the main, very impure and hygroscopic. The product turned brown in air. The yield was greater than with free pyrogen but the product was very impure.

Different organisms gave pyrogens with different physical characteristics. That from *P. vulgaris* was one of the most stable, while that from *E. coli* seemed less stable; but this was not an agreed subject. A variety of agents, including charcoal, asbestos pads and kieselguhr, adsorbed pyrogen and it was eluted from them with the greatest difficulty, involving large volumes of liquids. They were best adsorbed at *p*H 3 to 5. They could be eluted with a sodium phosphate buffer at *p*H 9 to 11. If left long in contact, decomposition was rapid.

The assay processes of the British and United States Pharmacopœias were limit tests; the use of clinical thermometers and the absence of a standard limited their value as biological tests. Such methods could not be used for research but were satisfactory for their purpose. Establishing a satisfactory standard preparation presented difficulties. Some of the factors involved would be dealt with by Dr. Dare. Mr. Whittet had experience both as a hospital pharmacist and as a research worker. Mr. Smith was engaged in day-to-day assay of pyrogen. Miss Dawson, together with the Chairman, was also engaged in research work on one aspect of pyrogen. Dr. Perry as Director of Biological Standards at the National Institute for Medical Research would speak on the preparation and use of an International Reference Preparation.

The following 3 papers were read:

THE OCCURRENCE AND IMPORTANCE OF PYROGENS

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Received December 11, 1953

THE fact that injections of distilled water can cause a rise in temperature has been known since as early as 1865 when it was reported by Billroth[1]. In 1875 the name "pyrogen" was given by Burdon-Sanderson[2], Professor of Physiology at this college, to a fever-producing substance which he had prepared from putrid meat and had shown to be free from living bacteria. During the latter part of the last century many cases of so-called injection fever were reported. In the early part of this century, when the intravenous route became customary for a number of drugs, other types of fever were reported, e.g. "protein fever," "salvarsan fever" and "salt fever". In 1911 Wechselman[3] showed that "salvarsan fever" was due to bacterial contamination of the water used for injection and that if freshly distilled bacteria-free water was used no febrile reactions occurred. Bennett and Beeson[4] quote Samuelson and Bergman as claiming that the so-called "salt fevers" were due not to salt but to water used for making saline injections. Hort and Penfold[5,6,7], in a series of papers, showed that the fevers due to the intravenous injection of salts, salvarsan and many other substances were all due to contamination of water used in

preparing the injections. They showed that freshly distilled water is apyrogenic but that it rapidly becomes contaminated with fever-producing material on standing. It was the classical work of Seibert[8,9,10] which finally established that all these so-called injection fevers were due to the presence in the distilled water used for injection of a filterable, heat-stable pyrogen of bacterial origin.

The tremendous increase in the use of the parenteral route for the administration of drugs and especially the frequent use of large volume intravenous infusions has made the need for the elimination of pyrogens of vital importance to both hospital and manufacturing pharmacists. There have been conflicting opinions about the type of precautions necessary in the preparation of parenteral injections. On the one hand we have the pharmacist who says he has been making infusion fluids for years without any special precautions and has never had complaints of untoward reactions. This person tends to minimise the danger of possible contamination with pyrogens. On the other hand there is the individual who is so obsessed with the danger of pyrogens that he considers that parenteral infusion fluids can only be prepared by manufacturers with strict controls at every stage. This type of mentality leads to the situation where a hospital authority has decreed that all the infusion fluids to be used in that hospital must be bought from manufacturers. The reasonable view lies between these two extremes. Apyrogenic solutions can be prepared with relatively simple apparatus in any well equipped pharmacy if adequate precautions are taken and a good, well baffled still and pure drugs are available; but some degree of control may be advisable in the absence of standards for pyrogen-free drugs.

Sources of Pyrogens

Berry[11] reported that the Cardiff Municipal water supply was pyrogenic in 1941, while Somers[12] has informed me that he has found the same with London tap water, and my own experiments have confirmed this observation. Brindle and Rigby[13], however, obtained no pyrogenic reaction with 8 samples of Manchester tap water but found that of another (unspecified) town to be strongly pyrogenic when distilled without baffle plates or traps. It has been definitely established that water distilled in a well baffled still and sterilised within 12 hours of distillation is apyrogenic. Thus the production of a suitable water for injection is not a serious problem. Since 1948 the British Pharmacopœia has included a test for freedom from pyrogens.

