guarantee for large quantities. As a rule, the manufacturers could take care of their own side of the work. They would probably have methods of eliminating pyrogen.

MR. P. J. FOWLER (Bristol) said that as a hospital pharmacist he was interested in avoiding pyrogens. Had Mr. Smith experience of samples of dextrose-salines which had been pyrogenic on the production line, and what was the source of the pyrogen? If it were the drugs, how were they stored?

MR. SMITH replied that no special treatment was used to render glucose pyrogen-free, but the sodium chloride used was heated almost to redness for a short time. It was more economical to throw away a whole batch of pyrogenic glucose-saline than to try to trace the source of the pyrogen.

MR. FOWLER thought the value of the information lay in avoiding pyrogen in other batches.

MR. SMITH added that if a batch of glucose-saline was pyrogenic a check would be made to determine if the same batch of glucose were involved. If it were, it would be discarded.

EVENING SESSION

Chairman: DR. H. O. J. COLLIER

The following 2 papers were read:

RABBIT RESPONSES TO HUMAN THRESHOLD DOSES OF A BACTERIAL PYROGEN

BY J. G. DARE and G. A. MOGEY

From the Department of Pharmacology, University of Leeds

Received February 11, 1954

THERE are very few quantitative data on the effect of bacterial pyrogens in man. Even less information is available on the relationship between effective doses in man and rabbit. Co Tui and Schrift[1] stated that the rabbit is one-third as sensitive to pyrogen as man; they suggested, in consequence, that to test intravenous solutions for human use 50 to 100 ml./kg. must be given to rabbits. But the number of observations on which their result is based is so small, and the variation of these responses can be so large, that the significance of this result is doubtful. Lees and Levvy[2], on the other hand, stated that a dose of 20 ml. per rabbit was sufficient to detect whether enough pyrogen was present to cause a response in man.

In view of the scarcity and the contradictory nature of the evidence available, and because of the differences in magnitude of the rabbit response in varying experimental conditions, or from repeated administration of pyrogen, we decided to determine the minimal effective dose

of a pyrogen preparation (Pyrogen Test Preparation No. 1[3]) in man, and to relate the results to the official pyrogen tests.

METHODS

A. MAN

Two series of experiments were performed in man—a preliminary set of 25 observations to establish a suitable dose range for the determination of the minimal effective dose, and a more extensive group of 67 observations to find this dose accurately. The pyrogen preparation was given intravenously, usually into a vein in the antecubital fossa, in a volume never exceeding 2 ml. As it was not known whether warmth and comfort might affect the response, half the subjects in the larger group were seated in deck chairs and half were put to bed; no differences were observed between these groups. The experiments were done in a room which was kept warm and as free from draughts as possible. The oral temperature was measured by clinical thermometer every 15 minutes. An all-or-nothing response was used, a positive response being recorded on the appearance of typical symptoms.

B. RABBIT

The response of both new and tolerant rabbits to intravenously injected P.T.P. No. 1 was recorded by measuring the increase in the rectal temperature. Some animals were rigidly restrained and their temperatures measured with clinical thermometers (method 1): others were only slightly restrained and their temperatures taken with electrical thermometers (method 2). These methods have already been described in detail[3].

RESULTS

A. MAN

In man, an effective intravenous dose of P.T.P. No. 1 produced no signs or symptoms for at least 45 minutes but, once started, these developed rapidly. In all subjects exhibiting a positive response, obvious symptoms developed within 75 minutes of the injection. The usual symptoms were: aching in the lumbar region and limb joints, a feeling of cold, spasms of shivering, nausea and headache. The subject lost colour and often turned ashen-grey. A really positive response was very distressing. When the reaction was allowed to continue, an increase in temperature usually occurred about 15 minutes after the onset of uncontrollable shivering; but pyrexia was not often found, probably because each experiment was stopped as soon as the typical symptoms appeared. Hence the effect in man had to be recorded as a quantal response. The signs were so characteristic that it was not necessary to continue an experiment once they were observed, while the symptoms were so distressing that the subjects were only too anxious to have them relieved quickly. 1 g. of aspirin, taken with a cup of tea, suppressed the symptoms within 20 minutes in all except the worst cases. After severe reactions further doses of aspirin, at intervals of one or two hours, were required. It was only because aspirin was so effective in suppressing the symptoms in

the earlier experiments that we felt justified in asking for volunteers for a large scale investigation.

