# Fever

**The Scientific Basis of Medicine**

**With Professor WI Cranston, St Thomas's Hospital Medical School, University of London.**

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**Introduced by Dr Ian Gilliland.**

**Produced by Peter Bowen.**

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<**Dr Ian Gilliland to camera**>

Professor Cranston is Professor of Medicine at St Thomas's Hospital Medical School. He is an honours graduate of Aberdeen who spent some years in the Department of the Regius Professor of Medicine at the Radcliffe Infirmary, Oxford, before taking over the Department of Medicine at St Thomas's. He is known for many fundamental observations, amongst which is the study of fever, the subject of this lecture. Professor Cranston.

<**Professor WI Cranston to camera**>

Fever has been a recognised accompaniment of infection for many centuries, but it's only in the last 20 years or so that we've begun to have some understanding of the

mechanism whereby it's brought about. It's true that in the middle of the last century some German observers, notably Billroth and Weber, carried out some experiments in which they injected pus from febrile animals into other animals, but the results of these experiments were extremely variable and contradictory. Thereafter, little experimental work was done until the beginning of this century. At that time, with the increasing use of intravenous injections, it began to be noticed that practically any kind of injection could be followed by fever. This led to a series of investigations, culminating in the discovery by Seibert, in 1925, that these fevers were due to gram-negative endotoxins, part of the envelope of gram-negative bacilli which were present in the infusions. This didn't require the presence of living organisms and these endotoxins, or bacterial pyrogens, were extremely resistant to destruction by heat or by chemical processes.

For the succeeding 20 years or so, it was tacitly assumed that these perhaps explained the fever due to infections, although this hypothesis really didn't explain a number of other aspects. First of all, there was fever due to gram-positive organisms – infection with, which didn't have endotoxins; and secondly there's fever in association with necrotic processes, with sensitivity reactions and so forth.

If I may jump a little bit ahead in time, the proposition that circulation of these endotoxins was responsible for fever in gram-negative infections was conclusively disproved by Greisman who, with his colleagues, in 1965 injected endotoxin into patients suffering from typhoid fever. These were volunteer patients and this is an example of one such experiment.

<**Cranston over graph showing results of experiment in which endotoxin was injected into patients with typhoid fever and temperature levels measured, then to camera**>

Here we have the rectal temperature in degrees Fahrenheit and here we have time in hours. The patient began with a temperature of about 100 and a half degrees Fahrenheit and at this point here, a continuous infusion of typhoid endotoxin was begun. In about an hour and a half there was a considerable rise in temperature, due

to the endotoxin. But although the infusion was continued during the succeeding 5 hours, the temperature slowly began to fall and even when the infusion rate was doubled – multiplied by 5, multiplied by 10, the temperature continued to fall, down towards its original level. At this point, the infusion was stopped.

Well here we have a situation in which a patient has typhoid fever and is infused continuously with typhoid endotoxin. Although this produces a transient fever, the effect is not sustained and this makes the argument that the fever of this disease is due to continual circulation of the endotoxin an untenable one so that there must have been some other explanation.

The next important step came in 1948 when Beeson, in a carefully controlled study in which he excluded possible contamination by endotoxin, showed that polymorphonuclear leukocytes, from sterile exudates, were capable of producing a pyrogenic material. This pyrogen had properties which were quite different from endotoxin. Repeated endotoxin injections give rise to smaller and smaller responses; a phenomenon known as tolerance. The repeated injection of material from white cells, which we'll call leukocyte pyrogen, did not do this. Also, as I have mentioned, bacterial endotoxins were resistant to destruction by heat, whereas leukocyte pyrogen was destroyed by heating to 70 degrees centigrade.

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At about this time, we had been doing some experiments on temperature regulation and had inadvertently infused some bacterial pyrogen into a volunteer. And we noticed at that time that it was about an hour and a half after the injection before the patient developed any manifestations of fever and we weren't quite sure what was happening during this delay period. In a perhaps rather naïve fashion, we imagined that perhaps there might be some interaction between the bacterial pyrogen and the peripheral blood and we therefore did some experiments in which we injected pyrogen alone and pyrogen incubated with whole blood intravenously. And this slide shows the results of our experiments.

