Wellcome Film Project

Cellular and Molecular Actions of Anaesthetics

The Scientific Basis of Medicine

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Produced by David Sharp.

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Black-and-white

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<**Opening titles**>

<**Dr John Nunn to camera**>

In the previous programme, we considered the molecular basis for the action of the inhalational anaesthetic agents. And you will recall that there was strong evidence that the action was physical rather than chemical. And furthermore, that the site of action was likely to be hydrophobic. There were two main contestants for the hydrophobic site of action: the first was the lipid of the cell membrane, and the second was hydrophobic receptor sites in macromolecules, such as, for example, proteins.

In this programme you will see that there is evidence for action of anaesthetics at many different sites. Now, the site, or sites of action which produce narcosis are still unidentified. However, in the case of the side actions of anaesthetics, you will see that our knowledge is a good deal more complete. So today, we are going to

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undertake a more detailed consideration of some of the actions of anaesthetics at cellular, sub cellular and molecular level.

<**Nunn over table listing features to be discussed, then to camera**>

Now the first slide will show those features which we are going to discuss. First, depolymerisation of labile microtubules. Secondly, interference with cell division. Thirdly, interference with some enzyme systems and other macromolecules. Fourthly, interference with cell motility and finally interference with membrane transport.

Now, all of these effects with the exception, I suppose you could say, of cell division have at some time or another been thought to play a part in the production of narcosis. And indeed, this may well prove to be the case in due course. However, time will tell. But these factors are certainly of importance in the production of the side effects of anaesthetics.

We're now going to start straight in with actions of anaesthetics on microtubules. And we will start with a reminder about the structure of microtubules.

<**Nunn demonstrates a model of a microtubule, then to camera**>

Now this is a rather rough-and-ready molecular model of a microtubule. These wooden balls here each represent the protein molecules. If we look at the tubule endways, you will see that the protein molecules are polymerised to form a ring containing 13 members. Inside view: the rings are stacked at a distance of 80 angstrom units to form a tube of indefinite length and very considerable structural rigidity. The diameter of the whole structure is about 230 angstrom units and so it can only be seen with the highest resolution of the electron microscope. As far as we know, there is nothing of great importance inside the microtubule and also the area around is apparently free – certainly there is no lipid associated with a microtubule.

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Now these structures are very widely found in plant and animal species and in particular in neurones. They are responsible for structural rigidity but are also associated, in many instances, with cellular motility. Now in the study of microtubules, a very convenient model system is the heliozoan Actinosphaerium. The next slide shows a picture of one of these fresh water pond creatures.

<**Nunn over slide showing heliozoan Actinosphaerium**>

You can see the multi-nucleate cell body and extending from this a series of radiating axopods. Now these are stiffened by an array of microtubules and these microtubules are known to be particularly labile and are sensitive to colchicine, to cold and also to very high hydrostatic pressure. We were able to study the effect of anaesthetics on this system thanks to the kindness of Professor Kitching at the University of East Anglia, and we were able to make a film of the effects with Dr MacDonald, Dr Allison and Dr Hulands. And now we're going to show this film, speeded up by a factor of 10.

<**Nunn narrates over film showing effects of anaesthetics on heliozoan Actinosphaerium**>

First of all, you can see one specimen, suspended in a hanging drop, the control state, exposed to air. Axopods clearly visible. During equilibration with 2% halothane you can see dramatic collapse of the axopods, rather like the melting of was tapers. And then, in the recovery phase – this is actually speeded up by a factor of 40, a rather slower process of recovery, you can see gradually the axopods reform. And ultimately, within about 90 minutes, the creature is completely recovered and apparently none the worse for this experience.

00:05:47:00

<**Nunn to camera**>

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Now, the appearance by light microscopy of the effects of anaesthetics upon this creature suggested very strongly that there was a direct action upon the microtubules. Electron micrographs were prepared across the base of axopods, and the first of these is taken from the work of Susan Craig's and shows the normal appearance of the array of microtubules.