The results are shown in Figure 1. Here, the lower abscissa-scale shows the dose in μg./kg. and the left hand ordinate-scale the percentage responding. The points mark the percentage response at each dose

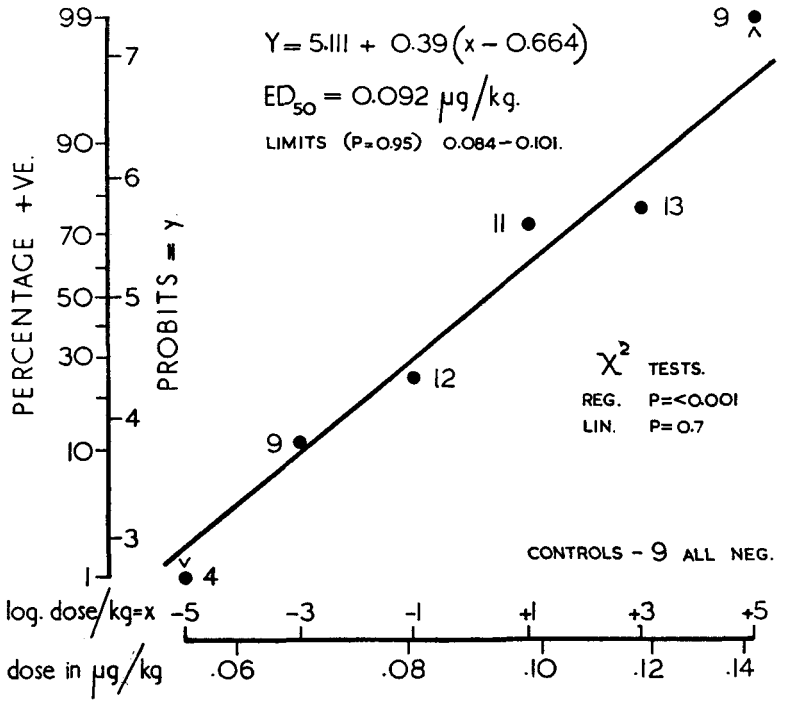

FIG. 1. Log dose/quantal response curve for man to P.T.P. No. 1. For detailed explanation see text.

level and the numeral beside each shows the total number of experiments done at that dose level. The 9 control experiments, in which only pyrogen-free saline solution was given, were all negative. The linear regression equation given at the top was calculated by using the log dose and probit scales. The χ^2 tests show that the slope of the regression line is highly significant and that there is no evidence of any departure from linearity. The line shown is the calculated regression. The calculated ED50 is 0·092 μg./kg., estimated to lie between 0·084 and 0·101 μg./kg. with P = 0·95.

B. RABBIT

A family of log.-dose/temperature response linear regression lines, obtained from experiments done on rabbits under various conditions, is given in Figure 2. The ED's 5, 50 and 95, for man, are also shown in Figure 2.

C. Correlation of Human and Rabbit Data

As the human responses were measured quantally and the rabbit responses quantitatively they can only be related at arbitrarily chosen levels. A mean rise in temperature in the rabbit of 0·6° C. has been commonly used in the past as the criterion of a positive response, and is in fact used in the B.P. test. From the data given in Figure 2, the dose which elicits a mean response of 0·6° C. in each of the different experimental circumstances can be obtained, and the ratio of this dose to the ED50 in man may be used as a measure of the relative sensitivities of man and rabbit to P.T.P. No. 1. These ratios, on a weight-for-weight basis, are given in Table I.

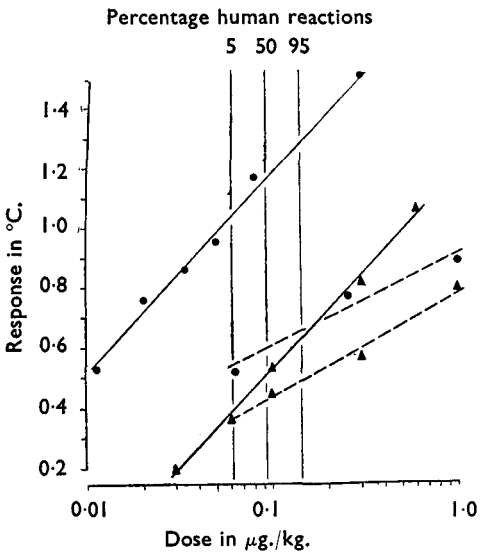

FIG. 2. Dose-response curves for the rabbit to P.T.P. No. 1 in various experimental circumstances; and doses causing various percentage reactions in man. Mean responses with Method 1—▲; and Method 2—●. Solid lines—new rabbits; broken lines—tolerant rabbits. The details of each dose/response curve are:

Method 1, new rabbits, 56 experiments,
$Y = 0{\cdot}54 + 0{\cdot}66\,(x - 1{\cdot}01)$. $S.D._y = 0{\cdot}32$
Method 1, tolerant rabbits, 80 experiments,
$Y = 0{\cdot}54 + 0{\cdot}34\,(x - 1{\cdot}32)$. $S.D._y = 0{\cdot}32$
Method 2, new rabbits, 90 experiments,
$Y = 1{\cdot}02 + 0{\cdot}66\,(x - 0{\cdot}76)$. $S.D._y = 0{\cdot}28$
Method 2, tolerant rabbits, 54 experiments,
$Y = 0{\cdot}73 + 0{\cdot}31\,(x - 1{\cdot}40)$. $S.D._y = 0{\cdot}28$
(log dose × 100 = x. y = response in °C.).

Of more practical interest is the efficiency with which the B.P. and U.S.P. tests will detect, say, a human ED5 of P.T.P. No. 1 present in a volume of solution to be given to man. For this we first need to know the average amount of pyrogen which will cause 5 per cent. of men to react. This is given in Table II along with the total dose for other response levels. Once the volume of the injection containing any one of these total doses has been specified, one can calculate how much pyrogen is present in 10 ml. of the injection. This is the volume given per kg. of rabbit in the B.P. and U.S.P. tests. The mean response of the rabbit to such doses can be calculated from the regression equations given with Figure 2. The expected frequency with which the B.P. and U.S.P. tests will fail to detect the pyrogen can then be calculated in the manner described previously[4]. Values calculated in this way for various volumes of solution containing one human ED5 are given in Table III.

The product of the percentage failure to detect pyrogen in any particular set of circumstances and the probability that the dose present will cause

TABLE I

RATIOS OF EFFECTIVE DOSES OF P.T.P. NO. 1 IN MAN, TO EFFECTIVE DOSES DETERMINED IN SEVERAL DIFFERENT EXPERIMENTAL CIRCUMSTANCES IN THE RABBIT

Experimental conditions	Dose/kg. causing pyrogenic response in rabbit	Ratio: Human dose Rabbit dose
New rabbits, minimal restraint, electrical thermometers	0·013 μg.	7·08
Tolerant rabbits, minimal restraint, electrical thermometers ..	0·094 μg.	0·98
New rabbits, severe restraint, clinical thermometers	0·127 μg.	0·72
Tolerant rabbits, severe restraint, clinical thermometers	0·295 μg.	0·31
ED50/kg. in man	0·092 μg.	—

TABLE II

AVERAGE TOTAL DOSES OF P.T.P. NO. 1 IN MAN AT VARIOUS EFFECTIVE LEVELS

Percentage effective dose level	Dose in μg./kg.	Mean weight of experimental subjects in kg.	Total dose in μg.
5	0·0614	68	4·175
50	0·0920	68	6·256
95	0·1370	68	9·316

TABLE III

EXPECTED EFFICIENCIES OF B.P. AND U.S.P. TESTS IN DETECTING PYROGEN IN VARIOUS EXPERIMENTAL CIRCUMSTANCES WHEN VARIOUS VOLUMES OF SOLUTION CONTAIN A HUMAN ED5 OF P.T.P. NO. 1

Volume containing one human ED5 of P.T.P. No. 1 (4·175 μg.)	Expected per cent. frequency of failure to detect pyrogen							
	B.P. test				U.S.P. test			
	Method 1		Method 2		Method 1		Method 2	
	New rabbits	Tolerant rabbits	New rabbits	Tolerant rabbits	New rabbits	Tolerant rabbits	New rabbits	Tolerant rabbits
1,000 ml.	97·38	96·4	3·9	79·73	91·62	88·56	0·23	41·41
333·3 ml.	51·47	79·81	0·03	41·85	18·09	51·07	very small	12·40
111·1 ml.	2·89	42·45	very small	16.25	0.13	12·34	,,	2·10
37·03 ml.	0·01	11·68	,,	3·73	very small	1·12	,,	0·22
12·34 ml.	very small	1·44	,,	0·54	,,	0·05	,,	very small

