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Chairman, 1955

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BACTERIAL PYROGENS

FOREWORD

DR. SEIBERT: "I started to work with the pyrogen back in 1923 and I can really say that I have never found any more difficult work than the work with pyrogen. In fact, I used to call it my little blue devil because it was there and wasn't there. I was impressed with the elusiveness of it, and the fact that it might be everywhere. It appears in all your flasks, all your water and in everything you work with. I am wary of the possibility of contaminating what I am working with, with a pyrogen. I have to wash all my glassware with freshly distilled water, make all my chemical reagents up with freshly distilled water, and I have to use special filters in order to eliminate the pyrogen.

Pyrogens exist in very small concentration and give such a tremendous reaction. I am so much impressed with all this work that is being done, but I wonder, has it been done that carefully? Are some of these pictures that you get mixtures, due partly to what you are giving but also due to contaminants?"

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I AM going to speak to-day about bacterial pyrogens. I am doing so because recently they have attracted renewed interest as therapeutic agents. I have said "renewed" because in different forms they were used with some success earlier in this century for the treatment of a number of disorders. I would also like to speak about them because I have had many tussles with them, especially in blood transfusion work, and many of the problems encountered then are still not solved. I feel too that in this country we are thinking too much about their nuisance value and not enough about their potentialities in the treatment of disease. I would like to discuss their potential value as non-specific therapeutic agents and to show that we have now reached the stage where bacterial pyrogens in pure form can, with advantage, replace the older materials and methods for producing a general stimulation of the defence mechanisms of the body.

In this country great interest used to be taken in the therapeutic uses of materials such as typhoid vaccine, used non-specifically, which were of value in the treatment of certain diseases although they had some disadvantages such as uncertainty of action and undesirable side effects. But during the last 30 years the older preparations have gradually been falling out of use and the flood of modern chemotherapeutic agents has hastened this process. Like many another old remedy they are again exciting interest, especially in the United States of America, and on the Continent, since their active principles have recently been isolated and purified and their effects and dose can now be readily controlled. The undesirable side effects are almost entirely absent in the new forms.

I should like also to consider the nature and chemical structure of these purified pyrogens so far as it is known and their behaviour when injected into the body and then to make brief reference to their uses in the treatment and amelioration of a variety of disorders.

I shall not be able to devote any time, to the purely pharmaceutical problems which these substances present as contaminants in parenteral preparations. This was the subject of a recent Symposium on Pyrogens[1].

Bacterial pyrogen appears to be capable of effecting, safely and rapidly, a general mobilisation of the body defences to an extent seen only when the body has been insulted by the harmful effects of trauma, infection and other forms of injury.

The stimulation and mobilisation of body defences which follows bacterial infections or trauma, or the injection of irritant substances, or excessive heat or cold has for long been known to produce a state of alarm and stress in the animal body. No complete understanding has yet emerged of the complicated "chain-reactions" which Selye has, perhaps too simply, called the "alarm reaction[2]." The substance or condition which produces it has been called the "stressor[2]." The same or similar effects to those produced by the injection of bacterial pyrogen are produced by a variety of stressors. Pyrogen differs from the others in that its effects are produced without disagreeable or unpleasant aspects such as being ill or injured. One would hesitate to induce a general stimulation by infecting a sick person with an organism causing malaria or other disease to alleviate the patient's sickness if another less drastic method were available, or to inject an intensely painful and irritating substance like turpentine or sulphur deep into a muscle, but this is still practised in certain places for the benefit to the patient which ensues in certain disorders[3]. These general methods of stimulating the body's defensive mechanism, including the injection of bacterial pyrogen, are forms of nonspecific therapy as opposed to specific therapy seen in the use of diphtheria antitoxin to treat diphtheria.

One of the forms of non-specific therapy practised shortly after the beginning of the century was called "protein shock," because it was believed that protein when injected was capable of acting as a non-specific stimulating agent or stressor. I first became acquainted with this form of therapy when working with the late Professor Ralph Stockman in the 1920's. Stockman was a great clinician and research worker, and many people besides myself are grateful for his influence at the formative period of our lives. Professor Sir David Campbell of Aberdeen University, one of our guests at this Aberdeen Conference and the President of The General Medical Council, was one of Stockman's lecturers at that time, and he also was interested in protein shock therapy and published at least one paper on its use in the treatment of rheumatoid arthritis.

At that time it was believed that almost any protein from almost any source was effective, and this led to the injection of milk protein, tumour extracts, horse serum and many others. It is believed now that the stimulating effect of the injection of protein from many different sources was really the result of contamination with bacteria or their metabolites

and was, in fact, a reaction caused by bacterial pyrogen. Ordinary household milk was a popular source of protein for shock therapy at that time as it was easily available, and one writer[4] describes how he obtained better effects by the injection of "market" milk. This supports the view which was even then gaining favour, that the effect was the result of bacterial contamination, especially when it was later shown that protein from milk obtained aseptically was not pyrogenic and was not effective.

