Testing of acid-citrate-dextrose anticoagulant solution for absence of pyrogens

Acid-citrate-dextrose (A.C.D.) anticoagulant solution is an ingredient of Whole Human Blood B.P. and, as such, may be administered intravenously in large quantities during blood transfusion. This suggests that a test for pyrogenicity would be a worthwhile precaution.

The British Pharmacopoeia does not require that this solution be tested for pyrogens, while the United States Pharmacopoeia describes a dilution method of testing and the Swiss Pharmacopoeia refers to the use of calcium gluconate to eliminate the risk of tetany in rabbits. A.C.D. solution has a pH of 5 and, as well as the discomfort this causes, tetany-like spasms have been noticed in rabbits after intravenous injection of 10 ml/kg.

To overcome these effects the use of a basic calcium salt that would neutralize the acidity and supply calcium ions has been investigated. Calcium carbonate was chosen and when added to A.C.D. solution (0·5 g/120 ml) containing sodium acid citrate (2 g) the salt raised the pH to about 6·5 and on intravenous injection the solution no longer produced signs of discomfort or tetany at 10 ml/kg in the rabbit.

The salt was sterilized by heating 0·5 g (B.P. grade) quantities, weighed into Universal bottles capped with aluminium foil, and heated at 250° for 1 h in a hot-air oven. This treatment was also expected to destroy any pyrogen. Immediately before making a pyrogen test, 0·5 g of the sterilized calcium carbonate was added to each 120 ml quantity of A.C.D. solution and the containers were placed in a water-bath at 40°. The slight excess of calcium salt settled out quickly leaving a clear supernatant available for injection.

To confirm that this was a reliable test of pyrogens in A.C.D. solution, 0·036 μg of Organon "E Pyrogen" in solution was added to 360 ml of a sterile anticoagulant solution which had been prepared using full precautions against the development of pyrogens. Sterile calcium carbonate (1·5 g) was then added and a pyrogen test was made on the supernatant fluid. Three rabbits were each given 10 ml/kg and this produced a total rise in temperature of 2·4° which was within the range expected from this pyrogen in doses of 0·001 μg/kg rabbit.

The same amount of the "E Pyrogen" solution was added to 1·5 g of calcium carbonate. This was immediately freeze-dried to eliminate the water and the dry calcium carbonate with pyrogen was heated in a hot-air oven at 250° for 1 h. The heated material was then added to 360 ml of the above batch of sterile A.C.D. anticoagulant solution and a pyrogen test was made as before. The total rise in temperature for three rabbits was 0·4° which indicated that the pyrogen had been destroyed.

Thus a satisfactory pyrogen test can be made on A.C.D. anticoagulant solution, using 10 ml/kg, if 0·5 g of calcium carbonate powder, previously heated at 250° for 1 h is added for each 2 g of sodium acid citrate in the solution. The addition of heated calcium carbonate does not add pyrogen nor does it inhibit the expected response to a known dose of a standard pyrogen. The test has the advantage that no dilution is required as in the U.S.P. method and that unlike calcium gluconate, calcium carbonate can be rendered pyrogen-free by heating.

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