<**Cranston over graph comparing temperature readings of a volunteer following injection with a bacterial pyrogen; firstly in saline and then after having been incubated in blood. Then to camera**>

Here's a normal volunteer with the rectal temperature in degrees centigrade and heat loss from one hand measured by calorimetry. At this point, here, a bacterial pyrogen in saline was injected intravenously. And you can see that it took something like an hour before there was a reduction in heat loss from the hand before there was a rise in temperature. However, when the same subject was injected with bacterial pyrogen which had been incubated beforehand for 3 hours with blood, we see that after the injection, here, there was a delay of only about 15 minutes before there was a fall in heat loss from the hand and the development of a fever. So, it suggested, then, that there was some interaction between the bacterial pyrogen and blood and that we could very considerably shorten the incubation period, if one likes to call it that, for the development of fever.

It was also shown that this acceleration of the response to bacterial pyrogen depended upon the presence of leukocytes in the peripheral blood. If the pyrogen was incubated with plasma or with red cells, this did not accelerate the onset of fever. Now, we also were able to examine the properties of this new product which had many of the properties of the leukocyte pyrogen that Beeson had discovered.

<**Cranston over charts comparing temperatures in a volunteer given bacterial and leukocyte pyrogens. Then chart looking at response of leukocyte pyrogen to heating. Then to camera**>

Here we have the responses to repeated injections of bacterial pyrogen and to endogenous or leukocyte pyrogen. This is the magnitude of temperature rise, expressed as a percentage of the temperature rise following the first injection. This is the response on the second, third, fourth, fifth, sixth and seventh injections; the injections being separated by intervals of about 48 hours. And you'll see here that when bacterial pyrogen is given repeatedly, the average response to the same dose of pyrogen falls off until at the 8th injection it's only about 30% of the response to the

first injection. Conversely, however, when leukocyte pyrogen is injected repeatedly in the same way, the average response does not significantly fall over this period of time.

We were also able to examine the response of this product to heating. Here we have the fever index, a measure of the size of the fever after incubating plasma containing leukocyte pyrogen at 37 degrees, at 56 degrees and at 70 degrees. This is the responses of 4 different individuals and you'll see here that heating to 70 very considerably reduces the response. As a control, here is the same material as this with added bacterial pyrogen which has been heated to 70 degrees and it's clear that the bacterial pyrogen, the activity due to the bacterial pyrogen, is not influenced by heating at this level.

So, here we have evidence which again suggests that man can produce a pyrogen with similar properties to those of the leukocyte pyrogen which had been demonstrated in the rabbit.

Now, if one looks at circulating leukocytes, if one just takes blood out of the patient and either incubates the white cells with saline, or even if one fragments them by ultrasonic disintegration, no pyrogen is present preformed within the cell. But it's possible, in white cells from animals, to demonstrate the release of leukocyte pyrogen, either by incubating with endotoxin, by taking cells from inflammatory exudates, by allowing the white cells to phagocytose gram-positive bacteria – an explanation possibly for the fever due, for example, for the fever due to streptococcus pneumonia – if leukocytes from sensitised animals are exposed to the antigen, they can produce endogenous pyrogen and so can viruses. So, it appears as though this might be a possible common factor in the production of diseases of different kinds.

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Well the next question that's been considered by a number of investigators is: how is this done? What is going on within these white cells to produce this leukocyte

pyrogen? Well, on exposure to a suitable stimulus it seems that at least a two-stage process is involved. And this slide, taken from the work of Atkins and his colleagues, shows what we know about the formation of leukocyte pyrogen from white cells which have phagocytosed, killed, gram-positive organisms.

<**Cranston over charts detailing the manner in which leukocyte pyrogen is formed from white cells, then to camera**>

Initially, during the first hour or so, there is an increase in oxygen consumption of the cells, an increase in $CO_2$ production, and an increased glucose utilisation. There's lysis of granules within the white cells and at the end of 2 hours, we have a situation in which the cell has now been activated, although if those cells are now broken up by ultrasonics and the product injected, there is no pyrogen actively present at this time. So this activating process can be blocked by actinomycin D, puromycin[1] or cycloheximide implying that probably protein synthesis is going on, whether this is synthesis of an enzyme which subsequently releases leukocyte pyrogen, or whether it's synthesis of a pre-pyrogen is not yet certain. It's also possible to block this process with sodium fluoride or iodoacetic acid.