It is not easy to prepare protein material or derivatives such as blood plasma or protein hydrolysates or even milk for intravenous injection without bacterial contamination and it is no reflection on the competence of the earlier workers to say they were mistaken as to the agent causing the reaction. Simple protein as such does not produce a specific stimulation although it does possess its own special effects. But we must not dismiss protein altogether from our picture since some bacterial proteins may function as carriers of the pyrogenic grouping under certain conditions which we shall discuss later. Anyone interested in this period when protein shock treatment was at its zenith can read about it in a book published by Petersen[4].

Bacterial vaccines, notably typhoid and TAB, were also used in this connection as a protein source with surprisingly good results; but it was not then suspected that what is now believed to be the active component of the vaccines, namely bacterial pyrogen, belonged to the same group of substances as those which at that time were causing trouble in injection fluids—substances to which Hort and Penfold had drawn attention in 1912[5–7], and which Seibert[8–11] was investigating in the early 1920's. It is now tolerably certain that the active substance in our vaccines and pharmaceutical injections and the very active substance now being supplied for clinical trials are the same, or differ in minor characters only.

By means of any of the agencies we have mentioned as well as by physical methods and tissue injury, many bodily changes including high fever can be produced. These are accompanied first by a fall in the white blood cells which is called a leucopenia, then by an increase in the white cells called a leucocytosis, and by other changes which are characteristic of the "alarm reaction" of Selye. We are chiefly concerned to-day with bacterial pyrogen and its various properties, but before finally leaving these other methods of stimulation I would like to refer again to the use of sulphur or turpentine injections.

Menkin[12–14] and Abderhalden[15,16] have shown that there exist in body tissues and cells, endogenous substances which are capable of causing the characteristic fever and white blood cell changes produced by bacterial pyrogen. To distinguish our bacterial pyrogen from the endogenous pyrogen of the body tissues, we usually refer to it as "exogenous pyrogen." We do not know what relation, if any, exists between our exogenous bacterial pyrogen and the endogenous factors of the body as described by Menkin and Abderhalden but it is widely felt that either they or other endogenous substances must be concerned.

When, for example, sulphur is injected into a muscle a great deal of local

inflammation and œdema is produced and local cell damage is caused resulting in a high and prolonged fever accompanied by the blood cell changes already mentioned. A distressing feature of this method is the great pain and discomfort caused. It appears here as if some endogenous pyrogen arising from the damaged tissue cells or white blood cells had been liberated after the injection. It is possible, therefore, to inject an extremely irritating substance either intravenously or intramuscularly, and so to reproduce the effects characteristic of a highly pyrogenic reaction when in fact no pyrogen is injected at all. How many of the reported reactions which had stimulated Hort and Penfold and Florence Seibert to study pyrogenic reactions in injections and which led to the conception of bacterial pyrogen, previously described by various names such as "injection" fever, "salvarsan fever" and so on, were in fact due entirely to pyrogen and how many to the irritation of the medicament or the method of injection. We must not, of course, exaggerate this point, but it is perhaps worth remembering as it may sometimes explain an unexpected reaction.

In this connection Dr. Favez[17], head of a large tuberculosis clinic in Lausanne, has described the effect of PAS when given in massive doses by vein, as is the practice in Switzerland and in the west of Scotland in the treatment of tuberculosis. Favez's patients were so much benefited by a stimulating side effect of his undoubtedly non-pyrogenic material that he conceived the idea of the simultaneous administration of a purified pyrogen to increase and extend even further this effect. In certain types of tuberculosis he obtained highly beneficial results.

It is generally held that stimulation therapy is contra-indicated in tuberculosis patients since it often liberates dormant organisms from resistant foci which can be a dangerous procedure. This view, however, belongs to the period when the chemotherapy of tuberculosis was much less advanced that it is to-day. In any case, Favez is convinced of the value of the method and has much evidence to support his view. It is known that bacterial pyrogen has a fibrinolytic action[18] and Favez is of the opinion that this fibrinolytic effect may bring about the liberation of the tubercle bacilli from resistant foci, so exposing them to attack by chemotherapeutic agents which otherwise would be ineffective.

Pyrogen has effects other than the production of fever. In fact for therapeutic purposes the title is no longer very suitable, and its retention is justified only because any change would cause confusion. Westphal in Germany calls it "Reizstoffe" or "irritating substance". In fact, from recent clinical reports it appears that in a great many cases the pyrogenic (thermal) effect is unnecessary and undesirable, and it has become the custom either to suppress the fever by the administration of antipyretics or by using a smaller dose. In any case fever is only one of the effects produced. But perhaps if we do not take the name too literally it is on the whole better to retain it if only for the sake of tradition.

