BRITISH MEDICAL JOURNAL

LONDON SATURDAY NOVEMBER 6 1954

THE EXPLORATION OF THE CELL IN HEALTH AND DISEASE*

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Quietly but relentlessly a revolution in cellular pathology is taking place. No longer must we be content to regard the cell as a self-contained unit, complex and seemingly imponderable; the first timid attempts at dissecting its structure are now being replaced by bold, nay outrageous, efforts to take the cell to pieces in order to find out what each part does. I will show that the cell membrane, the nucleus, and even the smallest formed elements—the mitochondria and microsomes—no less than the by-products of cell activity, can be separated from their usual habitat without any too violent disturbance and studied as entities. And there are signs that an even more remarkable procedure is on the way to exploitation. Not only can the all-important nucleus of a cell be removed but another nucleus from a closely related cell can be put in its place, thereby enabling that cell to carry on its function for at least some time.

A new Form of Pathology

Side by side with these micro-anatomical developments have come astonishing inventions in the field of biochemistry and biophysics which are invigorating the momentous domain of function. We now may contemplate a new form of pathology based on an intracellular physiology which in turn is built up at a submicroscopic, indeed a molecular, level. Let me show what this revolution means by a reminder of the landmarks in the history of the cell.

The first vague impressions that life might derive from simpler, unitary forms took on substance when the imperfections of the early microscopes were corrected around about 1830. Cells were then quite clearly seen, and within 20 years assumed new rank as living, dynamic units. At this time, too, arose cellular pathology, which was welded with astonishing prescience into a vigorous science. A century of progress followed which was devoted largely to the microscopical scrutiny of the cell, especially its interior and its derivatives. The harvest was a remarkable one, for the morphological elements were discovered, something was learnt about their functions, and the first steps towards an understanding of organization and chemical constitution of the parts were taken. Methods, however, were relatively crude and for the most part indirect.

This century has seen the invention of new tools which are still in process of exploitation. Isolated single and small groups of cells are now accessible to respiratory and metabolic investigation as the result of the ingeni-

ous experiments of Linderstrom-Lang, Holter, Zeuthen, and O'Connor. Microsurgery and differential centrifugation enable us to separate the various morphological constituents from the parent cell and with the aid of chemical refinements subject them to analysis. Cytochemistry and enzymology are being pursued vigorously with the object of learning more about intracellular respiration and metabolism. With the perfection of the electron microscope we may now learn about the molecular dimensions of the cell and its organelles; in this task we are assisted in a less specific fashion by the discoveries of polarization optics and x-ray diffraction. To the pharmacologist and immunologist we owe a great debt for the laying bare of unsuspected regions of the cell that are vulnerable to drugs, hormones, and antigens, while few can consider unmoved the ingenious researches of the neurophysiologist whose analyses of electrical responses penetrate to the very heart of specialized cellular function.

Now, I am sure that pathologists will not allow this surge of discovery to pass unnoticed over their own particular province; indeed, I see many signs that the impact is already producing steady results. But I fear that progress is slow and the young worker may be overwhelmed by the heavy burden of facts with which he is faced when he decides to take up pathology as his life's work. For these reasons I am encouraged to say something of what I am learning through contact with the fresh young minds of my assistants and other friends who are taking an active part in this field of endeavour. We shall embark upon a voyage of exploration of an ocean which is to a large extent uncharted. I trust that the end of our voyage will see us a little clearer about the direction in which we are going and encourage us to venture still further into the unknown. Clearly, in making this journey we must have a compass by which to steer, something that will give us a steady sense of balance and direction. Let this be our endeavour to discover "identity amidst diversity," or the Kantian doctrine "to understand nature by means of exact concepts." Plato said that if he met the man who could detect the one in the many, he would follow him as a god. May that be our precept in the short time we spend together.

The Cell Membrane

Much that we know about the cell membrane comes from studies on the passage of diffusible solutes such as common salts and solvents, especially water, into cells and, above all, on the exchange of molecules and ions between cells and their environment. It is precisely at the cellular

*The first Matthew J. Stewart Lecture, delivered at Leeds, June 17, 1954.

surface that the first obstacle to penetration of the cell is encountered, and we are led to ask why this should be.

Direct examination of the cell membrane has, up to the present, proved possible only in the mammalian red corpuscle, from which most of the cytoplasm can be removed as the result of haemolysis, leaving behind what appears to be a true membrane almost free from intracellular material. Under microscopical examination with dark-ground illumination or with polarized light this thin structure stands out brilliantly, displaying no signs of internal structure. Chemical analysis of these ghosts tells us that much lipid and protein is present; the ratio of total lipid to protein by weight in the ghosts of beef red cells turns out to be 1 to 1.76. All types of lipid are found, though phospholipids form the highest proportion and constitute, with cholesterol, over 90% of the total lipid. Apparently the phospholipids are strongly bound to the protein, but portions of the cephalins, for instance, are loosely bound and can be readily extracted.

