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

intended for use in man, and we have heard a lot about the difficulties of detecting pyrogenic contamination in such materials. Nevertheless, it should not be forgotten that the pyrogens themselves are extremely active substances and as such have a very considerable interest in pharmacology. While, therefore, I must first of all discuss the standardisation of pyrogens as contaminants, I propose to describe also the more interesting pharmacological effects of these substances.

My title, "Standards of Pyrogenic Activity," is intentionally ambiguous. The word "Standards" may thus be read as implying a specification of tests for the detection of pyrogens, or, alternatively, as implying unique standard preparations of pyrogens. Now let us consider very briefly the present position. At the moment, there does not exist any standard preparation of pyrogen, and control in the various countries throughout the world depends upon the specification of tests to control the level of pyrogenic contamination. All the various pharmacopœial tests 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, definite dose of the substance under examination. An attempt is made in designing these tests to adjust the dose of the substance in such a way that the rabbit will receive on a weight for weight basis a dose approximately equivalent to that normally given to man, but Dr. Dare has now produced the first convincing evidence that the existing pharmacopœial tests do in fact bear a definite relationship to the incidence of reactions in man.

Nevertheless, however well—given proper design—this type of test may work in practice, it implies, albeit tacitly, a return to a rabbit unit, a return that is, to an outmoded way of describing the contents of active principles in a drug, and as such it is open to the criticisms which for many years have been levelled against the use of all such animal units. These criticisms are now well recognised and depend upon the variability of the animals used for the test, both within themselves at any one period of time and within a colony by seasonal trend or other such variation; and, to my mind, many of the difficulties in assay which have been pointed out so adequately are merely a reflection of this criticism.

Thus, one colony of rabbits develops a tolerance to pyrogens on repeated administration of these substances; using another colony no such tolerance can be demonstrated. This discrepancy might be due to differences in dose levels, to different strains of rabbits, or to different conditions of housing the animals or carrying out the tests. What is important, however, is that such discrepant findings are probably inexplicable only because testing is not related to a standard preparation.

It was partly with these considerations in view that the Expert Committee on Biological Standardisation of the World Health Organisation decided to investigate the possibility of providing an International Reference Preparation of pyrogen. However, there are two other reasons for providing such a Reference Preparation, namely, the standardisation of preparations sold as hyperthermic agents, and the furtherance of basic

research on pyrogens—both being legitimate reasons for action on the part of the World Health Organisation.

There are many difficulties in deciding the type of material to be used for such a preparation. Pyrogens are elaborated by a large number of bacteria; it is not clear whether they represent a single chemical entity mixed with impurities of different kinds, whether they represent a family of substances of closely allied chemical structure, or whether, in fact, they may represent a collection of materials of widely different chemical structure, perhaps basically polysaccharides but with widely differing side substituents. This possible heterogeneity may well imply that difficulties will arise in assaying one pyrogen preparation against another because of differences in dose response lines and so on. It has, therefore, been decided as a first step in setting up such a reference preparation, to obtain two materials from different bacterial sources and to have them examined on a collaborative scale throughout the world, to determine the suitability of one or the other or both as reference preparations; the idea is that each laboratory should assay the one against the other, and also against any local laboratory pyrogen which they have been using as a working standard and whose behaviour in their own laboratory is well characterised. In this way it is hoped to obtain a large volume of evidence about the behaviour of pyrogens of different types under widely different conditions. This proposed scheme of study is still at an early stage of development. We have, however, obtained two pyrogens, one a crude extract of *Proteus vulgaris* prepared by my colleague Dr. J. H. Humphrey, and the second, a more highly purified preparation derived from *Serratia marcescens*, kindly provided for international use by Dr. M. J. Shear of Bethesda. Both of these materials have now been ampouled in suitable quantities and it is hoped that the study will begin very shortly. I am not, however, able to anticipate any of the probable results of this study, and there is consequently very little that I can usefully say about the International Reference Preparation of Pyrogens.

I would like, however, to mention a few ways in which an International Reference Preparation could be used to help to solve some of the outstanding difficulties we are meeting with to-day.