Before going on to consider the source and nature of bacterial pyrogen let me conclude this section by saying that there is a great deal of published evidence to support the view that the older methods and materials used in

non-specific therapy had many virtues. Now, with the advent of the purified active principles capable of exact dosage and predictable effect, it is possible to reassess the value of pyrogen in medical treatment. I do not think we can ignore its possibilities.

The Source and Nature of Bacterial Pyrogen

All the evidence suggests that only the Gram-negative organisms need be considered as fruitful sources of the pyrogenic and stimulating substances we have been discussing and that the pyrogen is associated with the endotoxin. If Gram-positive organisms are killed by heat they exert little or no pyrogenic action, whereas either alive or dead the Gram-negative bacteria have a powerful action when injected[19]. In general the Gram-positive types allow soluble exotoxins to pass into the medium whereas the Gram-negative types retain the complete endotoxic principles in or on the cell surface and only soluble fractions including pyrogen are found in the medium[20].

The endotoxin was first extracted in undegraded form by Boivin and his colleagues[21–24]. Since then it has been further studied by many workers who approached the problem chiefly from the immunological and biochemical aspects and were not concerned with these substances as sources of pyrogen. Later, groups of workers examined the water-soluble fraction to study another curious property, that of causing necrosis or break-down in tumour tissues, a property of bacterial extracts which had been known for many years. The endotoxin exists in all types of Gram-negative organisms so far investigated and, in practically all, the general structure and properties are very much the same.

Its characteristic properties are not destroyed by heating in water at 100° C., and it thus differs sharply from the exotoxins of the Gram-positive forms which, with few exceptions, are quickly inactivated by heat.

In most Gram-positive organisms the exotoxins are largely composed of protein, which readily suffers denaturation, whereas Boivin found that the Gram-negative endotoxins are complexes of polysaccharides and other constituents. Immunologically they behave as the dominant O-somatic antigens and because of their toxicity they were originally called bacterial endotoxins, so that either name may be met. In far-reaching researches into the nature of this antigenic complex, Morgan and Partridge[27] showed that it consists of a complex of protein, active lipopolysaccharide and inert lipid.

Goebel and others[30] had found that Flexner dysentery organisms, which are also Gram-negative, yielded a strongly antigenic and toxic endotoxin which went into solution in pyridine and water. Palmer and Gerlough[25] devised the useful phenol process of deproteinisation which, in modified form, has in recent times yielded such valuable results in the hands of Westphal, Luderitz and their colleagues in Germany.

The pioneer endotoxin work of Boivin[21–24], Morgan and Partridge[27], Miles and Pirie[28,29], Goebel[30] and many others, paved the way for recent workers such as Westphal, who studied these substances mainly as sources of pyrogen, and for others such as Shear[32–34], who studied them because

of their tumour-necrotising action. The result of the work of this group made it clear that most Gram-negative organisms contain a similar complex made up of a protein, a toxic factor bound to a polysaccharide and an inert lipid of the cephalin type. The toxic factor, which appears also to contain phosphorus, is the factor in which pharmacists are chiefly interested, as it appears that this substance is mainly responsible for the pyrogenic and the other related effects. The toxic factor when isolated from the bacterial complex seems to be attached firmly to the polysaccharide, which is therefore described as a lipopolysaccharide. The toxic lipid, usually found firmly bound to the polysaccharide, is different in structure and properties from the inert lipid previously mentioned which is not at all toxic, and it is also a more complex substance. The whole endotoxic complex appears to constitute, or to be closely connected with, the surface of the bacterial cell in smooth varieties, the polysaccharide moiety resembling the capsular membrane of the pneumococcus in this respect. The amount of the lipopolysaccharide appears to vary in R-forms of the organism and this seems to be devoid of the O-specific characteristics. These lipopolysaccharides from the R-forms are almost as pyrogenic as those from smooth forms but are devoid of some sugars especially the chromatographically quickly moving desoxy-sugars. It appears that a certain amount of toxic lipid is synthesised which is bound to the polysaccharide; if this is not synthesised in sufficient amount some is bound to the protein instead, giving lipoprotein. Smooth forms have been found which contained toxic protein as well as lipopolysaccharide and on the other hand R forms have been examined which contained besides toxic and pyrogenic protein, variable amounts of lipopolysaccharide[57].

It is an oversimplification therefore to say, as is often done, that pyrogens are lipopolysaccharide, since in rough forms of the organisms the pyrogenic constituent can be separated along with the protein and the polysaccharide constituent is present only in small amount. We can, in fact, extract from R-forms a pyrogen which is associated with the protein of the R-types of organisms. This pyrogenic protein is, however, much less active than the pyrogenic lipopolysaccharide, suggesting that the degree of activity is related to the particle structure and that protein is a less suitable carrier for the activity-conferring lipid than is polysaccharide.

