

A Tentative Test for Pyrogen in Infusion Fluids.

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Since Seibert¹ extended the work of Hort and Penfold² on the occurrence of pyrogen in distilled waters, various tests for the detection of this substance have been proposed. A chemical test which has been in use depends upon the decoloration of potassium permanganate added to the solution to be tested. But this test is unsatisfactory because, on the one hand, it can be used only in solutions in which medicaments do not decolorize this oxidizing agent and, on the other hand, it is not sensitive enough to detect the minute amount of pyrogen necessary to cause even a severe physiologic response. Resort must therefore be made to biologic test.

Test Animals. Seibert in her work used the rabbit as the test animal. This practice has been followed by other workers, among them Banks,³ Lees and Levvy,⁴ and by commercial houses in routine tests of infusion fluids. Sabin and Wallace⁵ used the dog in their study of the chill-producing substance in pneumonia serum. While in all our published work⁶ we have used the dog, we have had just as extensive experience with the rabbit. It is pertinent here to discuss the advantages and disadvantages of these 2 test animals.

The rabbit has a labile thermoregulatory mechanism and frequently gives false positive tests. Because of this, a negative test is more significant than a positive one in the rabbit. These animals must be conditioned to handling and to the taking of rectal temperatures for several days before they can be used, as untrained rabbits exhibit a rather bizarre temperature pattern. Feeding is followed by a mild pyrexia which lasts several hours and therefore they should not be fed for at least 6 hours before use as test animals.

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¹ Seibert, F. B., *Am. J. Physiol.*, 1923, **67**, 90; 1925, **71**, 621.

² Hort, E. C., and Penfold, W. J., *Brit. M. J.*, 1911, **2**, 1589.

³ Banks, H. M., *Am. J. Clin. Path.*, 1934, **4**, 260.

⁴ Lees, J. C., and Levvy, G. A., *Brit. M. J.*, 1940, **1**, 430.

⁵ Sabin, A. B., and Wallace, G. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 198; *J. Exp. Med.*, 1931, **53**, 339.

⁶ Co Tui, McCloskey, Schrift, and Yates, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 297; *J. A. M. A.*, 1937, **109**, 250.

The dog has a much more stable thermoregulatory mechanism but is less sensitive to pyrogen than the rabbit. A positive test in the dog is so characteristic, however, with the additional symptoms of leucopenia, vomiting and diarrhea, that it is unmistakable. For this reason, a positive test in the dog is much more significant than a negative one.

To summarize, the rabbit is the better animal to test for the absence of pyrogen, while the dog is the better animal to establish the presence of pyrogen. Both animals must be used to complete the picture.

Sabin and Wallace⁵ have shown that dogs react repeatedly to repeated intravenous injections of chill-producing pneumonia sera. In our experience both dogs and rabbits have been used for several positive pyrogen tests without developing manifest immunity or tolerance. Yet this question of immunity and tolerance has not been definitely settled, and until this is done it seems better to use a fresh animal for each test.

Another factor which has a direct bearing on the test is the sensitiveness to pyrogen of rabbits and dogs as compared with man. In preliminary studies with the purified pyrogen from typhoid vaccine, to be reported elsewhere, we found that the rabbit was approximately 3 times and the dog at least 6 times less sensitive than man.

Test Dosages. Assuming for the present that the above figures on comparative sensitiveness hold for pyrogen from other sources than the typhoid bacillus, the rabbit should receive as much as 3 times the maximum per kilo dose in man; and the dog 6 times.

Test doses of 5 to 20 cc per rabbit are employed by the commercial houses. On the assumption that the rabbits weigh 3 kg, this dosage would correspond to a 100 to 600 cc dose in man. If, in addition, comparative sensitiveness is taken into consideration, the dose is equivalent to $33\frac{1}{3}$ to 200 cc. As a patient may receive as much as 2000 cc at a time, this test dose is manifestly inadequate. In order to approximate the dose in man, the rabbit test dose must therefore be not less than 50 to 100 cc per kilo body weight. This amount of fluid almost approximates the total blood volume of the animal, but if the dose is injected in 10 to 30 minutes, the rabbit can easily take care of the plethora. In any event, the symptoms of plethora are different from those of a pyrogenic reaction. As the dog is to be used as a positive test animal, any dose which elicits the reaction shows the degree of pyrogenicity of the solution. This can be arrived at by trial and error, starting from a minimum dose of 250 cc.