a reaction in man, gives the probability of the pyrogen escaping detection *and* actually causing a reaction in man. This is only of theoretical interest because every injection is not tested: the usual commercial practice is to make a batch of injections and then to test a sample. Hence, even if a test will detect, with an efficiency of 99 per cent., a dose of pyrogen which will cause a response in 50 per cent. of human subjects, an occasional batch will escape detection and the probability of a clinical reaction to each injection from that batch will be P = 0·5. The batch producer is

more interested in ensuring that if the test lets him down occasionally it will only be in circumstances when the pyrogen is present in such small amounts that the incidence of reactions to that batch will be small. For this reason the results given in Table III have been limited to the frequency with which an ED5 in man will be missed by the B.P. and U.S.P. tests.

The results for the efficiency of the B.P. test are illustrated in Figure 3 and, for comparison, the efficiencies of the B.P. and U.S.P. tests in the best and worst conditions described here are illustrated in Figure 4.

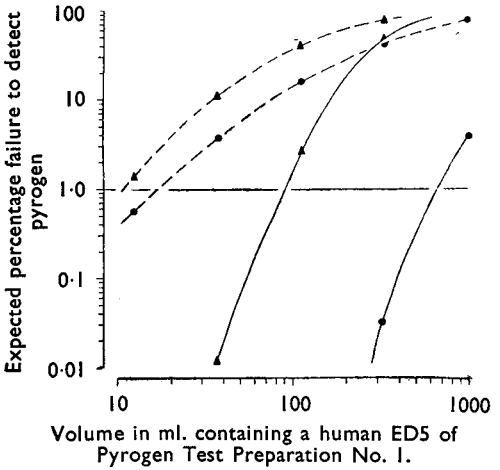

FIG. 3. Showing the expected efficiency of the B.P. test for pyrogens, when a human ED5 of P.T.P. No. 1 is contained in various volumes of solution. Percentage failures with Method 1—▲, and Method 2— ●. Solid lines—new rabbits; broken lines—tolerant rabbits.

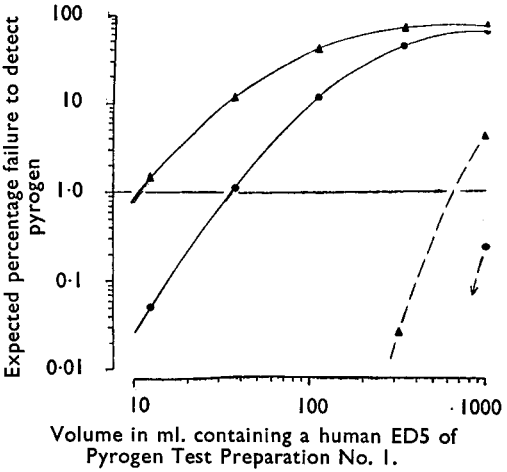

FIG. 4. Comparison of expected efficiencies of B.P. and U.S.P. tests for pyrogens in similar experimental circumstances. Percentage failures with B.P. test—▲, and U.S.P. test— ●. Solid lines—Method 1, tolerant rabbits; broken lines—Method 2, new rabbits.

DISCUSSION

From the ratios given in Table I it appears that with the criteria used the sensitivity of the rabbit may vary from one-third to 7 times that of man, depending on the experimental circumstances.

Lees and Levvy[2] found that an intravenous infusion which, in clinical use, was "usable but unsatisfactory" caused a mean rise of 0·93° C. when 20 ml. was given to each of 27 rabbits. An intravenous infusion containing an ED5 in 1000 ml. could reasonably be described as "usable but unsatisfactory". It is interesting to note that in the best experimental circumstances described here, when 10 ml./kg. of such a solution is given to rabbits (i.e. 0·042 μg./kg. of P.T.P. No. 1), the mean response is 0·94° C.

In the best experimental circumstances, the expected efficiency of the B.P. test in detecting pyrogen when one ED5 is contained in 1000 ml. of solution is about 96 per cent. The reasons for the efficiency being so low, with a dose causing a mean increase in temperature of 0·94° C., are (i) the very large variation in response of the rabbit and (ii) the very small number of rabbits used in the B.P. test. The expected efficiency

of the U.S.P. test in these circumstances is better than 99 per cent. Reasons for the greater efficiency of the U.S.P. test have been discussed elsewhere[4].

