TESTS WITH A PLANT FOR STERILISING, DEMINERALISING AND DEPYROGENISING WATER

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The German Pharmacopoeia 6, 3rd Edition, Supplement 1959, lays down that solutions for injection must be free from bacteria and pyrogens.

A necessary prerequisite for obtaining germ-free and pyrogen-free injection preparations is the removal of bacteria and pyrogenic substances from the water which is used for making and diluting the solutions as well as for rinsing ampoules and apparatus.

Today the achievement of sterility with the help of various filters is no longer a problem. Likewise the requirement of freedom from pyrogens for all infusion and injection solutions is today relatively easy to fulfil. The only requirement of pharmacopoeias both at home and abroad which gives difficulties to manufacturers is that only distilled water should be used for preparing these solutions, particularly when it is a question of preparing fairly large quantities of pyrogen-free water. Even though quite a number of substances which differ very considerably from the chemical point of view are capable of inducing fever, nevertheless when it comes to their importance as a contamination of injection and infusion solutions, as well as their pyrogenic action, the first place is occupied by bacterial Lipopolysaccharides.

The purpose of the researches reported below is to attempt to find out whether it is not also possible to depyrogenise water which has been prepared by ion exchange, provided suitable means are employed.

Although as long ago as in 1936 Co Tui and co-workers found that pyrogenic factors are retained by Seitz sheets, it was nevertheless Wilke and Voss who were the first to investigate systematically the retention of pyrogens by different materials which can effect sterilising filtration. They found that bacterial Lipopolysaccharides are removed from water by Seitz filter sheets, but not by other germ-proof filter media such as Berkefeld filter candles, membrane filters or Schott frits G5.

Since bacterial polysaccharides are not eliminated by filters whose effect is based in the main on a sieve effect, Wilke and Voss concluded that pyrogens are absorbed by the Seitz sheets. The binding capacity of Seitz sheets for pyrogen dissolved in water was found to be very great. Thus Wilke and Voss passed the pyrogens formed by 5×10^{12} Coli bacteria through 1000 sq. cm. filter area without any breakthrough of the feverproducing substance taking place. In practice filter sheets will certainly not be subjected to pyrogens in such a quantity.

The high adsorptivity of Seitz sheets for bacterial Lipopolysaccharides was confirmed by Brock, Geks, and Lorenz, and also by Whittet and Fischer.

The results of these experiments led one to think of producing pyrogen-free water from distillation or demineralisation plants by installing a Seitz filter in addition.

Since distillation plant has a very adverse effect on the finished costs because of the high cost of purchase of the plant and high operating costs, Messrs. Braun, Melsungen, Seitz of Kreuznach, and Berkefeld of Celle, suggested to us that germ-free demineralised and pyrogen-free water could be obtained in a single operation from water containing pyrogens by arranging in series germ-proof filters, demineralisation plant and Seitz sheets. Demineralisation installations are considerably more economical than distillation plant; moreover, ion exchange installations, when suitable resins are used, supply water which is very much purer chemically than even can be obtained by repeated distillations with high-grade stainless steel apparatus.

Harrison, Myers, and Herr as well as Whittet had already described that water containing pyrogens no longer possesses any pyrogenic properties, or only little, after it has been demineralised. It was therefore to be expected that in our experiments too pyrogens would be retained by cation and anion exchangers.

Experimental arrangement

The plant was arranged in such a way (see illustration) that tap water could be charged with certain quantities of pyrogens and was then passed through a Berkefeld filter vessel with three filter candles for protecting the plant from contamination with bacteria, before it passed through the Berkefeld demineralisation apparatus, type M II V and through a Seitz filter, type "Pilot-Z".

The sampling points for testing the water were located before the introduction of the tap water into the research plant, after the point of introduction of the pyrogens, after the Berkefeld filter vessel, after the preliminary filter and after the mixed bed of the Berkefeld demineralisation apparatus and after Seitz filter.

The charging of the demineralisation apparatus, first of all with a standard packing of Levatit resins*, later on with Amberlites**, as cation and anion exchangers, depended on the nature of the tap water employed.

