SUMMARY

1. A new method for the determination of 1:2-dithiols is described, depending on the fact that cyanogen chloride reacts with 1:2-dithiols to give a compound which readily liberates thiocyanate in alkaline solution.

2. 1:3-Dimercaptopropanol and the oxidized form of BAL do not react. Of many thiol compounds, only glutathione, ergothioneine and thiolacetic acid interfere.

3. A method for the determination of BAL in blood is described, but a satisfactory method in the case of urine has not been found. The results obtained are discussed.

4. The mechanism of the reaction is discussed.

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The Estimation of Urethane (Ethyl Carbamate) in Blood

By H. E. ARCHER, L. CHAPMAN, EVA RHODEN AND F. L. WARREN

The Chester Beatty Research Institute, The Royal Cancer Hospital (Free), Fulham Road, London, S.W. 3 and The Pathological Department, Wellhouse Hospital, Barnet

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In connexion with the recent use of urethane in the treatment of leukaemia (Haddow & Sexton, 1946; Paterson, Ap Thomas, Haddow & Watkinson, 1946), the necessity arose for a method for estimating this substance in blood.

The very simplicity of the chemical structure, $\text{NH}_2\text{COOC}_2\text{H}_5$, and its close resemblance to urea, indicated that attempts to estimate urethane by reactions based on the amino group were unlikely to be successful. Attention was therefore directed to the characteristic ester grouping. It was found that urethane is quantitatively hydrolyzed to ethanol, ammonia, and carbonate, by boiling with sodium hydroxide solution. The ethanol can be estimated by distillation into acid potassium dichromate solution followed by titration of excess dichromate with sodium thiosulphate after addition of potassium iodide.

METHOD

The estimation involves two stages:

(1) *Determination of volatile reducing substances in blood before alkaline hydrolysis.* Folin-Wu blood filtrate (10 ml.) is measured into a 100 ml. flask containing a few glass

beads and connected with a Jackson's condenser which has its delivery tube extended downwards for about 15 cm. The delivery tube dips below the surface of a mixture of potassium dichromate and sulphuric acid (1 ml. of 0.1N- $\text{K}_2\text{Cr}_2\text{O}_7$ and 5 ml. A.R. conc. H_2SO_4) which is contained in a tube of 20 ml. capacity. The tube containing the mixture is well cooled in ice. With no water cooling on the condenser, about 8 ml. of the flask's contents is distilled over into the oxidizing mixture. A glass stopper is sealed into the tube with a drop of conc. H_2SO_4 and the contents mixed and heated to 80° for 20 min. The tube is then cooled and the contents washed into a flask with about 100 ml. of water, 5 ml. of 5% KI solution are added and the liberated iodine titrated with 0.1N-sodium thiosulphate.

The above determination usually yields a small blank value.

(2) *Determination of volatile reducing substances in blood after alkaline hydrolysis.* A clean flask is fitted to the condenser, and a mixture of 10 ml. of blood filtrate together with 5 ml. of 10N-NaOH is refluxed for 15 min. The water supply to the condenser is then stopped and 8-10 ml. of the flask's contents distilled into acid dichromate mixture as in (1). The subsequent procedure is as described in (1) above.

The difference between the dichromate reduced in (1) and in (2) is a measure of the urethane content of the blood. 1 ml. of 0.1N-dichromate is equivalent to 2.225 mg. of urethane.

RESULTS

As an illustration of the use of the method the results obtained on specimens of blood from two patients with leukaemia who received urethane orally are given.

Case 1. The patient had been receiving 1 g. of urethane daily for 50 days, the last dose being administered at 4.30 p.m. on the 50th day. On the 51st day, a sample of blood was drawn at 1 p.m. At 1.20 p.m. the patient ingested 2 g. of urethane. Blood samples were taken after this dose at 0.5, 2, 5.5 and 22.5 hr. The urethane content of these samples is shown in Table 1.

Table 1. *Blood urethane values of case 1*

Sample	Time (hr.)	Urethane (mg./100 ml.)
1	0	10
2	0.5	38
3	2	36
4	5.5	22
5	22.5	21

The urethane content of the first sample was produced by the preliminary treatment with urethane. Administration of 2 g. raised the level of urethane in the blood by 28 mg./100 ml. in the first 0.5 hr.

Case 2. Samples of blood were obtained before the patient had started urethane treatment. Urethane (3 g.) was then given daily for 14 days and the content of the blood measured at intervals. The results of these estimations are shown in Table 2.

Table 2. *Blood urethane values of case 2*

(Samples 1 and 2 were taken before starting urethane treatment.)

Sample	Day of treatment	Urethane (mg./100 ml.)
1	- 4	4
2	- 2	5
3	1	10
4	3	31
5	7	27
6	14	27

If the pretreatment blank values (samples 1 and 2) are taken as 5 mg./100 ml., then the daily ingestion

of 3 g. of urethane maintained in this patient a blood level of about 22 mg./100 ml.

DISCUSSION

Theoretically, the method of estimation described above has the disadvantage that blood may contain compounds other than urethane that, on hydrolysis with alkali, may yield volatile oxidizable substances. In practice, however, it was found that normally this blank value was only about 3-5 mg./100 ml. of blood and did not interfere appreciably with the estimation. Care must be taken, however, that the patient is given no other drugs that may be so hydrolyzed, nor must the blood be preserved with such a substance. Heparin was found quite satisfactory as an anticoagulant for this purpose. It is essential that the blood samples should be as fresh as possible.

Attempts were made to shorten the procedure by carrying out the control and the urethane determinations on the same sample of filtrate. Blood filtrate was distilled down without collecting the distillate in order to remove volatile oxidizable materials. The volume was then restored by addition of water, 10N-NaOH added, and the mixture refluxed for 15 min. before distillation into dichromate. This method proved to be unreliable owing to the volatility of urethane. Up to 50% of the total urethane was lost in the preliminary removal of the volatile substances. In view of this the standard procedure adopted was that described above in which the total urethane and the blank value are determined on separate samples of blood filtrate.

SUMMARY

1. A method is described for the estimation of urethane in the blood of patients undergoing therapy with this drug.

2. The urethane content of the blood can be maintained at a relatively high level for a prolonged period by the daily administration of 1-3 g. of urethane orally to an adult subject.

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