WATER FOR INJECTION BY ION-EXCHANGE

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THE purification of water by means of ion-exchange resins has been recognised as satisfactory for general purposes by a number of Pharmacopoeias (B.P., 1958; U.S.P., 1960; see also Saunders, 1954). The disadvantage of the method is that the freshly purified water may be contaminated by bacteria and consequently cannot be used for preparing solutions for injection (Whittet, 1961).

The object of this work has been to study the conditions required to give a reliable source of apyrogenic deionised water which can be used for all purposes where bacteriologically clean water is required. The advantage of the ion-exchange purification is that large quantities can be prepared rapidly as required, the small plant used in these experiments could supply 40 litres/hr.

Bacterial Counts on Water Samples

These were performed by spreading five 0·5 ml. water samples over the surface of over-dried tryptone soya agar plates. The more heavily contaminated samples were diluted 100 times with sterile distilled water before counting. For the less heavily contaminated waters, five samples each of 20 ml. were inoculated into 20 ml. of double strength liquid culture medium and the results read as growth or no growth. This latter procedure is a test several times more stringent than the British Pharmacopoeia test for sterility.

All samples with appreciable contamination, showed a mixed flora with Gram-negative rods and micrococci predominating; many contained Gram-positive sporing rods. The sample of London tap water used as influent in the later experiments came from a roof storage tank and was contaminated with a Gram-positive sporing organism which provided an efficient test for the subsequent experiments since it appeared to be resistant to ultra-violet and chemical sterilisation and readily grew in purified water on storage at room temperature for several days.

The results showed that stored distilled water often gave counts of above 10^4 organisms/ml.

Ion-Exchange Purification of Water

To prepare purified water with negligible bacterial contamination by ion-exchange, the de-ionising equipment must be completely freed from micro-organisms. It is then preferable to have a sterile feed water supply otherwise colonies begin to grow in the resin column and eventually appear in the effluent.

The de-ioniser used in this work was a modified Elgastat B112 UV in which the influent water passed first through the outer jacket of a double

jacketted ultra-violet lamp, then to a polythene bottle containing 8 litres of mixed ion-exchange resins and on through the inner jacket of the lamp and a conductivity cell to the effluent pipe, the tip of this pipe was immersed in a 1 per cent formaldehyde solution when not in use. The thickness of each of the water layers surrounding the lamp was about 1 mm.

Sterilisation

At reasonable flow rates the ultra-violet lamp alone could not be relied upon to give a sterile effluent. Initial chemical sterilisation of the whole apparatus was achieved by filling it with 1 per cent formaldehyde solution and leaving overnight. Subsequent washing with about 60 litres of water was necessary to remove the formaldehyde.

With London tap water feed, purified water with a count of less than one organism per 20 ml. and a negligible pyrogen reaction was obtained. When the water was stored for a week, however, an appreciable growth of micro-organisms occurred due probably to the presence of spores in the feed water which had survived passage through the ultra-violet lamp.

To overcome this difficulty, the feed water was treated with an ionic sterilising agent—acidified sodium hypochlorite—which is removed by the resins. The influent tap water was run into a 20 litre polythene aspirator and sodium hypochlorite solution acidified with hydrochloric acid was added to give a concentration of about 80 p.p.m. of free chlorine. The chlorinated water stood overnight before use.

The resulting purified water collected one week after the formaldehyde sterilisation had a count of less than one organism per 100 ml. and a negligible pyrogen reaction. One month after the formaldehyde sterilisation the pyrogen reaction of the purified water was still well within the Pharmacopoeia limits (average temperature rise of 0·23°) but it gave a small count of about five organisms/ml. This effect must have been due to some highly resistant spores in the feed water and could be avoided by using a higher concentration of chlorine.

Conclusions

The total capacity of the ion-exchange cartridge for London tap water is about 250 litres; of this about 40 per cent had to be invested in washing the apparatus free from formaldehyde and in removing the sodium hypochlorite.

The technique described could be used with an ion-exchange plant in which regeneration of the resins is carried out. After regeneration the whole plant should be sterilised with formaldehyde and then washed through with chlorinated water, subsequently all water admitted to the apparatus should be chlorinated and ultra-violet irradiation of both influent and effluent water is recommended.

The following arrangements should give a reliable source of apyrogenic, purified water with a negligible bacterial count.

1. A column of mixed ion-exchange resins is supplied with chlorinated feed water from a tank of volume equal to the capacity of the resins for

the raw water used. Both influent and effluent water are irradiated by ultra-violet light.

2. The whole ion-exchange apparatus is sterilised either by filling it with 1 per cent formaldehyde solution or by drawing moist formaldehyde vapour through it, the latter method reduces the volume of wash water required.

3. When not in use the outlet pipe of the apparatus is kept immersed in formaldehyde solution to prevent entry of micro-organisms.

The apparatus should be sterilised at weekly intervals and the resin bed volume should be such that the volume of purified water required per week is three-fifths of the capacity of the de-ioniser, for the feed water used.

The authors consider that a modification might now be made to the monograph in the British Pharmacopoeia on Water for Injection, so as to permit its preparation by ion-exchange under suitably controlled conditions.

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Discussion

The paper was presented by Professor Cook. The following points were made in the discussion.

The authors were not satisfied that the formaldehyde was killing all the spores in the column. Chlorination was the most efficient method of sterilisation. Other processes with chemicals were unsuitable. Irradiation with ultra-violet light was only an aseptic precaution; there was a risk of ozonolysis or peroxide formation with ultra-violet light, and this might be significant in the preparation of injections of phenothiazine compounds having a free sulphur atom because the sulphoxides formed would inactivate the compounds. Water was more likely to be contaminated by colonies or organisms rather than by a single organism, and for that reason a filtration method of sterilisation would probably be more reliable than ultra-violet light. Multi-layer filters with built-in heaters were available and these could be sterilised *in situ*. On one occasion, gamma-radiation had been used successfully to sterilise an ion-exchange column. The conditions in a mixed-bed resin, which had been reported to give pyrogen-free water, could differ from those in a cartridge type of apparatus such as had been used. The frequency of flow through the column was of

importance; if the flow was stopped for 24 hr. pyrogenic water was produced. The water from the apparatus as well as being pyrogen-free was also satisfactory both physically and chemically. Tests for pyrogenicity were made on the feed water, the first effluent, the last effluent, and the first effluent after storage.