The analysis of the tap water showed the following:

pH	7.05
Total hardness	6.72 German degrees
Carbonate hardness	6.72 German degrees
Non-carbonate hardness	–
Free CO_2	5.0 mg/litre
Aggr. CO_2	less than 0.05 mg/litre
Lime (CaO)	54.5 mg/litre
Magnesia (MgO)	22.0 mg/litre
Iron, unfiltered	below 0.05 mg/litre
Iron, filtered	below 0.05 mg/litre
Manganese	Nil
$KMnO_4$ consumption, unfiltered	4.5 mg/litre
$KMnO_4$ consumption, filtered	4.5 mg/litre
Chlorides	10.5 mg/litre
Sulphates	21.8 mg/litre
Nitrates	6.0 mg/litre
Nitrites	Nil
Ammonia	Nil
Silicia	7.5 mg/litre

Before the commencement of the experiment the Berkefeld filter vessel was treated with sterilising salt in accordance with the instructions, the demineralisation apparatus was regenerated and the Seitz filter was sterilised in accordance with instructions after inserting 9 Seitz EkS sheets (20 x 20 cm., corresponding to a filter area of 0.29 sq. m.). After the Seitz filter there was inserted a Seitz fibre catcher.

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* In the preliminary filter: strongly acid cation exchanger on a styrene resin basis. In the mixed bed: mixture of the cation exchanger used in the preliminary filter with a strongly basic anion exchanger of type I on a styrene resin basis.

** In the preliminary filter: Amberlite JR 120. In the mixed bed: Amberlite JR 120 and JRA 410.

After the regeneration of the Berkefeld demineralisation apparatus, the tap water which passed through had a conductiv ty of 0.01 µS per cm^1. The demineralisation apparatus consisted of a preliminary filter and a mixed bed. The demineralisation output for our tap water was in the case of the prefilter between two regenerations 5,500 litres x 11° d, that of the mixed bed 2,500 litres x 11° d for an hourly output of 50 to 250 litres. The Seitz filter with a filter area of 0.29 sq. m. required under normal load a maximum speed of flow of 165 litres per hour corresponding to 500 litres/m^2 hour.

In order to see whether pyrogenic substances are dissolved out of the apparatus, particularly out of the Seitz filter, by water, before starting the dosed feed of pyrogens we allowed 250 litres of water to pass through the plant, we collected it at the points described and tested its content of pyrogenes by means of the rabbit test. The first filtrate fractions and also samples of water taken later at intervals contained no pyrogenic substances.

The charging of the tap water with pyrogens was carried out by means of a dosing pump. The dosing was selected in such a way that about two threshold doses for rabbits weighing 3 kilograms were given to 5.0 mls of tap water. Also before the commencement of the experiment such a quantity of pyrogens was fed to the preliminary filter and the mixed bed of the Berkefeld demineralisation apparatus, each of which contain approximately 20 litres of water, that 5.0 mls contained 2 threshold doses for rabbits weighing 3 kilograms. (A threshold dose is to be understood to mean that quantity of pyrogens which brings about a temperature rise of approximately 1.5° C in rabbit weighing 3 kilograms).

The content of pyrogens in the samples of water and the calculation of the temperature rise were carried out in accordance with the instructions shown in the commentary to the German Pharmacopoeia 6, 3rd Edition, Supplement 1959, on pages 50 to 58.

The pyrogenic substance we used was Lipopolysaccharides which we produced from a strain of Coli bacteria according to the process described by W e s t p h a l and co-workers and a bacterial Lipopolysaccharide which Professor W e s t p h a l kindly made available to us. *

We obtained a temperature rise of approximately 1.5² C in rabbits weighing 3 kilograms by the intravenous injection of 0.01 γ of the pyrogen dissolved in 5.0 mls of pyrogen free saline.

During the experiment approximaley 120 to 150 litres of water passed through the plant per hour. At intervals of about 2½ hours samples of water were collected at the points mentioned.

Result

We confirmed that the ceramic diaphragm of the Berkefeld candles does not retain the pyrogen which we used. The water which was collected after the Berkefeld demineralisation apparatus already contained a small quantity of pyrogens after the passage of 24,400 threshold doses (approximately 61.0 litres of water), but only contained a large quantity of pyrogens after the passage of 340,000 threshold doses (= 850 litres of water). By the saturation of the ion exchange capacity 1,080,000 threshold doses (= 2,700 litres of water) had passed through the plant, that is to say slightly more than the calculated demineralisation output between two regenerations. The water collected after the Seitz filter was still free from pyrogens. In order to determine how much pyrogenic substance the Seitz filter was capable of retaining, the experiment was continued in the same manner without the prior regeneration of the demineralisation apparatus. In order to save animals, the water was tested for its pyrogen content after the passage of a further approximately 880,000 threshold doses (approximately 2,200 litres of water). The samples of water collected in between were stored in sterile testtubes at — 40° C. Only after 16,720,000 threshold doses (= 42,000 litres of water) had been passed through did the water after the Seitz filter contain pyrogens.

In a further experiment we replaced the Levatite by Amberlite.

