The standarization of pyrogens / by W.D.M. Paton.

UNITED NATIONS

64

NATIONS UNIES

97

WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTÉ

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

WHO/BS/90
30 October 1950

ORIGINAL: ENGLISH

THE STANDARDIZATION OF PYROGENS

by

W. D. M. Paton, M.A, National Institute for Medical Research, London N.W. 7

(1) Bacteria capable of producing pyrogens are widely distributed in natural waters and in other sources,[1,3,6,7] and pyrogen can be formed by them within a few hours.[4] As a result, contamination of drugs, particularly of antibiotics prepared by bacterial action, and of solutions for parenteral administration (such as blood substitutes) can occur very easily. Once such contamination has occurred, the pyrogens are not easily removed, for many of them resist the autoclave and pass ordinary filters.[7,8,10] It has thus become necessary for certain pharmacopoeial preparations to pass a test for freedom from pyrogenicity. Since the actual amounts of pyrogen present are usually minute, of the order of microgram or less, this test is necessarily a biological one.

(2) The tests in current use are based on the definition by Co Tui[5] of the "Minimal Pyrogenic Dose": this is the dose per kilogram of body weight which, when injected intravenously into a rabbit, does not cause a rise in body temperature of more than 0.5 - 0.6°C. within four hours. The United States Pharmacopoeia and British Pharmacopoeia tests stipulate that a given amount of test material should not cause a rise of more than 0.6°C. above the initial body temperature, with readings taken during the three hours after the injection. In all these tests a "biological unit" is adopted, that is a standard based on the assumption that the sensitivity of the rabbit to pyrogens is constant from one laboratory to another.

This assumption is certainly unjustified. Tests of the same material usually give different results in different laboratories, sometimes so far as to give a definitely positive test at one, and a definitely negative test at another laboratory. Causes for this are several, and include:

(a) Variation in sensitivity between different species of rabbit.(9)

(b) Individual variation in sensitivity within a species.(9,12)

(c) Differences in experimental technique; such as the method of temperature recording, the environmental temperature, and the frequency with which a given animal is used.(2)

(3) It is evidently important that there should be available some reference standard of pyrogenicity by which these variables can be controlled. There are, indeed, already certain facts favourable to the establishing of such a standard.

(a) Pyrogenic preparations, active at 1 ug. or less per kg. body weight, are in general straightforward to prepare, and they are reasonably stable for long periods of time.

(b) The pyrogenic response can be made quantitative. There is a linear relationship between the rise in temperature and the logarithm of the dose of pyrogen, such that for each twofold increase in dose, there is on the average an additional 0.25°C. rise in temperature (approximately). This slope of the dose-response curve is the same for pyrogens from Proteus vulgaris, Br. abortus, T.A.B. vaccine, and for a pyrogen that occurred in an early batch of penicillin.(9)

(4) It is not possible, however, at present to select a reference pyrogen, to be used as a standard with which to compare unknown materials possibly pyrogenic to man, for the following reasons, amongst others:

(a) Pyrogenic substances have been obtained from at least fifty different sources (see Appendix). No adequate attempt has been made to determine which of these are important for the contamination of injections and infusions administered to man.

(b) Pyrogens vary considerably among themselves in the character, duration and latency of the febrile response which they arouse, and in their stability to destructive agents.(9,11,13) There are, therefore, probably several types of

yrogen, and it is certainly unsafe to assume at present that all these types will etain the same activity relative to each other when the technique of the test is aried.

(c) There is considerable uncertainty as to the sensitivity of man to yrogens, in comparison with that of animals. The scanty evidence published uggests that man may be about three times more sensitive than the rabbit,(5) ut other experience indicates the contrary. Nor is there any information about he dose-response curve in man for even one pyrogen, or whether different pyrogens ave the same relative activity in man as in rabbit.

5) The immediate need, therefore, is for a reference standard simply for nvestigative purposes. Without this, it is doubtful if satisfactory and ufficiently general knowledge about the problems just mentioned will ever be ained. The selection of a pyrogen for such an investigative reference standard s clearly an arbitrary matter, and it would probably be useful if several epresentative pyrogens were chosen rather than one. Preparations from Proteus ulgaris, Br. abortus, S. typhi, Pseudomonas aeruginosa, and Micrococcus etragenes, purified until active in a dose of 1 ug/kg. body weight of test animal, ould provide suitable material for the investigations.

APPENDIX

Organisms from which Pyrogenic Substances have been obtained. (1,3,6,8,9,12)

Achromobacter pinnatum, solitarium, candicans, refractans, tiogense, punctatum, lacticum, W14B, W14C, 2WS,
Serratia marcescens, keilensis.
Bact. coli, formica, cloacae.
Salmonella typhi.
Alkaligenes fecalis.
Pseudomonas fluorescens, aeruginosa, ureae, scissa.
Vibrio cholerae, comma.
Brucella abortus, melitensis.
Proteus vulgaris, morganii.
Pasteurella pestis,
Pfeiferella mallei.
Haemophilus influenzae, bronchisepticus.

Neisseria gonorrhoeae, meningitidis.
Micrococcus catarrhalis, tetragenes.
Staphylococcus albus, citreus, aureus.
Streptococcus pyogenes, lactis.
Bacillus mycoides, subtilis, megatherium, anthracis, aerosporus.
Corynebacterium acnes, diphtheriae.
Lactobacillus casei.
Azotobacter chroococcum.
Actinomyces albus.

Viruses: Influenza A (P R 8); Influenza B (Lee); Newcastle Disease ("B" strain).

REFERENCES

(1) Banks, H.M. (1934). Amer. J. clin. Path. 4, 260.

(2) Beeson, P.B. (1946), J. clin. Invest. 25, 917.

(3) Bourn, J.M. & Siebert, F.B. (1924). Amer. J. Physiol. 71, 652.

(4) Brindle, H. & Rigby, G. (1946). Quart. J. Pharm. 19, 302.

(5) Co Tui, Hope, D., Schrift, M.H. & Powers, J. (1944). J. Lab. clin. Med. 29, 58.

(6) Co.Tui, Schrift, M.H. (1942) J. Lab. clin. Med. 27, 569.

(7) De Merre, L.J. & Probey, T.F. (1945). Les Pyrogenes. Actualités Medico-Chirurgicales, No. VIII. Bruxelles.

(8) Hort, E.C. & Penfold, W.J. (1912). J. Hyg., Camb. 12, 361.

(9) MacIntosh, F.C. & Paton, W.D.M. (1947). Unpublished.

(10) Siebert, F.B. (1923). Amer. J. Physiol. 67, 90.

(11) Wagner, R.R., Bennett, I.L. & LeQuire, V.S. (1949). J. exp. Med. 90, 321, 335.

(12) Wylie, D.W. & Todd, J.P. (1948). Quart. J. Pharm. 21, 240.

(13) Wylie, D.W. & Todd. J.P. (1949). J. Pharm. Pharmacol. 1, 818.