<**Nunn over electron micrographs of heliozoan Actinosphaerium microtubules**>

And here you can see the very elegant array, these are cross sections of many hundreds of microtubules formed in a two-starred helix and a twelve-rayed symmetry. Under the influence of an anaesthetic, next slide, the microtubular structure is lost and we're left with this rather crystalline residue of, presumably, microtubular components which have been depolymerised from their normal format.

Now removal of the anaesthetic resulted in rapid reformation of the microtubular arrays, 2 to 3 minutes being sufficient for the reappearance of structures such as you see here: again two-starred helix with twelve-rayed symmetry but such was the haste of repolymerisation here that you can see that some rather abnormal form has appeared at the top. But there is no doubt that in general this depolymerisation of microtubules that we have seen under anaesthetics is entirely reversible.

<**Nunn to camera**>

And in this respect it is very similar to the action of colchicine. It is known that colchicine binds with microtubular proteins and by inference it would appear that anaesthetics probably do the same. I would not want to give any impression that all microtubules, wherever they may be found, are affected by anaesthetics in the same manner. We shall see, in a few moments, that the microtubules of cilia, for example, are highly resistant to the effects of anaesthetics and it's also been demonstrated by Sauberman that the neurotubules in the optic nerve, which are reasonably close to brain in their configuration, that these are resistant to the effects of anaesthetics.

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Hinkley, however, has reported that extracellular preparations of microtubules can be dispersed, reversibly, by clinical concentrations of anaesthetics. So we are left with a conclusion that there is a good deal of target specificity in this matter and that some microtubules are sensitive and some are not. Now this phenomenon is related to the effect of anaesthetics on cell division, which is the next topic to be considered.

It is an old established observation that anaesthetics interfere with the cell division of most species, particularly studied by Martin during the last century. In botanical specimens it can be shown, very easily, that cell division is grossly impaired by anaesthetics in a reversible manner, and the type of interference is very similar to that, again, of the drug colchicine.

<**Nunn over slide of broad bean root tip exposed to halothane**>

The next slide shows a preparation from the root tip of the broad bean, *Vicia faba*, a preparation made with Dr Lovis of the University of Leeds. Now, this bean was exposed to 1% halothane and instead of the normal appearance of mitosis in the root tip, these chromosomes here can be seen to display the abnormality of mitosis which is typical of that produced by colchicine. Colchicine, as you remember, binds with microtubular protein and since the mitotic spindle consists of microtubular protein, the inference is that the anaesthetic here has bonded and disrupted the mitotic spindle.

<**Nunn over slide of mitotic spindle in fertilised sea urchin egg before and after exposure to halothane**>

Now the mitotic spindle is not visible in this preparation but in the next slide we have been able to visualise the mitotic spindle by means of birefringence in the fertilised sea urchin egg, a study again by the kindness of Dr Forer and Dr Allison, and here you can see the normal appearance of the mitotic spindle in the fertilised sea urchin egg. On exposure to 2% halothane, the mitotic spindle disappeared completely as can be seen in the next slide – here is the complete egg with no sign of the mitotic

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spindle, and on withdrawing the anaesthetic, the mitotic spindle reappeared and division of the cell continued normally.

<**Nunn to camera**>

Now I don't want to give the impression that interference with mitosis by means of a spindle poison action is the only effect of anaesthetics on cell division. It seems very likely indeed, at least in mammalian cells, the predominant effect is not interference with the mitotic spindle but with DNA synthesis. Studies of Bruce have shown this quite clearly. So that there is a certain species difference in the way in which anaesthetics interfere with cell division, although it is interesting that whatever the mechanism concerned, cell division is most certainly interfered with in almost all species by anaesthetics.

Well now, at this stage we're going to move on. Mike Halsey is going to consider the action of anaesthetics on enzymes and other macromolecular systems.