In the first place, the pyrogens obtained from different bacterial sources, show a widely different degree of activity. Thus, while a crude extract of *Proteus vulgaris* may be active in doses as small as 0·01 μg., a highly purified preparation from *Serratia marcescens* requires a dose of some 1 or 2 μg. to produce a response in the rabbit. Furthermore, different dosage-response relationships may also reflect differing kinds of pyrogens. It is perhaps not too sanguine to hope that comparisons of the potency and dosage-response relationships of the different preparations throughout the world against the common standard, may yield further evidence of the basic chemical structure necessary for activity.

In the second place, and perhaps the most important, the establishment of a basic standard for use throughout the world should, as I have suggested before, help to solve some of the difficulties which have arisen with varying sensitivity of animal colonies and with the design of routine

tests for pyrogenic contamination. And, finally, the provision of such a standard may well help to elucidate the mechanism of action of pyrogens quite apart from their control as contaminants in drugs designed for entirely different purposes. Thus, in my view, although enormous difficulties in the use of such a preparation will arise, all such difficulties may be pointers to the ultimate solution of the problem. This is in sharp contradistinction to the present position where the difficulties are the result of the lack of such a standard preparation and their occurrence in no way helps, and indeed hinders, the advance of our knowledge concerning these substances.

I would now like to turn to the research side and tell you a little of what we have been doing as part of a study of the mode of action of pyrogens. All the work which I report has been done in collaboration with my colleagues Dr. J. H. Humphrey and Dr. W. W. Douglas. In the first place, we were interested in the discrepancy, which I have already mentioned, between the tolerance apparently developed by some colonies of rabbits to pyrogens, and the lack of tolerance in other laboratories. It is known that pyrogens are themselves antigens, and it seemed to us possible that the tolerance was a reflection of the developing immunity to pyrogens. We have tested this point, using as our test preparation a very highly purified preparation of pyrogen made by Dr. Humphrey, who also made an antiserum to this pyrogen. Some of the results are shown in Figure 1.

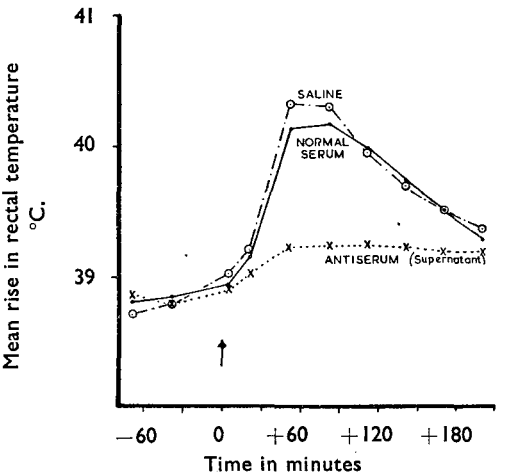

FIG. 1. Pyrogenic responses in rabbits. Each point is the mean of 3 animals. Each animal received at zero time, an intravenous injection of 0·02 μg. of pyrogen.

○—·—○ pyrogen solution in saline.

●——● pyrogen solution in normal serum.

× - - - - × pyrogen solution in antiserum. Precipitate removed by centrifugation.

It will be seen that a dose of 0·02 μg. of this material produces a rise of temperature of approximately 1·5° C. when given in saline solution, intravenously, to a group of 3 animals; each point on the curve is the mean of these 3 animals. If the pyrogen is mixed with normal serum instead of with saline solution, no change is detectable in the animal response. However, when the pyrogen is mixed with the anti-serum, it precipitates and the precipitate can be removed by centrifugation. When the supernatant is then given to a group of a further 3 animals, it produces

much less response. It is thus possible to remove, by an antibody-antigen reaction *in vitro*, almost all the pyrogen from the solution. Incidentally, we have examined this particular preparation fairly closely over a large number of months, in the same-rabbit colony, and Figure 2 shows the dose-response relationship which has been constructed for it. We do not claim that this represents a proper determination of such a dose-response line, since some points were determined on different occasions in time. Nevertheless, the responses of all these animals have remained remarkably constant throughout the last 18 months.