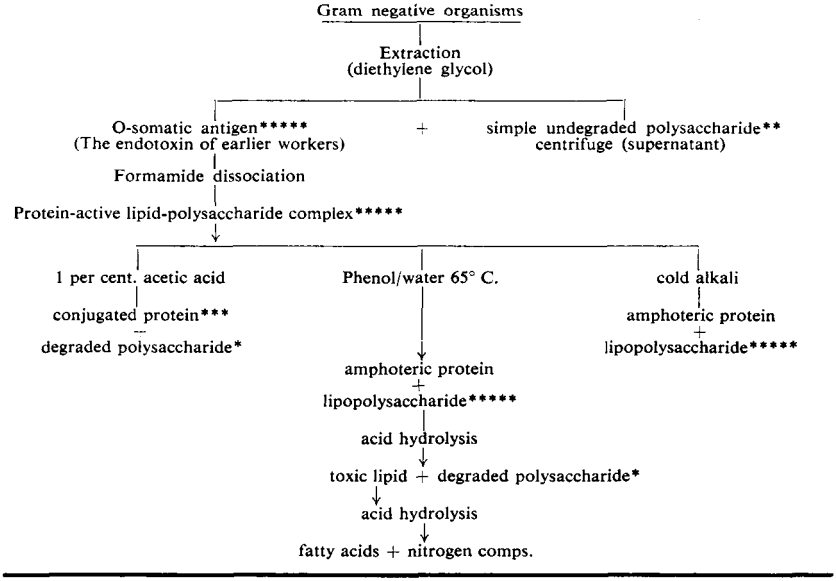
This is also shown if the toxic lipid is separated by acid hydrolysis from either the protein or the polysaccharide when it occurs as a fatty or waxy substance very insoluble in water but soluble in chloroform, and, is not active for the purely physical reason of insolubility. Westphal[35] has shown that if the separated lipid is dispersed by means of a surface-active substance such as Tween, it regains some, but not all, of its activity. The removal of this lipid from the bacterial complex removes also the toxic and pyrogenic properties leaving either degraded polysaccharide or simple amphoteric protein.

If the bacterial endotoxin, which is composed of a complex of bacterial protein, lipopolysaccharide and inert lipid, is split by the method of Goebel by hydrolysis in alkaline alcoholic solution, we obtain products, one of which is a toxic lipopolysaccharide and the other a non-toxic

protein; whereas with gentle acid hydrolysis we obtain a toxic protein and a degraded non-toxic polysaccharide. We see from this that the toxic pyrogenic factor may occur along with a polysaccharide or a protein carrier, according to conditions. These reactions are summarised in Table I.

TABLE I

THE RELATION BETWEEN THE PROPERTIES AND ORIGINS OF BACTERIAL PYROGEN



Increase in pyrogenic potency expressed by increased number of asterisks.

TABLE II

THE RELATIVE TOXIC, ANTIGENIC AND PYROGENIC PROPERTIES OF THREE POLYSACCHARIDES FROM *Shigella dysenteriæ*

Degraded polysaccharide	..	Non-toxic, non-antigenic	Pyrogenic, 2–5 μg./kg.
Undegraded polysaccharide	..	Poorly toxic, weakly antigenic	Pyrogenic, 0·05 μg./kg.
Lipopolysaccharide ..	..	Toxic, weakly active in producing agglutinins or precipitins in rabbits. Strong heterophile (Forssman), antigen	Strongly pyrogenic, 0·002 μg./kg.

Davies, Morgan and Record[106].

Workers who have investigated the problem with a view to isolating a pyrogenic factor generally isolate the toxic lipopolysaccharide, largely because of the methods adopted and the fact that the lipid fraction is firmly bound to the polysaccharide. Recently (June, 1955), Davies, Morgan and Record have separated from *Shigella dysenteriæ* a polysaccharide in three forms (Table II). The first, a degraded form with a molecular weight of about 25,000, which proved to be non-toxic, non-antigenic but was pyrogenic in relatively large doses. The second, an undegraded

polysaccharide, extracted from the organism with diethylene glycol, with a molecular weight of the order of one million. This material was poorly antigenic but was pyrogenic in doses of 0·05 μg./kg. The third was a lipopolysaccharide isolated from the protein-polysaccharide complex with phenol. The lipopolysaccharide was of very large particle size and was a powerful heterophile (Forssman) antigen, but was only weakly active in the production of specific agglutinins and precipitins in rabbits. This lipopolysaccharide appears to be of the same order of pyrogenic activity as the pure lipopolysaccharides of Westphal.