00:10:55:02

<**Dr Michael Halsey to camera, then demonstrates a model of a haemoglobin molecule**>

Well actually, the first macromolecule that we're going to consider is haemoglobin. This has, in fact, been termed by one eminent authority to be a perfect example of an allosteric enzyme.

If one first looks at the co-ordinates of this model which you'll remember is based on the co-ordinates of Brookes and his colleagues and compares, first of all, simply its size – this little thing down here is, in fact, on the same scale, diethyl ether. Here is the haemoglobin molecule and, in fact, it is only one half of it, there are a total of 4 subunits and this illustrates only 2 of them. The interesting thing, when one's concerned with the interaction of haemoglobin with various substances, is that oxygen, for example, interacts over in this part of the molecule, with the haem

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system, whereas at least 1 anaesthetic interacts in this part of the molecule. And thus the idea has grown up that in some cases anaesthetics can interact with haemoglobin and cause very specific changes. For example, most anaesthetics appear to totally un-affect the oxygen combining capacity of haemoglobin, but there have been reports that the carbon monoxide combining capacity is affected by anaesthetics.

<**Halsey to camera**>

Now, not all enzymes are affected equally by anaesthetics. As an example of this, I've taken the recent work done by Hulands and Brammall on the enzymes in the Embden-Myerhof pathway. They have shown that 5 of these enzymes are totally unaffected by very high concentrations of Halothane. However, in contrast, another enzyme, related to the Krebs cycle, glutamate dehydrogenase, has been shown to be reversibly inhibited by concentrations of halothane and of a number of other anaesthetics. And they are currently studying this reversible inhibition and the kinetics that are involved in it. And so one has the idea that certain enzymes are particularly sensitive to anaesthetic but other enzymes are not. Even within one enzyme system there appears to be specificity.

As an example of this I would like to consider the effects of anaesthetics, which are shown up here on the bioluminescent bacteria. It is known that anaesthetics, in fact, can cause, can reversibly depress, the output of light from bioluminescent bacteria.

<**Halsey over diagram showing effect of anaesthetics on bioluminescent bacteria**>

The enzyme system involved has been studied in great detail and the particular interaction with the anaesthetics has been studied by two groups of workers over the years, and the names particularly of Brian Smith and David White are associated with this work. It's known, for example, that the luciferase light-producing reaction is a by-product of the main electron transport chain, and the main electron transport chain is totally unaffected by clinical concentrations of anaesthetics. Furthermore,

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this particular interaction to form this reduced enzyme intermediate I, and the long-lived intermediate II are also unaffected by clinical concentrations of anaesthetics. But by a variety of biochemical techniques it's been shown that the anaesthetic interaction with this whole enzyme system must be very specific, occurring somewhere between II and L* because from here on it's unlikely that anaesthetics are interacting.

<**Halsey to camera, briefly refers to diagram showing anaesthetic interaction, then back to camera**>

Now, so far we have considered the interaction of anaesthetic molecules with macromolecules with a view to understanding more about the anaesthetic interaction and the type of changes that can occur. But people have also studied biochemical changes in the body with a view to suggesting that this may be the primary cause of anaesthesia.

Probably one of the first people to suggest this was Vervoorn, many years ago, who proposed that anaesthesia suppressed what he called oxygen carriers. This idea of anaesthesia producing an enzymatic inhibition has been particularly studied by Questel and his colleagues and here is the familiar electron transport chain, and it was discovered by them that anaesthetics, in fact, interact and block this chain at only one point in the system, namely between NAD and flavoprotein. This has led to the idea that anaesthesia may be related to a suppression of ATP formation. This whole area is extremely complex and at the moment controversial because people have attempted to measure the ATP levels in the brain during anaesthesia. It's now possible, using some techniques developed by Krebs' group in Oxford to study, to produce brain samples, in less than 1 second. Using this technique Bibek[?] showed that during anaesthesia, the ATP levels in the brain, in fact, slightly rose rather than fell as would be supposed by the proponents of the biochemical theories. This, however, is a rather complex situation because it's still possible that there could be local suppression of ATP and, in particular, there have been the proposals of so-called controller cells in the brain.