It is estimated from this dose-response line that the amount of the original dose of 0·02 μg. of pyrogen left in the supernatant in the experiment illustrated in Figure 1 was about 0·002 μg. —i.e., the antigen-antibody reaction *in vitro* has precipitated about 90 per cent. of the pyrogen. The threshold dose of this material lies in the neighbourhood of 0·005 μg. This dose of pyrogen was therefore administered to two groups of animals (Fig. 3). One group had received an injection of normal serum the previous day, and the other group had received an injection of the antiserum to this preparation at the same time. The dose of antiserum given was sufficient to neutralise *in vitro* 500 times the dose of pyrogen given the following day, and yet the response to the pyrogen is completely unaffected.

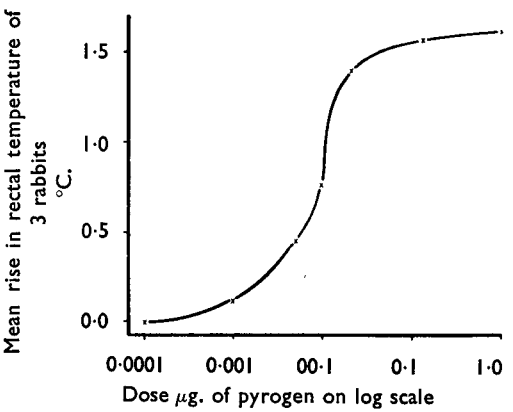

FIG. 2. Log.-dose response curve for pyrogen (same preparation as that used in experiment illustrated in Fig. 1). (N.B. The experiments were *not* all done concurrently.)

We are faced with two possible explanations. In the first place, the pyrogen, on administration, may set in motion the train of events leading to a rise in temperature very rapidly, i.e. before it has time to combine with the existing antibody. Alternatively, the antigen-antibody complex may itself be pyrogenic. In any case, one can conclude that the circulating antibody does not diminish the pyrogen response, and hence the antigenicity of pyrogens cannot play a part in the tolerance described.

Another factor of great interest in the study of pyrogens is the latency which exists before the onset of the rise of temperature. There are 2 possible explanations of this latency, which amounts to some 20 to 30 minutes. In the first place, pyrogens appear to have a central action. My colleague, Dr. Douglas, has shown quite convincingly that there is, for instance, no change in the degree of vasodilatation of a rabbit's ear unless the ear is sympathetically innervated; furthermore, if pyrogens

are given to animals whose ears are constricted in any case, the temperature still rises, on this occasion not by heat conservation but by heat creation —i.e., the animals start to shiver. If the action is a central one, it is possible that the latency is attributable to the blood-brain barrier; in other words, the pyrogen may require to gain access to cells of the central nervous system before producing an effect. To test this possibility, we have made use of the preparation devised by my colleagues Dr. W. Feldberg and Dr. S. L. Sherwood in the Division of Physiology at the National Institute for Medical Research. They inject drugs intraventricularly in cats through a metal cannula with its tip in the lateral ventrical and its base fixed in the skull. Through their kindness we were able to use one of their cats and to inject a dose of pyrogen by this route.

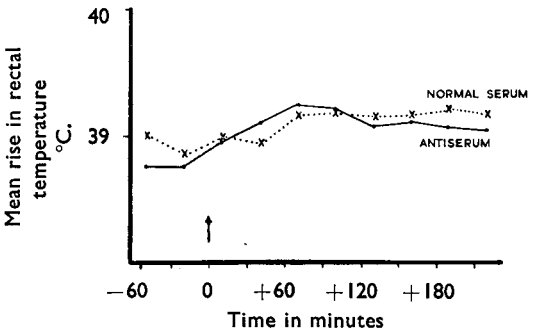

FIG. 3. Pyrogenic responses of rabbits. Each point is the mean of 3 animals. Each animal received at zero time, an intravenous injection of 0·005 μg. of pyrogen (same preparation as that used in experiment illustrated in Fig. 1). All animals were given serum 24 hours previously.

× - - - - × normal serum given.
●——● antiserum given (500 times enough to precipiate 0·005 μg. of pyrogen).

