(1955), and Isaacs (1957) all found a high degree of side-effects or patients' disapproval of a barbiturate compared with other drugs even though the barbiturate might be more effective in producing sleep. In contrast many workers have commented on the lack of hangovers with meprobamate (Selling, 1956; Kessler and Barnard, 1956; Gardner, 1957) and with chloral (Lasagna, 1956; Imboden and Lasagna, 1956)—the active component of dichloralphenazone—as compared with barbiturates. Perhaps this is even more likely to occur in elderly patients, who are reputed to tolerate barbiturates poorly.

Apart from the drug preferences by the patients, the main point of interest in the trial was the significant effect of the order of administration of the drugs. This trend may be an artifact due to an alteration in the nurses' or patients' attitude as the week progresses or may represent a genuine change in the pattern of sleep of patients in the ward. The order-effect was in fact greater than the drug-effect but did not influence the validity of the drug comparisons. This illustrates the necessity of a balanced design in this type of trial.

In conclusion, barbiturates in the dosages given do not appear to be satisfactory as routine hypnotics for the elderly, because they are not liked by the patients. It is obviously desirable to give drugs that have an effective hypnotic action and are liked by patients; meprobamate 800 mg. and dichloralphenazone 1.3 g. fulfil these requirements.

Summary

Amylobarbitone sodium 200 mg., dichloralphenazone 1.3 g., promazine resinate 50 mg., meprobamate 800 mg., and a placebo have been compared as hypnotics in a blind controlled trial in 65 elderly patients in a geriatric ward. Objective assessments were made by hourly checks and subjective assessments by asking patients for an overall evaluation of the night's rest. The importance of including

a subjective assessment of this sort in symptomatic therapy is stressed.

Analysis of the results demonstrated that all active drugs had hypnotic activity as compared with the placebo when judged by the nurses' hourly check. Meprobamate and dichloralphenazone were, however, preferred by the patients to the two other drugs and to the placebo. At the dosages used, meprobamate and dichloralphenazone are considered satisfactory hypnotics for use in elderly patients.

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REFERENCES

- Armitage, P. (1960). *Sequential Medical Trials*, Table 3, p. 7. Blackwell, Oxford.
- Brigstocke, M. O. (1961). *Brit. med. J.*, **2**, 827.
- Brit. med. J.*, 1962, **1**, 1691.
- Cox, G. H., and Marley, E. (1959). *J. Neurol. Neurosurg. Psychiat.*, **22**, 57.
- Felsinger, J. M. von, Lasagna, L., and Beecher, H. K. (1953). *J. Pharmacol. exp. Ther.*, **109**, 284.
- Foltz, E. L., Dracos, F., and Gruber, C. M., jun. (1955). *Amer. J. med. Sci.*, **230**, 528.
- Gardner, A. (1957). *Amer. J. Psychiat.*, **114**, 524.
- Goodnow, R. E., Beecher, H. K., Brazier, M. A. B., Mosteller, F., and Taguizi, R. (1951). *J. Pharmacol. exp. Ther.*, **102**, 55.
- Hare, E. H. (1955). *Brit. J. prev. soc. Med.*, **9**, 140.
- Hinton, J. M. (1961). *Brit. J. Pharmacol.*, **16**, 82.
- and Marley, E. (1959). *J. Neurol. Neurosurg. Psychiat.*, **22**, 137.
- Howell, T. H. (1960). *Practitioner*, **184**, 45.
- Imboden, J., and Lasagna, L. (1956). *Bull. Johns Hopk. Hosp.*, **99**, 91.
- Isaacs, B. (1957). *Lancet*, **1**, 556.
- Kessler, L. N., and Barnard, R. D. (1956). *Med. Tms (N.Y.)*, **84**, 431.
- Lasagna, L. (1954). *J. Pharmacol. exp. Ther.*, **111**, 9.
- (1956). *J. chron. Dis.*, **3**, 122.
- Llewellyn, A. (1961). *Brit. J. clin. Pract.*, **15**, 839.
- Selling, L. S. (1956). *J. clin. exp. Psychopath.*, **17**, 7.
- Stewart, J. S. (1956). *Brit. med. J.*, **2**, 1465.
- Straus, B., Eisenberg, J., and Gennis, J. (1955). *Ann. intern. Med.*, **42**, 574.