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Now the basic unresolved question concerning the biochemical theories of anaesthesia is whether these changes are the primary cause of anaesthesia or whether they are simply secondary effects or possibly a consequence, say, of anaesthesia. The old analogy is the chicken and the egg – which came first? Thus, the question is: do biochemical changes cause the inhibition of synaptic transmission, or are they themselves caused by the general depression of the nervous system?

On this note of uncertainty, I think it's best to leave the question of enzymes and return to John.

00:16:36:00

<**Nunn to camera**>

Well, now we shall take up something which is rather more clear cut, at least in the observations, that is to say the effect of anaesthetics upon motility. From the practical point of view, the most important effects of motility are upon cardiac muscle and to a lesser extent upon skeletal muscle. But there are, in fact, interferences with all forms of motility that so far have been investigated under the influence of anaesthetics, and I'm going to start with a film of the effect of anaesthetics on the motility of lymphocytes.

This is a film made with John Sharp of the University of Leeds; it is a phase contrast film of lung epithelium in which we were fortunate in having lymphocytes which had invaded the field and thereby we were able to study the effect of anaesthetics upon them. Now, we could start the film.

<**Nunn narrates over film showing the effect of anaesthetics on lymphocytes**>

This is time-lapsed and is being run at 30 times real speed. You can see the background of lung epithelial cells grown in culture. The rounded cells, moving rather

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briskly over a limited area are, in fact, lymphocytes. The spiky cells are macrophages and they can be ignored for the purposes of this presentation.

You will notice two lymphocytes in the upper part of the central part of the frame. Now at the moment the culture is in equilibrium with air, no anaesthetic is present and there you can see normal motility. The medium is now replaced with one which has been equilibrated with 2% halothane vapour and if you concentrate on the two lymphocytes, again in the central and upper of the field, you will see that their motility is strikingly reduced. They have rounded off and are showing a curious bubbling motion on the surface. During recovery, when the medium is again saturated with air, one of the two lymphocytes recovers rapidly to its normal motility, the other, I'm sorry to say, appears to have died under the anaesthetic. Throughout this you may have noticed that the macrophages were unaffected.

Now, turning to a culture of thymus. There are a great many lymphocytes in this preparation and we are dealing with a statistically more manageable number of cells. Now here you see many lymphocytes of which a high proportion are actively motile and may be seen darting across the field with the characteristic type of motion. This film is also time-lapsed, with a time factor of about 30 and you're seeing now a 30 minute anaesthetic condensed to 1 minute. And during this period you can see that the motility of the lymphocytes is very greatly reduced. They still move a little, making short bursts, which is followed by a period of immobility with vigorous bubbling on the surface of the cells. This is the final few minutes of the anaesthetic when almost all movement has ceased. And now in the recovery phase, when the medium is again saturated with air, you will see a very clear, obvious and statistically highly significant return of motility of about 50% of the cells which were anaesthetised. The remaining 50%, again, failed to regain their normal motility.

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<**Nunn to camera**>

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Now this very dramatic effect on lymphocytes is similar to the effect which has been seen on the cellular slime mould, *Dictyostelium discoideum*. Studies by Allison and Wiklund have shown that anaesthetics will stop the movement of these normally very active cells, round them off, and give them an appearance which is very similar to that which may be obtained with the drug cytochalasin B. Now, cytochalasin B is known to interact with actomyosin and prevent movement in this manner – and by implication it would seem likely that anaesthetics may well be acting directly on actomyosin in this situation.