When we did this the construction of the experimental installation was left unchanged, as well as the nature of the tap water. The latter was continuously charged with pyrogens so that approximately two threshold doses were contained in 5.0 mls of water flowing through.

Result

The salt breakthrough took place after 2900 litres of our tap water had passed through.

The samples of water taken after the demineralisation apparatus and after the Seitz filter after 3000 litres of water (approximately 1,200,000 threshold doses) had passed through were found to be free from pyrogens.

From this experiment it can be seen that the individual makes or even types of resin show a different adsorption behaviour in regard to pyrogens.

It will not be possible until further tests have been made to say whether with the different types of resins a desorption of the pyrogens takes place during the regeneration and whether the further working cycles give the same results both qualitatively and quantitatively or whether saturation phenomena occur as a result of the character of individual resins, or else whether the absorption is wholly or partly irreversible and results in the known "fouling" of the resin.

Captions to illustration, as numbered in pencil on the original:

1. Pyrogen solution
2. Dosing pump
3. Tap water
4. Candle filter
5. Demineralisation apparatus
6. Preliminary filter
7. Mixed bed
8. Seitz filter
9. Fibre trap

General Summary

The idea was to find out whether by arranging in series a Berkefeld filter vessel, a Berkefeld demineralisation apparatus, and a Seitz filter type "Pilot-Z" it was possible to make water of varying composition not only free from bacteria and free from salts but also free from pyrogens. In order to do this first of all such a quantity of a pyrogenic substance was introduced into tap water that 5.0

* We would like to express our grateful thanks to Professor W e s t p h a l for handing to us the purified bacterial Lipopolysaccharide.

mls of the water contained quantities of pyrogens which represented approximately 2 threshold doses for rabbits weighing 3 kilograms.

The Berkefeld filter vessel arranged in front of the plant was there in order to protect the subsequent apparatus from mechanical impurities and to inhibit the floating in of bacteria. As was expected, the ceramic diaphragm of the Berkefeld candles did not retain pyrogens.

The demineralisation apparatus, charged with Levatites, was capable of demineralising approximately 2,400 litres of our tap water. After the passage of 61 litres of tap water containing dissolved in it approximately 24,400 threshold doses, we were able to detect a slight presence of pyrogens in the sample of water taken after the demineralisation apparatus. After 850 litres of tap water (approximately 340,000 threshold doses) had passed through, the sample of water taken after the demineralisation apparatus had a high content of pyrogens, that is to say the quantity of pyrogens added to the tap water passed undiminished through the resins.

The samples of water taken after the Seitz filter "Pilot-Z" were free from pyrogens until 34,600 litres of tap water had passed through, corresponding to about 138.5 mg of pyrogenic substances or 13,850,000 threshold doses. Only after a further about 9,500 litres approximately of water containing pyrogens had passed through was it possible to detect pyrogens in the filtrate. From this one can calculate an absorption capacity of 550mg of pyrogenic material on 1 sq. m. of Seitz-EkS sheets. This figure coincides in order of magnitude with the one found by Wilke and Voss who were able to observe no breakthrough with a charge of 215 mg of Coli pyrogen per sq. m. of EkS sheets. This means to say that sterilisation filters with 1 sq. m. EkS sheets can depyrogenise more than 100 cu. m. of tap water if this water contains what is for normal conditions the high concentration of 2 threshold doses per 5 mls. These throughput quantities are considerably greater than the degree of utilisation which is generally usual for sterilisation filter sheets.

When Amberlites were used in the demineralisation apparatus larger quantities of pyrogens were retained. The experiment was broken off after charging with 1,200,000 threshold doses, corresponding to about 12 mg of pyrogenic substances.

From the above experiments it can be seen that when suitable exchanger resins are used in conjunction with filter sheets which retain pyrogens it is quite possible to produce pyrogen-free water. Obviously this means that it is essential to comply with the instructions given for operation and maintenance.

Our experiments also provided proof that the binding capacity of complete demineralisation installations for pyrogens also depends on the quality of the resins and evidently is not related to the ion exchange capacity of these resins.

It is not possible to give a final judgment regarding the capacity of synthetic resin ion exchangers because in order to do this it will first of all be necessary to obtain further information by means of experiments with more types of resin regarding the absorption capacity, the regeneration behaviour and particularly as regards the question as to whether pyrogens are dissolved away from synthetic resin particles during regeneration, and information, regarding entrainment and possible "fouling".

However, in view of the high adsorption capacity of Seitz sterilisation sheets which we have been able to prove, there is already available for practical use a sufficiently sure process for the production of a perfect water in the combination of complete demineralisers and Seitz filters.

Summary

(A translation of the summary is already provided on the original leaflet)

Literature

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