Wellcome Film Project

Non-Specific Immunity

The Scientific Basis of Medicine

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<**Opening titles**>

<**Narrated by Gilliland to camera**>

Dr John Humphrey is head of the Division of Immunology and Deputy Director of the National Institute of Medical Research at Mill Hill, London. He has contributed very widely to the subject of immunology. He has contributed books and articles to journals on this subject and is an international authority. Today he is dealing with a general problem – non-specific immunity. Dr John Humphrey.

<**Humphrey to camera**>

I chose as my title for this talk non-specific immunity, despite the fact that I personally haven't done much experimental work in this field, for two reasons. Firstly, because I

think that non-specific immunity is important and secondly, because nowadays when such rapid advances have been made in the knowledge of specific immunity, I thought it would be good for me, and I hope for you, to get a sense of proportion correct on this topic.

Now, it's easy enough to say what I mean by specific and non-specific immunity. Specific immunity can be defined as the immunity due to increased levels of antibody or increased numbers of sensitised cells which occur as a result of prior contact with the specific antigen. Non-specific immunity is the immunity which does not result from prior contact with a specific antigen. But although it's easy enough to define, you will see that it's quite hard to identify.

Although, you or I could not manage if we really had a deficient, specific immunological mechanism, for example if we had hypogammaglobulinaemia we would be subject to recurrent infections. And had we been born with a combined deficiency with lymphocytes and of antibody- forming mechanisms, we should have lived for a very short time. The fact is that even children born with severe immune deficiencies don't succumb all that quickly considering that they are surrounded by a hostile environment with microbes ready to penetrate at any time. Furthermore, in the 1950s Ashley Miles and his colleagues did some very important studies of what happens when small numbers or varying numbers of a wide range of microbes were introduced below or into the skin of guinea pigs. They could affect whether or not there was a local circulation at the time, whether cells could arrive, whether antibodies could arrive and so on, by means of adrenaline and other mechanisms which would prevent the local infection. And what they found was, whether or not the organisms grew, produced lesions, was decided within the first two to five hours after inoculation of the organisms. Most organisms were killed or prevented from multiplying locally within this period of time which is much shorter than would be required for mobilisation of a specific immune response. And this happened even when the animals had not been specifically immunised against the microbes; that is, the microbes were what you would call non-virulent, though of course if the animals had been dead they would have proliferated perfectly well in their blood. Some organisms, and these are the ones we would call virulent, survived this decisive

period and went on to multiply and produce lesions. He called this phenomenon primary lodgement and I think it's a very important thing to bear in mind.

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Now, in this talk I'm going to discuss knowledge, some of it old, some of it fairly recent, about ways in which microbes may be hindered from penetrating into the body, may be destroyed or prevented from multiplying, without involving specific immunological mechanisms. Now, I can't cover the whole field in one talk and I won't do more than mention, for example, mechanical barriers. Mechanical barriers are the self-renewing skin, gut lining and so on, and they are of course extremely effective.

<**Humphrey over series tables listing subjects under discussion, camera returns briefly to him occasionally**>

Then we have chemical factors such as the unsaturated fatty acids which are present in the skin and render healthy skin an almost self-sterilising area. Of course, if you have a sodden, wet skin, say a charwoman's skin in the old days at least, then things would grow quite well. But healthy skin can destroy and prevent bacteria from growing. Then there are lysozyme in tears and other secretions which destroys the bacterial cell wall and is a very potent local disinfective.

Another non-specific mechanism of chemical nature, which is not often remembered, is the fact that bacteria require to have inorganic irons in suitable quantities in their neighbourhood and they can't, for example, get on without iron. And there are metal chelating proteins such as transferrin which bind iron and lower the iron level so much that bacteria cannot multiply when they're around – and I shall come back to this later on in the talk. Then there is the sialoglycoproteins, the mucins on mucus surfaces which are in fact themselves receptors for quite a number of viruses and can divert the viruses from entering the receptor or from attaching to the receptors on cells and therefore to some extent prevent infection by small numbers of organisms.