It is known that cats have approximately the same latency after

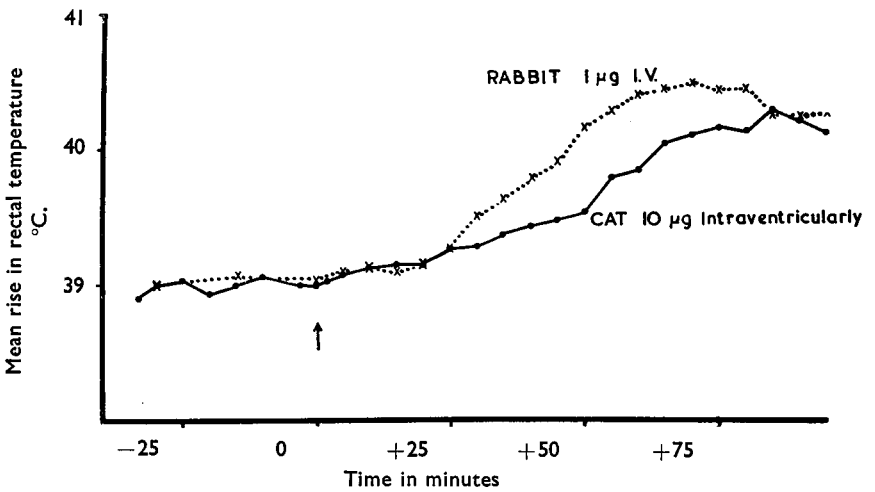

FIG. 4. Effect of route of injection of pyrogen. At zero time, 1 μg. pyrogen given intravenously to one rabbit (× - - - - - - ×) and 10 μg. of same preparation given into the lateral ventricle of one cat (●——●).

intravenous injection of a pyrogen as do rabbits. Yet Figure 4 shows that the pyrogenic response in this cat had the same, or perhaps an even longer latency than the pyrogenic response of the same material given intravenously in a rabbit.

The second possibility is that pyrogen as elaborated by the bacterium is not itself an active material but requires to be metabolised in the body before it can produce a typical pyrogenic reaction. A fair amount of work has already been done along these lines in the United States, where pyrogen has been incubated with plasma and reduction in latency has been obtained. Dr. Douglas and I have begun some experiments to study this point, and Figure 5 shows some very preliminary results.

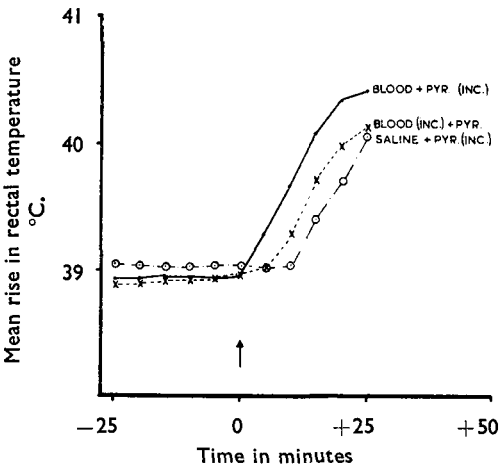

FIG. 5. Effect of incubation with rabbit blood on latency of pyrogenic response. Each point is mean of same 8 rabbits. Each animal received a dose of 1·5 μg. of pyrogen at zero time.

○—·—○ pyrogen solution in heparinized saline after incubation for 1½ hours at 39° C.

× - - - × pyrogen solution in incubated whole rabbit blood (heparinized) (incubation of blood for 1½ hours at 39° C.).

●——● pyrogen solution in whole rabbit blood (heparinized). The solution was incubated for 1½ hours at 39° C.

We have incubated pyrogen with whole blood, we have added pyrogen to incubated blood, and we have incubated saline solution containing the same quantity of pyrogen. It will be seen that pyrogen incubated with blood appears to have a shorter latency and perhaps a slightly higher activity than does the same dose of pyrogen given in saline. The work is still in progress.

I have not done any more than hint at some of the possible investigations that might well be pursued in the study of the mode of action of pyrogens. It will be obvious to all that these studies, carried out as they are in different laboratories throughout the world, would be much more comparable if the same reference preparation were used by all. Here again, is a very valid reason for the provision of an International Reference Preparation, apart from all questions of control.