Numerous drugs have been reported as being contaminated with pyrogens. Lesser[14] quotes Riber[15] as finding evidence of bacterial contamination in chemicals used for making parenteral medicaments. Allport[16] reports the presence of pyrogens in dextrose injection and in calcium gluconate injection. Collier and Paris[17] found pyrogenic samples of physiological saline, injections of dextrose, sodium sulphate and sodium lactate, Darrow's solution and Hartmann's solution. Co Tui and Wright[18] report the presence of pyrogens in dextrose, in sodium chloride and in sodium lactate.

Dorche[19], on the other hand, has stated that commercial sugars are generally apyrogenic and Davis[20] stated that in a period of 10 years at University College Hospital about 1 ton of anhydrous dextrose had been used in the preparation of dextrose injections without any cases of pyrogenic reactions. Dorche and Castaing[21] and Berry[22] were unable to find pyrogenic samples of dextrose and Brindle and Rigby[13] had to prepare their pyrogenic dextrose injections from pure dextrose and pyrogenic water. Furthermore, these authors found that pyrogenic samples of dextrose became apyrogenic after 6 months storage in a bottle on the laboratory bench. Thus it seems likely that good quality dextrose is free from pyrogens. It has been shown, however, that injections of dextrose prepared from pyrogenic water and dextrose can become heavily contaminated with pyrogens if they are not sterilised shortly after preparation. Todd[23] reported the development of pyrogenicity of a sample within 3 hours of preparation, whereas Brindle and Rigby[13] never found it to develop in less than 12 hours although it did so after a longer period. Todd stated that it was always in dextrose solutions that pyrogens were found to develop and then only when there was a departure from the usual procedure. It has always been my practice to ensure that intravenous solutions are sterilised as soon as possible after preparation and that they are sterilised on the same day that the water was distilled.

Sodium citrate, because of its preparation by a fermentation process, is liable to contamination with pyrogens. Brindle[24] quotes an American report that of 30 samples examined 15 were pyrogenic. One of the difficulties of testing this drug for pyrogens is the fact that it produces profound shock on intravenous injection into animals. Charonnat and Lechat[25] have described a method of overcoming this by mixing the sample with apyrogenic calcium chloride before injection.

Many antibiotics are also liable to contamination, due to their preparation by growing moulds in culture media. The British Pharmacopœia now includes a test for absence of pyrogens for amorphous and benzylpenicillin, aureomycin, the three salts of streptomycin and dihydrostreptomycin. A similar test is included in the United States Pharmacopeia for potassium and sodium penicillin G and in the French Codex for the penicillins and dihydrostreptomycin sulphate.

Another biological drug liable to contamination with pyrogens, due to its method of manufacture, is heparin[18] and the British Pharmacopœia now includes a test for absence of pyrogens in both the solid substance and the injection. The French Codex includes a test for absence of pyrogens in injectable liver extracts. This has been described by Cheymol and Lechat[26]. In the French Codex also is a test for absence of pyrogens in normal dried citrated human plasma.

Charonnat and Lechat[25] have pointed out that many of the reactions attributed to calcium gluconate are actually caused by contaminating pyrogens and a test for their absence in the injection of this salt has recently been added to the British Pharmacopœia. The same authors mention corticotrophin as being liable to pyrogenic contamination.

Both the British and United States Pharmacopœias include a test for

absence of pyrogens in water for injection, but, whereas the former does not control any of the large volume intravenous injections, the latter subjects the following injections to test: injection of sodium lactate; Ringer's solution; Ringer-lactate solution; physiological saline; injections of dextrose and sodium chloride, dextrose, sodium acid citrate and injection of sodium citrate.

Another drug which is often contaminated with pyrogens is inulin[18,27,28], and, since this is often used in high dosage for the measurement of glomerular filtration rate, it is important that pyrogens should be removed Protein hydrolysates, made by the acid or enzymic hydrolysis of casein, are also very liable to contamination[29] and dextran, being made by a fermentation process, may be pyrogenic unless carefully purified.

The above examples show that distilled water is liable to contamination with pyrogens unless carefully prepared and that numerous drugs may be pyrogenic from their method of manufacture. Furthermore, even if the materials for preparing injections are pyrogen-free, the finished product can become contaminated if the technique of preparation is not satisfactory.

Co Tui and Wright[18] have stated that non-pyrogenic chemicals, even in the "dry" state, can become pyrogenic on standing in unsterile conditions. As might be expected, the better the medium the chemical is for bacterial growth, the more easily it becomes pyrogenic.