Then there are natural inhibitors of a variety of bacterial products – such as inhibitors of hyaluronidase, ribonuclease, many trypsin-like enzymes (though these are the trypsin-like enzymes that come from the so-called non-pathogenic organisms), inhibitors of streptolysin S and these are in general materials whose function is to inhibit destruction of the body by its own enzymes which have been released from its own cells, and I think it's an accident in a way that they inhibit bacterial products, but it's quite difficult to show that a number of the toxins produced, alleged toxins produced by bacteria, are in fact in any way involved in infection by those bacteria and the reason is, probably, that there are these natural inhibitors which prevent them from having any effect. And then I should mention interferon. Interferon is, as you know, a substance which is released from many cells when they are infected with virus or when the get double-stranded RNA into them, and which causes cells which have contact with interferon, or make interferon, to generate another material which inhibits the translation of bacterial RNA and prevents the virus from growing.

<**Humphrey stands by series of charts which are then shown in close-up, uses indication stick**>

Now, it was shown by Dr Alec Isaacs, when he first described interferon, that the production of interferon in a mouse, which was artificially infected with influenza virus, was very rapid, that the proliferation of the influenza virus fell down and the numbers in the mouse diminished at the time when interferon was produced, well when I say long before antibodies, well when I say long before, a few, a day or two before, actual antibodies were produced. And he regarded this as evidence that interferon was very important in protecting against viral infections.

<**Humphrey to camera**>

It's been very much harder to show that the interferon mechanism also works in man and I must say it is not quite sure what its true significance is. But I don't think it can exist for nothing.

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Now I shall, what I shall talk about are firstly antibodies, although partly to dismiss them. Now, antibodies arise largely by specific stimulation, by prior contact with the antigen – these are what we call acquired antibodies. Then they can be, are, acquired passively from the mother and a child is born with a fair complement of antibodies from its mother which lasted for six to nine months, this time depending on the fact that the immunoglobulins have a half life of just over twenty days and they gradually are eliminated from the baby unless it has made some more.

But apart from specifically stimulated antibodies and those acquired from the mother we have what are called natural antibodies, that is those that are present without any known stimulation having taken place. Now often, perhaps usually, these are in fact the result of prior stimulation which we don't know about; for example, by cross-reacting antigens derived from bacterial products absorbed from the gut, which is acknowledged now to be the source of the blood group agglutinins which we all possess and which pretty certainly come from stimulation with gram-negative organisms during early life, and also may be acquired as a result of unapparent infections. But even omitting that it's quite, I think, certain that we start with antibodies against a most remarkable variety of materials, if you look for them by sufficiently sensitive methods. For example, the method of attaching small chemical groups, haptines, to bacteriophage, and then using these as a test for antibody against these chemical groups which can inhibit infection of the bacteria by the bacteriophage, it's been possible, for example Professor Mercola[?] in Finland, to show that even arctic reindeer have quite measurable amounts of antibodies against highly unlikely determines such as para-hydroxy-iodo-nitrophenol groups, the ones which he used for convenience – they can hardly have met this naturally, and the inference is that these antibodies, present in minute amounts, are what one would expect from Burnet's clonal selection theory of antibody formation, namely they are derived from just a very few cells present in the body with the particular specificity which would recognise this particular determinant secreting minute amounts. I may say that this sort of antibody is nearly always macroglobulin IVM and it's a very nice confirmation, in a way, of the clonal selection theory of antibody formation.

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Now, this being so, it's very hard to rule out the existence of true, natural antibodies, even in the absence of specific stimulation, except in very special circumstances; for example, if you take piglets which have been born and reared under sterile conditions and have been deprived of colostrum from the mother, because they start with no immunoglobulin derived from their mother and none made by themselves, so if they have immunity one can regard this as natural immunity.