DISCUSSION

DR. F. WOKES (King's Langley) said that Westphal and his colleagues (Westphal, Luderita and Keiderling, *Bull. Schweiz. Akad. med. Wiss.*, 1952,

8, 100) had obtained very potent preparations of pyrogen. Their experiments had a bearing on some points raised in the earlier papers. They had published the details of two highly purified pyrogens, one obtained from *Salmorella abortusequi* and the other from *E. coli*. In the *Salmonella abortusequi* preparation 80 to 83 per cent. of the total solids were sugars—glucose, lactose, rhamnose, glucosamine, 3 to 5 per cent. ribosenucleic acids, some phosphoric acid, a small amount of acetyl compounds—2 or 3 per cent. This seemed to be more complete data on composition than had previously been published. Both preparations were free from protein and amino-acids, and there was very little nitrogen. It appeared that the pharmacological properties were due to the polysaccharides. The most potent preparation obtained was effective in man at a dose level similar to those quoted earlier, 0·0 to 0·1 μg./kg. The lymphocyte response was found to be fairly parallel with the effect on temperature. Doses of the *E. coli* pyrogen were effective when administered subcutaneously as well as intravenously, and there had been experiments with oral administration. The preparations were not suitable for administration to very young persons.

MR. G. A. STEWART (Dartford) said that, using a preparation of pyrogen made by the method of Dr. Dare, he had observed an increase in sensitivity in female rabbits when the animals were injected each day for up to 6 days. After that with continued daily injections the animals became less sensitive. Male rabbits had not been tried. Had anyone else found that effect?

DR. DARE said that he had not given daily injections. Beeson (*J. exp. Med.*, 1947, **86**, 29), and also Tennant and Ott (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 614), said there was a progressive diminution from day to day, but none of them described any differences between sexes. Dr. Dare's experiments had been carried out on equal numbers of males and females at any one time, and he had not tried to analyse sex differences.

MR. G. MILNE (Glasgow) referred to Dr. Dare's comments on the effect of anæsthetics on pyrogen. From the point of view of blood transfusion work, the opposite effect was noticeable. Very few reactions to pyrogen in blood or plasma were reported while the patient was under the influence of an anæsthetic. If the transfusion was finished before the patient recovered consciousness, there was no evidence of any pyrogenic reaction. Sometimes, if the patient recovered consciousness while the transfusion was going on there was a typical pyrogenic effect. From the point of view of the 5 per cent. level in humans, which Dr. Dare mentioned, Mr. Milne quoted some figures from the transfusion service in Glasgow for the months of August, September and October 1953, in which 2800 people had been transfused. The incidence of pyrogenic reactions reported from the hospitals was 2·45 per cent. He had noticed that the incidence in plasma transfusion was lower. There were 69 instances of pyrogenic reaction, classified as severe rigor, rigor with rise of temperature, slight rigor, and rise of temperature only. The variation from individual to individual was striking. The temperature ranges reported varied from

99° to 103° F. Was there a satisfactory method for testing such protein-containing fluids as plasma and blood for pyrogen?

DR. PERRY said he thought that was what they were trying to find out.

DR. DARE commented on temperature responses during anæsthesia. The anæsthetised rabbit did not respond to injected pyrogen with a rise of temperature. It was possible that there might be other effects from pyrogen given during anæsthesia.

PROFESSOR TODD said Professor Westphal's results, which had been mentioned, agreed with many statements made during the discussion. He did not know whether they disagreed with some of the things he himself had said about antigenic properties. Professor Westphal gave figures for his results with intravenous injections at intervals of two days. He stated that tolerance took the form of the necessity to increase the dose at 2-day intervals to maintain the same temperature level. In a series of injections extending over 18 days at 2-day intervals it had been found necessary to increase the amount of pyrogen injected from 0·5 μg. to 2·0 μg. to maintain the increase in temperature. Was this a case of antigenic substances or of the machinery getting a little tired? Dr. Perry might, or might not, be able to decide whether this was antigenic response.

