A Method for Quantitative Determination of Tetraethylthiuram Disulphide (Antabuse, Abstinyl) and Its Reduced Form, Diethyldithiocarbamic Acid, as Found in Excreta

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In recent papers by danish authors the action of antabuse (*t*etraethyl*t*hiuram *d*isulphide, here called T.T.D.) has been studied and a method for its determination was developed [1]. T.T.D. was determined as the amount of sulphur present in ether extracts from the sample to be tested, using the common gravimetric method of determining sulphate in solution. This method has some disadvantages, however, as it is rather laborious and not specific as other ether-soluble sulphur compounds may interfere.

A new rapid and more specific method was developed, based on the intense colour of the cupric diethyldithiocarbamate, which makes it possible to trace even small amounts of T.T.D. and also offers a possibility to determine its reduced form, which can be expected to occur in the excreta.

1. *Determination of tetraethylthiuram disulphide.* A solution of T.T.D. in an organic solvent, *e. g.* benzene, carbon tetrachloride or chloroform, reacts with *cuprous* iodide, probably according to

$$CuI + (C_2H_5)_2N\cdot CS\cdot S\cdot S\cdot CS\cdot N(C_2H_5)_2 \rightarrow$$

$$(C_2H_5)_2N\cdot CS\cdot S\cdot Cu\cdot S\cdot CS\cdot N(C_2H_5)_2 + \tfrac{1}{2}I_2$$

The solution has an intense brown-yellow colour suited for photometry [2]. The reaction is fast and seems to proceed practically quantitatively. Cupric diethyldithiocarbamate isolated from the solution showed the correct m. p. (193—194°)[2]. Positive iodine reaction with starch solution can be obtained, but the iodine liberated does not interfere with the photometric determinations as described below.

Cuprous chloride and bromide also react at first with T.T.D. to give the brown-yellow cupric salt but subsequent reactions (not studied as yet) change the colour of the solutions.

Procedure. The sample (urine or a water suspension of faeces) is extracted with a known volume of carbon tetrachloride and centrifuged, if necessary, to get a clear carbon tetrachloride layer. The carbon tetrachloride is withdrawn, shaken with 0.5 g of pulverized cuprous iodide for 5—10 minutes and filtered. The extinction can be determined in a Pulfrich photometer, filter S 66 (violet), 2 cm cuvettes. A reference solution is prepared from an antabuse solution of known concentration. When the extinction is plotted against the concentration a straight line through the origine is obtained.

2. *Determination of diethyldithiocarbamic acid.* The reduced form of T.T.D. is the diethyldithiocarbamic acid, which at the pH values in question is present in ionized form. In this form it is water-soluble but insoluble in organic solvents. Thus it is possible to separate the two forms: when extracting with carbon tetrachloride, the diethyldithiocarbamate ions remain in the water phase while the disulphide is transferred to the carbon tetrachloride phase. The sodium diethyldithiocarbamate is used as reagent in the common method for micro determination of cupric ions [3] and we have made use of a reversal of this method.

Procedure: Cupric sulphate solution, sodium citrate solution (buffer) and a suitable volume of carbon tetrachloride are added to a defined amount of the sample (urine or a water suspension of faeces) or the water phase left from the

extraction procedure under 1. The mixture is shaken thoroughly until all the complex cupric salt is dissolved in the carbon tetrachloride and centrifuged. The carbon tetrachloride solution is then filtered and the extinction is determined as under 1.

3. *Application of the method.* The method was used to determine T.T.D. qualitatively and quantitatively in experiments on rabbit and man.

Rabbits were kept in metabolism cages and urine was sampled as a rule during 24 hours periods. T.T.D. in varying doses was given as a suspension by stomach tube. In one rabbit 0.3 g T.T.D. was given on three successive days. 27 per cent of the T.T.D. given was recovered in the urine in the reduced form while only traces of unaltered T.T.D. were found. In another rabbit, given 0.05 g T.T.D. in a single dose, 12 per cent of the dose given was recovered in the reduced form. 48 hours after the dosage practically no T.T.D. could be recovered in the urine. A third rabbit was given 0.1 g T.T.D. on three successive days and killed 6 hours after the last dosage. 3 per cent of the T.T.D. given was recovered in the urine in the reduced form. The unaltered form of T.T.D. was found in the contents of the digestive tract. No T.T.D. could be traced in the blood serum or in the liver.

Two healthy men were given T.T.D. per os during three days (1 + 0.5 + 0.5 g). On the third day urine and faeces were sampled. No T.T.D. could be traced in the urine. In the faeces 117 respectively 56 mg of unaltered T.T.D. were recovered and only minute amounts of the reduced form (0.5 and 0.2 mg respectively).

A closer study of the accuracy of the methods, their applicability under different conditions *etc.* is in progress.

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A Simple "X-Ray Colorimeter"

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When performing a quantitative microchemical analysis often the problem of determining small amounts of an element with a high atomic number in the presence of elements with low atomic numbers arises. A typical case is that of a protein reacting with a heavy metal such as silver. By determining the amount of silver bound to a protein the number of the silverbinding groups in the protein can be calculated. In the example mentioned the quantitative estimation by ordinary methods of analysis of the small amounts of silver bound to the organic substance offers difficulties.

This paper describes a method permitting the rapid determination of a high atomic element bound to an organic compound. As the absorption of X-rays increases with the fourth power of the atomic number, an organic compound to which a small amont of a high atomic element is bound gives greater absorption of X-rays than the organic compound alone. This is the same principle as utilized in the determination of tetraethyl lead in gasoline[1].

The principle of the method can be seen from Figure 1. Primary X-rays are generated in the X-ray tube A. Philips' commercial diffraction unit was used as a source of X-rays. The voltage of the X-ray tube and the filtering of the X-rays may be

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