THE SPECTROPHOTOMETRIC DETERMINATION OF THALIDOMIDE IN BODY FLUIDS

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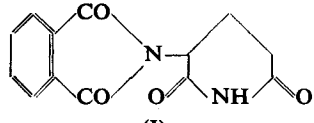
An ultra-violet spectrophotometric method is described for the determination of thalidomide in blood, plasma or urine. Thalidomide is extracted from the sample with chloroform, and the drop in extinction measured when solutions are treated with alkali. The method has been used to determine the amount of drug present in animal and human blood.

The ultra-violet absorption spectrum of thalidomide (I) in 0·1N hydrochloric acid exhibits maxima at 220 mμ and 299·5 mμ (Fig. 1) and the concentration of this material may be measured with high sensitivity using the first of these two peaks where E (1 per cent, 1 cm.) = 1,950. In absolute ethanol the spectrum shows but minor changes, $\lambda_{max.}$ appearing at 218 mμ (E, 1 per cent, 1 cm. = 2,010), and 292·5 mμ. When solutions of thalidomide in either of these solvents are treated with alkali there results a decrease in extinction at the peak wavelengths. This decrease is proportional to the concentration of thalidomide present, and provides a basis for an assay of this substance.



(I)

During this work Beckmann and Kampf (1961) reported a practically identical method for estimating thalidomide in body fluids. Our results discussed below confirm the work of the German authors and underline the usefulness of such a procedure for the estimation of this substance. It is suggested by Beckmann and Kampf that treatment of thalidomide with alkali results in the hydrolysis of the phthalimide ring to an *N*-substituted phthalamic acid. We are in agreement with this suggestion.

Experimental

All spectral determinations were made using a Unicam SP500 spectrophotometer.

Extraction of thalidomide from aqueous solutions in the pH range 1–7·4 may be accomplished with chloroform, and this solvent is also suitable for extracting thalidomide from body fluids. Basic impurities may be removed from such extracts by washing with dilute acid, but attempts to remove acidic impurities with dilute alkali lead to destruction of the thalidomide.

Samples of blood, plasma or urine (5–10 ml.) were extracted with 50·0 ml. of chloroform in a stoppered flask by shaking vigorously for approximately 5 min. The solvent extract was run through a Whatman No. 41 filter paper and washed once by shaking with 5–10 ml. of 0·1N hydrochloric acid. The acid wash was discarded and the solvent was again filtered through Whatman No. 41 paper; 40 ml. of the clear filtrate was evaporated to dryness in a vacuum desiccator.

The dry residue was dissolved in 0·1N hydrochloric acid at 60–65° and filtered through an acid washed Whatman No. 1 paper, then made up to a final volume of 8·0 ml. The extinction (E_1) of 3·0 ml. of this

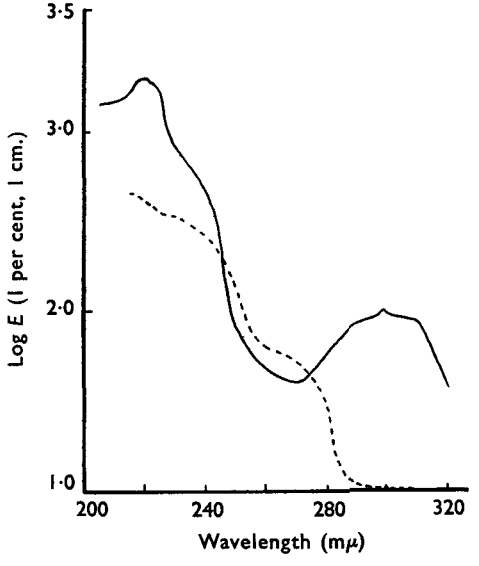


FIG. 1. The ultra-violet absorption curve of thalidomide in 0·1 N hydrochloric acid. ——— Thalidomide in acid solution. – – – – – After treatment with alkali.