It is possible to transfer the lipid to other carriers by a method devised by Morgan[36,37], who showed that artificial complexes could be made by coupling the active lipopolysaccharide or the conjugated protein of dysentery or typhoid organisms to a variety of substrates such as agar or mucin or to proteins such as vitellin or serum globulin. Westphal[35] finds that such coupling only occurs when the toxic lipid is present, and has succeeded in transferring the lipid to a casein carrier, so producing a highly active artificial pyrogen.

Little is known about the nature of the pyrogenic substance in pharmaceutical solutions. The substance must be present in a very active form since the few bacteria originally present are represented only by their soluble by-products, usually much diluted. The active substance may be the lipopolysaccharide already described, or perhaps a more active form containing the active grouping favourably presented by a suitable carrier.

Co Tui[38] was the first worker to attack this problem from the angle of pyrogenic activity. He succeeded in isolating from aqueous cultures of the dead bacterial bodies, a polysaccharide substance which was free from protein and had high activity; but the greatest amount of light yet thrown on this subject has undoubtedly come from the work of Westphal and his colleagues in Germany and Switzerland during the last year or two. They improved Palmer and Gerlough's phenol process by extraction with water and phenol at the high temperatures when they are miscible; these separated on cooling to an aqueous phase containing lipopolysaccharide and nucleic acid and a phenol phase containing bacterial protein and inert lipid. Westphal has in this way produced what is probably the purest and most pyrogenically-active polysaccharide yet extracted[39–42]. From the material obtained by the phenol extraction of the acetone-dried bacterial bodies he separated the active substance from the nucleic acid by fractional precipitation with alcohol, taking advantage of the nucleic acid absorption at 258 to 260 mμ, and finally by purification using the preparative ultracentrifuge. He found that this method was applicable to all the Gram-negative organisms he examined.

He and his colleagues were able to show that the lipopolysaccharide is electrophoretically homogeneous with a molecular weight of about one million. Other workers examining active polysaccharides from similar organisms have given values of up to 10 million. It is probable that molecular weight is largely influenced by the condition of extraction and degree of polymerisation which has occurred.

There is a need for a standard pyrogen, and at present Westphal's

preparation is probably the most suitable for this purpose and could well be adopted until further knowledge is forthcoming about the nature of the active fraction or active grouping. Workers in America[43,44] have also produced highly purified preparations, but according to our measurements of relative potency it is doubtful if those we have been privileged to examine approach the activity of Westphal's preparation. The great difficulty lies in separating the nucleic acid. It has been shown that Westphal's *E.coli* lipopolysaccharide contains about 40 per cent. of active lipid tightly bound to the polysaccharide carrier. The polysaccharide portion from the lipopolysaccharides of Gram-negative bacteria is composed of amino-sugars including glucosamine and chondrosamine with pentoses and methyl pentoses. The amino-sugars are acetylated and phosphorus is bound to the toxic lipid in small amount, and to the polysaccharide in greater amount, probably in the form of esters. The complete structure of the active lipid is still unknown, but it contains phosphorus and is a phospholipid. The active lipid can be separated from the undegraded polysaccharide by hydrolysis with dilute mineral acid as was shown by Miles and Pirie and other workers with the O-antigen of brucella some years ago.

Niemann[45–48] has examined a pyrogenic lipopolysaccharide isolated in a study of the tumour-necrotising action of a strain of *E. coli*, and has shown the presence of a number of fatty acids such as lauric and myristic along with glucosamine, ethanolamine, phosphoric acid and a curious substance not hitherto reported which consists of a paraffinoid chain with two substituting amino groups. He calls this substance "necrosamine" and this may prove to be of great pharmacological interest. It is seen that we are on the verge of interesting discoveries and that there remains a great deal of work still to be done.

Westphal has endeavoured to produce active preparations for sub-cutaneous use by acetylation of the hydroxyl groups of the polysaccharide, and many other interesting attempts to modify the molecule with a view to modification of the pharmacological characters have also been made with some success. Acetylation of the sugars reduces the toxicity and modifies some of the stimulating actions, but the acetylated pyrogen prepared from *E. coli* is still undergoing clinical trials.

Mode of Action

The mode of action of bacterial pyrogen is not yet completely understood but it is believed that the fever and other effects produced as a result of the injection of bacterial (i.e., exogenous) pyrogen, are largely due to a stimulation of the central nervous and other systems by an endogenous factor or factors liberated into the bloodstream. Rather surprisingly, evidence has been produced to show that the hypothalamus is not necessarily involved in this reaction[49,50]. After injection and before the rise in temperature takes place there is a great increase in the intake of oxygen; but the rise in temperature which follows is produced by conservation of heat by the constriction of surface blood vessels rather than by an increased heat production by shivering[51,52].

During a pyrogen test most workers have noticed that the rabbits' ears and pads become very cold as a result of this local vasoconstriction which it is said, is mediated by the sympathetic nerves. Anti-pyretics will abolish the temperature response without interfering with the other effects of pyrogen and this offers some therapeutic advantages but dictates in these instances the use of some other index of pyrogenic activity, (e.g., a white blood cell method). This is important also in the testing of anti-pyretic substances for the presence of pyrogen.

