OBSERVATIONS ON THE INTRAVENOUS TOXICITY AND CHELATING ACTIVITY OF SODIUM STIBOGLUCONATE

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The optical activity, calcium chelating power and intravenous toxicity, but not the leishmanicidal activity, of a sodium stibogluconate solution varies with pH and age. As the optical rotatory power increases, the calcium chelating property decreases, and the solution becomes less toxic. Solutions of sodium stibogluconate injected intravenously into mice produce hypocalcaemia, which may be the cause of death. Production of hypocalcaemia may be a phenomenon common to many substances with chelating powers, a factor to be considered in the design of comparative toxicity tests.

The lack of relation between the analytical and toxicity specifications in the B.P.C. monograph for sodium stibogluconate led to this investigation. Goodwin[1-4] has demonstrated that the acute toxicity of a sodium stibogluconate solution decreased with pH, whereas its leishmanicidal activity was unaffected. We had observed that traces of excess chelating agent increased the intravenous toxicities of metal chelates in mice. We therefore investigated the techniques for assessing the toxicity of sodium stibogluconate, and also certain of its physical and chemical properties with the object of relating the observations.

Experimental Methods

Solutions for toxicity studies, unless otherwise stated, were prepared at room temperature by grinding the solid to a paste with water and then diluting to the required volume. Concentrations were chosen to give the LD50 dose in approximately 0·2 ml. of solution. The doses were injected intravenously into GFF male mice, each of between 16 to 22 g., in groups of 5. A constant rate of injection of 1 ml./30 seconds was used. Mortalities occurring after 1 hour were not included.

Blood samples of 1 ml. in paraffin waxed glass tubes, 3 in. long, 0·375 in. internal diameter, were slowly inverted at 30 second intervals and their clotting times observed. Leishmanicidal activity was tested by Goodwin's[1] method as follows.

Hamsters which had been infected four weeks previously with *L. donovani* were grouped in fives. The animals were anaesthetised and spleen biopsies made. Smears were made from the tissue removed. The following day the animals received a single subcutaneous injection of sodium stibogluconate. A week later a second biopsy was made and spleen smears again made.

The effectiveness of the sample was estimated by the comparison of the number of parasites per 100 spleen cell nuclei before and after treatment. Counts of approximately 1000 spleen cell nuclei were made in each case.

The power of the solution to chelate calcium was determined by the technique of Mehltretter and others[5]. Sodium oxalate, 2 ml. of a 2 per cent solution, was added to a suitable quantity of sodium stibogluconate, 20 to 50 ml. of 10–30 per cent solution. The mixed solution was titrated with calcium acetate, 10 per cent, until a permanent white cloudiness appeared within 2 minutes.

Infra-red absorption spectra of Nujol mulls were recorded on a Perkin-Elmer, Model 21 double-beam spectrophotometer with a sodium chloride prism.

The rotatory powers of 10–30 per cent solutions in 2 dcm. tubes were measured with sodium light. pH values of similar solutions were measured potentiometrically.

RESULTS

The LD50 value calculated by a log probit method for a solution of sodium stibogluconate freshly prepared at room temperature increased with time. The results are shown in Table I. The decrease in toxicity with increasing age of solution was confirmed by the figures in Table II.

TABLE I

REDUCTION OF INTRAVENOUS TOXICITY OF A FRESHLY PREPARED 10 PER CENT SOLUTION OF SODIUM STIBOGLUCONATE WITH TIME

Dose mg./kg.	Age of solution									
	1 min.	15 mins.	1 hr.	2 hrs.	3 hrs.	5½ hrs.	8 hrs.	1 day	2 days	13 days
900	1/5									
1000		0/5								
1100	1/1									
1200		1/5								
1300	1/1	0/4								
1400			1/5							
1500		5/5	3/5	0/5						
1700				0/5						
1850				2/5						
2000			1/1	2/5						
2150				4/5	2/5					
2300					2/5					
2500					3/5	2/5	1/5	0/1		
2600							6/10	1/5		
2700						4/5	7/10	5/10		2/5
2850							5/5	5/11	0/2	
3000						3/3		6/7	2/5	4/5
3200								1/1	3/5	
3400									2/3	
3600									1/1	
Graphical LD50 (common slope)	1000	1300	1500	2000	2300	2500	2600	2800	3100	2800