Now, I shall also talk about complement, that is a complex of, it's a family of at least nine components which can be triggered sequentially by interaction with antibody which has been deformed by combination with the antigen or by other means and become activated in a sequence and produce, as I shall discuss later, biologically active by-products and eventually damage to cell membranes.

Now, complement represents a supremely ingenious natural mechanism for producing a final common pathway as a result of the interaction of antibodies, of all sorts of specificities, with antigens, of all sorts of specificities, with each other. Obviously the body couldn't have separate mechanisms for each kind of specific combination with antigen-antibody and what in fact it's done is to devise the complement system whereby the consequences, the biological consequences, are channelled through a quite limited number of final common pathways which involve, for example, causing inflammation, increased phagocytosis and, as I have mentioned, cell lysis. I shall come back to complement later.

And then the third thing I'm going to talk about is the phagocytic cells which are principally the granulocytes, what Metchnikoff called the microphages, and the main ones are the neutrophil, polymorpholeukocytes and the macrophages, the big eaters of Metchnikoff which include tissue histiocytes, although less is known about them than about the macrophages which one can extract, for example from the peritoneal cavity or the lung. What is known is that these macrophages, those in the peritoneal

cavity, those in the blood, those in the liver in the form of Kupffer cells, are all derived from common precursors and can convert to one another.

Now, the question for this talk is how far they can get along without the help of specific immunity. Now let's consider first of all granulocytes; granulocytes that is another term for polymorpholeukocytes. Large numbers present in the circulation, as soon as any foreign substance is introduced, let's say beneath the skin, they're the first to arrive on the spot. They arrive within minutes, locally. They are attracted there by what are now called cytotaxins which are sometimes materials released by the foreign substance itself or sometimes material generated from other factors in the plasma as a result of contact with the foreign substance. Now, the great thing about the granulocytes is that they ingest particles and bacteria.

<**Humphrey stands and moves over to wall to show sequence of stills, uses indication stick**>

And I have here a sequence of film taken by Professor Jim Hirsch at the Rockefeller Institute which shows human granulocytes, neutrophils, ingesting *Bacillus megaterium* and you'll notice that the time frames are only ten seconds apart and that this granulocyte, within seventy seconds, has completely ingested the *Bacillus megaterium*, wrapped itself around and that bacillus is now on its way to being dead.

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<**Humphrey to camera**>

Now, granulocytes are a very important part of defence even in the presence of a fully functioning specific immune system. For example, agranulocytosis occurring as a result of exposure to drugs, or after experimental depletion with drugs such as nitrogen mustard which can selectively kill off the polymorphs, one gets severe local bacterial invasion spreading cellulites at the site of introduction. This happens very readily and of course it can spread so that the numbers of organisms are overwhelming and you get a septicaemia as well. But the chief thing is the very much

increased local invasiveness of the bacteria because they're not ingested by the polymorphs. This can overwhelm pre-existing specific immunity. But it's interesting to know that even if an animal has been deprived experimentally of all its granulocytes, if one infuses a constant stream of organisms intravenously and measures the rate of clearing of these organisms, it's really undiminished. This was shown in the 1950s, early 1950s, by Grace Kirby and it indicates that the other phagocytic cells which one would collect together, I suppose, in the reticular and epithelial system are also very important for the removal of systemic organisms.

<**Humphrey over table revealing main points of content discussed**>

Now, I'll discuss something about the way neutrophil polymorphs, the granulocytes, do their work. They ingest particles as you saw on that picture just now, and they ingest very readily things like latex or avirulent bacteria when these are, what is called, opsonised.

<**Humphrey to camera**>

Now, opsonin is a word coined by Sir Almoth Wright to describe serum factors which coat foreign particles, coat bacteria and enable them to be ingested more readily by the phagocytic cells. These were first popularised, I suppose, in *The Doctor's Dilemma* when Sir Ralph Bloomfield-Bonington explains Sir Colenso Ridgeon's discovery, Sir Colenso Ridgeon being a thinly disguised character of Almoth Wright, by saying that the secret of defence was to butter the bacilli, this was in fact a description of opsonins.