The rise in temperature after intravenous injection of pyrogen is preceded by a latent period of no temperature rise of up to 90 minutes in man and rather less in rabbits, after which there is a fairly sharp rise in temperature. As was stated this is proportional to the dose given within a limited dose range. Pre-injection temperature is gradually reached again after several hours.

The explanation of the latent period is still a matter for some speculation, but it has been shown that if plasma is taken from a normal animal and incubated with pyrogen and then reinjected, the ensuing latent period is shortened[53,54]. This, together with the work of Grant[55] suggested that during the latent period a new substance is being formed in the body or that the bacterial pyrogen is being modified in some way to produce an endogenous substance which if re-injected acts more rapidly. Other workers believe that plasma alone will not transform exogenous to endogenous pyrogen. It seems more likely that the exogenous pyrogen is first phagocytosed by leucocytes which later release the endogenous pyretic mediator[107]. The important role of the leucocytes for the initial phase of pyrogenic action in higher animals has also been impressively shown by Braude and his colleagues[108] who by using ^{51}Cr-labelled endotoxin from *E. coli* found that more than 90 per cent. of the injected endotoxin is very quickly taken up by the buffy coat layer.

We already have mentioned that Menkin[12–14] and also Abderhalden[15–16] have described substances occurring in body fluids which display some of the properties of endogenous pyrogens and which are liberated more abundantly from tissues and cells on injury; it has not been proved that the reaction following the injection of exogenous (bacterial) pyrogen is due to the liberation of endogenous pyrogen from the tissues although some workers believe this to be the case.

If repeated daily injections of pyrogen are given to experimental animals the dose has to be increased in order to maintain the same level of response because the body seems quickly to become tolerant to the effects of pyrogen. This tolerance, however, disappears in 2 or 3 weeks in rabbits[56] and this has to be remembered in carrying out routine British Pharmacopœial limit tests. Man also becomes tolerant to the effects of injected pyrogen and this was a major difficulty in using vaccines as sources of pyrogen.

The mechanism of the production of tolerance is not clearly understood, but it is fairly well agreed that it is not directly related to the production of antibodies. This does not mean that the pyrogenic lipopolysaccharide is completely non-antigenic; it appears to be a hapten or incomplete

antigen and it seems to be more antigenic in some animals than in others[57] It is, however, a potent heterophile (Forssman) antigen[106]. Even in those instances in which the presence of circulating antibodies to pyrogen have been claimed, no diminution in the pyrogenic response was found; and where pyrogens have been administered together with antigens the appearance and disappearance of tolerance of pyrogen did not parallel the appearance and disappearance of antibodies[58–61] whose production was stimulated by the antigens. Barry Wood and Atkins[109] have now shown that specific bacterial immunity does not diminish pyrogen activity. It does not seem that production of antibodies is involved in the phenomenon of pyrogen tolerance.

Beeson[58] has shown that an induced tolerance to pyrogen may be broken down by blocking the reticuloendothelial system with colloidal thorium dioxide. This suggests that in the tolerant animal it is the reticulo-endothelial system which has developed enhanced ability to eliminate or destroy endogenous pyrogen. This rapidly acquired tolerance, necessitating an increased dose to maintain the level of response, was one of the factors which discouraged the use of vaccines to produce a pyrogenic reaction which would stimulate the body defences. The dosage of successive injections had to be rapidly stepped up in order to maintain an effective response. One worker writes of administering a milky fluid[62] heavily loaded with bacterial bodies in an attempt to maintain the same level of reaction. As vaccines are not without other toxic components this incidental increase in the toxic substances also administered, had undesirable side effects. The new highly purified pyrogens are free from side effects of this kind because they are free from impurities, and while increasing dosage must be given to offset induced tolerance the initial dosage is so minute, (of the order of 0·1 to 0·2 μg. for Westphal's pyrogen, "Pyrexal," prepared from *Salmonella abortus equi*, and 1 to 2 μg. for "Piromen," the American preparation,) that even the largest dose given contains little, if any, extraneous toxic material.

While on the subject of tolerance it is appropriate to mention our own experience with purified pyrogens. We have found that the tolerance developed with a pyrogen preparation from *Proteus vulgaris* is also valid for a salmonella and a pseudomonas pyrogen, indicating a high degree of cross tolerance and a common active component in all three.

The rise in temperature which occurs is always accompanied by changes in the white blood cells, although the reverse is not always true[63–65]. With an ordinary pyrogenic dose there is first a disappearance of the white blood cells, that is leucopenia, followed by a rapid increase in the total number of white cells, a leucocytosis, especially of young polymorphonuclears leucocytes with undivided nuclei. This is accompanied by an eosinopenia and a sustained lymphopenia. This appearance of the young polymorphs giving what is called a "shift to the left" is also seen in many conditions of stress, for example bacterial infections, and injury. With pyrogenic stimulation the degree of shift to the left is proportional to the dose of pyrogen[66].