DR. J. H. HUMPHREY (Mill Hill) said that, at Mill Hill, it had been shown without doubt that antibody was formed against the pyrogen itself, but the fact that it was present did not prevent pyrogenic response taking place. The difference between tolerance and antibody formation had been forcibly brought to his attention 10 years before when he tried to keep a woman's temperature above 105° F. for 5 days by continuous intravenous infusion of typhoid bacilli. Although they started with a thin suspension they ended with something like milk, and yet she did not develop antibodies during that time. Could Dr. Dare shed some light on a report of recent work on the subject in the United States, in which it was stated that if thorotrast were given in sufficient doses—10 ml. of 25 per cent. suspension—any rabbit which had become tolerant to pyrogen lost its habituation and, furthermore, appeared to lose its tolerance to the Shwartzman phenomenon in which they were originally interested? They had used the pyrogen mentioned in Dr. Perry's paper to produce Shwartzman reactions once a week for about 14 weeks in a set of rabbits, by which time they had an enormous amount of antibodies circulating, but they still responded to approximately the same dose as that to which they originally responded. Thus in a different, but associated, phenomenon produced by the same sort of materials, there was revealed a similar ability to develop antibodies, without the antibodies being effective.

DR. J. I. M. JONES (Park Royal) asked for information about the reliability of the method of testing for pyrogen. He had a record of 10 rabbits used in a test in which the responses ranged from − 1·1° to + 2·7° F. The first 4 rabbits passed the B.P. test, the second 4 did not, and the last group did. If anybody had had wide experience of the reliability of the tests, perhaps they would give him some information.

Dr. DARE said he had no experience of negative response, but the standard deviation of a single observation carried out on rabbits, in circumstances where no allowance could be made for between-rabbit variation, was between 0·3 and 0·35°. In other words, when using only 3 rabbits, the mean response could vary within very wide limits. A response, which should be about 0·9 was liable to vary under any particular set of experimental conditions within normally accepted limits of 0·5 and 1·25. Thus for individual responses they had a very large variation which could embrace all Dr. Jones's suggested figures, except where he had a negative response.

DR. TIGHE (Oxford) said because of numerous transfusion reactions promethazine was being given before blood transfusions. Did the anti-histaminic drugs neutralise or in any way act on pyrogens?

MR. WHITTET said chlorpromazine which is closely related chemically to promethazine was similarly used to produce hypothermia. He had noted a slight fall in temperature with Dare's pyrogen preparation occurring shortly after the injection was made; it was followed by the usual rise 20 minutes later.

DR. DARE replied that if the response of a number of rabbits was plotted in a control experiment a certain amount of irregularity in the observations was seen. Taking observations from the injection time, he usually found a slight increase for 3 or 4 minutes and then a slight fall, and then it began to follow the usual path. This happened whether pyrogen was given or not: it seemed to be concerned with the mechanics of injection.

MR. WHITTET agreed that this was so, but added that with Dr. Dare's preparation of pyrogen the fall was more marked than otherwise.

DR. DARE replied that he had not observed that. Reverting to the discussion of Shwartzman reactions, the issue might be simplified by the recent publication of Bennett and Beeson (*J. exp. Med.*, 1953, **98**, 493) who had described the isolation of what they called "pyrexin" from leucocytes and pyrogen. Their suggestion was that pyrogen was released from the leucocytes.

Dr. HUMPHREY said that astonishing changes in the leucocytes and in the blood stream were observed after injecting these substances. One of the effects, when anything like a large dose—2 μg. or 4 μg.—intravenously, was used, was to bring the temperature down instead of sending it up. When that happened the rabbits had a very prolonged fall in blood pressure, which might last for 16 hours or so. Had any blood pressure measurements been taken in the human experiments, before the humans became so unpleasantly ill that the experiments had to be terminated?

DR. DARE replied that blood pressure measurements were taken. They had examined as many subjects in each experiment as could conveniently be done in 15 minutes, so that observations could be repeated every 15 minutes. No difference was found in blood pressure, but they did not take the blood pressure after the reactions because many of these were so violent that they had to suspend operations and administer aspirin and

cups of tea. They found no differences in pulse rate or in bleeding time and no difference in the blood pressure up to the time of the onset of the obvious symptoms.

