THE PREPARATION OF TWO BIS-DIAZO DYES FOR INTRAVENOUS INJECTION

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TRYPAN red and vital red, two closely-related bis-diazo sulphonated dyes, originally prepared by Ehrlich, are used clinically for the estimation of blood volume and as spirochæticides. Trypan red has been shown to protect animals against herpes fibrilis, psittacosis and equine encephalomyelitis[1], and both trypan red and vital red are claimed to have a therapeutic value in the treatment of amyotrophic lateral sclerosis in humans. Apart from a statement by Aird[2], which mentions the use of Seitz filtration, we have found no information on methods of preparing solutions of these dyes for injection purposes. From a practical point of view, the pharmacist prefers a heat sterilisation procedure to methods involving filtration and aseptic distribution. It seemed important therefore to investigate whether these solutions could be autoclaved without destruction of the dyes or altering their toxicity. We have examined solutions of trypan red and vital red before and after autoclaving and compared them with solutions which have been sterilised by passing through a bacteria proof filter.

METHODS

Preliminary experiments indicated that 4 per cent. solutions of both dyes were required for the toxicity tests. Solutions were prepared by dissolving the dyes in water for injection with the aid of heat, followed by filtration through a No. 1 Whatman filter paper and then through a No. 3 sintered glass filter. This process was common to all solutions. Some were then autoclaved without further treatment, some were sterilised by passing through a 5/3 sintered glass filter and some were centrifuged before autoclaving. The concentration of dye in the final solutions was determined colorimetrically in a photoelectric colorimeter previously calibrated with a standard solution of the dye which had been clarified by centrifuging at 3,000 r.p.m. for 30 minutes. The *p*H values of the solutions were also measured before and after autoclaving in order to detect any possible decomposition involving the sulphonyl groups. The trypan red solution had *p*H 6·2 and the vital red solution *p*H 6·5.

The samples were tested for acute toxicity by the intravenous route in mice, the person doing the toxicity tests being unaware of the respective treatments the different solutions had received. Albino mice weighing between 16 and 24 g., which had been deprived of food overnight, were randomised into groups of 10 mice. The groups were injected intravenously at ascending dose levels with the solutions previously warmed to 37° C. and the mortalities observed over 24 hours, from which the LD50's together with estimates of their limits of error were calculated[3].

RESULTS

The colorimetric determinations showed that there was no loss in colour through autoclaving and the *p*H values of the solutions remained constant. The solutions could therefore be safely autoclaved for 30 minutes, at 10 lb. without affecting the dye. Filtration of the solutions through a No. 5/3 sintered glass filter resulted in the loss of dye, due to adsorption on the glass filter bed. Solutions for the toxicity tests were therefore made on the strong side and adjusted to the correct strength before being sterilised. Similarly with centrifuging, a loss of dye occurred due to the removal of undissolved particles if insufficient time was allowed for the dye to go into solution. Solutions which had been passed through a No. 3 filter, but not through a 5/3 filter showed a very fine deposit of solid particles on the bottom of the ampoule after a week's storage. Solutions sterilised by 5/3 filtration and which had been centrifuged did not show this deposit. The deposit was of a dirty orange hue and was insoluble in water, dilute sodium hydroxide or dilute hydrochloric acid and may be presumed not to be undissolved dyestuff.

The results of the toxicity tests are shown in Tables I and II. The solutions of both trypan red and vital red showed no real difference in toxicity after the different treatments so that the solutions for therapeutic use could be sterilised by filtration or by heating in an autoclave.

TABLE I

TOXICITY IN MICE OF 4 PER CENT. TRYPAN RED SOLUTIONS

Solution	LD50 ml./20 g.	Limits of error P = 0·95
1. Autoclaved	0·38	93 to 107 per cent.
2. Centrifuged before autoclaving	0·46	96 to 104 ,,
3. Filtered through 5/3 sintered glass	0·40	95 to 105 ,,
4. Filtered through 5/3 sintered glass before autoclaving	0·42	95 to 105 ,,

TABLE II

TOXICITY IN MICE OF 4 PER CENT. VITAL RED SOLUTIONS

Solution	LD50 ml./20 g.	Limits of error P = 0·95
1. Autoclaved	0·37	89 to 111 per cent.
2. Centrifuged before autoclaving	0·36	96 to 104 ,,
3. Filtered through 5/3 sintered glass	0·38	92 to 108 per cent.

DISCUSSION AND CONCLUSIONS

The solutions used in these investigations were at a concentration of 4 per cent. though they are normally used clinically at a concentration of 1 per cent. The weaker solution is easier to prepare, but we strongly advise filtration through a No. 5/3 sintered glass filter to ensure the absence of subsequent deposition of solid particles. Loss of dye is liable to occur during filtration procedures due to adsorption on the glass and this should be allowed for by making the initial solutions on the strong side and adjusting the final concentration from the result of colorimetric estimations before the final ampouling. For small volumes centrifuging

provides an alternative method of clarification but again losses of dye can occur if complete solution is not ensured. Final sterlisation can be carried out in the sealed ampoules by autoclaving at 10 lb. for 30 minutes without destruction of the dye or an increase in toxicity.

Summary

1. Aqueous solutions of vital red and trypan red can be sterilised by autoclaving for 30 minutes at 10 lb. without a change in colour or an increase in toxicity.
2. Solutions should be clarified by filtration through a No. 5/3 sintered glass filter or by centrifuging.
3. Loss of dye can occur through incomplete solution or by adsorption to the glass filter.
4. The concentration of the dye in the final solution should be determined colorimetrically and suitably adjusted prior to autoclaving in the final containers.

References

1. Wilson-Hurst, Peters and Melvin, *Brit. J. Pharmacol.*, 1952, **7**, 455.
2. Aird, *Arch. Neurol and Psych.*, 1948, **59**, 779.
3. Finney, *Probit Analysis*, 2nd Ed., Cambridge University Press, 1952.

Correction.

THE FAILURE OF BROMAZINE HYDROCHLORIDE TO AFFECT THE OUTPUT AND COMPOSITION OF RESPIRATORY TRACT FLUID

By Eldon M. Boyd and R. Noreen Hicks

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Fig. 1, p. 47. Values for chloride content of respiratory tract fluid shown in the Figure must be multiplied by 0·176 to obtain the correct concentrations.