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The Estimation of Suramin in Plasma

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The colorimetric estimation of suramin (Antrypol, Bayer 205) by conversion of the hydrolytic fission products to an azo dye has been described by several investigators. Dangerfield, Gaunt & Wormall (1938) have summarized the earlier work, and have described in detail a method in which the drug is subjected to acid hydrolysis; the fragments are then diazotized and coupled with methyl-α-naphthylamine. Spinks (1948) has used alkaline hydrolysis and *N*-β-sulphatoethyl-*m*-toluidine as a dyestuffs end component. Balaban & King (1927), in their investigation of the affinity of suramin and other complex ureides for cellulose, devised a roughly quantitative method in which the concentration was determined from the turbidity produced by the addition of a basic protein such as clupeine sulphate; for this method the authors claimed a sensitivity of 1 in 100,000.

In this investigation the possibility of a direct, colorimetric estimation by a dye-laking procedure has been explored by examining the behaviour of a range of basic dyes in the presence of the drug. Certain styrylquinoline dyes have been found to exhibit a marked colour change under these conditions, and 2-*p*-dimethylaminostyryl-6-acetamidoquinoline methochloride has been selected as the

most suitable for the estimation. This compound was described by Browning, Cohen, Ellingworth & Gulbranson (1926); it is 'styrylquinoline 24' in their series and will be referred to here as *SQ* 24.

In the presence of excess suramin the deep-red colour of *SQ* 24 is converted to a pale yellow; the change in absorption spectrum is shown in Fig. 1.

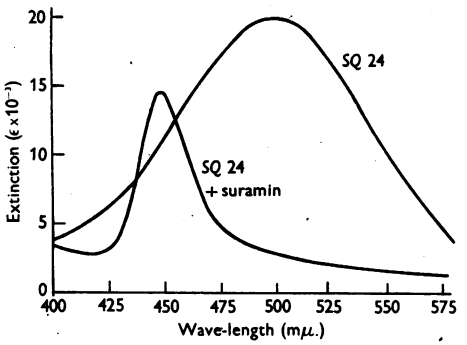


Fig. 1. Absorption spectra of *SQ* 24, with and without added suramin.

SQ 24 has a broad maximum at 495 mμ., and the complex with suramin has a sharp maximum at 450 mμ. The greatest difference between the two curves is to be found at 505 mμ. The complex cannot be extracted by organic solvents, and on standing overnight the yellow aqueous solution deposits a flocculent brown precipitate. If a sufficient concentration of water-miscible organic solvent, such as ethanol or cellosolve, is added to the yellow solution the original red colour of the reagent is regenerated. Plasma proteins do not interfere with the suramin reaction, and the concentration of the drug in plasma may be determined by comparing the optical density of the dye solution, to which has been added diluted plasma (in sufficient 0·9 % NaCl to prevent globulin precipitation), with that of the dye solution without suramin.

METHOD

The analytical procedure described below for plasma is suitable for serum or for aqueous solutions of suramin. The quantities given are for plasma samples containing 0·5–15 mg./100 ml.; for greater or lesser amounts of suramin the ratio of sample to dye solution should be suitably modified.

Reagents required. (*a*) 2-*p*-dimethylaminostyryl-6-acetamidoquinoline methochloride (*SQ* 24) solution; 1 mg./100 ml. distilled water. (*b*) Isotonic sodium chloride (0·9 % NaCl in water).

Apparatus required. (*a*) 6 × ⅝ in. test tubes with graduation mark at 10 ml. (*b*) Suitable colorimeter. If none is available, an approximate estimate of the suramin concentration may be made by comparison with a set of freshly prepared standards. In this investigation the Coleman spectrophotometer at a wave length 505 mμ. has been used employing cylindrical cells. Satisfactory results have also been obtained with the Spekker absorptiometer using an Ilford 603 blue-green filter and 1 cm. cells. To avoid adsorption of the dye on the surface of glassware it is important that the tubes and colorimeter cells should be free from surface etching; they should be well cleaned in chromic-sulphuric acid mixture before use.

