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Studies on Suramin (Antrypol, Bayer 205)

6. FURTHER OBSERVATIONS ON THE DETERMINATION OF SURAMIN IN WHOLE BLOOD AND SERUM

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The amount of the trypanocidal drug suramin present in the blood serum or plasma of man, and any other animal which has received an injection of the drug, can readily be determined by a method previously described (Dangerfield, Gaunt & Wormald, 1938; Boursnell, Dangerfield & Wormald, 1939). The technique of this determination has deliberately been made as simple as possible in order that the method might be of use in tropical countries, even in regions where the simplest laboratory facilities are not available. The possibility has always been kept in mind that medical officers in regions well removed from the central hospitals might require blood suramin determinations on some of their treated trypanosomiasis patients. The drug has a very definite prophylactic value, and plasma suramin determinations might be made in future on large groups of people who have been injected with the drug before being exposed to infection.

Serum and plasma from suramin-containing blood contain the same concentration of suramin, and either material serves for the determination of the amount of the drug in the blood. Should the serum or plasma become grossly infected, however, the resulting bacterial changes might produce amines and other compounds which would vitiate the results. Experiments have, therefore, been carried out to determine whether any simple antiseptic can be added to the serum or plasma to preserve it for subsequent suramin determinations without interfering with the colorimetric determination. Experiments have also been carried out to investigate the reliability of suramin determinations on whole blood samples. Although these investigations were primarily designed for the above-mentioned purposes, they have also furnished useful information about the distribution of the blood suramin between the cells and the plasma.

METHODS

Suramin determinations. These were carried out as described previously (Dangerfield *et al.* 1938; Boursniell *et al.* 1939). The volume of each sample was made up to 3.0 ml. with distilled water before the acid hydrolysis. Blank determinations were made on blood serum or plasma to which no suramin had been added, and these control solutions were placed in the 'backing' tube of the comparator to compensate for a small amount of 'non-suramin' amine precursor in blood samples.

Some commercial samples of suramin when hydrolyzed, diazotized, and coupled with methyl- α -naphthylamine, gave a slightly stronger pink colour than did the sample used originally for the preparation of the Tintometer discs, i.e. they gave a 'recovery' of 101–108%, but this difficulty was readily overcome by standardizing each fresh batch of suramin. These slight differences between different suramin preparations might be partly due to differences in their water content (see also Hawking, 1941). Since, however, all our samples were dried *in vacuo* over CaCl_2 , it seems more probable that there is a slight variation in the composition of commercial suramin.

RESULTS

The recovery of suramin added to whole blood

Varying amounts of suramin were added to defibrinated or oxalated blood (ox, rabbit and human) and determinations were carried out on the whole blood, and in some cases on the separated cells and plasma. The results of typical experiments (Table 1) show that the recovery of the added drug is low and very variable. Duplicate determinations usually gave good agreement, but the percentage recovery

with any one sample of blood varied with the amount of suramin added. The latter observation excludes the use of a 'recovery' factor for calculating the true suramin content of whole blood.

It was thought possible that the recovery of suramin might be improved if the acid hydrolysis of the whole blood sample were more vigorous, e.g. by the use of a larger volume of HCl or by hydrolyzing for 9 hr. instead of the customary 6 hr. Where 5 ml. of HCl were used, the recovery (69–97%) was higher than that obtained with 3 ml., but the difference was not sufficiently marked to justify this change of technique. An increase in the duration of acid hydrolysis led to slight or negligible increases in the recovery of suramin added to whole blood, and more prolonged acid hydrolysis is not desirable since it has been found that further heating beyond the normal 6 hr. period leads to loss of the hydrolyzed suramin (Dangerfield *et al.* 1938).

The recovery of suramin added to washed red blood cell suspensions. Since there is approximately complete recovery of suramin added to serum or plasma, it was concluded that the lower recovery with whole blood samples was due to constituents of the red cells, or more probably to products produced by acid hydrolysis of these cells. Suramin determinations have therefore been made on mixtures of suramin and suspensions of washed red blood cells (ox and human); again the recovery was very low.

Possible reasons for the low recovery of suramin added to whole blood. The low recovery of suramin from whole blood samples is apparently due to some constituent of the red cell or a product formed by

Table 1. *Recovery of suramin added to whole blood*

(Suramin was dissolved in 0.9% (w/v) NaCl and 1.0 ml. of the solution was added to fresh defibrinated ox or human blood or oxalated rabbit blood.)

