THE ACTION OF ION EXCHANGE RESINS ON PYROGENS

PART I. EFFECT ON THE PYROGENICITY OF TAP WATER

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WATER purified by ion exchange materials is now included in the British and United States Pharmacopœias under the title of "Purified Water". The monographs in both these publications state that purified water is unsuitable for the preparation of injections, although several workers have reported that treatment with ion exchange resins can reduce the pyrogenicity of solutions.

Harrison, Myers and Herr,[1] in one of the earliest papers on demineralised water for pharmaceutical purposes, carried out the U.S.P. test for pyrogens on samples of New Brunswick City water before and after treatment with ion exchange resins.

The untreated water gave a mean rise in temperature in five rabbits of 0·86° F. (0·48° C.), whereas the treated sample gave practically no rise in temperature, 0·12° F. (0·07° C.), in five rabbits. The authors stated that it could not be presumed that water once contaminated with pyrogenic material would be purified by treatment with ion exchange resins, but that the treatment did not result in water acquiring pyrogenicity. They did not, at that time, recommend the use of demineralised water for preparing parenteral solutions but considered it possible that future investigation might show it to be a practical application of the process.

Smith and Pennell[2] used an ion exchange agent "Decalso" for reducing the pyrogenicity of concentrated protein solutions. They showed that it had appreciable effect by itself and was very effective in conjunction with Seitz filter pads. "Decalso" is a synthetic sodium aluminium silicate, in the form of 60–90 mesh granules, and acts as a cation exchange material. It is not so stable as the resins and can be used only in neutral solution.

Reid and Jones[3] used ion exchange resins in the production of human blood plasma protein fractions. They reported that a pyrogenic solution (*Aerobacter cloacae* pyrogen), which gave a strong reaction when diluted 100 times and a weak reaction when diluted 10,000 times, after being passed through a 107 cm. column of mixed anion and cation exchange resins and then through an 84 cm. column of cation exchange resin gave no detectable response. These workers did not state the types of resins used. Dr. R. Heiz, in a personal communication, has informed me that the ion exchange resins Amberlite IR120 (cation exchanger) and Amberlite IR410 (anion exchanger) used as mixed columns will remove from solutions quantities of a lipopolysaccharide "Pyrexal" (Wander), a pyrogen from *Salmonella abortus equi.*

Fischer[4] described two plants intended for the production of bacteria-free and pyrogen-free water using the same Amberlite resins together with Seitz filtration. He tested three samples of water after they had been passed through a Seitz filter, sealed into ampoules and sterilised. All three samples were apyrogenic.

Unfortunately he did not record whether he tested samples before Seitz filtration, so it is impossible to tell whether pyrogenicity was removed by the resins or by the filter pads. Co Tui[5] has shown that these pads can absorb appreciable quantities of pyrogens. Wilke[6] has described a similar ion exchange-filtration apparatus.

Hatta and others[7], working with pyrogens from several organisms, including moulds, stated that the pyrogenic substances were completely eliminated by passing solutions through beds of ion exchange resins. The results given in their paper, however, do not show complete removal of pyrogenicity in every case.

The papers quoted above give a considerable amount of evidence that the pyrogenicity of solutions can be markedly reduced or even abolished by means of ion exchange resins. On the other hand, Suzuki[8] reported that treatment with both cation and anion exchange resins was practically ineffective for depyrogenising a glucose injection contaminated with a pyrogen of fungal origin.

In view of these conflicting reports, experiments were carried out to determine whether demineralised water is apyrogenic and, if so, to ascertain which resin is responsible for removal of the pyrogen.

Experimental

The resins used were Zeocarb 225 (cation exchanger) and Deacidite FF (anion exchanger). They were placed in glass tubes resembling percolators with stopcocks at the lower end. Glass wool was used to prevent the resins from entering the stopcock.

