Response Due to 0 Somatic Antigen of *Shigella dysenteriae* in Rabbits

The various pharmacopeial tests for the absence of pyrogens in injectible drugs are arbitrary in that they choose some fixed level of response in the laboratory animal, namely the rabbit, as the maximum permissible response to be obtained with a fixed dose of the substance under examination. Earlier it was shown[1] that the existing pharmacopeial tests do in fact bear a definite relationship to the incidence of reactions in man. But this type of test is nothing but an outmoded way of describing the contents of active principles in a drug, and discrepancies may arise due to differences in dose levels, to different strains of rabbits or to different conditions of housing the animals or carrying out the tests. Such discrepant findings are probably inexplicable because testing is not related to a standard preparation. On the recommendation of the Expert Committee on Biology Standardization, 0 somatic antigen of *Shigella dysenteriae* (OSASD)[2], strain K.624, 'smooth' was prepared and recommended to be used as an International Reference Preparation. We have used the antigen to find its suitability as a potent pyrogen that can be used as a reference preparation.

Methods. 12 healthy rabbits (albino) of either sex, each weighing not less than 1.5 kg and having a rectal temperature not below 38.5 or above 39.5 °C were selected and maintained on a uniform unrestricted diet. They were kept in the test room for at least 18 h before test. Food was withheld during the test. A spot galvanometer-cum-thermocouple was used for continuous record of temperature without disturbance of the animal. The animals were placed in suitable holders and some 90 min were allowed for the animals to become accustomed to the restraining device before taking the initial temperature-recording. Thermocouples were inserted in the rectum to a depth of not less than 6 cm and not more than 9 cm. 5 temperatures were recorded prior to the beginning of injection. The 'mean initial' temperature of each rabbit was recorded according to British Pharmacopeia (B.P.)[3]. Syringes, glass wares and sodium chloride were rendered pyrogen-free by heating at 250 °C for 60 min. B.P. procedure was followed in preparing test samples for injection and in calculating response of each rabbit. Control test was carried out for each rabbit by recording its response following i.v. injection of pyrogen-free water. This test was repeated several times at an interval of 10 days. After 3 days following the last control test, 0.003 μg of the reference preparation was injected i.v. to each rabbit and individual response was noted. After 3 weeks the rabbits were subjected to control tests. Following 3 days' rest they were given each an i.v. injection of 0.006 μg of the antigen and their responses were recorded. The animals were allowed to take rest for 3 weeks and again conditioned with control tests. After 3 days an i.v. injection of 0.03 μg of the antigen was administered to each rabbit and corresponding responses were noted. The antigen, before each test, was dissolved in pyrogen-free water and made isotonic with pyrogen-free sodium chloride. Response in the control tests was the average of all such tests with individual animal.

Results. It is evident from the Table that there was a general increase in response following injection of 0.003 μg of reference preparation. This observation was in conformity with that of HUMPHRY et al.[4] who reported that 0.003 μg of the antigen was sufficient to raise the temperature of the rabbits. Except in 2 cases, very little change in response was observed by increasing the dose from 0.003–0.006 μg. But a significant increase was observed following injection of 0.03 μg of the antigen. However, 0.003 μg of OSASD was found sufficient to produce satisfactory response in rabbits. Individual response in the control tests fluctuated between 0.04 and 0.23 °C, while that in the tests with 0.003 μg of the antigen varied between 0.25 and 0.45 °C.

In the United States Pharmacopeia[5] any material which when injected in rabbits (10 ml/kg) produces an individual response of 0.6 °C or more is regarded as not satisfactory so far as the absence of purogens is concerned. In our investigation the upper limit for individual response with 0.003 μg of the antigen was found to lie somewhere between 0.40 and 0.45 °C. It may be assumed that in the event of OSASD being used as a reference standard, an individual response of 0.45 °C or more may be regarded as not satisfactory so far as the absence of pyrogens is concerned[6].

Response of rabbits with and without OSASD

Rabbit No.	Control test mean ± s.e. (°C)	Test with OSASD 0.003 μg (°C)	0.006 μg (°C)	0.03 μg (°C)
1	0.12 ± 0.018	0.28	0.30	0.58
2	0.12 ± 0.018	0.45	0.45	0.94
3	0.04 ± 0.009	0.30	0.32	0.64
4	0.13 ± 0.018	0.42	0.46	0.91
5	0.13 ± 0.004	0.32	0.33	0.83
6	0.08 ± 0.009	0.30	0.37	0.87
7	0.14 ± 0.013	0.28	0.37	0.70
8	0.23 ± 0.018	0.40	0.43	0.72
9	0.18 ± 0.009	0.45	0.70	0.93
10	0.07 ± 0.018	0.25	0.30	0.60
11	0.13 ± 0.009	0.33	0.81	0.97
12	0.09 ± 0.009	0.30	0.32	0.92

Zusammenfassung. Hochgereinigtes Antigen von *Shigella dysenteriae* bewirkte eine signifikante Temperatursteigerung in Kaninchen.

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