There is very great selectivity in this field. If we take, for a moment, the difference between cardiac and skeletal muscle, it should be pointed out that skeletal muscle is almost entirely insensitive to anaesthetics and responds normally to electrical stimulation throughout the clinical range of concentrations of anaesthetics. In contrast, cardiac muscle is very sensitive and shows substantial depression within the clinical range of anaesthetics. Different anaesthetics, interestingly enough, act differently in this respect. And so here, again, there is great target selectivity and also differential sensitivity to different anaesthetics. From the point of view of mechanisms of narcosis, there is always the possibility that actomyosin may be responsible for the reduction release of transmitter substances in the pre-synaptic region and that anaesthetics could act by interference with actomyosin at this particular site.

Well now let's look at another example of cellular motility, cilial beat. An old observation of Claude Bernard in the last century that the swimming of species such as paramecium was inhibited by anaesthetics. Now we have used *Tetrahymena pyriformis* which is a very convenient organism to study.

<**Nunn over slide of *Tetrahymena pyriformis*, then short film showing the effect of anaesthesia on the organism**>

The next slide shows a portrait view; a pear-shaped organism covered with cilia and swimming very rapidly with the sharp end first. Now, Jean Sturrock and I have studied this creature and measured its swimming velocity at different concentrations of a range of different anaesthetic agents; a preparation which is highly quantitative.

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Now, we're going to show you a short length of film where you can see clearly the rapid action of anaesthetics and also see how it is totally reversible. This is shown in real time.

Now there you can see the field of Tetrahymena swimming briskly and now the anaesthetic is acting and you can see a very clear reduction in velocity bringing them practically to a standstill with no really effective forward movement. Now the anaesthetic has come off and there is an instantaneous resumption of velocity; this is entirely reversible as you can see. We'll give them one more shot of the anaesthetic: there again they're reduced in their swimming velocity, and then withdraw the anaesthetic and away they go. This has been timed with care by analysis of single frames and, in fact, it takes about 6 seconds for full effect.

<**Nunn to camera, then over diagrams charting the effects of different anaesthetic drugs on swimming speeds of Tetrahymena**>

Now from films of this nature, one can measure the velocity of individual Tetrahymena and from this means we've been able to construct dose response curves for different anaesthetics.

Now if I can turn to the diagram over here. On this axis I've got the concentration of six anaesthetics as percentages of one atmosphere. And on this axis, here, I've got the effect of the anaesthetic represented as percentage of control swimming speed. This is a log logit plot. Now, for the six anaesthetics you will see there are dose response curves of roughly equal slope with the possible exception of diethyl ether; taking halothane, the most familiar anaesthetic nowadays, you can see that 50% effect takes place at 1% of an atmosphere. That is to say that this is an effect which does occur within the clinical concentration.

However, notice the three most potent agents – the ranking order is not the same as for narcosis. Methoxyflurane, you'll remember, was the most potent anaesthetic as regards narcosis, but here it ranks number 3, being displaced in first place by trilene

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(or trichloroethylene) and in the second place by chloroform. Now this means that the correlation of lipid solubility should be lost and, indeed, so it is.

In this diagram here, I have lipid solubility on this axis, the ED 50 for reduction of swimming velocity here, and our anaesthetics arranged here, and you can see that the correlation is very much less satisfactory than for narcosis and the departure of these points from the line of best fit is very highly significant.

00:25:05:11

<**Nunn to camera**>

Now, what does this mean? Well, it might, on the one hand, mean that the anaesthetics are acting in cilial beat in a lipid which is of different solubility parameter to that of olive oil which, as you remember, is the oil which has been used in determining the correlation with lipid solubility in relation with narcotic potency; but on the other hand there remains the possibility that the action here is not so much in a bulk lipid phase but rather in receptor sites, in macromolecules, and in this situation it is conceivable, so far as I can understand the situation, that factors other than pure lipid solubility may be relevant. One thinks, perhaps, of the size and shape of molecules and this might distort the correlation as you can see in that diagram. And a loss of true correlation with lipid solubility, in fact, turns up repeatedly when side effects of anaesthetics are quantitatively investigated.

Now one final point on cilial beat. You will recall that with *Actinosphaerium nucleofilum* there was a very marked change in the ultra structure of the microtubules – it was tempting to see whether the ultra structure of the cilia should be altered by anaesthetics.