The size of the Zeocarb 225 bed was about 30 cm. long by 7 cm. diameter and that of the anion bed about 60 cm. long by 7 cm. diameter, these being the proportions of the resins necessary for demineralisation. These sizes were chosen to give columns of about the same size as in some of the commercially available plants.

The source of pyrogen was London tap water which has always been found to be highly pyrogenic[9]. Before use the columns were thoroughly washed with several bed volumes of freshly distilled water from a still known to give pyrogen-free water.

The rabbits used were New Zealand whites of 2·0 to 3·0 kg. They were known from previous experiments to give a marked response to injections of pyrogens and had not shown signs of tolerance.

All the water samples were adjusted to isotonicity with sterile pyrogen-free concentrated salt solution. All the injections were made into the ear veins and the dose given was in every case 10 ml./kg. body weight.

During the experiments the rabbits were kept in the usual type of restraining box and their rectal temperatures were measured by means

of thermistor electrical thermometers. Their temperatures were recorded for at least one hour before and three hours after injection.

Effect of Uncharged Resins

To determine whether the resins were capable of removing pyrogens by simple absorption or adsorption, tap water was passed through uncharged columns.

The effluents were injected immediately after collection into four groups of three rabbits.

The maximum rise in temperature for each animal is shown in Table I and the mean temperatures for all the animals before and after injection are plotted in Figure 1.

TABLE I

EFFECT OF TREATING PYROGENIC TAP WATER WITH ION EXCHANGE RESINS

Effect of untreated tap water (control)		Uncharged Zeocarb treated water		Uncharged Deacidite treated water		Zeocarb–Deacidite treated water	
Rabbit	Response °C.	Rabbit	Response °C.	Rabbit	Response °C.	Rabbit	Response °C.
468	1·30	139	1·30	140	0·55	148	−0·05
573	1·40	141	1·52	131	0·78	400	0·13
466	1·00	143	1·60	133	0·83	436	0·10
573	1·63	140	1·65	123	1·75	122	0·03
444	1·50	139	1·73	124	1·75	143	0·18
466	0·95	141	1·90	131	2·00	426	0·18
483	1·25	148	1·68	573	0·40	467	0·05
150	0·80	400	0·33	837	0·60	482	0·00
145	1·63	430	1·53	978	0·28	444	−0·02
145	1·68	466	0·30	426	0·65	837	0·60
143	1·83	439	2·20	148	0·93	141	−0·25
147	1·43	436	1·56	444	1·00	145	0·13
Total ..	15·52	Total ..	17·30	Total ..	11·52	Total ..	1·08
Mean ..	1·29	Mean ..	1·44	Mean ..	0·96	Mean ..	0·09

Dose in all cases 10 ml./kg. body weight, injected intravenously.

The maximum total rise in temperature in twelve rabbits must not exceed 6·6° C. for water to pass the B.P. test for absence of pyrogens.

Effect of Charged Resins

The columns were then charged and tap water was passed through the cation and anion exchange columns. The resulting deionised water was injected, immediately after collection, into four groups of three rabbits. The rate of flow through the columns was standardised at about the optimum for columns of this size as suggested by the manufacturers of the resins.

The four groups of three tests were carried out on different days and the resins were not regenerated between tests. The columns were kept closed between tests and, before use on each occasion, were washed with several bed volumes of freshly distilled water. At least two bed volumes of the water to be tested were passed through the columns before taking the test samples.

As controls, samples of tap water from the same source were injected in the same dose into an equal number of rabbits.

The results of all these tests are shown in Table I and Figure 1.

Effect of Individual Resin Columns

As tap water deionised by passage through these two ion exchange columns appeared to be completely freed from pyrogens, experiments were carried out to ascertain which of the resins is responsible for this effect.

Tap water was passed separately through the same cation and anion exchange columns used in the previous experiments and the effluent from each was injected into different groups of three rabbits.