Now, the question is what opsonins are. They involve complement, that's pretty certain and they require, to work, the presence of some irons like calcium, magnesium which are essential for the working of complement. But in the case of virulent organisms, capsulated organisms, they certainly involve antibodies, specific antibodies as well. But there's some quite good work showing that what are called monoclonal immunoglobulins, that is materials produced by someone with myeloma, which is certainly not antibody against latex particles or carbon particles or we'll say

an avirulent organism, that these can coat the particles perfectly well, in other words that they're attaching, via a mechanism, which does not involve the specific combining site of the antibody, but what is more in the nature of a physicochemical absorption to the surface. We don't know the mechanism but it doesn't, to my mind, involve specific immunity.

<**Humphrey over table revealing main points of content discussed**>

Well, when it comes to capsulated bacteria and ones which are called virulent because, for the very reason that they are not ingested and killed without <*unidentified word*> these are opsonised, coated by a specific antibody and are helped by the addition of complement as well. And then of course, antigen-antibody complexes are readily taken up by neutrophil polymorphs.

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When this has taken place, the granules in the polymorphs fuse with the vesicle into which the phagocytosis has taken place and they rupture locally. Now, I have here another picture, taken from a paper by Professor Jim Hirsch […]

<**Humphrey over still images, uses indication stick**>

[…] which shows chicken granulocytes, chickens because they have rather particularly large granules, ingesting some yeast particles. And you'll see the time frame is very rapid here, three seconds, ten seconds, twenty seconds, and that by twenty seconds the yeast particles have been ingested and what I want you to notice is that the granules in the neighbourhood of the ingested particles disappear and in fact become, they look like clear holes, because the contents have ruptured into the vesicle containing the ingested yeast particle.

<**Humphrey over table revealing main points of content discussed**>

As a result of this release of the contents of the granules into the vesicle containing the particle, the particles are digested unless they are either indigestible or unless they are toxic in the sense that the bacteria themselves release something which interferes with the mechanism, with the metabolism with the granulocyte and kills it.

Accompanying the ingestion of foreign particles there's an increased consumption of glucose and liberation of carbon dioxide and lactic acid. This is also accompanied by production of hydrogen peroxide and of a low acidity which is important as I shall mention in a moment. As I have said, the granules fuse with the phagosomes and after that not only are the granules activated, lysed, but some of the granules are extruded from the polymorph into the surrounding medium and their contents are released there. And this release of the polymorph granules into the surrounding medium is an important part in the generation of the local inflammation which occurs when polymorphs arrive on the scene and start ingesting, because, for example, they can activate, they release cytotaxins and they release endogenous pyrogen – endogenous pyrogen is a material which comes out of polymorphs, it also comes out of macrophages, which will raise the temperature produced to fever by acting on the temperature regulation centre by mechanisms which I can't go into at the moment.

This question of generations of cytotaxins causes a self-multiplying process whereby more neutrophils and macrophages are attracted onto the scene.

<**Humphrey to camera**>

We know a little bit about what are the mechanisms of the action of these cytotaxins; they appear to act as almost local hormones on the cells which they attract causing activation of esterases, increased movement, which will move them in the direction of a gradient of increasing concentration of the cytotaxins.

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Now I want to turn to the question of what these granules contain and I have a list of some of the enzymes that have been identified.

<**Humphrey over tables revealing main points of content discussed, camera returns to him intermittently**>

We have acid phosphatase, alkaline phosphatase, nucleotidase, ribonuclease, deoxyribonuclease, esterase and the list goes on: cathepsin, trypsin-like β-glucuronidase, aryl sulphatase – that's a thing which will split sulphate groups attached to fatty acids, elastase, lysozyme which we've mentioned already. They also contain in other granules, well, they contain peroxidase – now I should say a bit more about peroxidase. As I've mentioned, hydrogen peroxide is generated in activated polymorphs and hydrogen peroxide with peroxidase is a potent killer of microorganisms provided they themselves do not produce too much catalase which destroys hydrogen peroxide. And if you have conditions where the generation of hydrogen peroxide within the granulocytes is defective, these granulocytes are very often unable to fulfil their function, and it is probably at least one of the causes of chronic granulomatous disease which is a deficiency of neutrophils.