It is believed that this white blood cell effect is at least in part mediated

by the liberation of ACTH from the hypophysis with subsequent increased secretion of the adrenal cortical hormones[63,67,68]. It is interesting to speculate to what extent a course of pyrogen injections could replace a course of injections of either ACTH or cortisone in certain conditions. Several of the effects of ACTH, for example white blood cell effect, are similar to the effects of pyrogen and it was this finding which first prompted the replacement of ACTH by pyrogen in allergies. Nevertheless one very important difference exists: ACTH causes a profound temperature-fall in normal animals (hypothermia) when injected at the rate of 1 unit per kg. We have found that added pyrogen will reduce this hypothermia, and will elicit the typical pyrogenic response with a delayed peak if large amounts are present. If smaller amounts are present the response does not correspond to the amount added.

Some rabbits appear to be unduly sensitive to the action of ACTH and a state of semi-collapse follows the injection of even 1 unit/kg. In these the hypothermia is so severe and prolonged that no pyrexia occurs and the test fails to detect the presence of added pyrogen. In our experience with rabbits in testing ACTH for pyrogen, the test which uses fever as an index is extremely unsatisfactory, but we can as yet suggest no alternative. ACTH also interferes with the white cell response to pyrogen, so that this too is rendered unsuitable as an index of pyrogen present in the ACTH.

It is believed that the white blood cell changes following injection of pyrogen are largely mediated by the adrenal cortex as they are substantially altered in adrenalectomised animals[69–71]. This stimulation of the adrenal cortex appears to be in response to the increased secretion of ACTH which may in turn be the result of a fall in circulating corticoids which may follow the demands of the tissues after injury or stimulation of the tissue cells by pyrogen. Evidence of pituitary adrenal stimulation by pyrogen is also found in the increased urinary excretion of corticoids[72] and in the fluctuation of plasma ascorbic acid levels[73]. Whatever the mechanics of the process there is adequate evidence of the activation of the pituitary adrenal cortex cycle.

Clinical experience has shown that long-continued administration of cortisone or ACTH has disadvantages; the withdrawal effects of these hormones are also common and are sometimes evidenced by resistant exacerbations[72]. The advantages of stimulating the adrenal cortex with pyrogen thus becomes apparent. The withdrawal effects and endocrine disturbances seen with ACTH and cortisone do not occur in pyrogen therapy.

Besides a well marked stimulation of the pituitary adrenal systems the influence of injected pyrogen is seen on connective tissue and on the reticulo-endothelial system. The dermis is an essential part of the reticulo-endothelial system, and the stimulation of this system by pyrogen has been shown dramatically in the healing effect of pyrogen on burned, wounded and frostbitten skin[58,73–77]. In experiments on the regeneration in the central nervous system it has been shown that by enhancing vascularity of the area, pyrogen inhibits the process of gliosis and so facilitates

regeneration of nerve fibre. Evidence for structural regeneration has been supplied and in laboratory studies some functional regeneration has been reported[78–82]. This is new evidence in a problem which has been perplexing neurologists for years.

It has been known for many years that Gram-negative bacterial extracts when injected have the power to cause breakdown and necrosis in tumour tissue. A preparation known as Coley's fluid was in use for some time for this purpose. Dr. Shear in America has investigated the purified lipopolysaccharide which he has obtained from *Serratia marcescens*[32,34] (*Chr. prodigiosum*). In addition to its ability to cause breakdown of neoplastic tissue this substance also proved to be a potent pyrogen[83] and there is evidence of a common tumour—necrotising activity in many of the Gram-negative endotoxins.

We have seen in this section that apart from being a potentially useful remedy with remarkable effects, the lipopolysaccharide is proving to be a useful tool in revealing how many of the mechanisms of the various body systems function. Much work remains to be done to explain these actions fully and to decide, for example, whether there exist substances in the body each separately capable of stimulating one or other of the actions discussed.

Pyrogens as Medicaments

I cannot close without some reference to the various disorders which have been successfully treated by means of either vaccines or pure pyrogen. I shall mention only a few, but there is much published evidence concerning favourable effects in many disorders.

Let us be perfectly clear about one point: pyrogen is not a specific therapeutic agent like an antibiotic which has its own bacterial spectrum nor for that matter is it like an antitoxin. Its action is not an attack but a vigorous stimulation of the natural defences of the patient; and this is the rationale for its use in so many diseases. It may not be too bold to say that it has been the absence of such a defensive agent which has directed medical research towards finding attacking agents such as serologicals and antibiotics. Now that we have both there is no reason why they should not be used together—the one to help the other.