The effluent from the cation exchange column had a pH of about 2·5 and was highly pyrogenic, whilst that from the anion exchange column had a pH of about 10·5 and was apyrogenic. To make sure that the physical effect of the larger bed of the anion exchange column was not responsible for the difference in action, a column of the cation exchanger of the same size as the anion exchange column was tested and was still found to be ineffective in reducing pyrogenicity. In later work anion exchange columns as small as 15 cm. long and 3 cm. in diameter were found to be capable of removing large quantities of pyrogens.

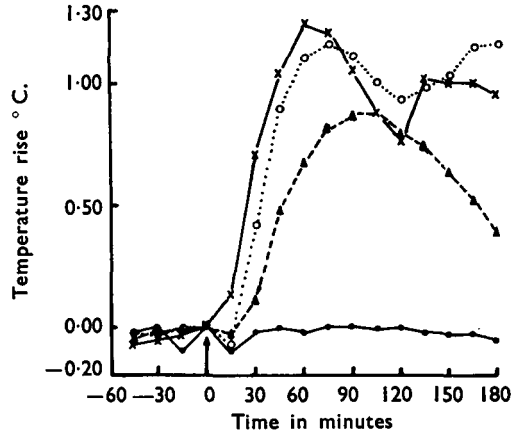

FIG. 1. Effect of ion exchange on the pyrogenicity of water.
●——● Tap water passed through charged columns.
×——× Untreated tap water.
○....○ Tap water passed through uncharged Zeocarb (cationic) column.
▲- - -▲ Tap water passed through uncharged Deacidite (anionic) column.
All points are the mean of 12 tests.

To confirm that acidity of the cation exchange effluent was not the cause of the rise in temperature, physiological saline, known to be pyrogen-free, was adjusted with autoclaved 0·1 N hydrochloric acid to pH 2·5 and was injected into an equal number of rabbits as a control. The results of all these tests are shown in Table II and Figure 2.

To show that the alkalinity of the water from the anion exchange column was not causing destruction of the pyrogenicity, tap water was adjusted to pH 10·5 with autoclaved 0·1 N sodium hydroxide solution and was injected into an equal number of rabbits.

To ensure that the temperature rise caused by the intravenous injection of tap water is not due to living or dead organisms, one sample was steamed for 30 minutes to destroy living vegetative organisms and another was sterilised by passing through a bacteria-proof sintered glass funnel. Both of these samples were found to be highly pyrogenic on injection into rabbits. The sterility of the filtered sample was confirmed by the usual tests.

TABLE II

EFFECT OF INDIVIDUAL CHARGED ION EXCHANGE RESINS ON PYROGENIC TAP WATER

Zeocarb treated water		Deacidite treated water		Acidified apyrogenic saline pH 2·5 (negative control)		Alkalised tap water pH 10·5 (positive control)	
Rabbit	Response °C.	Rabbit	Response °C.	Rabbit	Response °C.	Rabbit	Response °C.
409	1·18	439	0·08	439	0·05	400	0·60
426	1·65	467	0·10	575	0·18	468	1·28
482	1·53	495	0·80	978	0·15	837	1·43
400	0·68	148	0·20	468	0·00	426	1·55
436	1·60	467	0·18	483	1·03	466	0·93
495	1·03	483	0·23	837	0·48	444	1·10
148	1·13	409	0·35	145	0·38	439	1·40
436	1·43	482	0·23	445	0·28	573	1·08
467	1·43	468	0·43	466	0·13	978	1·08
122	1·13	483	0·75	148	0·08	409	1·73
139	1·40	978	0·20	400	0·20	443	0·45
469	1·65	141	−0·15	436	0·10	148	0·98
Total ..	15·84	Total ..	3·40	Total ..	3·06	Total ..	13·61
Mean ..	1·32	Mean ..	0·28	Mean ..	0·25	Mean ..	1·14

Dose in all cases 10 ml./kg. body weight, injected intravenously.