Entries in the Table: no. of deaths/no. of mice injected. The correlation coefficient for LD50 and age of solution in the period from 5 minutes to 8 hours is $r = 0{\cdot}897$ ($n' = 5$), a value which is improbable by chance. ($0{\cdot}01 > P > 0{\cdot}001$.)

The toxicities of samples of different pH values are given in Table III. They show that toxicity decreases with increase in acidity and also that the solutions became less toxic with time.

Since the toxicity studies suggested that some change occurred in sodium stibogluconate solutions, measurements of optical activities against time, pH value and temperature were made. The results are summarised in Figure 1.

The curves show mutarotation positively to an equilibrium value that increases with fall of pH. The figures for rotation of the freshly prepared solutions are of little value because of the difficulty in deciding zero time. Equilibrium was rapidly reached in warmed solutions.

Infra-red spectra of solids, that in solution had pH values 6·3, 6·0 and 5·5, showed that the carbonyl band of the COONa group (*c.* 1595 cm.$^{-1}$) progressively diminished and the COOH band (*c.* 1670 cm.$^{-1}$) increased.

TABLE II

ACUTE TOXICITY TO MICE OF 15 PER CENT SODIUM STIBOGLUCONATE SOLUTIONS 1 AND 24 HOURS OLD

Dose mg./kg.	Solution 1 hour old							Solution 24 hours old						
	Lab. std.	399/3A	415A/2	416A/2	424/B	425/B	S.W.B.	Lab. std.	399/3A	415A/2	416A/2	424/B	425/B	S.W.B.*
750	0/1	0/3	0/5		0/5	0/1								
900		0/2	3/5		5/10	0/5								
1050		0/5	5/5		6/6	5/5								
1125				3/10								1/10		
1200					2/2	1/1								
1275	0/5	2/5	2/2	7/10								5/10		
1350					2/2	1/1		0/1		0/5	2/10	7/10	0/5	
1500	5/5	5/5	2/2	2/2	2/2	1/1		0/1		9/10	5/10	4/5	6/10	
1650										5/5	8/10	4/5	5/5	
1800									0/1	1/1	1/1	2/2		
1950									4/10		1/1	1/1		
2100								3/10	6/10	1/1	1/1	1/1		
2250	2/2							6/10	6/10					
2400								7/10						
2550							0/1							
2880	2/2							1/1						
3000							0/3							0/3
3840							2/5							0/5
4130							3/5							4/5
4420							4/5							3/3
4800														
Graphical LD50	1390	1310	880	1200	900	975	4280	2400	2210	1580	1650	1420	1610	4230

Entries in the table are deaths out of number of mice injected.
*Solution was at pH 5·4 and was warmed during preparation.
$F = 62{\cdot}95$, $n_1 = 1$, $n_2 = 5$ (P 0·001)
The toxicity at 24 hrs. is less than that at 1 hr.

For a solid precipitated from a solution of pH 3·5 there was a strong COOH band, but none for lactone nor COONa group, although the sodium content of the dry solid was 70 per cent of that present in a material of pH 6·3.

After thus establishing that the toxicities and rotatory powers of freshly prepared sodium stibogluconate solutions change with time and pH, their powers to chelate calcium were investigated. The results summarised in Figure 2 show chelating power to decrease with increasing rotatory power.