MR. A. L. BACHARACH (Greenford) said that normal human subjects had been used in Dr. Dare's experiments. How would it be possible to obtain evidence on the effect of pyrogen on sick persons who, after all, were the only people who were likely to be injected with pyrogenic material in quantity?

DR. DARE said it did not seem justifiable to give pyrogens to sick people to see what happened.

DR. HODGES said he was interested in the remarks about the differences in pyrogenic response between animals anæsthetised with urethane and those anæsthetised with pentobarbitone. Was this because the barbiturates had a specific depressant effect on the heat-regulating centre.

DR. DARE said pentobarbitone was chosen because according to Dr. Pickford's first report (personal communication) every rabbit died. He found that if the animals were nursed carefully over the first 2 or 3 days some recovered. It seemed that because of prolonged anæsthesia the animal died from lack of food and water or the general condition resulting from the continued anæsthesia. He tried pentobarbitone to see whether with a different and short-acting anæsthetic the duration of anæsthesia after giving pyrogen would be extended or not. Doses as great as 10 μg./kg. of pyrogen were given at intervals of up to 30 minutes after the administration of the anæsthetic, but no effect on the duration of the anæsthesia was found.

Dr. G. BROWNLEE (London) asked about recovery rates from anæsthesia in rabbits with 1·4 g./kg. Urethane gave a fall of blood pressure and a low recovery rate, whereas the barbiturates, particularly some of them, gave a reasonable blood pressure and a good recovery rate.

DR. DARE said with the barbiturates the dose was 35 mg./kg. and with urethane it was 1·4 g./kg. In 10 rabbits which he urethanised, 4 out of the first 5 died and 2 of the second 5 died. Very careful attention was given to nursing in the second group, the animals being wrapped up and kept warm. The anæsthesia in rabbits which had pyrogen and recovered was about 24 to 36 hours longer than in the controls which recovered.

MR. P. J. FOWLER (Bristol) asked whether there were any thermostable pyrogens and, if so, approximately what proportion of normally occurring pyrogens were thermostable?

DR. DARE said that it was generally accepted that pyrogens were thermostable. Because he did not want to run the risk of the solutions of pyrogen becoming contaminated with pyrogen from unknown sources he autoclaved the solutions for 30 minutes. He did not know whether there had been any destruction of his pyrogen preparation but it was still very active. Whether some pyrogens were thermolabile and destroyed in autoclaving he did not know.

PROFESSOR TODD, on the question of thermostability, referred to a paper read at the British Pharmaceutical Conference in 1948 (Wylie and Todd, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 240). It appeared that one source produced pyrogen which seemed to be relatively stable. It was a fact that the rate of destruction fell off very rapidly.

CHAIRMAN'S CLOSING ADDRESS

DR. COLLIER said that judging from the discussion at this and previous meetings the subject of pyrogens lent itself to debate and he was surprised that there was not more warmth in the argument. The variety of the opinions expressed showed the uncertainty of the state of our knowledge. From the excellent papers presented and from the discussion they might conclude that much remained to be established.

He emphasised, as had been said by Professor Todd and Dr. Perry, that they were interested in pyrogens for several reasons. First, for the reason which Mr. Whittet had so forcibly outlined—that they represented an important group of contaminants which, if included in medicaments, might have serious effects on patients, secondly, they might be interested in pyrogens as therapeutic agents, thirdly, they were interested in their biological actions, or how they raised the body temperature, when given in such extraordinarily minute doses as those mentioned by Professor Todd, Dr. Perry and Dr. Dare.

He turned first to the most important practical question. How were pyrogens to be excluded from preparations administered to man? This was only one aspect of a larger question—how were they to exclude all the toxic products of microbial contamination from such preparations? It seemed possible that some microbial toxins were not pyrogenic; and this probability gave especial value to the work such as that of Miss Dawson and Professor Todd on other biological responses to bacterial toxins. For practical purposes, however, there was good reason to concentrate on pyrogens as the most important contaminants produced by microbes because of their commonness and their serious effects on the human body. When they did so they were faced with everyday problems which had been presented so calmly by Mr. Smith and commented on so provocatively by Dr. Dare.