The maximum total rise in temperature in twelve rabbits must not exceed 6·6° C. for water to pass the B.P. test for absence of pyrogens.

A column of 15 cm. length and 3 cm. diameter was made from a sample of Deacidite FF which had been in continuous use for two-and-a-half years. This was also found to give complete removal of pyrogenicity.

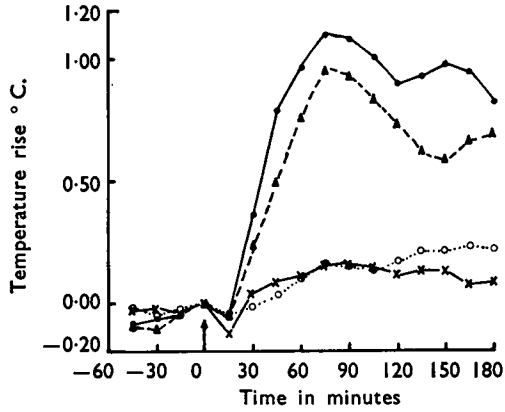

FIG. 2. Effect of individual ion exchange resins on the pyrogenicity of tap water.

●——● Tap water passed through charged Zeocarb (cationic) column.

×——× Tap water passed through charged Deacidite (anionic) column.

○....○ Apyrogenic water adjusted to pH 2·5 (Negative control).

▲- - -▲ Tap water adjusted to pH 10·5.

RESULTS

The results in Tables I and II and Figures 1 and 2 show that untreated tap water and tap water adjusted to about pH 10·5 are highly pyrogenic. Treatment with the uncharged ion exchange columns does not remove this pyrogenicity, showing that pyrogens cannot be removed by adsorption on the resins.

On the other hand, water deionised by passing through both the columns or treated by the anion exchange resin easily passes the B.P. test for absence of pyrogens. This is a severe test, as untreated tap water gives a mean rise of about 1·3° C. in the dose used for these tests and a response of about 0·6° C. in a dose as low as 0·4 ml./kg. body weight. Acidification of pyrogen-free saline does not render it pyrogenic. This confirms the observations of Seibert[10]. A sample of the

anion exchange resin which had been in continuous use for two-and-a-half years is still capable of completely depyrogenising tap water.

Discussion

Several groups of workers have shown that water deionised by ion exchange resins can give water which will comply with the B.P.[11], D.A.B.6[5], Pharm. Helv.[5,12] and U.S.P.[1] tests.

Saunders[11] showed that, provided a correct technique is used and only water of specific resistance greater than 1 megohm per cm. is collected, the purity of demineralised water prepared from London tap water is at least equal to that of distilled water B.P.

The results given above and some experiments carried out with a commercially available ion exchange plant containing the same resins in single columns indicate that deionised water of this chemical purity may also be apyrogenic.

The effect of operating technique on the bacteriological content of water from deionising plants has been studied by Cruikshank and Braithwaite[13] and by Eisman, Kull and Mayer[10].

The results in this paper also show that the removal of pyrogens from London tap water must be a function of the charged anion exchange resins, since uncharged resins of both types and the charged cation exchange resin are without effect. This suggests that some pyrogens, at least, must be negatively charged molecules. It is interesting to note that Caillaud and Vincent[15] were able to depyrogenise water completely by means of an electro-osmotic cell.

Work is proceeding to test the effect of several different ion exchange resins on as many different pyrogens as possible. All pyrogens so far tested have been removed by anion and unaffected by cation exchange resins. Some evidence has been obtained that the anion exchange resins are more effective against some pyrogens than others.

It seems probable that deionised water of sufficient chemical purity to comply with the pharmacopœial requirements for purified water may also be apyrogenic, but whether the demineralising plants at present available will be suitable for the production of water for injection remains to be determined. It should be possible to construct an apparatus to give deionised pyrogen-free water.