Now, after 2 hours, release of leukocyte into the surrounding medium begins and is maximal by about 6 hours. At about 3 hours it's possible to show the presence of leukocyte pyrogen within the cells and thereafter it accumulates in the medium. After 2 hours, addition of actinomycin, puramycin, cyclohexamide, sodium fluoride or cyanide to the incubating medium makes no difference to the subsequent release of leukocyte pyrogen. So it looks as though the latter part of the process is not an energy-requiring activity. There is a little bit of evidence to suggest that there's a feedback inhibition of the production of leukocyte pyrogen by pyrogen which has already been released into the medium. If white cells are incubated in small volumes, the release of leukocyte pyrogen, the total released, is rather less than if they're incubated in large volumes. So there may possibly be some feedback inhibition. Iodoacetic acid, in fairly high concentrations, will apparently limit the production of

[1] Standard spelling. It is spelt 'puramycin' on chart in this film.

leukocyte pyrogen at this stage although it doesn't block it completely. However, there is some evidence that this sort of concentration of iodoacetic acid can perhaps interfere with the pyrogenic properties of leukocyte pyrogen itself once it's been formed and this evidence cannot be taken as cast iron at the moment.

Now, the same sort of processes appear to occur after incubation with endotoxin, which has been examined, though the other stimulants to leukocyte pyrogen production have not been examined in this way. It's now known also that the polymorph is not the only cell which can act in this way. There's evidence that monocytes can produce leukocyte pyrogens with properties identical to those produced by the polymorph. And there is also evidence that Kupffer cells can do the same thing.

Now, in animals, during experimental infections with pneumococci or with viruses, it has been possible to show in the peripheral circulation that leukocyte pyrogen is present at the time when the animals are febrile. The same is true of fever due to hypersensitivity. So that in the animal, at least, it appears as though we've got a situation in which it's possible that there is local release of leukocyte pyrogen in inflammatory areas but this then travels, possibly in the lymph, for there's some evidence for this, and in the circulation to produce its effect elsewhere.

Chemically, the nature of leukocyte pyrogen has not been particularly established. There's evidence that it's a protein molecule with a molecular weight of around 20,000 and that it is extremely potent and the quantities of nanogram amounts, given intravenously, will produce quite a marked fever. But as it has not been produced completely pure yet, there is, as yet, little evidence on immunoassay or other possible methods of approaching this problem.

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Now, where does this leukocyte pyrogen act to produce a fever? A large number of studies have established that there is no action on any peripheral structure and that the action appears to be at some site within the brain. Corroborative evidence for this

is first of all that if leukocyte pyrogen is infused via the carotid artery, it produces a larger temperature rise than if it's infused intravenously. It's also true to say that if it's injected into the cerebral ventricles, it causes a very much larger temperature response, dose for dose, than given by any other route. Therefore, attempts have been made to determine where this material acts within the central nervous system by micro-injecting small quantities of leukocyte pyrogen into different parts of the brain.

<**Cranston over diagram showing which areas of the brain react to infusions of leukocyte pyrogen, then to camera**>

And this slide shows the kind of results that have been obtained with the rabbit. In this slide, the white circles indicate the areas of diffusion of injections that produced positive responses. The dark circles indicate areas where the responses were negative. But since this slide was produced there has been suggestive evidence that there may perhaps be another area in the periaqueductal grey matter in which small injections of leukocyte pyrogen can produce a temperature response. So that, however, the most important site does appear to be the pre-optic area of the hypothalamus which, of course, is the area normally responsible for central thermo-regulation.