TABLE III

DECREASE IN TOXICITY OF SODIUM STIBOGLUCONATE WITH DECREASE IN pH VALUE AND AGE OF SOLUTION

Material* injected	Concentration of injected solution per cent	pH	LD50 mg./kg. at hours after dissolution of solid	
			1 hr.	24 hrs.
399/O	15	6·3	1455	2360
399/P	30	5·4	2775	3375
424 B/O	15	6·57	1130	2360
424 B/P_1	30	5·67	2200	3500
424 B/P_2	30	3·48	4800	5930
415/O	15	6·45	880	1760
415/P	30	5·4	2250	2580
416/O	15	6·35	—	1840
416/P	30	5·6	—	2550
425/O	15	6·35	—	1950
425/P	30	5·6	—	2775
M.R.C. standard	30	5·35	—	4125
	30	6·0	—	2440
	15	6·6	—	1610

* O = Original; P = pH adjusted and reprecipitated. The probability of finding this correlation between pH and toxicity by chance is approximately 1 in 100.

The calcium chelating power of freshly prepared solutions *in vivo* were then assessed. A 15 per cent solution (w/v) of sodium stibogluconate was more toxic than the same solution with the addition of 1 per cent of calcium chloride. The two solutions were compared with the standard preparation by the B.P.C. test for undue toxicity. The solution with no calcium killed 90 per cent of the animals injected, that with calcium only 10 per cent. A 2·2 kg. rabbit injected rapidly with 4·4 ml. of a freshly prepared 30 per cent solution of sodium stibogluconate convulsed violently but quickly recovered. A blood sample, taken from the ear immediately after injection, took 60 minutes to clot; one taken before the injection took 8 minutes.

Another rabbit given four times the dose received by the first, but in doses of 1·1 ml. at 8 minute intervals, showed no distress. Its blood clotting time doubled after the first injection, increased to fourfold with further injections and then began to fall.

No difference in leishmanicidal activity was observed for solutions between pH limits 6·25 and 5·5.

Discussion

Since mutarotation is a manifestation of molecular change, solutions exhibiting this phenomenon may change their biological properties with age. These properties will depend on the relative proportions of epimers present at the time of measurement.

The mutarotation curves show (Fig. 1) that the modification occurring in a freshly prepared solution of sodium stibogluconate is not of the first order[6]. They suggest the possible existence of at least three components in the equilibrium mixture, but insufficient is known about the binding of the antimony in the material to make it possible to determine the mechanism of the changes. However, from the infra-red spectra and sodium contents of the dried material it can be deduced that unlike that in sodium gluconate most of the sodium is not attached to the carboxylic group.

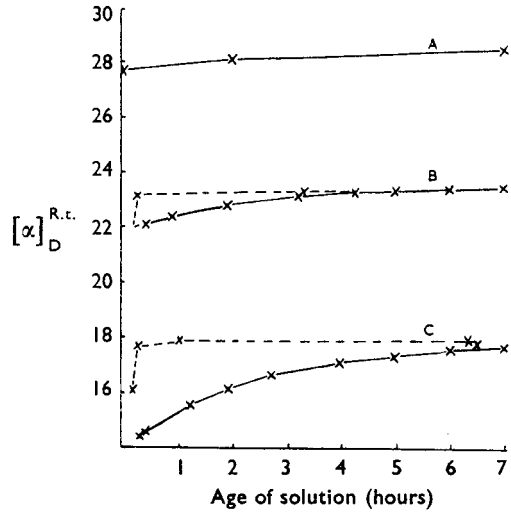

FIG. 1. Changes in molecular rotation of sodium stibogluconate with pH and age of solution: A, at pH 3·5, B, at pH 5·5 and C, at pH 6·4.
x———x Solution prepared and kept at room temperature (R.t.)
x- - - -x Solution prepared at 50° and kept at room temperature (R.t.)