Summary

1. The effects of two ion exchange resins (Zeocarb 225 and Deacidite FF) on the pyrogenicity of London tap water have been examined.

2. It is possible to produce large quantities of pyrogen-free deionised water from London tap water by passing it successively through columns of the charged cation and anion exchange resins.

3. The uncharged resins and charged cation exchange resins have practically no effect on the pyrogenicity.

4. The anion exchange resin is responsible for removing the pyrogenicity from the water.

I wish to thank the Permutit Company for generous supplies of resins and Mrs. C. Friedenthal for translating several papers from German.

REFERENCES

1. Harrison, Myers and Herr, *J. Amer. pharm. Ass., Sci. Ed.*, 1943, **32**, 121.
2. Smith and Pennell, *J. Bact.*, 1947, **51**, 715.
3. Reid and Jones, *Amer. J. clin. Path.*, 1949, **19**, 10.
4. Fischer, *Pharm. Industrie*, 1955, **17**, 129.
5. Co Tui, *Ann. Surg.*, 1937, **106**, 1089.
6. Wilke, *Pharm. Industrie*, 1953, **15**, 224.
7. Hatta, Aoyama and Tanji, *Jap. med. J.*, 1950, **3**, 125.
8. Suzuki, *J. pharm. Soc. Jap.*, 1953, **73**, 615, through *Chem. Abst.*, 1953, **47**, 9562.
9. Whittet, *J. Pharm. Pharmacol.*, 1954, **6**, 304.
10. Seibert, *Amer. J. Physiol.*, 1923, **67**, 90.
11. Saunders, *J. Pharm. Pharmacol.*, 1954, **6**, 1014.
12. Büchi and Soliva, *Pharm. Acta. Helvet.*, 1954, **29**, 221.
13. Cruikshank and Braithwaite, *Industr. Engng Chem.*, 1949, **41**, 472.
14. Eisman, Kull and Mayer, *J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 88.
15. Caillaud and Vincent, *Ann. pharm. franc.*, 1949, **7**, 276.

DISCUSSION

The paper was presented by THE AUTHOR.

DR. L. SAUNDERS (London) asked if the author had any information on the effect of the ion exchange resins on pyrogenic distilled water. Was the removal of pyrogens connected with the removal of salts from tap water? The author suggested that some pyrogens must be negatively charged molecules, but there was another possibility. If the pyrogens were polysaccharides, catalytic hydrolysis might destroy the pyrogenic properties.

MR. J. W. HADGRAFT (London) pointed out that the author did not state whether the water was drawn directly from the mains supply or from a tank supply. That might be of some importance since his own experience with London mains water did not support the author's statement. One sample taken from his hospital's mains supply and tested for pyrogens was described as being the least pyrogenic preparation tested for some considerable time.

DR. J. C. PARKINSON (Brighton) said he was puzzled about the origin of pyrogens in tap water. London tap water was often surprisingly free from bacteria, and he wondered whether it was possible that chlorination, for example, could leave matter in the water which might give a "pyrogenic" response.

DR. J. G. DARE (Leeds) hoped that the author would continue his work in a more quantitative way and establish whether the resins were effective with heavily contaminated water.

MR. T. D. WHITTET, in reply, said he had not yet tried the effect of the resins on pyrogenic distilled water, but he hoped to do so. He agreed that removal of pyrogens might be connected with catalytic hydrolysis of polysaccharides. It was a point of interest that resins in the borate form would absorb large quantities of polysaccharides. His experience

was that London tap water was always pyrogenic provided it was not sterilised, but he had found in the course of his work that the thermal stability of pyrogens in London tap water was very low. It was difficult to think of a reason why any chemical in water should affect pyrogenicity. Dr. Windle Taylor, whom he had consulted, was of the opinion that there was not enough residue from chlorination to have any effect on the temperature of rabbits. He had compared the water from the mains taps with that from other taps in the department and had found no difference in any of them before sterilisation.