Now they also contain in distinct granules, a substance called phagocytin. Phagocytin is at least six different arginine-rich cationic (that is, positively charged) proteins which have been shown to have a high bactericidal activity and this bactericidal activity, interestingly enough, is different for the different members of this group. The effectiveness of phagocytin, which is released at a slightly acid pH which is achieved in these polymorphs, and works at a slightly acid pH, is against gram-positive organisms among others, and this is one of the reasons why polymorphs are very good at dealing with gram-positive organisms provided that they don't have materials which kill the polymorphs themselves.

Now, I should mention the eosinophil polymorphs, although not so much is known about them. They behave similarly to neutrophils in many ways, although they lack phagocytins and apparently they don't produce lysozyme. However, they do have a very high peroxidase content and have shown to be rather good at killing organisms when they've ingested them. They are, interestingly enough, influenced and attracted by distinct cytotaxins and that's why you can get an eosinophil invasion without a

neutrophil invasion at a site, for example, after a local anaphylactic reaction has occurred. But I can't go into that any more at the moment.

Now, I want to talk about macrophages, or, in the latest nomenclature, mononuclear phagocytes. Now, these cells are distributed ubiquitously, collectively known as the reticular endothelial system, where they are concentrated in the lymphoid organs and the liver but are present throughout the tissues, in the body cavities, and circulate in the form of monocytes in the blood. Because peritoneal or alveolar macrophages are the easiest to get hold of, they have been studied much more intensively than those elsewhere.

<**Humphrey stands and moves to show electron micrographs on board, uses indication stick**>

Now I would like to show you an electron micrograph of a fairly typical macrophage which has not been activated in any sense and has not been spread out in culture on glass. And what I want you to notice is that it contains a large number of dark, round bodies which are called the dense bodies which are in fact the lysosomes of the macrophage and contain the enzymes which are in many ways similar to those of the polymorphonuclear leukocytes. A higher power shows these in greater detail – here they are, and it also shows other round bodies which are in this case, you can see, losing their contents into the cytoplasm and these are, in fact, present in most sections of macrophages that one looks at and I think they represent the lysosomes which are undergoing a process of release for reasons which I don't really know about.

<**Humphrey over tables revealing main points of content discussed, camera returns to him as he expounds on each point raised in the table**>

Now, these macrophages have on their surface receptors for a number of different biologically active materials. They have receptors for what are called cytophilic immunoglobulins and these are immunoglobulins of which the γ_2 is more cytophilic than the γ_1, that means they attach to the surface of macrophages, absorb there in a

relatively specific way, and are able to coat the macrophage with antibody or just general immunoglobulin so that it has on its surface already something which can recognise foreign particles. There, I've noted that IgM, the new immunoglobulins are also cytophilic, they attach at separate sites and there's a lot to be learned about the nature of the receptors on the macrophages for these cytophilic immunoglobulins. They have receptors for what I have called Fc, that is the Fc part of immunoglobulin molecules of all kinds when they are aggregated as for example complexed with antigens in the form of specific antigen-antibody complexes, or when they are complemented by chemical means or by heat. So that aggregated immunoglobulins attach to the surface of macrophages by separate receptors. They have receptors for the activated third component of complement, which I shall be mentioning later, but that means that anything that has got activated third component of complement is bound to the macrophage surface by this particular receptor. And then I have mentioned that they have receptors for M1F, the macropage inhibitory factor, or activating factor which is released in cell-mediated immunity when the sensitised lymphocytes come into contact with the antigen, or when they are activated by other means. And I've put that in because it's interesting that peritoneal macrophages, not alveolar macrophages, appear to have such receptors.