Type of blood	Volume of blood (ml.)	Suramin added (mg.)	Acid hydrolysis		Recovery* (%)
			Amount of 10.3 N-HCl (ml.)	Duration (hr.)	
Ox	0.50	0.44	3.0	6	86
			3.0	9	86
			5.0†	6	97
	2.0	0.44	3.0	6	69
			3.0	9	75
			5.0†	6	83
Rabbit	2.0	0.50	3.0	6	52
			5.0†	6	69
	2.0	2.0	3.0	6	60
			5.0†	6	76
Human	2.0	1.03	3.0	6	66
			5.0†	6	76

* In this and subsequent tables the results recorded are average values for two duplicate determinations.

† When 5.0 ml. of 10.3 N-HCl were used, the hydrolyzed solution was diluted with distilled water to 16.7 ml. (as against 10.0 ml. when 3.0 ml. of HCl were used) to give the right acidity (approx. 3 N) for satisfactory diazotization and coupling.

hydrolysis of the cells, which inhibits the diazotization or coupling, or which reacts with the amines derived from suramin. Experiments were therefore carried out to determine the recovery of suramin after its addition to HCl-hydrolyzed blood or red blood cells. The results showed that quantitative recovery was obtained under these conditions. The loss appears to occur, therefore, when the suramin is heated with HCl in the presence of red cells.

The preservation of serum for suramin determinations

Many experiments have been carried out with a variety of commonly used antiseptics to determine which are satisfactory for the preservation of serum for subsequent suramin determinations. The following antiseptics were added to serum from rabbits injected with suramin, or to normal human serum to which suramin had been added: ether, chloroform

Table 2. *Distribution of suramin added to whole blood*

Mixture	Defibrinated ox blood (ml.)	Suramin added in 1.0 ml. of 0.9% NaCl (mg.)	Suramin found (mg./21 ml. of the mixture, or fraction obtained from 21 ml. of the mixture)		
			Whole blood	Cells*	Plasma
A	20	20.0	12.8	1.4	17.0
B	20	3.5	2.4	0.2	3.2

* For reasons given in the text the cells were not washed, and the small amount of suramin found in them was probably present in the plasma adhering to the cells.

Distribution of added suramin between the red cells and plasma of whole blood

As a preliminary to an investigation on the distribution of suramin between the plasma and cells of the blood of animals injected with the drug, experiments have been carried out to determine how suramin added to the whole blood is distributed. Suramin (dissolved in a little 0.9% NaCl) was added to fresh defibrinated ox blood, and samples of the well shaken mixture were centrifuged and the plasma and red cells separated as completely as possible. The cells were not washed in this experiment, in order to avoid the loss of any suramin which might be loosely combined with the cell membrane, or any which might diffuse through the cell wall during the washing. The results (Table 2) show that the added suramin became associated completely or almost completely with the plasma. The small amount present in the red cell fraction in this experiment can almost certainly be accounted for by the small amount of plasma remaining in the unwashed red cell fraction.

The recovery of the suramin from the whole-blood mixtures (64% for A and 69% for B) was similar to that obtained in other comparable experiments. The total recovery from the separated plasma and red cells, i.e. the sum of the values for the two fractions, was quite satisfactory (92 and 97%), the slight loss being due to incomplete recovery of the small amount of the drug present in the red cell fractions.

In other experiments, suramin was added to suspensions of red blood cells to determine whether any of the drug became attached to the cells either immediately or when the mixtures were allowed to stand, with gentle shaking, for 3 hr. The results of these experiments, some of which are given in Table 3, show that no significant amount of the added drug became attached to the red cells.

and toluene (0.3, 0.2 and 0.2 ml. respectively/2 ml. of serum); phenol and merthiolate (to give concentrations of 0.2–0.4 and 0.018% respectively); 10.3N-HCl (3 ml./2 ml. serum). Suramin determinations made on these treated sera after they had been standing at 17–25° for periods of 1–5 weeks showed that ether,

Table 3. *Addition of suramin to suspensions of washed red blood cells*

(Fresh defibrinated blood was centrifuged and the red cells washed several times with 0.9% NaCl solution, and then suspended in sufficient 0.9% NaCl to give a volume equal to that of the original blood sample.