solution was measured at 220 mμ against the 0·1N hydrochloric acid used as solvent (in 1 cm. cells). To both solution and solvent cells, 0·50 ml. of N sodium hydroxide was added. The solutions were stirred, and after 15 min. the extinction (E_2) at 220 mμ was again determined. This procedure was repeated on a second 3 ml. aliquot of the test solution, and the mean of the two values for E_1–E_2 gave ΔE_T for the test solution. A blank determination was carried out in an identical manner on blood containing no thalidomide to give ΔE_B for the blank solution. Then $\Delta E_T - \Delta E_B = \Delta E$, the extinction difference due to thalidomide. The weight (W) of thalidomide which this represented was read from a standard curve, and the concentration (C) of the drug in the test sample then = W × 8 × 50/40 × volume of sample in ml. Owing to the low solubility of thalidomide in water the standard curve was obtained as follows. A saturated aqueous solution of thalidomide was prepared. To 9 ml. of this solution was added 1 ml. of dimethylformamide, and the extinction

of this solution at 299 mμ was determined. For a 10 per cent w/v solution in dimethylformamide the E (1 per cent, 1 cm.) at 299 mμ = 93·2, and this value was used to calculate the concentration of thalidomide in

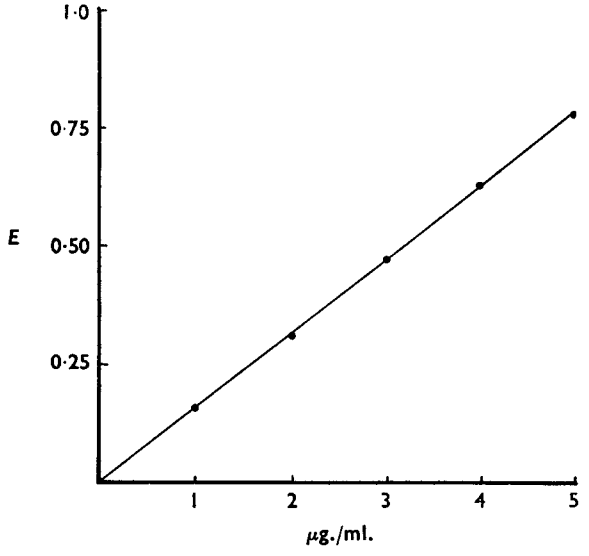


FIG. 2. Calibration curve for thalidomide in 0·1 N hydrochloric acid. $E = E_1 - E_2$ at 220 mμ.

the saturated aqueous solution. By stepwise dilution of the saturated aqueous solution with 0·1N hydrochloric acid, concentrations ranging from 1–5 μg./ml. were obtained and the standard curve constructed from these (Fig. 2).

As an alternative assay procedure, the dry residue was dissolved in 8·0 ml. of absolute ethanol and centrifuged, ΔE values were determined on aliquot samples of this solution at 218 mμ essentially as described above, using 0·2N potassium hydroxide as alkali. Solutions for the

TABLE I

RECOVERY OF KNOWN AMOUNTS OF THALIDOMIDE

No. of experiments	5 ml. sample	Thalidomide added μg.	Solvent	Calculated recovery and standard error
1*	Water	5	Acid	5
13*	Whole blood	5	Acid	4·5 ± 0·32
3*	Water	8·5	Acid	8·33
3	Plasma	10	Acid	9·72
11	Whole blood	10	Acid	9·65 ± 0·5
12	Whole blood	20	Acid	19·22 ± 1·57
10	Whole blood	50	Acid	40·5 ± 3·05
2	Whole blood	100	Acid	68·35
11	Whole blood	10	Ethanol	9·69 ± 0·56
12	Whole blood	20	Ethanol	19·59 ± 1·27
8	Whole blood	50	Ethanol	46·23 ± 2·16
4	Whole blood	100	Ethanol	93·1 ± 5·5
4	Urine	20	Ethanol	18·6 ± 0·95

* In these experiments the dry residue was dissolved in 5 ml. of acid.

construction of the standard curve were obtained by dissolving a known weight of thalidomide in absolute ethanol, and then diluting to give the desired concentration range.

Results and Discussion

The accuracy of the analytical procedure was checked by adding known amounts of thalidomide to 5 ml. aliquots of blood, plasma or urine and then carrying out the assay described. Details are given in Table I.