By making certain assumptions about the molecular weight of the protein—for example, that its molecular weight is not less than 70,000—we can calculate that there are some 70 lipid molecules for every protein molecule present in the membrane, and, of these, 32 molecules are cholesterol and 38 phospholipid. The latter are further divided into 27 cephalin, 7 lecithin, and 4 sphingomyelin molecules per protein molecule. The plasma membrane of red cells thus has a relatively large number of lipid molecules loosely or firmly bound to each protein molecule.

Calculation also gives some idea of the thickness of the plasma membrane concerned in permeability phenomena, and it seems to be between 50 and 60 Å. This may well be made up of a double layer of lipids accounting for 30 Å of thickness and a protein layer which when dried measures 20–30 Å. To these must be added an unknown quantity of water.

Through such a membrane water diffuses with great rapidity along aqueous channels in the membrane. There are indications, too, of other kinds of space, for if the haemolysis of red cells during unfavourable osmotic conditions is followed by means of high-speed photography, it is found that haemoglobin molecules escape uniformly all over the cell surface not unlike water through a sprinkler. Under other conditions, however, the surface of the cell can be made to burst with extrusion of haemoglobin at one point. Uniform loss from many points requires that the channels through which haemoglobin diffuses to the exterior be uniformly spread over the surface of the cell.

These facts may be reconciled in a three-dimensional model, composed of an interlacing meshwork of protein molecules, possibly two molecules thick, with interspersed lipid molecules bound to the protein or to each other and forming a porous entity. These pores vary in diameter, show a water channel near to the protein and phospholipid molecules which leads from the external environment to the cell interior, and probably number 3,000 to 300,000 in each cell membrane. Quite likely, too, there are water pools in addition to these channels, which account for the speed with which haemoglobin molecules leave the red cell during haemolysis. Lipid molecules bound to protein have been pictured as swinging out of position during haemolysis so that the pools become continuous with the water of the external environment and of the cell interior (Parpart and Ballentine, 1952). The strongly bound lipid molecules in the plasma membranes may thus behave in the manner of a swinging door, hinged by their head attachment to the protein and caught at their outer edge by interaction with long-chain fatty acids of weakly bound lipid. In this way pores can vary from time to time in their dimensions in the same cell. So, too, they may vary in size, number, and water content from species to species, thus accounting for the manifold permeability differences that have been noted by many workers.

A wealth of observation, accumulated since the pioneering studies of Pfeffer and Overton, has taught us some

of the principles that underlie this business of penetration of cells by solutes. Overton deserves the credit for recognizing that an organic molecule can be so changed that its affinity for certain organic solvents as compared with water tends likewise to increase with its ability to enter living cells, even though there is at the same time an increase in its molecular weight and volume (Jacobs, 1952). If, for instance, chemical groups such as CH_3 , C_2H_5 , or C_6H_5 be attached to the molecule, then that molecule will, in general, penetrate the cell more rapidly although it is enlarging. The cell surface invariably contains large amounts of lipid which serves as a solvent for many hydrocarbons. In other words, possession of lipid-soluble characteristics promotes the power of penetration through the cell membrane by the compound under consideration. So, too, a non-aqueous barrier presented to the external environment by the bound-lipid molecules would normally confine the passage of lipid-insoluble molecules to the aqueous channels.

The size of a soluble molecule may also exert an important influence on its capacity to enter cells. A porous membrane would thus behave as a sieve, allowing some molecules to pass through and holding others back. The water pools together with the aqueous channels thus may well determine the rate of penetration of lipid-insoluble molecules and the limits of molecular size for penetration.

Another possibility exists in the form of specific affinities possessed by certain compounds for the surface of some particular cell or cells independent of lipid solubility and molecular size. Urea, for instance, has a very high permeability value for mammalian red blood corpuscles which has nothing to do with lipid solubility or molecular value. So far little has been learnt about these affinities. In some cases special enzyme mechanisms may be concerned as with the entry of glycerol into bird red corpuscles (Jacobs, 1952). Thiamine, too, is first adsorbed to the surface of yeast cells to be incorporated into the cytoplasm only after phosphorylation (Sperber, 1941). Selective ion passage across the cell membrane most probably is a matter of selective charge on the aqueous channels (Michaelis, 1925).

But even the penetration of water into many cells is not the straightforward matter of osmosis, since it now turns out that it is exceptional for cells and their contents to be in osmotic equilibrium—that is, isotonic—with their extracellular environment (Opie, 1949). Largely owing to the recent researches of Krebs and his colleagues and Robinson (1950, 1952) we suspect that cell respiration governs the water balance because some of the energy which it releases is used for active transport of water out of the cells through the mediation of compounds containing high-energy phosphate bonds. Robinson points out that if the contents of cells are hypertonic then there must be an active transport of water outwards across the cell membrane, for inward transport of solutes through a membrane freely permeable to water could hardly account for a sustained difference in osmotic pressure and would cause the cell to swell continuously. Active transport probably also applies to loss of potassium and other ions. Thus there would appear to be pumping mechanisms bound up with cell respiration situated quite likely in the cell membrane, but we have no knowledge of their precise location.