Some of the recent reports on the newer preparations advocate their use in sub-febrile doses; in others, febrile doses have been recommended but the fever has been suppressed with antipyretics. On the other hand some workers believe that the actual production of fever is necessary for the full beneficial effects to be produced. There is little doubt that febrile doses are essential in certain conditions. In general, pyrogen appears to be most usefully used in conjunction with other specific therapies when such a combination appears rational.

The similarity of the effects of pyrogen, ACTH and cortisone on the white blood cell picture pointed to involvement of the pituitary-adrenal system in the pyrogen reaction, and the known beneficial effects of ACTH and cortisone in the treatment of allergic conditions suggested the use of pyrogen in a variety of conditions[63]. It seems that pyrogen is capable of filling a role similar to that filled by these hormones in allergic treatment

but without their dangers. And this is important; for dangers such as resistant exacerbations on withdrawal and endocrine imbalance often follow their use, while pyrogen therapy has, so far as is known, neither withdrawal symptoms nor long-term side effects[84].

It is reported that sub-febrile doses are no less effective in giving relief in many conditions[84–88], and moreover some patients report a sensation of relaxation and well-being after pyrogen treatment. It has since been found that sub-febrile doses of pyrogen are particularly useful in relieving the depression which often accompanies the allergic state[87]. It should be remembered however that pyrogen, like ACTH or cortisone, can act only as an adjunct to specific diagnosis and therapy in allergy. In this role pyrogen has been most useful especially in stubborn cases of multiple allergies.

Several authors have reported favourably on pyrogen in dermatological practice using febrile and sub-febrile doses, and febrile doses with antipyretics. Superiority over vaccine therapy in this field has been well established[90] when febrile doses are administered. Fever can be avoided by utilising the subcutaneous or intra-muscular routes of administration and pyrogen has been used successfully in this manner in various dermatological conditions as an adjunct to topical therapy[88]. More than one author has been impressed with the striking results obtained in the pyrogen treatment of otitis externa[88–91]. Complaining of the dangers and disadvantages attendant on the use of ACTH and cortisone in dermatological practice, Guerrieri, reporting a successful trial of pyrogen alone (Piromen) (alone) in neurodermatitis[84], comments on its safety in use and the absence of any "post-treatment rebound." Other workers[92–94] have confirmed the beneficial effects in varied dermatoses.

The findings that in cats and dogs pyrogen aids nerve regeneration and that in transected spinal cords good anatomical regeneration takes place with enhanced vascularisation and absence of glial scarring are interesting[78–82], and prompted one author[95] to use it (Piromen) in 118 cases of spinal cord injury and disease. Beneficial results were obtained in 10 per cent. of the patients, but he felt that further investigation was warranted as the dosage and duration of treatment had been inadequate. Improvement with pyrogen therapy has also been reported in other diseases involving the nervous system[96–100].

The effect of pyrogen in stimulating the repair of damaged tissue has been investigated in the treatment of duodenal ulcers. The value of this treatment can easily be assessed from the results which are reported[101] as relief in 19 out of 25 cases with complete healing of the crater in 18 out of the 19. Recurrence was less than one-third.

The fibrinolytic action of pyrogen[18] has already been mentioned, but this localised action of pyrogen has another interesting application which has been known for many years. This concerns the use of pyrogen[102] for the detection of unsuspected foci in, for example, an infected gall bladder or other organ or tissue. Frequently, patients have complained of localised pain while undergoing pyrogen therapy and this has led to the detection of a septic focus at the site of pain.

One of the oldest uses of pyrogen has been the non-specific antibacterial effect; diptheria carriers[103] have been cleared and other infections such as typhoid have been aided by fever therapy. The action of pyrogen as an adjuvant in sulphonamide and chloramphenicol treatment has also been reported[105]. Cases of agranulocytosis[104] caused by drug treatment have been successfully treated and it has been assumed that the leucocytosis which follows the administration of pyrogen is responsible for this success.

This very brief review of the clinical applications of pyrogen will enable my audience to appreciate how the physiological effects of this remarkable substance have been systematically applied to the treatment of various disease states with considerable success.

We have reviewed the properties of bacterial pyrogens and have shown that they bring about a stimulation of the body's natural defences which are immediately mobilised when the body is attacked. Although our description of this attack and this mobilisation of defences is perhaps inadequate, and although we speak of stimuli, injury and irritation, which barely express the full meaning, it is clear that in pyrogen we have a tool which, if used intelligently has great powers for good, not in one, but in a wide variety of conditions. It would be foolish to suggest that pyrogen therapy is a panacea or will render any existing useful drug unnecessary, but it is suggested that it will act as a vigorous adjuvant to the well-tried medicament. Pyrogen therapy, I feel, is an ally and not a substitute for specific therapy.

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