

## Effects of weight and sex of rabbits on the results of the testing of chorionic gonadotrophin injection according to the European Pharmacopoeia

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The test for pyrogens specified by the European Pharmacopoeia (1971, 1980) consists of measurement of the rise in rectal temperature evoked in rabbits by intravenous injection of a sterile solution of the substance to be examined. It is recommended to use '... healthy, adult rabbits of either sex weighing not less than 1500 g...'. It was observed in our previous work that reconstituted freeze-dried chorionic gonadotrophin (Gonadotraphon L. H. Paines & Byrne) gave irreproducible results when the tests were performed in groups of rabbits complying with the compendial criteria but otherwise selected on a random basis. A study of the effect of weight and sex on the test results was designed to investigate the causes of the irreproducible behaviour; a preparation of Gonadotraphon L. H. (1000 i.u.) which previously failed in a pyrogen test performed with a 'random' group of rabbits was used. In the new investigation, the animals were selected as follows:

Group	Sex	Weight
1	male	'low' (1.5-1.6 kg)
2	male	'high' (2.1-2.3 kg)
3	female	'low' (1.5-1.7 kg)
4	female	'high' (2.1-2.5 kg)

\* Correspondence.

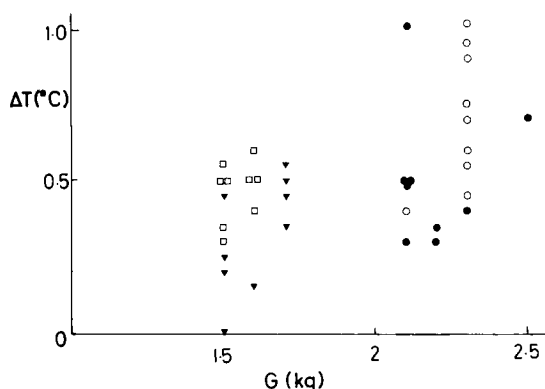


FIG. 1. Maximum increase in body temperature,  $\Delta T$ , against body weight, g, over a period of 3 h (European Pharmacopoeia, 1971, 1980) in 4 groups of animals which received 1 ml kg<sup>-1</sup> Gonadotraphon L.H. injection (1500 i.u. ml<sup>-1</sup>) intravenously. Males: 'low' weight (□), 'high' weight (○); females: 'low' weight (▼), 'high' weight (●).

Otherwise, the study was conducted as specified by the European Pharmacopoeia (1971). Each group contained 9 animals which received single doses of 1 ml of injection per kg. Their temperature was followed every 30 min for 3 h. From Fig. 1 it is apparent that the rabbits in the 'high' weight male group exhibit greater increases in temperature than the animals in the other three groups. According to the European Pharmacopoeia (1971, 1980), the material would 'pass' the pyrogen test in groups 1 and 3, it would 'fail' in group 2, and it would be re-tested if the results in group 4 only were obtained. Whilst the reasons for the observed differences are not clear, the implications seem obvious; without special precautions the compendial test for pyrogens when applied to Gonadotrophin injections may give false positive, or negative, results depending on the sex and weight of the test animals. Our findings are in agreement with a detailed study of Lipton & Ticknor (1979) who showed that the febrile response of the rabbit to an intravenously administered pyrogen varied with both age and sex. Their study, however, was not concerned with the effect of weight on the febrile response, and their animals were generally much older than those used in our work. It is possible that a different animal species may show a more uniform reaction. Other possibilities are to use leukocyte counts instead of temperature rise as a measure of pyrogenicity (Dawson & Todd 1952, 1954; Dawson et al 1954; Todd 1955) or the *Limulus* Amoebocyte Lysate test proposed by Hochstein et al (1973).

Although the European Pharmacopoeia does not specify the experimental procedure for pyrogen testing in great detail, the interested reader may obtain the actual method used in this work from the authors on request.

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