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Now, when a particle has adhered to the surface, and I have noted on this slide especially when it's charged, because if you can treat, for example erythrocytes with formaldehyde, altering their charge, they will adhere to macrophages in the absence of any opsonising material, although if they haven't been treated with formaldehyde they require opsonins before they adhere. This is followed by a separate process which is ingestion and both ingestion and subsequent digestion are increased by the presence of opsonins.

Now, again the question comes up: what are opsonins? The opsonins are certainly specific antibodies when these are available, they are activated complement when this is activated, but as in the case of ingestion of polystyrene particles, carbon particles and so on by neutrophils, so in the case of macrophages it appears that

there's a non-specific property of immunoglobulins which allows them to coat these particles and look as though they've been coated by specific immunoglobulins.

Now, phagocytosis and pinocytosis which is the ingestion of things which are smaller than the particles which require wrapping around of the cell surface, the cell membrane, but these enter by little tiny pinocytotic vesicles which travel in without any great wrapping around, is followed by fusion of the phagocytotic vesicle or the pinocytotic vesicle with the lysosomes – those rounded bodies which I showed you in the electron micrograph just now. The enzymes in the lysosomes are similar to those in the neutrophils except that there is no peroxidase present and this is probably quite important because the macrophages are less effective than antibody and complement present than are neutrophils in dealing with gram-positive organisms. In fact macrophages are not all that efficient at dealing with ingested bacteria unless they have the aid of opsonins and complement. And sometimes, as in the case of tubercle bacilli, virulent tubercle bacilli or toxoplasma, ingestion of these microbes is not followed by fusion of the lysosomes with the phagosome and the fusion seems to be prevented by something released by the microbes and so none of this digestion takes place. It's an interesting question why this fusion is presented because it means the organisms live quite happily within the macrophage.

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Now, as I have said, ingestion of particles, ingestion of antigen-antibody complexes, leads to activation of the lysosomes and production of more lysosomes. Then there are various conditions in which lysosomes activity is increased by, in a sense, exogenous agents. One is adjuvants, now I can't go into what adjuvants are, but one of the functions is that they increase the activity of the macrophages in a general way. Then the existence of a cell-mediated immunity reaction going on in the neighbourhood also activates, I hate to use the word activates because it is so vague but that's all I can really say at the moment, the macrophages, perhaps it does this through the mediation M1F. Peptone is another agent which can activate macrophages. And the activation is shown […]

<**Humphrey stands and moves to show electron micrograph, uses indication stick**>

[…] by, for example, an increase in the ruffled membrane of the macrophages – here is one growing on glass and this is its ruffled membrane, by increase in the numbers of lysosomes, increase in the motility and phagocytic capacity of the macrophages. And macrophages activated in this way, for reasons which we don't understand, are better at killing off […]

<**Humphrey to camera and narrates intermittently over series of tables listing main points discussed**>

[…] intracellular organisms such as *Brucella,* tubercule bacilli and they are also, incidentally, better at killing off tumour cells – it's quite easy to show what appears to be a non-specific increase in immunity to allergenic tumour cells when macrophages are activated in this way.

Now, this is in a sense a non-specific activation, it doesn't depend upon the identity of the organism which is being ingested and it's really quite important because, for this marginal increase may make all the difference for the proliferation of the organism or its killing. Then I should say that macrophages, when they ingest material, release interferon whose function we don't quite know and release endogenous pyrogen, again whose function we don't quite know. I could add that macrophages are able to synthesise the second component of complement, probably the third component of complement and probably the fourth component of complement and maybe some others and therefore carry around with them part of the complex, part of the complement complex which I shall be talking about in a minute.

Now, macrophages are relevant to professional immunologists in a whole variety of other ways but I think I shall have to leave them at this stage and turn to the question of complement.

