

stained sections (MB: A₂, lipid colourless or pale green) with 96% alcohol, pH 10.0. This removes some stain from the section, but the previously pale green or colourless lipid droplets now take on an obvious blue-black colour.

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A modified fluorimetric procedure for the rapid estimation of oxytetracycline in blood

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Chemical methods of determination of terramycin (oxytetracycline or OTC) described in the literature make use of either fluorimetric or colorimetric measurement of a degradation product (Chiccarelli, Woolford, and Avery, 1959; Hayes and DuBuy, 1964). The method described by Hayes and DuBuy is based on the conversion of tetracycline to anhydrotetracycline by heating in an acid medium and the resultant fluorescence of anhydrotetracycline (Hayes and DuBuy, 1964). This method is especially sensitive for the determination of oxytetracycline in biological tissues. Ibsen, Sanders, and Urist (1963) have described a general method for the determination of tetracyclines making use of Mg⁺⁺-OTC chelate formation. In the present communication we describe a modification of the method of Hayes and DuBuy (1964) adapted for estimating oxytetracycline in serum or plasma obtained from clinical blood samples.

Experimental

All reagents used were AR grade. Terramycin HCl was a gift from Pfizer Ltd. The trichloroacetic acid (TCA):HCl reagent used was a modification of the reagent used by Hayes and DuBuy (1964) and was prepared by mixing 3 volumes of 5N TCA with 1 volume of concentrated HCl. In place of TCA:HCl reagent, an NaWO₄:H₂SO₄ reagent prepared by mixing 2 volumes of 10% NaWO₄ and 1 volume of concentrated H₂SO₄ may also be used without altering the sensitivity of the method.

Procedure

To 1 ml aliquot of human serum (or plasma) obtained at various intervals after administration of an oral amoebic antibiotic² preparation containing terra-

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²A preparation containing terramycin, chloroquin phosphate, and diiodohydroxyquin in the proportion of 167 mg, 85 mg, and 500 mg respectively.

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mycin or 1 ml of standard terramycin solution (containing 0.2 to 1.0 μg terramycin HCl in 0.1 N HCl), 0.1 ml of 1.1 N HCl is added. The resulting mixture is shaken for five min and immediately deproteinized by the addition of 0.3 ml TCA:HCl reagent. Alternatively 0.3 ml of $\text{NaWO}_4:\text{H}_2\text{SO}_4$ reagent may be used for deproteinization (Folin and Wu, 1920). The bulky precipitate formed is broken up by vigorous mixing and the resultant mixture centrifuged at $2000 \times g$ for 15'. The amount added is sufficient to precipitate all the proteins in the serum. If plasma is used in place of serum, the process is repeated by a further addition of 0.3 ml of the same reagent to ensure complete precipitation of plasma proteins. In our experience, this has not affected the reading in any way other than changing the dilution factor. The transient opalescence, produced after precipitating with the tungstate reagent, vanishes after cooling during the procedure and does not interfere with the method described. The rest of the procedure is the same as described by Hayes and DuBuy (1964).

Care should be taken that the serum samples are not extensively haemolysed. Highly icteric serum samples interfere with the assay procedure by giving spuriously high values (see table).

Measurements are made using a Turner model 110 fluorimeter with a temperature stabilized door and a built-in high sensitivity attachment (no. 110-655) and employing a no. 110-812 primary filter (Corning 7-51 + Wratten 2-C) and a no. 110-817 secondary filter (Wratten 8) combination. Matched Pyrex test tubes supplied by the manufacturers of the instrument serve as micro cuvettes.

Results and Comment

SENSITIVITY OF THE METHOD

The standard curve for oxytetracycline employing this method is shown in the figure. It can be seen that identical results are obtained when either TCA:HCl or $\text{NaWO}_4:\text{H}_2\text{SO}_4$ reagent is employed for deproteinizing. It is reported that the sensitivity of measuring oxytetracycline is 1700 times that of tetracycline measurement (Hayes and DuBuy, 1964). The sensitivity is further enhanced five to 10-fold with the use of a high sensitivity attachment which makes it possible to estimate micro quantities of the antibiotic present in small aliquots of blood. It is pertinent to note that Ibsen *et al* (1963) have used 10% TCA to extract added oxytetracycline from human and rabbit serum with a recovery approaching 100%. In the light of this finding, our method has considerably simplified the extraction procedure for oxytetracycline from serum samples. It has been possible to estimate as low as 0.2 $\mu\text{g}/\text{ml}$ of the