The nature of the action of leukocyte pyrogen on the cells in this area still remains quite obscure. There is evidence that cells in this area are the main sites in the brain at which noradrenaline and 5-hydroxytriptaline are found. There's also evidence that micro-injections of noradrenaline and 5-hydroxytriptaline into these areas will produce temperature responses in experimental animals. But it is still not at all clear whether leukocyte pyrogen acts on the cells in these areas by any mechanism which involves the participation of noradrenaline or of 5-hydroxytriptaline and this is a question which is still open. There's also some evidence that prostaglandins, if injected into these areas, may also alter temperature and, again, there is no evidence whether this is or is not of general physiological importance.

So, we can produce, on the basis of animal experiments, a fairly simple hypothesis.

<**Cranston over diagram showing hypothesis detailing how leukocyte pyrogens can produce a raise in temperature, then to camera**>

That cells, leukocytes, monocytes or Kupffer cells or possibly other cells which we don't yet know about, in response to inflammatory change or in response to endotoxin or to antigen, if the cells are stimulated, will produce leukocyte pyrogen, which is carried by the circulation to the brain which produce an action by a mechanism of which we're uncertain, in the anterior hypothalamus, possibly in the periaqueductal grey matter and that this, then, turns on effector mechanisms for heat conservation which causes the temperature to rise. The original hypothesis, that endotoxin may have a direct effect on the hypothalamus in spontaneous fever, seems to be largely excluded by the evidence I've already quoted from Greisman and his colleagues.

Well, this is all very fine as far as animals are concerned but what about the situation in human fever? Have we got any evidence that the same kind of circumstance can arise in man?

Well, I've already shown some evidence that human white cells can produce a leukocyte pyrogen with properties very similar to those of the animal white cells. The next obvious step is to look at patients who are febrile because of disease and to attempt to determine whether leukocyte pyrogen is present in the peripheral blood. Originally, Snell undertook a number of experiments along these lines by bleeding patients with infections, treating the infection and then re-transfusing the blood and he was generally unsuccessful in detecting any circulating pyrogen. There have been very few successful experiments of this kind but this represents one of them.

<**Cranston over graph showing temperature readings plotted against leukocyte pyrogen levels in a patient with malaria, then to camera**>

Here we have the rectal temperature of a patient with malaria. Time, in hours along the abscissa, and you will see that during a spontaneous attack of malaria his

temperature rose from 37 degrees centigrade to 41 over a period of about 3 hours. At this point in time, when he was not febrile, but when he had parasites in his circulation, 300ml of blood was drawn from a peripheral vein. Again, at this point, when his temperature was rising rapidly, a further 300ml of blood was withdrawn. These two blood samples were centrifuged and separated into plasma on the one hand and red and white cells on the other hand. His malaria was treated and, on subsequent days, the cells and plasma were re-injected subsequently at time zero, the time now is 40, 80, 120 minutes after injection and the rectal temperature was measured. Injection of the cells or the plasma taken from blood when the patient was afebrile didn't really cause any significant rise in rectal temperature. The plasma obtained when the patient was febrile also didn't produce any very significant effect. But the cell, when injected at this point, caused a subsequent rise of rectal temperature of about 1.4 degrees centigrade and the onset of this temperature rise was about 30 minutes after injection and you'll remember we showed before that with leukocyte pyrogen injection, the time of onset of fever is short, of the order of 15 to 30 minutes.

So this might be acceptable as evidence compatible with the circulation of leukocyte pyrogen in human febrile disease. This is a very rapid rise in rectal temperature and it may of course be that the failure to find leukocyte pyrogen in the circulation in other febrile illnesses was because it just wasn't possible to remove enough blood in otherwise febrile people in order to demonstrate the presence of the pyrogen.

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Well, we have attempted to see whether this is so. And here we have the results of an experiment in a normal individual.

<**Cranston over graph showing results of an experiment to measure temperature after leukocyte pyrogens are infused into a healthy individual, then to camera**>

Temperature, rectal temperature, in black; ear temperature, open circles; and time in units of 30 minutes along here. At this point, time zero, 30ml of leukocyte pyrogen were injected intravenously and this was followed by a continuous infusion of leukocyte pyrogen at a dose of a tenth of a millilitre given intravenously. And here, about an hour and a half after the injection of the pyrogen, the temperature reached a plateau at about 1 ½ degrees higher than its control level and it then remained fairly stable. Now at this point when the subject had a stable pyrexia of about 1 ½ degrees centigrade, 500ml of venous blood was withdrawn. Now, this is a situation in which we have a normal subject who has been made febrile by the intravenous infusion of leukocyte pyrogen; we knew he had leukocyte pyrogen in his circulation and therefore if we could not find this by re-transfusing the blood in this patient, it means that it's very unlikely that we're going to be successful in finding this in most other febrile disease.