<**Humphrey stands and moves to show a large projection of a table, uses indication stick**>

Now complement, as I have said already, consists of nine or more materials present in the blood in varying amounts which can be triggered in sequence to undergo a series of biologically important events. Now, in the so-called classical pathway, the one that's been well worked out and which we think we understand in fact and in which most of the members of the complement sequence have been isolated and characterised, is activated by immunoglobulins – in humans it would be immunoglobulin G of the subclasses 1,2 and 3; when these are complexed with antigen or when they are aggregated in one form, they activate the first component of complement, so does immunoglobulin M. The first component of complement is converted to an enzyme for enzyme chemically activated form which we've delineated C1 with a bar over it, this in turn activates the second and the fourth to form a second enzyme which is C4,2 with a bar over it and that is perhaps better understood as the enzyme which converts the third component of complement in the following way.

The action on C3, that's the third component of complement, leads to the liberation of biologically active peptide products, and it also leads to the activation of the subsequent members of the complement sequence – C5, 6 and 7, and they seem to be activated together and one consequence of this is materials which are cytotaxic for polymorphs, materials which can increase local vascular permeability and in fact produce inflammation. If the next component, C8 and C9, are activated on the membrane of a cell, that cell membrane is damaged and usually irreversibly with the death of the cell.

Now, this has been known for quite a long time but more recent knowledge is concerned with what's known as the alternate pathway. Now, the alternate pathway appears not to involve specific antibody in its activation. It's known that the endotoxins from gram-negative organisms for example yeast and yeast cell walls, substances like inulin, agar, are able without eminently interacting with specific antibody to act on an unidentified precursor present in the plasma which becomes a

substance which, I'm sorry about this name, is the proactivator convertase of the third component of complement. Now the proactivator is fairly well identified and this proactivator convertase enzymically converts this into an activator of C3 and the activator of the third component is able to mediate all the subsequent changes which would be otherwise initiated through the classical pathway.

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Now, this activation of C3 is effective, for example, attachment for C3 on membranes and so on […]

<**Humphrey to camera**>

[…] and is, I think, a genuinely non-specific trans-mechanism. It's a bit difficult to prove it because we find it hard to eliminate all antibodies, as I've said they're very hard to prove that absolutely none is present, but there are strains of guinea pigs which are deficient in the fourth component of complement and in which the classical sequence cannot be activated up to the stage of the C3 convertase. And yet, the plasma of guinea pigs lacking C4 are able to be activated through the alternate pathway perfectly efficiently. And I can show you here […]

<**Humphrey stands and moves to show an electron micrograph, uses indication stick**>

[…] an electron micrograph taken by negative staining of a gram-negative organism which has been acted on by the complement sequence going, as I said, right through to the stage of C8 and C9, the result being the production of membrane lesions which are of the form, more or less, of a [?]cell in the lipid layer on the outside of the organism. And this particular microbe was damaged by serum containing the complement components taken from a sterile colostrum-free piglet which almost certainly could not have occurred through the classical pathway but must have been due to activation by the surface endotoxin to produce precisely the same damage to the bacterium.

<**Humphrey to camera**>

So that we have what looks like an ingenious mechanism of non-specific immunity against those microbes which are able, by means which we don't understand, to activate the alternate pathway.

Now, this sounds fine, but it's not really, it's not enough by itself. For example, it's recently been shown by my colleagues Dr Bullen and Dr Henry Rogers, who are at Mill Hill, that even when a microbe is damaged by complement in this way, it may not be killed provided there's enough iron around in the neighbourhood, and there normally is not much iron because the transferrin keeps it low enough, but if there's haemorrhage, production of haematin level is raised, then even this complement mechanism appears not to be effective.

So, the whole story is complicated. But then so are most biological phenomena because they have to be prepared for an almost infinite range of different situations, often contradictory in their requirements.

When I set out to learn about non-specific immunity I became fascinated by it, but I also realised that we were lagging behind in our understanding of it compared with our knowledge of specific immunity, and I hope that one of the consequences of this talk will be to stimulate the interest of some of you in looking more closely into this question of non-specific immunity.

<**End credits**