Procedure

(*a*) Pipette 0·2 ml. plasma into 2 ml. isotonic NaCl solution in a tube graduated at 10 ml. Add 5 ml. *SQ* 24 solution and make up to 10 ml. with distilled water.

(*b*) Pipette 5 ml. *SQ* 24 solution into 2 ml. isotonic NaCl and make up to 10 ml. with distilled water.

(*c*) Pipette 0·2 ml. plasma into 2 ml. isotonic NaCl and make up to 10 ml. with distilled water. The solutions are allowed to stand for a few minutes and the optical densities measured in a suitable colorimeter. The density of the plasma blank (*c*) is subtracted from that of (*a*), and the difference subtracted from the dye blank (*b*). The amount of suramin present in (*a*) may then be determined by applying this difference to a standard curve, constructed from solutions containing known amounts of suramin. Fig. 2 shows standard curves constructed by the above method; curve *A* is for dilutions of suramin in water, while to obtain curve *B* the solutions contained a total concentration of 20 % (v/v) serum. With the amounts of plasma or serum used in the method as described above, the standard curve coincides with the water curve *A*.

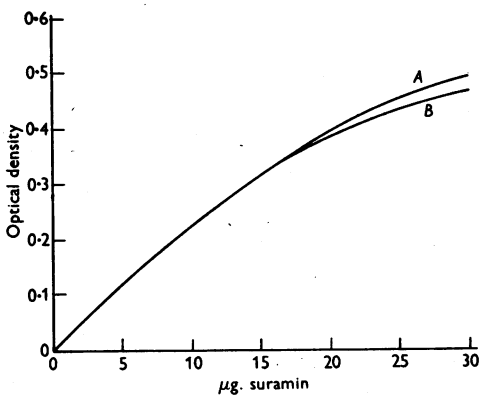


Fig. 2. Standard curve for suramin estimation, *A* in water, *B* in 20 % serum.

DISCUSSION

Comparison with the hydrolysis method

Samples of oxalated plasma, taken at intervals from a rabbit dosed intravenously with 100 mg./kg. of suramin, were subjected to the above analytical procedure and also to the hydrolysis method of Dangerfield *et al.* (1938), modified by the use of the Rose & Bevan (1944) end component *N*-β-sulphatoethyl-*m*-toluidine. The results by the two methods are compared in Table 1.

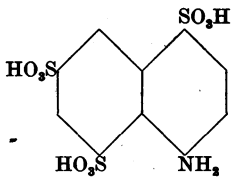
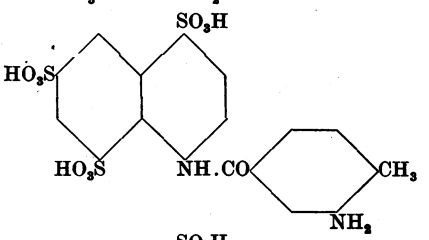
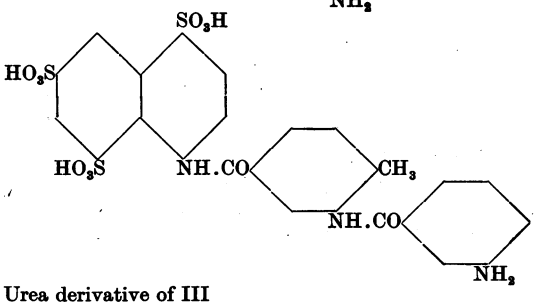
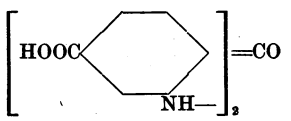
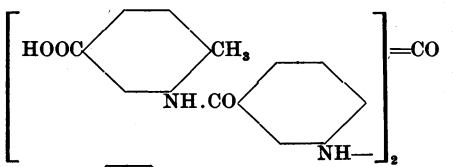
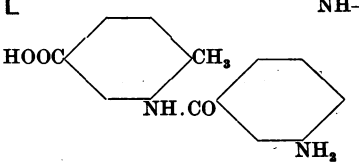
Table 1. *Concentration of suramin in the plasma of an injected rabbit, by dye-laking and hydrolytic methods*

Time after dosing	Plasma suramin (mg./100 ml.) Dye-laking	Hydrolysis
15 min.	71·2	61·6
5 hr.	25·2	21·6
1 day	16·2	15·2
4 days	6·1	5·7
10 days	2·4	2·5
20 days	2·3	3·2

The blank value of the rabbit plasma prior to dosing was 1·3 mg. by the dye-laking method, and 0·96/100 ml. by the hydrolysis method.

