

LETTER TO THE EDITOR

BARBITURATE ESTIMATION IN BODY FLUIDS BY GOLDBAUM'S  
SPECTROPHOTOMETRIC METHOD

Sir,

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Barbiturates in the body fluid are estimated by various methods, e.g. gas liquid chromatography, radioimmunoassay, enzyme-immunoassay and spectrophotometry. From the view point of easy applicability we have experimented with Goldbaum's (2) spectrophotometric method to define its utility and limitations.

*Method of barbiturate estimation* : Analytical grade reagents were used. One ml of plasma or serum was introduced in 25 ml Erlenmeyer flask, with ground-glass stopper. The pH was brought to 4-5 by adding 1 ml of N/10 HCl. Then 0.5 gm sodium chloride powder was added and dissolved by shaking. To the flask 8 ml chloroform was added and shaken for 20 min. The contents were transferred to a centrifuge tube and the two layers were allowed to separate, if necessary, by centrifuging. 5 ml of the chloroform layer was transferred to a 15 ml glass stoppered centrifuge tube and 2 ml of 0.45N NaOH was added and shaken for 5 min. From this tube 1 ml NaOH layer was taken in a 5 ml test tube and kept in hot water to remove traces of chloroform. The aliquot was transferred to quartz cuvette and optical density measured at 260 m $\mu$  using ultra-violet lamp, in spectrophotometer (1st reading). Then 0.5 ml of borate buffer (6.2 gm boric acid and 7.45 gm KCl in 100 ml distilled water) was added to the NaOH solution in the cuvette. Crystals appear in the stored borate buffer, therefore, it was warmed gently just before use. The contents were mixed by inversion and optical density was again measured at 260 m $\mu$  (2nd reading). For blank, 1 ml distilled water was used and processed as above. 0.1 ml of 0.04% w/v phenobarbitone sodium in 0.9 ml distilled water was processed like plasma or serum and used as a standard. Results were derived as follows:—

Difference of optical density = (1st reading) - (2nd reading  $\times$  1.5);

$$\text{Barbiturate level (mg/100 ml)} = \frac{\text{Difference of optical density of unknown sample}}{\text{Difference of optical density of standard}} \times 4$$

A series of experiments was performed using the above technique which revealed the following information :

- (a) The Goldbaum's method can detect phenobarbitone, pentobarbitone, secobarbitone and hexobarbitone in a sample of serum or plasma, down to a concentration

of 1  $mg/100\text{ ml}$  (i.e., 10  $\mu g/ml$ ). The plasma values were about 20% higher than the corresponding serum sample values.

- (b) Recovery of phenobarbitone in plasma or serum in 6 experiments ranged between 72 to 90%. In our opinion this constitutes a satisfactory recovery considering possible presence of various interfering substances that may be present in body fluids. Although diazepam, diphenylhydantoin and paracetamol (which are likely to be used with phenobarbitone) have considerable absorbance near 260  $m\mu$ , they do not interfere in the above barbiturate estimation procedure as they are eliminated during the extraction steps. Further, use of heparin (as anticoagulant) allows about 10% higher recovery of phenobarbitone than when ammonium oxalate, sodium fluoride, E.D.T.A. or sodium citrate is used (6 experiments).
- (c) Phenobarbitone values did not decline in blood samples kept at room temperature for upto 48 hr and in sodium hydroxide aliquot for upto 24 hr. The method can be used as such for the estimation of barbiturate in 2  $m/$  volumes of cerebrospinal fluid and saliva.
- (d) Since urine is an easily available body fluid, Goldbaum's method was modified for urine barbiturate estimation. It was found that normal human urine contains some substance which is not removed in the procedure described above and interferes because of extensive absorbance at 260  $m\mu$ . This interfering substance was removed by washing the chloroform layer with phosphate buffer and filtering it through Whatman filter paper no. 41, as recommended by Braughton (1) for urine and gastric juice. With this modification we could estimate phenobarbitone in urine. The recovery was in the range of 65–70%.
- (e) Phenobarbitone levels in 6 patients on antiepileptic therapy were in the range of 10–30  $\mu g/ml$  plasma/serum, indicating that Goldbaum's method is suitable for the monitoring of barbiturate levels in such patients. However, it is advisable to take 2  $m/$  of body fluid instead of 1  $m/$  for the extraction procedure as the levels are likely to be lower in these patients.

Determination of barbiturate levels in body fluids has now acquired sufficient credit to be considered clinically useful in the treatment of barbiturate intoxication. It is also desirable to monitor phenobarbitone levels at suitable intervals to facilitate the safe and effective use of this drug in the epileptic patients. By using Goldbaum's method, during the last seven years we have estimated barbiturate levels in 190 patients at this Institute (3). Our experience suggests that considering the simplicity and specificity of the method barbiturate estimation in body fluids can be established as a useful routine chemical pharmacology service in Indian hospitals.