<**Nunn over slide showing transverse section of cilia exposed to halothane**>

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The next slide shows a transverse section of a group of cilia exposed to 6.7% halothane, and this, I would remind you, is approximately 10 times the normal anaesthetic partial pressure required for anaesthesia in man.

Now this EM, prepared by Ted Wills and Joan Richmond, shows cilia under these circumstances retaining their normal structure. You can see the outer line doublets of microtubules and the inner central pair, unchanged. Other features such as the dynein arms, the nexin and the central sheath and the microfilaments are also all preserved and we have not, so far, been able to determine any gross change in the ultra structure, forming, I think, a very nice example of target selectivity between the microtubules in the cilia and the microtubules in *Actinosphaerium* nucleofilum.

<**Nunn to camera**>

Well now, the last and perhaps the most important aspect of cellular effects of anaesthetics which we are going to consider, is the effects on membranes and for this I'm going to pass you over to Mike Halsey.

<**Halsey to camera**>

We've already considered that enzyme specificity as far as anaesthetic effect, but there is also the great specificity of action of anaesthetics on membranes. Now as an example of this, I would like to choose some work done some years ago by Terry Wood, Brian Smith and myself on the effects of anaesthetics on red cells. You know that when the red cells are incubated, the sodium concentration which is built up during carbon storage tends to decrease. This is illustrated over in this diagram, over here.

<**Halsey stands, refers to diagram showing** >

Here we've plotted on the vertical axis, the sodium concentration inside the red cell and we have, in fact, incubated it in various concentrations of diethyl ether. The top curve, the incubation was done in the presence of ouabain and therefore it

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represents the passive permeability of the ion. However, in the bottom curve we've got the addition effect of the active transport of the ion because the sodium pump has not been inhibited. And furthermore, the interesting fact is that this concentration of diethyl ether can be related to that occurring in general anaesthesia. And the concentration of ether in general anaesthesia, in this particular case, would be in this region, way down here. Whereas the effects that are particularly marked occur in this region at some 10 times the anaesthetic concentration. In this particular region, incidentally, lysis of the cell also occurs.

<**Halsey to camera, then over diagram**>

Now since that time, there has been considerable investigation of the effects that occur during at the anaesthetic concentrations in that particular system. But to turn from the general effects on red cells to the more specific ideas about how anaesthetics might block synaptic transmission, and presumably interfere with the membrane processes associated with it. It's quite possible to envisage that the process of synaptic transmission is associated with ion fluxes throughout pores in the membrane. And although everybody talks about pores, it's important to realise they've never actually been seen, and therefore all the evidence for them is of necessity indirect. However, it's quite possible to speculate about them. And this diagram over here is one speculation that's taken place.

Here we have two situations of a rather crude and simple membrane that's been simply represented by this part here and here, and also in the lower part here and here. And you will see that we have a hole coming through the membrane at this point. Now in the resting state, one part of the membrane, which has been crudely marked the gate is, in fact, possibly the polar portion of phosphatidyl tails in the lecithin molecules, is bonding right across the holes with calcium as a bridge head. And this is effectively interrupting the interaction of the sodium ions on the outside of the membrane who would, during the depolarising phase, normally move straight through the system as the calcium is removed and the gate opens up. However, in this particular diagram I have made the system potentially anaesthetised and

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therefore these letters here, A, represent anaesthetic molecules in both situations which are, in fact, effectively blocking the passage of sodium ions.

00:30:43:00

<**Halsey to camera, interspersed with reference to diagrams**>

One could, of course, have similar ideas concerning the release of neurotransmitters. But one particular thing that we emphasised in the last programme was the expansion of membranes by the anaesthetic molecules dissolving. And this next diagram over here, of an equally rather crude and naïve nature of a membrane, illustrates that particular point.

