SYMPOSIUM ON PYROGENS

AFTERNOON SESSION

Chairman: PROFESSOR J. P. TODD

CHAIRMAN'S OPENING ADDRESS

PROFESSOR TODD said that the term "pyrogen" was now limited to substances derived from living sources. Chemicals were known which elevated the temperature, but the expression was now specialised to mean substances derived from bacteria. Some had claimed algæ, yeasts and moulds to produce fever when injected; others doubted whether pure cultures, free from epiphytes were, in fact, pyrogenic. In his own hands, pure cultures of yeasts and moulds were not pyrogenic; he had no experimental evidence with algæ, pure cultures of the latter having only recently become available to him. Most research had been made with pyrogen of Gram-negative bacterial origin.

One aspect of pyrogen work concerned their use as therapeutic agents, the other their occurrence adventitiously in preparations for medical use. It was the latter aspect and the consequent "nuisance value" which had attracted most attention in this country, but elsewhere preparations for therapeutic purposes were in use.

The introduction of salvarsan in 1909 by Ehrlich made intravenous injections more common, since this drug could be used with effect only by the intravenous route, and the frequency with which fever followed the injection attracted attention. In 1923, Florence Seibert showed that the cause was microbial contamination and that by distillation of the water in a suitable still, the water could generally be rendered non-pyrogenic.

It was now known that pyrogens belonged to the group known as bacterial polysaccharides of high molecular weight; hydrolysis yielded a mixture of sugar residues containing a glucosamine radical, phosphoric esters and sometimes ribosenucleic acid. If the ribosenucleic acid were split by ribosenucleases it was said the substance was no longer pyrogenic. These extremely potent substances were difficult to purify, and isolation in quantity for chemical investigation was a most laborious undertaking.

Pyrogens were found in a liquid culture in two forms, free and as a constituent of the cell, namely, "bound pyrogen". Free pyrogen, from organisms grown on a purely inorganic medium, was separated by centrifugation or filtration and isolated by precipitation with organic solvent, or by freeze-drying. A fairly pure, active, non-hygroscopic pyrogen could be obtained from some bacteria in this way. The product was thermolabile so that evaporation, even under reduced pressures, was not possible without great loss of potency. Variations of *p*H from 7 were also destructive. The yield was small, about 1·0 mg./l., and represented about 1000 doses, i.e., a material response was obtained with 1 μg. On the other hand, the substances prepared from whole or digested cells by the classical methods for separating bacterial polysaccharides were, in

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

BY T. D. WHITTET

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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