RAPID METHOD OF SCREENING FOR BARBITURATES

BY

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Barbiturates are the most common poisons at the present time in the United Kingdom. Although ultra-violet spectrophotometry is necessary for absolute assay and hydrolysis procedures and paper chromatography for identification, there is an urgent need for a simple rapid screening test which demonstrates the presence or absence of any barbiturate on a small volume of blood. It is the purpose of this paper to describe such a method which gives an unequivocal answer on 2 ml. of blood in five minutes and requires no apparatus more elaborate than a magnetic stirrer and a water-pump. In addition there is no dirty glassware to be cleaned at the end of a determination, the apparatus being instantly ready for further tests.

Zaar and Gronwall (1961) showed that barbiturates form mercury salts that are soluble in chloroform and insoluble in water. Using an ingenious but relatively lengthy extraction device, they took advantage of this property to assay barbiturates in blood. Although the same principle is followed here, the extraction of the barbiturate from the blood into chloroform, the reaction with mercuric ions, and the colour tests with dithizone for any mercury barbiturate

in the chloroform are all performed in a 100-ml. beaker (see Diagram). The other important feature of the method is that extraction of the barbiturate from the buffered blood is performed by magnetic stirring, using only 10 ml. of chloroform. By this technique emulsions, which are routine if shaking in a separating funnel is used, do not occur even with haemolysed post-mortem blood. The small volume of chloroform also avoids the necessity for evaporation stages. If a water-operated magnetic stirrer is used the apparatus can be made portable, dependent only on a water supply. The only other equipment needed is a suction device; a simple water-pump is most convenient. The glass suction-tube ($\frac{3}{16}$ in. (0.5 cm.) diameter) is taped on to a supporting stand so that it dips into the beaker, thus enabling an aqueous phase in the beaker to be drawn off, leaving 10 ml. of the lower chloroform layer *in situ*.

The concentrations and volumes of reagents are chosen to give a positive reaction to barbiturate levels in blood of 1 mg./100 ml. or higher. Positive results will be obtained on blood from epileptics who may have normal phenobarbitone levels of 5 mg./100 ml. or from patients on long-

term barbitone therapy who also may have levels of this order. Apart from these two exceptions normal therapeutic blood levels do not approach 1 mg./100 ml., and a positive reaction will clearly indicate barbiturate poisoning as a cause of a coma of undetermined origin. Arrangements can then be made for an exact identification and assay so that the possible use of an artificial kidney can be considered.

The technique works well on whole blood or serum and on urine or stomach washes at exactly the same sensitivity, if blood is not available for any reason. Salicylate, even in the highest toxic levels in blood or urine, does not interfere.

Method

Reagents.—Dithizone in chloroform: this should give an optical density at 605 $m\mu$ of approximately 2 in a 1-cm.

Diagram of assembled apparatus.

cell; it is important that this should be read in a colorimeter and not judged by eye, and it should be prepared freshly once every two weeks. **Mercury reagent:** 0.5 g. of mercuric chloride is dissolved in 50 ml. of water with 3 drops of concentrated nitric acid; 1 ml. of this solution is diluted to 50 ml. with water and 0.42 g. of sodium bicarbonate is added. **Phosphate buffer:** M/15 at pH 6.95: 0.624 g. of Na_2HPO_4 , 3 H_2O , and 0.363 g. of KH_2PO_4 are added to distilled water and the volume made up to 100 ml.