Again, one has a view of a membrane and in this case the top view is the unanaesthetised membrane whereas the bottom view is the anaesthetised membrane. The outer protein sheath is represented by the jagged line over here, whereas the lipid molecules are represented, more conventionally, on the inside. However, one additional feature is that protein molecules permeate right through the membrane and restrict expansion in this direction. And thus you will remember that when we talked about anaesthetic interaction one can see that there will be an expansion of the membrane but it is, in fact, tending to expand in this direction 2-dimensionally, rather than 3-dimensionally. And for this reason, the pore size, if that presumably exists, is closed. There's one additional feature of this membrane, which I pointed out to you, and that is from the neurophysiological data, it appears that the pores from the membrane are more in the nature of a hole in the membrane rather than a complete tunnel going through the membrane.

Now there are many other aspects of membrane structure that we could consider but there is just one further speculative idea that I would like to deal with. If we move over to this diagram, this is the traditional Davson-Danielli model, and you'll remember we used it last time in discussing the biomolecular lipid layer inside. However, ideas since those original proposals have now moved on and it's now thought that, in fact, proteins don't simply lie on either side of the biomolecular lipid

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layer, but they also pass right through the membrane and there is, in fact, great blobs of protein situated right in the membrane. Furthermore, it's known that these blobs of protein do, in fact, have lateral movement within the membrane and one can imagine that protein molecules are, in effect, floating in a sea of lipids.

Now for our last diagram we're looking not at a sideways view of the membrane but, in fact, from a bird's eye view, from outside in the extracellular fluid; one's looking down on the membrane and viewing both the proteins and the lipids in that area. And this is illustrated over in this diagram.

If one concentrates first on this part of the diagram one sees, as I've already emphasised, a bird's eye view of the protein molecules, represented by the large blobs, floating in these sea of lipids all around. However, from some general neurophysiological evidence, it's known that in the subsynaptic membrane which, after all, is a very relevant one to our discussion, the protein molecules are far more concentrated and it's been suggested, particularly by Dr Tony Allison, that these may be produced and linked together by what have been generally called specific products. However, further changes also occur when a neurotransmitter might be released and interact within this system. And in this case, in this part of the diagram, it's possible to conceive that the, let's say, acetylcholine molecules interacting here, here, here, here and here and forming the whole structure into a regular symmetrical array with an empty space in the middle through which the ion transport could come through. Incidentally, it's known in the synaptic system, the holes through the membrane are probably far larger than those that occur in the axonal membrane.

Now, one necessarily must speculate as to how anaesthetics could interrupt this system if, indeed, this system does exist. And this has been done in this bottom part of the diagram where, again, the anaesthetic molecules are intacted here, but instead of simply vaguely space occupying the system, they do in fact form specific interactions linking the protein molecules in such a way that they cannot form a regular array in spite of the fact that the acetylcholine still interacts with one part of the protein molecule.

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Of necessity, this discussion about membranes must be a very brief one and has been very selective and has left out much of the neurophysiological studies that are currently going on in this area. However, it is clear from these studies that this area of research is of potentially great importance and it certainly is a very exciting and fast-moving field of research. But at the same time, it's also clear that there is a very long way to go before we fully understand the processes involved in the interruption of synaptic transmission. And so finally, I'd like to hand you back to John for a summary of the programme.

<**Nunn to camera**>

Now we have taken you on a rapid tour through the field of studies of cellular and molecular effects of anaesthetics. Time has not permitted us to cover all the studies in this field and we have therefore selected those aspects which we feel to be of special interest. We have also been unable to take into account the actions of, for example, local anaesthetics, steroids, barbiturates etc. and we have concentrated on the inhalational anaesthetics. Even with these limitations, at the present state of our knowledge, the mechanism of synaptic block has still not been elucidated. We have, however, made some progress in our understanding of the ground rules by which inhalational anaesthetics may interact with biological systems and, in the process, we have gained some insight into our understanding of the mechanism of action of certain of the side effects of anaesthesia.

<**End credits**>

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