The decreases in acute toxicities of sodium stibogluconate solutions on standing or on acidification (Tables I, II and III) indicate the formation of a less toxic epimer. Moreover, since the calcium chelating powers of the solutions decrease *pari passu* with the toxicities to mammals, it is suggested that the phenomena are related. Therefore the molecular species present in a freshly prepared solution of pH say, 6·5, can chelate blood calcium more strongly than can the epimer produced on standing or by acidifying the solution.

The convulsions and seven-fold increase in blood-clotting time of a rabbit injected with freshly prepared sodium stibogluconate indicate that the blood calcium has been removed by the injected drug. But the rapid recovery by the first rabbit, and the tolerance shown by the second, which received repeated injections, suggest either that the removal of blood calcium is reversible or that calcium reserves may be mobilised rapidly. The rate of injection will affect toxicity if the rate of the reverse action is similar to the rate of chelation.

The mutarotation curves (Fig. 1) show that equilibrium is rapidly reached in warmed solutions, so that the toxicities of solutions warmed or

autoclaved before injection will not decrease with time. (Sample S.W.B. in Table II.)

Maffii and others[7] have shown that the intravenous toxicities of tetracyclines in mice are reduced if saline instead of water is used as the solvent. Nicolle and Weisbuch[8] demonstrated that potassium chloride decreases the rates of mutarotation of certain sugars. These phenomena may be relevant, for a single experiment has shown that potassium chloride modifies the rotation of a sodium stibogluconate solution. The phenomena also indicate that apparently minor changes in experimental conditions may affect biological activity and allow wrong conclusions to be drawn about an injected drug.

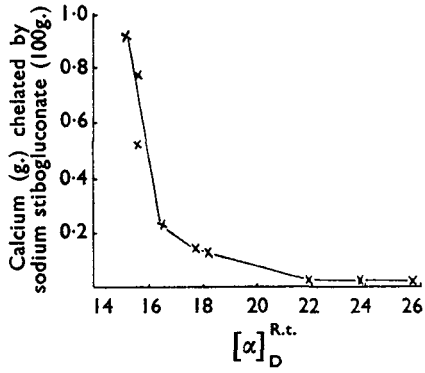

FIG. 2. Calcium chelating activity of sodium stibogluconate solutions plotted against optical activity.

It is therefore suggested that when drugs with chelating properties are compared for toxicity the conditions of the test should be such that the chelating activities are similar. Sodium stibogluconate is an example. At present its solutions are tested for undue toxicity according to the Pharmacopoeial test for neoarsphenamine. This seems hardly logical, as Neoarsphenamine solutions become more toxic with age, while sodium stibogluconate solutions become less so. It is suggested that test solutions should be equilibrated before injection and compared with a standard solution at the same pH and concentration: also the solid material should be dissolved under identical conditions and the solutions injected at the same rate.

REFERENCES

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DISCUSSION

The paper was presented by DR. J. GREEN.

The CHAIRMAN. It might have been better to have stated specifically a desirable method for achieving equilibrium before testing for toxicity and to have given the appropriate pH value. It was not possible from the results quoted to discover why the leishmanicidal activity did not vary with age and pH.

DR. G. E. FOSTER (Dartford) was able to confirm that the addition of calcium reduced toxicity, but not that toxicity decreased on keeping. Official monographs included a test for the presence of trivalent antimony since it was thought that toxicity was due to this, but it now seemed that a stringent test was not necessary. Tests to exclude calcium had been tried but failed probably owing to the chelating effect of the stibogluconate.

DR. J. GREEN replied that the point to which the Chairman had referred was being rectified. Specific recommendations about pH were not made because the matter was about to be discussed by the British Pharmacopoeia Commission. The present B.P.C. monograph stated that tests should be made on an autoclaved solution, when many of the phenomena observed were encountered. There was much smaller change in toxicity at the lower pH and it was at about pH 6 and above where chelating activity was the greatest. As the pH dropped the chelating activity became less and changes in toxicity also became less. All the samples in question were tested for trivalent antimony, but it was not responsible for the toxicity.