The apparatus is assembled as shown in the Diagram. The tube dipping into the beaker is clamped in position so that the small hole, which is purposely arranged to be at the side and not at the end, is approximately level with a line on the outside of the beaker marking a 10-ml. graduation. The stirrer bar must be glass or P.T.F.E. encased. Chloroform is added to the beaker and is reduced in volume to 10 ml. by turning on the suction. After turning off the suction, 2 ml. of blood and 2.5 ml. of phosphate buffer are added and the magnetic stirrer is turned on. After two minutes of vigorous stirring the layers are allowed to separate and the upper layer is removed by suction. By turning the beaker by hand all but a drop of aqueous layer can be easily removed. Complete removal of all traces of red cells is accomplished by adding about 50 ml. of distilled water and reapplying suction. Three or four washes are usually ample. Suction is then turned off and 1 ml. of mercury reagent added. By two minutes of vigorous stirring chloroform-soluble mercury barbiturate is formed if any drug is present. The stirrer is turned off and any remaining mercuric ion in the aqueous phase is removed by suction and water-washing. Two 40-ml. washes are ample. Then 1 ml. of dithizone solution is pipetted carefully under the chloroform remaining in the beaker and the colour distributed evenly by a slow rotation. The colour must be observed immediately; unchanged green implies absence of barbiturate and a clear orange is seen in the presence of drug. The apparatus is easily prepared

for further use by washing with water and chloroform, removing the solvents by suction, and raising the beaker slightly by hand to empty it completely.

Results

The Table shows a series of cases in which positive results were obtained and in which the actual levels were procured by ultra-violet spectrophotometry. Control experiments with amylobarbitone added to blood have confirmed the theoretical results expected from the quantities of mercury ion and dithizone used.

Table of Results

Barbiturate	Blood Concentration (mg./100 ml.)
Amylobarbitone	5
"	4.1
"	2.3
Butobarbitone and pentobarbitone mixture	2.8
Quinalbarbitone	1.7
" and cyclobarbitone mixture	3.6
Pentobarbitone	3.3
Amylobarbitone, butobarbitone, and quinalbarbitone mixture	0.8

Although rarely necessary the method can be scaled down by a factor of 10; 0.2 ml. of blood is then used with corresponding reductions in all reagents. The smaller volumes make magnetic stirring difficult, but if the reactions are performed in a 3 by $\frac{1}{2}$ -in. (7.5 by 1.3-cm.) glass-stoppered tube and extractions accomplished by mechanical rolling, emulsions do not occur. Washings have then to be done by use of a Pasteur pipette. A mechanical roller can easily be made with a driven roller wheel and an idler wheel with the tube placed in position between them.

Summary

A method of screening 2 ml. of blood for barbiturate in five minutes is described. If necessary the scale of working can be reduced so that only 0.2 ml. is necessary. No expensive equipment or reagents are required.

REFERENCE

- Zaar, B., and Gronwall, A. (1961). *Scand. J. clin. Lab. Invest.*, **13**, 225.
(The reader is also referred to a paper by G. Wallenius, B. Zaar, and E. Lausing, *Scand. J. clin. Lab. Invest.*, 1963, **15**, Suppl. No. 69, 252.)

Medical Memoranda

Candida Endocarditis After Cardiac Surgery

Only two cases of candida endocarditis have been recorded in Great Britain (Caplan, 1955; Conn *et al.*, 1959), but elsewhere the incidence, particularly after cardiac surgery, seems to be increasing. The following case occurred after mitral valvotomy.

CASE REPORT

On June 26, 1962, a 32-year-old joiner, who had had a valvotomy for mitral stenosis four months earlier, was admitted to Edgware General Hospital complaining of increasing breathlessness, diarrhoea, and vomiting. His temperature was 100° F. (37.8° C.) and pulse rate 96. Haemorrhages were seen in the right fundus and there was a slight exudate from the thoracotomy scar. The heart was enlarged, a tumultuous impulse being palpable in the sixth intercostal space in the anterior axillary line, and a soft apical systolic murmur, not previously audible, was present. There was no splenomegaly. His haemoglobin was 12.2 g./100 ml. and leucocytes 19,000/c.mm. (neutrophils 79%, lymphocytes 18%, monocytes 3%). The urine contained a few erythrocytes but culture was sterile. A swab from the thoracotomy wound grew *Staphylococcus pyogenes*, but blood cultures taken on admission were sterile. The antistaphylococcal alpha-haemolysin titre was normal, but the anti-Panton-Valentine leucocidin titre was considerably elevated.