The results in the present paper are all based on the relationship between effective doses, in man and rabbit, of a bacterial pyrogen from one species only, namely *P. vulgaris*. Care must therefore be taken in generalising from them. There is, however, some evidence that similar results might be obtained with a pyrogen from another species—as witness, for example, those of Lees and Levvy with an unknown pyrogen.

The results reported here for one pyrogen obtained from a very common species, reveal that stringent test conditions are necessary to ensure detection of minimum effective human doses in large-volume injections. A general pyrogen test that will not ensure certain detection of this particular pyrogen cannot be considered efficient; and, as the ratios of activity in man and rabbit for pyrogens from other bacteria may vary considerably, it is even more important to use the most efficient test when pyrogen from unknown sources may be present.

Throughout this discussion it has been implied that a pyrogen test is adequate if it results in the detection of the maximum amount of pyrogen which, when administered by itself, will just fail to cause a reaction in man. Whether this assumption is justified or not depends upon whether pyrogens administered with other substances potentiate or antagonise their actions, and on whether the action of the pyrogens is affected by such substances. There is some evidence on these questions:

(*a*) Pickford (private communication) found that rabbits anæsthetised with urethane and given P.T.P. No. 1 remained unconscious for 2 or 3 days, and that many of them died without regaining consciousness. One of us (J.G.D.) has confirmed this result but has failed to get a lengthening of the duration of anæsthesia with pentobarbitone sodium.

(*b*) In 1937, Wien[5] demonstrated that rabbits with experimentally produced pyrexia failed to show any lowering of the blood-sugar level after 0·5 unit/kg. of insulin. It is well known, too, that the dose of insulin has to be increased when a diabetic patient has a fever.

(*c*) Hort and Penfold[6] observed that when poor pyrogen-producing strains of bacteria were cultured by passage through a rabbit they seemed to produce more pyrogen. From human blood samples which had proved unsatisfactory in clinical use, Probey and Pittman[7] isolated pyrogen-producing bacteria. They found that when these organisms were cultured in rabbit serum there was a tenfold increase in their pyrogenic activity. These observations suggest that the increased pyrogen production resulted from some factor in the rabbit serum. However, Farr, Lequire, Schork and Gayhart,[8] showed that when pyrogen is administered in homologous plasma there is a potentiation of the pyrogenic response compared with that when saline is the vehicle. The importance of these observations in relation to the testing of water for use in reconstituting dried human serum is obvious.

It appears, therefore, that pyrogens may interact with other substances in a variety of ways and, if this is substantiated, tests should obviously

aim, as far as possible, at the total exclusion of pyrogenic material from injection solutions.

SUMMARY

1. The scarcity and conflicting nature of the existing evidence on the relationship between effective doses of bacterial pyrogens in man and rabbit is pointed out.

2. The dose/quantal response curve for man to a pyrogen prepared from *P. vulgaris* has been determined and the intravenous ED50 for this preparation is estimated to lie between 0·084 and 0·101 μg./kg. (P = 0·95).

3. The dose/temperature response curves for the rabbit to the same pyrogen preparation, in a variety of different experimental circumstances, have been determined.

4. The relative sensitivities of man and rabbit to this pyrogen have been calculated on a weight-for-weight basis. Taking as the criteria of pyrogenic response (*a*) a rise of 0·6° C. in the temperature of the rabbit and (*b*) shivering in man, it is found that the rabbit is one-third to 7 times as sensitive as man, depending on the experimental conditions.

5. The expected efficiencies of the B.P. and U.S.P. tests, in detecting a human ED5 of Pyrogen Test Preparation No. 1 in differing volumes of solution, have been calculated.

6. Some evidence that pyrogens may interact with other substances has been discussed.

These experiments would not have been possible without the kind and patient co-operation of many of our students and we are glad to acknowledge their help. We are indebted to our colleagues, Drs. Barbara G. Brown and K. A. Exley, for help with the administration of the pyrogen. Part of the expenses incurred in this work have been met from a grant by the Medical Research Council to J.G.D.

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STANDARDS OF PYROGENIC ACTIVITY

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THE main feature of interest in so far as pyrogens are concerned, seems to be that they are a nuisance. Many of the papers published stress this nuisance value. These substances crop up in all sorts of materials