Well, here we have the situation when we re-injected either 220ml of the plasma, this is rectal temperature along here – 36.5, 37.5 degrees centigrade, or 280ml of the white cells. In neither case was there any evidence of any febrile response at all.

Well this suggests that the reason for the failure to find circulating leukocyte pyrogen in febrile patients might be because it isn't present in a large enough quantity to demonstrate by cross-transfusion experiments in man. It is possible to assay human leukocyte pyrogen in rabbits which, of course, gives one now a great advantage. Previously the sort of experiments that had to be done were those in which blood was taken from a patient, or from a volunteer, and had to be re-infused into him. But it's possible to assay this material in the rabbit, provided it's not given for more than about 5 consecutive days; thereafter one does run into spurious hypersensitivity responses.

Using this kind of assay, it has been possible to demonstrate material with the properties of leukocyte pyrogen in a number of other disease states which had previously been unexplained. It's quite well known that some patients with renal carcinomas are febrile and that the fever disappears when the tumour is removed. We have been able to study a number of these patients now and on this slide there is

evidence for the presence of leukocyte pyrogen within the tumour in two of these patients.

<**Cranston over graphs showing temperature rise levels in patients injected with extracts of normal kidney or of cancerous kidney**>

The upper part of the slide represents the responses obtained in one patient; the lower part of the slide, those obtained in the other. The ordinate represents the temperature rise obtained, the average temperature rise, obtained in groups of 6 rabbits injected with extracts of normal kidney or of the tumour. In this patient, extracts of 1.2g of normal kidney produced no significant temperature elevation. In rabbits infused, or given injections of any type, there's often a small temperature response of up to about 0.2 degrees centigrade and this is the reason why, in all these series, we've included saline control groups of 6 rabbits as an indication of the sort of random temperature rise effect. Well, 1.2g of normal kidney extract produced no temperature rise, but 1.2g of the tumour produced a very significant increase in temperature in groups of animals of this kind.

Now, of course, this was obtained from a patient at operation and although all ordinary precautions were taken to ensure that it wasn't contaminated by bacterial endotoxin, this remains a possibility. It is possible to remove completely the response to bacterial pyrogen or endotoxin in rabbits by treating them 24 hours before with a large intravenous dose of endotoxin. This last column here represents the response to injection of extracts of 1.2g of tumour in rabbits which had been made refractory in this way. In other words, here we have animals which we know would not respond to bacterial endotoxin but they respond to much the same extent to the tumour extract as do the animals which had not been rendered refractory.

In the second patient we have a very similar situation where an extract from a normal kidney produced no significant temperature rise; the control injections produced rises of about 0.16 of a degree centigrade; extracts of the tumour in normal rabbits and refractory rabbits again cause a temperature rise.

We have now been able to do this in 5 patients with renal carcinoma and have been able to show the presence of leukocyte pyrogen in all these tumours. We've looked at 4 patients who had renal carcinomas but who were not febrile and similar extracts of their tumours did not contain any leukocyte pyrogen.

Well, in summary, it looks as though there is evidence compatible, at least, with the hypothesis that leukocyte pyrogen plays, if you like, a final common pathway in most kinds of fever. What we don't know yet is exactly the mechanism at a cellular level whereby it is produced and the other important question which remains to be solved is that of the mechanism of its effect within the brain. Apart from this, of course, the other general problem, that we still have no very satisfactory answer to, is why people become febrile at all. There is some evidence that febrile patients with pneumonia fare neither better, nor worse, than patients with relatively low temperature. There's evidence that patients with myocardial infarcts who are febrile fare neither better, nor worse, than those who are relatively afebrile.

And so, the sort of philosophical question about the general value of fever remains, I'm afraid, unanswered.

<**End credits**>