These results show that with thalidomide blood levels up to 4 μg./ml. the standard error of the assay procedure is $\pm$ 7·5 per cent with a

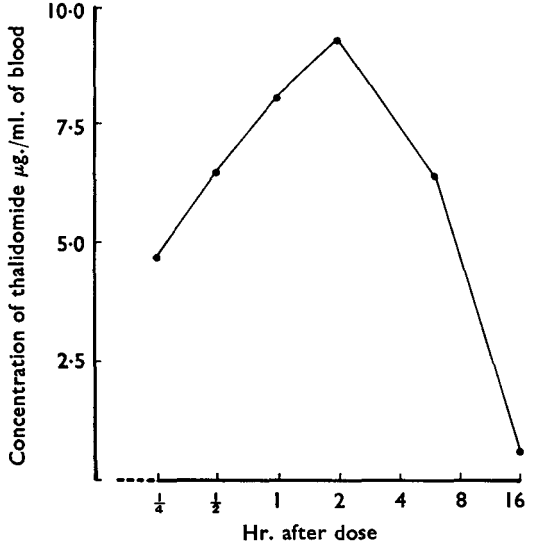


FIG. 3. The concentration of thalidomide in mouse blood after oral dosing. (10 mg./20 g. mouse).

recovery efficiency of over 90 per cent irrespective of whether ethanol or 0·1N hydrochloric acid is used. At blood concentrations above 10 μg./ml. better results are obtained with ethanol, as in the alternative procedure.

Absorption measurements from 180–250 mμ are liable to much error due among other effects to stray light. The fact that Beer's law is followed (Fig. 2) taken with the satisfactory recovery of thalidomide from biological fluids, however (Table I), demonstrates that the assay is satisfactory for the purposes stated.

Potassium hydroxide of the strength used in the alternative assay with ethanol has been reported as acting as a cut-off filter just below 218 mμ, and when the extinction value of the ethanol:0·2N potassium hydroxide mixture was measured against air at 218 mμ, a figure of 1·65 was obtained. In spite of this fact, that approximately 97 per cent of the incident light was absorbed by this solvent mixture, the method

yielded satisfactory results (Table I). Extinction measurements made on an ethanol:0·1N potassium hydroxide mixture gave a value of 1·0, whilst the 0·1N acid:N sodium hydroxide mixture measured at 220 mμ gave a reading of 0·220. These extinction values suggest that the use of a potassium hydroxide solution weaker than 0·2N would be advisable.

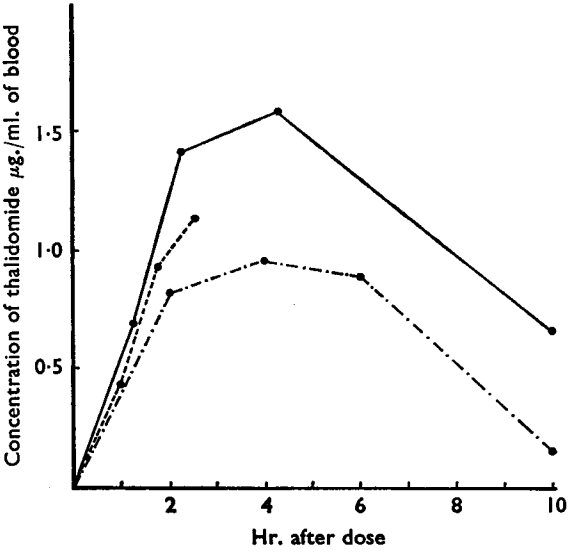


FIG. 4. The concentration of thalidomide in the blood of human volunteers; 3 volunteers, each given a 1 × 150 mg. tablet of thalidomide.

Blood determinations in man and experimental animals have been carried out by this method. In mice the curve shown in Fig. 3 resulted. Blood levels of the drug in three human volunteers were as shown in Fig. 4.

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REFERENCE

Beckmann, Von R. and Kampf., H. H. (1961). *Arzneimitt.-Forsch.*, **11**, 45–47.

The paper was presented by DR. GREEN.