He would concentrate on four of these practical problems, drawing on what had been said during the discussion and possibly from his own experience. In doing so it was worth stressing that in practice they were probably almost always presented with an uncertain mixture of unknown pyrogens.

The first question was that of tolerance or, put another way, how often should they use their rabbits? The results of Dr. Dare's researches were such as to cause anxiety in any laboratory doing the ordinary B.P. test—anxiety that in the near future they might have to use still more rabbits and change the stock still more often.

Secondly, what was the best form of test? Dr. Dare had provided convincing evidence that the U.S.P. test was more critical than the B.P. test. He did not think it could be denied that a test based on the individual

responses of 5 rabbits was more likely to reveal pyrogenicity than a test based on the mean responses of three. If they were to adopt the U.S. test, that meant more rabbits.

Mr. Smith had revealed that, in practice, pyrogen testing might be carried out on still fewer rabbits than the B.P. test and not miss serious pyrogens. They must ask themselves, why did it work? The answer might be that the routine pyrogen test carried out in many places was really a test of the efficiency of the production methods. It was the whole method of production which was being tested, and if they could test 24 rabbits on 12 samples, 2 rabbits on each sample, they were putting 24 rabbits to test the efficiency of the production method. That was likely to produce pyrogen in all or none of the samples, and for that reason they were detecting failures of the whole method. The test was therefore much more likely to find failures than one would imagine if one thought of it as a single test of a single solution. In practice, with certain exceptions, manufacturers found that the pyrogen test prevented them from issuing solutions which were seriously pyrogenic.

This led to the third question—what was the relation of the responses of rabbits and those of man? Dr. Dare had dealt extremely thoroughly with the matter and his results were reassuring in that if rabbits were tested in the optimum way they appeared to be more sensitive than man—and by "optimum way" he meant the way which Dr. Dare himself recognised.

The fourth problem was one which had scarcely been mentioned during the discussion, but which had been met in testing solutions of calcium gluconate in his laboratory, and which Douglas and Paton (*Lancet*, 1952, **202**, 342) had referred to in connection with tests on adrenocorticotrophic hormone. What if the pyrogenicity of the fluid, but not the toxicity, were masked by an antipyretic substance? They had found that calcium gluconate was antipyretic, and an injection of about 0·6 mg./kg. would lower the body temperature of rabbits by about 1° C. This was a serious difficulty in testing calcium gluconate solution. It might be that the pyrogenic response was masked, but that when the solution was injected into man other possible responses, such as malaise, were seen. It might also be that the antipyretic action of calcium gluconate was not seen in man. It was such a problem as that which gave special value to the work done by Professor Todd and Miss Dawson, because it might be in such a situation that the leucocyte type of test would be more informative than a pyrogenic test.

There was one more possible problem to which he felt sure they should pay attention. It was possible that in the future—not the distant future—they would have to deal with the threat of myxomatosis in the rabbit colonies. It might be difficult to keep the colonies and, if they could keep them, it might only be at the expense of seriously increased precautions to protect them. That would make rabbits still more expensive and valuable. That could be added to what Dr. Dare had said about rabbits becoming easily tolerant to pyrogen. In any case, rabbits were expensive. One wondered whether the Gordian knot might not be cut by devising

a mouse test for pyrogen. Five mice of good strain could be used in each test, injecting each with 0·5 ml. of test solution by the tail and measuring their rectal temperatures. Mice would be relatively cheap and could be discarded after the test to avoid any question of their becoming insensitive. A relatively large dose—25 ml./kg. could be injected. This sounded attractive; but he realised that it might well be unpractical as the possibility had already been partly explored.

In conclusion, it was a great pleasure to thank all who had contributed to the symposium. He thanked, too, those who had contributed to the organisation, the authorities of University College and Professor J. Z. Young for making possible the use of the theatre, as well as Mr. Whittet for helping him to make the arrangements.

The proceedings concluded with a vote of thanks to the Chairman for his work as Chairman of the Biological Methods Group.