Since the dye-laking method necessitates the measurement of a colour difference it might be expected to be rather less precise that the hydrolysis method, particularly with the lower suramin concentrations. The magnitude of this colour difference, however, makes the dye-laking method rather more sensitive; together with the far greater manipulation necessary for the hydrolysis method, this

Table 2. *Suramin 'equivalents' of possible metabolic fission products*

	Formula	Mol. compound equivalent to 1 mol. suramin
I		0·20
II		0·41
III		0·31
IV	Urea derivative of III	0·84
V		0·20
VI		0·24
VII		0

probably makes the precision of the two methods of about the same order. The very much simpler technique of the dye-laking procedure, and the shorter time required, should make the method of particular value if plasma concentrations are required to be estimated in the field with limited laboratory facilities.

Specificity of the dye-laking method

A number of the possible metabolic fission products of suramin have been subjected to the analytical procedure described above and the results are listed in Table 2.

These results indicate that the dye-laking reaction occurs with those fragments which contain the naphthylaminetrisulphonic acid group and also the bis-urea derivatives. It may be assumed from the results with (VII) that amides and the simpler amino-acids do not give the reaction. It is interesting to note that the order of suramin equivalents of this series of compounds approaches very closely to their order of persistence in the blood stream as found by Spinks (1948).

Table 1 indicates that the results obtained by the two methods are of the same order, the dye-laking method giving slightly the higher figures. This suggests the absence from the plasma of appreciable amounts of simple amino-acids and amides without the naphthylaminetrisulphonic acid group, since these would cause the dye-laking method to give lower apparent plasma concentrations. On the other hand appreciable amounts of naphthylaminetrisulphonic acid would cause the dye-laking method to give a higher apparent concentration, since according to Spinks (1948) the azo dye developed from this compound is weakly coloured. It is possible that the observed higher results with the dye-laking method are due to this cause. Spinks has also shown that persistence for a prolonged period in the blood after intravenous injection is only exhibited by symmetrical polyamides of high molecular weight, which contain naphthylaminepolysulphonic acids as end groups, and that after 10 hr. the amounts of the other products of hydrolysis of suramin in the blood are negligible. Moreover, since all the degradation products examined have no trypanocidal activity, it is very likely that after this period both methods are measuring true suramin concentration and any divergence between them is due to experimental error.

SUMMARY

1. A method for estimating suramin in aqueous solution or in serum and plasma is described. It utilizes the colour change which is observed when suramin is added to a styrylquinoline dye, 2-*p*-dimethylaminostyryl-6-acetamidoquinoline methochloride.

2. The method is of about the same precision as the hydrolysis method of Dangerfield *et al.* (1938) and rather more sensitive; it involves a much simpler and more rapid technique. The two methods yield similar values for the suramin content of the blood of a rabbit injected with suramin.

3. The behaviour of hydrolysis products of suramin has been investigated and the specificity of the method is discussed.

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Sexual Differences in the Storage and Metabolism of Iron

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It is now an accepted fact that hormones and/or vitamins have important parts to play in the exchanges and functions of sodium, chlorine, potassium, calcium and iodine, but their role in the metabolism of iron has not been so conclusively demonstrated or so generally recognized. Bunge (1889) suggested that young female animals put aside reserves of iron for the future needs of reproduction, and that the deviation of iron from the circulation to the storage organs at puberty might explain the high incidence of chlorosis in adolescent girls. In more recent years further evidence has been produced that the metabolism of iron is controlled, to some extent at any rate, by the sex hormones. Women, for instance, tend to have lower concentrations of circulating haemoglobin than do men, apart altogether from the obvious effects which menstruation must have upon their bodily require-