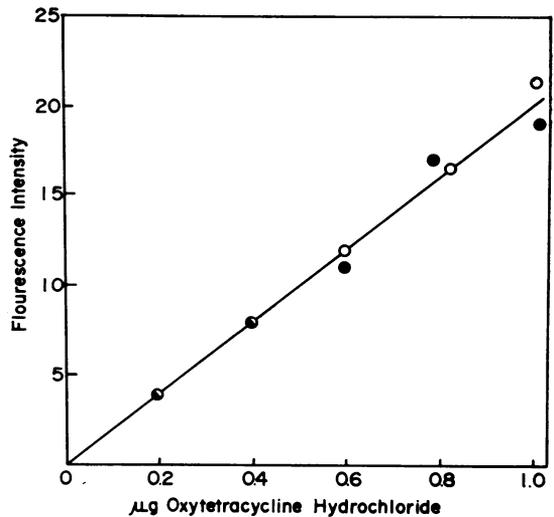


Fig Standard curve for analysis of oxytetracycline $\text{NaWO}_4:\text{H}_2\text{SO}_4$ reagent (●) or TCA:HCl reagent (○) used as deproteinizing agent. See text for details.

antibiotic and up to a concentration of 1 $\mu\text{g}/\text{ml}$ or more.

Stillman (1960) has reported that 0.6 $\mu\text{g}/\text{ml}$ of oxytetracycline as the minimum therapeutic level. Our study reveals that it is possible to measure therapeutic levels of oxytetracycline conveniently. Generally oxytetracycline has been assayed microbiologically for evaluating therapeutic levels in clinical studies. Our method has the distinct advantage over microbiological assay procedures in that it can be used with smaller quantities of serum samples.

APPLICATION

The method employing the modified TCA-HCl reagent to precipitate the protein has been successfully used to measure terramycin levels in blood after the administration of the amoebic antibiotic preparation containing terramycin to patients. Representative values for terramycin levels attained after the administration of the amoebic antibiotic are shown in the table. This method has also a selective advantage over other existing methods in that oxytetracycline can be preferentially estimated in the presence of other chemotherapeutic agents (chloroquin phosphate and diiodohydroxyquin in this instance).

Rapid analyses of blood samples for terramycin can be made using the simple procedure described and can be suitably adapted for automatic analysis in clinical research.

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Time (hr)	Case No.			
	1	2	3 ^a	4
0	0	0	0	0
2	Not detectable	0.34	0.45	0.12
6	1.10	0.78	2.40	0.60
12	1.40	0.33	1.20	0.06
24	1.40	—	—	—

Table Levels of oxytetracycline in serum samples of patients after oral administration of the amoebic antibiotic^{1,2}

¹Results are expressed in $\mu\text{g/ml}$ serum.

²Three capsules of the amoebic antibiotic were administered to patients suffering from amoebic dysentery. Zero hour serum samples served as controls. Samples were stored in a refrigerator till they could be analysed later on the same day.

³Icteric samples.

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The development of aluminium containers for the storage of blood in liquid nitrogen for the immunization of Rh⁻ volunteers

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The deliberate immunization of volunteers for the large-scale production of high-titre anti-D immunoglobulin is now a well recognized procedure in any blood transfusion programme, and efforts designed to reduce the risk of transmitting disease during this procedure have hitherto been restricted in this department to the careful selection of 'accredited donors', ie, those whose previous five donations have been transfused and the recipients monitored for the succeeding six months. This approach is limited as it does not exclude the possibility that the donor may be incubating a recently acquired transmissible disease at the time when blood is withdrawn. Glycerolized blood may be stored in a frozen state for prolonged periods during which time surveillance of the donor's health can be maintained. Despite the introduction of large volume frozen units for transfusion purposes (Pert, Schork, and Moore, 1965; Rowe, Eyster, and Kellner, 1968; Krijnen, Kuivenhoven, and De Wit, 1970; Jenkins and Blagdon, 1971; Mitchell and Muir, 1972) hitherto no small-volume system has been developed which is suited to the immunization of volunteers for the production of anti-D. The following communication describes the introduction of such a system, based on readily available components which can operate economically in any laboratory where liquid nitrogen is available.

Materials and Methods

Screw-cap aluminium containers (20 × 40 mm, New Style) were obtained from the Metal Box Co, Cowlares Industrial Estate, Glasgow, N1. Silicone rubber sheet (1/16 in. × 11 in. × 11 in. grade TC-156) was obtained from ESCO, 14-16 Great Portland Street, London, W1. A two-hole metal punch (5 mm dia. × 11 mm centres) was obtained from J. Hamilton, Star Works, Pollard Street, Manchester. Latex rubber fingerstalls (approximately 20 mm diameter) were obtained from a local surgical retailer. Storage trays with 36 spaces

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