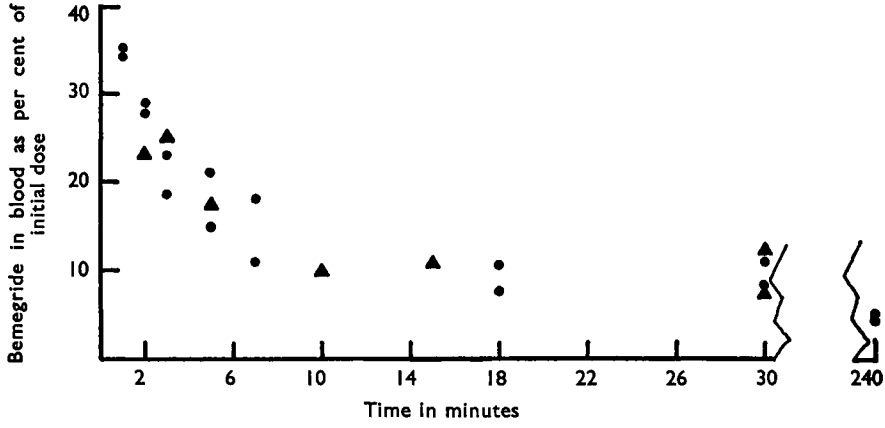
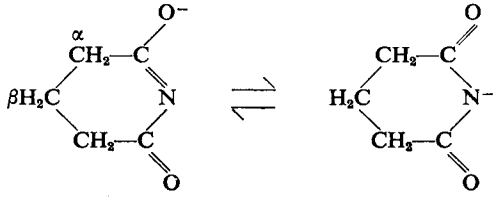


FIG. 3. The concentration of glutarimides in blood after intravenous administration. ● Bemegride; ▲ *β-spiro-cyclo*pentane glutarimide.

approximately 1/10th of the absorption of the parent compound at 230 mμ, and finally the substituted glutaric acid which showed no absorption in this region. However, two alpha substituted glutarimides, α-*n*-butyl- and α-phenyl-α-ethyl glutarimide, which had intact ring structures had no definite absorption peak at 230 mμ in alkaline solution and an αβ-substituted compound (α-methyl bemegride) had absorption characteristics only slightly, if at all different from, those of bemegride. Very little is known about the mechanism of ionisation of the glutarimides, but two forms are possible and it seems likely that they occur together in some sort of equilibrium:



Alpha substituent groups by virtue of their proximity would exert a greater influence on the mechanism of dissociation than β substituent groups—and so, depending on their structure may be the final factor controlling the optical absorption of the glutarimides at 230 mμ.

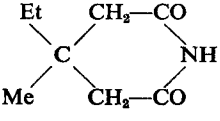
From consideration of the curves in Figure 2 and the means of the ratios $O.D._{230}/O.D._{240}$ in Table II, it seems clear that the absorption of the blood extracts at 230 mμ was due to the glutarimides.

Losses due to the instability of the glutarimides in 0·04N NaOH were minimised by holding solutions at temperatures less than 10°. The loss of bemegride in a solution which stood for one hour at room temperature and rose from an initial 10° to 18·5° was approximately 15 per cent. Curry has shown the loss to be about 50 per cent per hour in 0·5N NaOH at 37°[7].

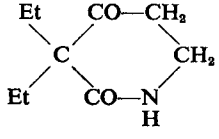
The rapid rate at which both compounds left the blood of guinea pigs after intravenous administration seems worthy of further comment. This disappearance was too rapid to be due to excretion alone. Therefore the glutarimides were either metabolised in the blood or stored within the organism. If a rapid metabolism caused the disappearance from the blood then this probably involved cleavage of the imide linkage which reduces or abolishes absorption at 230 mμ. The two likely metabolites resulting from biological cleavage of the bemegride ring are the β-methyl-β-ethyl glutaramic and β-methyl-β-ethyl glutaric acids, and it is interesting to note that while both have been claimed to be non-active as barbiturate antagonists[4,6] recent work in these laboratories by Mr. A. W. Macfarlane has established the presence of weak activity in the former.

On the other hand the glutarimides are lipid soluble and this together with their slight ionisation at biological pH suggests that they would pass rapidly through most biological membranes. Information on the tendency for the drugs to accumulate in various tissues awaits the development of a sufficiently sensitive tissue assay. However, the sedative "Dihyprylone" (3:3-diethyl-2:4-piperidinedione) which is chemically related to bemegride has recently been shown to be uniformly distributed in all tissues after oral ingestion[8] and the blood levels of bemegride after 10 minutes, when equilibrium had been established, were on the basis of Ancill's data[5] compatible with an even distribution through the entire animal body.

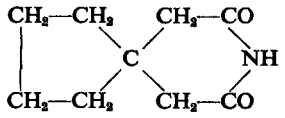
The blood levels of bemegride after four hours suggest a moderately slow rate of excretion. This may explain its ability to shorten barbiturate sleep 48 hours after a single oral dose in rats[1], and indicates that the drug is active at low blood levels.



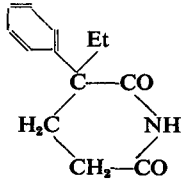
Bemegride: β-methy-β-ethyl glutarimide
(4:4-ethyl-methyl-2:6-piperidinedione)



Dihyprylone: 3:3-diethyl-2:4-piperidinedione



N.P.122: *β-spiro-cyclo*pentane glutarimide



Glutethimide: α-ethyl-α-phenyl glutarimide

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