Lees and Levy[30] showed that activated charcoal can remove pyrogens from aqueous solutions and Todd[31] and Brindle and Rigby[13] have suggested methods of preparing apyrogenic solutions with the aid of this adsorbent. One of the difficulties of this process is the removal of fine charcoal particles from the finished solution. In my opinion the routine use of charcoal is unnecessary unless the medicament is known to be pyrogenic. Recently Reid and Jones[32] have shown that some ion exchange resins will completely depyrogenise solutions and these might prove more convenient to use than charcoal.

Somers[33] has pointed out that glassware is very often contaminated with pyrogens and that in America strict regulations are laid down for the preparation of glassware to be used for preparing solutions for pyrogen tests.

Collier and Paris[17] have shown that many pyrogenic injections lose this activity on storage. In view of the stability of pyrogens it is probable that this is due to their adsorption on to the glass of their containers. If this is so, and infusion bottles are not properly cleaned, it is possible that the pyrogens may be released again on autoclaving later batches. In view of all these possible sources of pyrogenic contamination those of us who have responsibility for the manufacture of intravenous injections must exercise the greatest care in ensuring correct distillation of water, selection of suitable drugs and in supervising the preparation of the final solution.

Although pyrogenic reactions are rarely, if ever, severe enough to endanger the life of a normal subject, the patient who needs large volumes of intravenous fluids, or many of the drugs liable to be pyrogenic, is

usually seriously ill. In such cases the injection of a contaminated solution might mean a fatal result instead of aiding the patient's recovery. Even if this were not so we cannot be satisfied with any preparation which may cause a patient unnecessary discomfort, however slight.

Suggestions for Controls

It is obviously desirable that the Pharmacopœia should includes tests for the absence of pyrogens in any intravenous injection liable to be contaminated. This would involve all the large volume infusion fluids as well as injections of any drugs likely to be pyrogenic. Berry[22] has pointed out that, except for pyrogenic drugs, the presence of pyrogens in injections is most important in large volume infusion fluids. The French Codex subjects to control any intravenous injection with a volume equal to or greater than 125 ml.

It might be helpful to those preparing intravenous injections on a relatively small scale if the Pharmacopœia laid down standards for pyrogen-free sodium chloride, dextrose and other drugs in common use for large volume injections, as has been suggested by Hough[34] and by Myers and Armitage[35]. If such drugs became commercially available it might obviate the need for testing the final product in individual hospitals provided that the technique of preparation is adequately controlled. The only official pyrogen-free drug, apart from the antibiotics, heparin and calcium gluconate, is sodium chloride, included in the B.P. Appendix and intended for rendering water for injection isotonic before applying the official test for absence of pyrogens.

When large amounts of intravenous fluids are being prepared regularly as in the larger hospitals, especially the teaching hospitals, it is obviously desirable to have regular tests applied even if it is not feasible to test every batch. There is probably a good case for each of the larger teaching hospitals having its own facilities for pyrogen testing, but for the smaller hospital some central laboratory should be established to undertake this work at a reasonable cost. Grainger[36] has suggested that the Pharmaceutical Society should set up such a laboratory.

Summary

1. Pyrogen-free injections can be prepared without elaborate equipment if a good still and pure drugs are available and an adequate technique is followed.

2. Serious results may follow the use of unsatisfactory materials or careless methods.

3. It is suggested that the Pharmacopœia should include standards for pyrogen-free drugs which should become commercially available.

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ROUTINE PYROGEN TESTING

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Received March 8, 1954

THE British Pharmacopœia specifies that aureomycin, streptomycin, penicillin, heparin and injection of heparin, injection of calcium gluconate and water for injection should be substantially free from pyrogens and indicates that such freedom may be established by showing that their intravenous injection into rabbits is not followed by a marked increase in body temperature. The doses to be administered are specified. The injections are required to be made into 3 rabbits and the mean maximum temperature rise in the rabbits during the 3 hours following injection may not exceed the pre-injection temperatures by more than 0·6° C. Table I shows the doses prescribed by the British Pharmacopœia.

It would be expected that the application of this test to these substances even from large-scale manufacture would produce little difficulty and this is true in the case of the antibiotics, heparin and calcium gluconate, but some difficulties do arise in the case of water for injection.

The British Pharmacopœia includes a monograph on water for injection