Procedure of the Test. The test is to be run in two parts, the preliminary part on rabbits, the confirmatory on dogs.

Preliminary Test. Two albino rabbits of $2\frac{1}{2}$ to 3 kg are used. They must previously have been conditioned for several days to handling and taking of rectal temperature until these procedures provoke no struggling and their rectal temperature readings are constant within $.25^{\circ}\text{C}$ in 3 readings taken 15 minutes apart. Before injection, temperature readings taken at 15-minute intervals must not be over 39°C . In taking the temperatures, the thermometers must be inserted the same distance beyond the internal sphincter of the rectum and allowed to remain there for 2 minutes.

The test solution is warmed nearly to the temperature of the animals, and 50 cc per kilo body weight is injected into one animal and 100 cc per kilo body weight into the second animal, no faster than 10 cc per minute. The temperature readings are taken every half hour for 4 hours after the injection. A rise of over 5°C in 1 to 3 hours after injection is taken as presumptive evidence of the presence of pyrogen. A pyrogenic sample ought to give a more severe response with 100 cc than with 50 cc.

Confirmatory Test. This may not be necessary in situations, such as exist in commercial houses, where a positive presumptive test is enough to lead to the condemnation of a product as unfit for human use. In situations in which more positive evidence may be required, especially where there may be legal or medico-legal complications, the clear-cut result of a positive dog test is not easy to controvert. The test is performed as follows: One healthy dog of 15 kg body weight, conditioned to the taking of rectal temperature, with an initial rectal temperature of not over 39°C and blood count not exceeding 20,000 white blood cells per cu.mm, is used. Depending upon the response of the rabbits, from 250 cc to 1500 cc of the fluid is given intravenously through one of the external jugular veins, not faster than 30 cc per minute. The blood count is again taken 45 minutes and the temperature every half hour after the injection. The presence of a leucopenia (a fall of 5,000 cells per cu.mm or over) and temperature rise of $.5^{\circ}\text{C}$ from 1 to 3 hours after the injection are evidences of the presence of pyrogen. The occurrence of emesis, prostration and diarrhea are added checks.

Experimental Data. This two-fold test has been performed 214 times in this laboratory in a period of 32 months. Some of these tests were done for the New York University Surgical Division of the Bellevue Hospital, a few for some metropolitan hospitals, and the rest for the Comptroller's Office of the City of New York. Of

these tests, 172 were negative and the clinical use of these products has caused no pyrogenic reactions. Forty-two were positive, a result likewise confirmed by clinical use of the material.

The accompanying table embodies 5 sets of data obtained from the same commercial infusion fluid. Experiment 1 is the record of a pyrogenic reaction in a woman; Experiments 2 and 4 are the record of inadequate test doses in a rabbit and in a dog, respectively, while Experiments 3 and 5 are the record of adequate test doses in each of these 2 animals. The importance of using a test dose comparable to the human dose is shown by a study of Table I.

From the academic point of view it might be better to wait for more light on some of the factors previously mentioned before presenting the test. There is, however, a real need for a pyrogen test in the clinical world where inadequate tests are already in use. From this point of view, a test already given this amount of calibration and refinement would be better than the inadequately standardized tests now in use. Although this test may not always detect the presence of minute doses of pyrogen, it can be depended upon to detect the presence of moderately reactive amounts.

TABLE I.

Exp. No.	Test animal	Wt, kg	Dose per kg wt, cc	Total dose, cc	Change in WBC (in thousands)	Change in temperature, °C	Other symptoms
1.	F.C.C. ♀ Patient	55	11	600	8.-3.	37.7-41.8	Shivering, nausea, depression
2.	Rabbit	2.5	10	25		38.9-39.3	No symptoms
3.	Dog	12	20	240	21.5-14.4	38.7-39.1	No symptoms
4.	Rabbit	3	30	90		38.8-41.0	Depressed
5.	Dog	14	60	840	14.7- 5.2	38.7-40.9	Emesis, diarrhea, depression