Mixture A: 20.0 ml. of ox red blood cell suspension + 22.1 mg. of suramin in 1 ml. of 0.9% NaCl.

Mixtures B and C: 20.0 ml. of human red blood cell suspension + 21.6 mg. of suramin in 1 ml. of 0.9% NaCl.

The mixtures were kept at 21° for 15 min. (A and B) or 3 hr. (C) with occasional gentle shaking, and 5 ml. samples of each were then centrifuged. The cells were washed three times with 0.9% NaCl (3 ml. each time), and the washed cells suspended in 0.9% NaCl to give a final vol. of 5.0 ml. The supernatant solution and washings from each sample were combined, and 0.9% NaCl was added to give 20.0 ml.)

Mixture	Suramin added (mg.)	Suramin found (mg./21 ml. of A, B or C, or fraction obtained from 21 ml.)		
		Original cell suspension	Washed red cells	Super- natant plus washings
A	22.1	17.0	0.0	22.1
B	21.6	15.8	0.2	21.0
C	21.6	16.4	0.2	20.8

chloroform and toluene are not completely satisfactory for preservation; the results usually agreed with determinations made before the preservation of the serum, but occasionally low results were obtained. The HCl-treated samples were sometimes

satisfactory, as was noted by Hawking (1940 *a*), but they occasionally gave surprisingly low results; no explanations for this can be offered at present. The results with the sera preserved with phenol or merthiolate were invariably satisfactory. Serum samples to which no antiseptic had been added became grossly infected when kept at room temperature for 1-5 weeks, and they often gave high suramin values.

DISCUSSION

Determinations of the amount of suramin in the blood can yield useful information about the prophylactic value of the drug, and about the blood level which might reasonably be expected to confer immunity against trypanosomiasis. Vierthaler & Boselli (1939), for example, found that a concentration of 1.3 mg. of the drug/100 ml. of plasma, as determined by the method developed in our laboratory (Boursnell *et al.* 1939; Dangerfield *et al.* 1938), was sufficient to protect rabbits against infection when injected with *Trypanosoma brucei*. Hawking (1940 *a, b*) concluded that defective accumulation of the drug in the blood was one of the main factors responsible for relapses in three patients treated with suramin for African sleeping sickness; he also found that numerous living trypanosomes reappeared in the blood of one patient even though the plasma contained 4.5 mg. of suramin/100 ml.

It is possible that investigators in tropical countries might wish to make suramin determinations, by our method, on samples of whole blood, but the low results obtained in our experiments indicate that plasma or serum must be used to obtain a true picture of the blood suramin level. Where samples of sera or plasma have to be kept for some considerable time before suramin determinations can be carried out, it is advisable that some antiseptic should be added; gross infection sometimes produces appreciable amounts of amines, or amine precursors, which cause a high result to be obtained in the suramin determination. Of the limited number of antiseptics tested by us, phenol

and merthiolate have proved most satisfactory, and it is recommended that 0.2 ml. of 5% (w/v) phenol or 0.1 ml. of 0.5% (w/v) merthiolate should be added to 2 or 3 ml. of plasma or serum. The use of phenol for this purpose might be specially recommended, since it has been shown (Wormall, 1933) that phenol-treated serum can be used for the diagnostic red cell adhesion test for human trypanosomiasis (Duke & Wallace, 1930; Wallace & Wormall, 1931).

The long retention of injected suramin in the animal body can be attributed to the combination of the drug with the plasma and tissue proteins. In view of the ready combination of the drug with many proteins (for the literature see Boursnell & Wormall, 1939; Dewey & Wormall, 1946; Spinks, 1948), it seems rather surprising that no significant amount of suramin combines with the red blood cells. In our experiments, red blood cells took up no suramin when they were mixed with suramin solutions and left for up to 3 hr., and, in experiments which will be reported later, little suramin has been found in the red cells of the blood of rabbits injected with the drug.

SUMMARY

1. Suramin determinations made on whole blood samples, by the method previously found satisfactory for serum and plasma, show a low recovery of the suramin.

2. This low recovery is due to constituents of the blood cells.

3. Suramin added to whole blood is almost completely confined to the plasma, and none combines with the red cells.

4. The addition of phenol or merthiolate to serum samples is recommended if these samples have to be kept for some time before suramin determinations are made.

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