These modern views are of great interest for the pathologist, especially since Robinson has given us a new lead regarding the explanation of intracellular accumulation of fluids. Pathological experience shows that a poor oxygen supply to cells of most organs is followed by cloudy swelling and hydropic change, the main feature of which is water-logging of the cells. Robinson suggests that cloudy swelling might arise primarily from derangement of the active transport of water which normally maintains the volume and the hypertonicity of respiring cells. This ingenious theory would certainly account for the frequent association of cloudy swelling with anoxia.

The cell surface is probably a mosaic of patches composed of proteins, lipids, polysaccharides, extremely variable from species to species, but possessing, we have seen,

features common to all cells which control the interchanges between cell and environment. Unique among these components are the surface enzymes. Part, at least, of cell respiration, in the broad sense of oxidative breakdown, takes place at the cell surface; later on we shall see that part, too, is concentrated within the cell in the mitochondria. The cell surface has been likened by Runnström to a cell organ, but unfortunately we know nothing about its intimate topography. There is every reason to believe, too, that the cell surface is capable not only of secreting and expelling components, but also of releasing substances to the endoplasm which may influence the behaviour of distant regions within the cell. But we come up against an almost unexplored territory here, and we must await new discoveries before embarking upon new theories.

Pharmacological Discoveries

Before passing on to fresh regions of the cell, allow me to recall a few of the discoveries made by pharmacologists which have their lesson for the pathologist. I would remind you of the growing opinion that effective drugs react first with specific surface constituents, patches, or receptors, and that this association leads secondarily to changes in the pattern and function of other parts of the cell (Loewi, 1952). An elegant proof of such surface action is to be seen in some experiments carried out by Cook in 1926 and based on the use of methylene blue. This dye can penetrate heart cells to produce deep staining of the cytoplasm and also exerts an atropine-like action on the heart cell. If the heart be deeply stained with methylene blue and then washed thoroughly to remove any dye outside of the muscle cells the atropine-like action ceases, although the heart remains deeply stained. But the action returns precisely at the very moment a trace of methylene blue is added again to the preparation, which means that the cell surface is the region responsible for the atropine effect.

Further evidence in favour of specific surface patches comes from the calculation of the extremely minute quantities of drugs such as acetylcholine and adrenaline capable of exerting cell stimulation. Some years ago Professor A. J. Clark calculated that acetylcholine in effective amounts covers only one six-thousandth part of the cell surface, a conclusion difficult to assess except on the assumption of sensitive patches. No doubt such enzymes as cholinesterase are located at the cell surface in such patches.

Proteins, too, are supposed to act at the cell surface, though some reach the interior by phagocytosis or after injuring the point of contact. The latter seems to be the case with some viruses and toxins. Proteins marked with isotopes—for example, I^{131} —can penetrate renal tubule cells during excretion, as my assistant, Dr. W. G. Spector (1953) has shown, but it is not known whether such cells are normal or damaged. Most proteins, we would expect, would not be able to diffuse into intact cells because of their large molecular weight. Some sort of combination with surface constituents may initiate the cell response in these instances, as, for example, in the Dale experiment with the sensitized uterus reacting instantaneously to the specific antigen.

Let me here recall certain observations that may bear upon the action of poisons on cells. With refinements of quantitative methods we have learnt that cell permeability may be profoundly modified by changes in hydrogen-ion concentration and temperature variations. Extremely low concentrations of copper instantaneously lower the permeability of human red blood corpuscles for glycerol (Jacobs, 1950) due to inhibition of an SH-containing enzyme apparently concerned at the cell surface in a metabolic process which yields the energy for the uptake of part of the glycerol (Le Fevre, 1948). Again, it is surely suggestive that heavy metals such as arsenic and mercury inactivate the SH group of enzymes containing that group as an essential part. Other drugs interfere with enzyme activity by competing either with substances physiologically acted upon by enzymes—substrate competition—or with elements needed for the synthesis of enzymes. Hydrogen cyanide, for in-

stance, combines reversibly with the iron of the haem forming the prosthetic group of oxidizing enzymes and thus prevents those enzymes from being renewed. Tannic acid is an example of a poison that acts on the protein of the surface pores, decreasing the amount of water within the water channels, and profoundly modifying cell permeability (Jacobs).

Finally there is an interesting group of bacterial toxins whose precise action on the cell membrane is being elucidated by workers in this country. Foremost among these is Professor C. L. Oakley, whose researches have done so much to show that lecithinases are largely concerned in the action of certain clostridial toxins. *Cl. welchii* α toxin is now known to be almost certainly a specific lecithinase that can exert lethal, haemolytic, and necrotic effects in susceptible animals. The haemolysis of horse and sheep red corpuscles by this enzyme has been shown by Dr. Marjorie Macfarlane (1950), of the Lister Institute, to be always preceded by decomposition of some of the phospholipid of the cell, much of which, we have seen, resides in the cell membrane. The enzyme fits into the cell surface in which the substrate, phospholipid, is embedded. The lecithinases vary in their activity with different species of red cell (Oakley *et al.*, 1947), and this may well be due, as Dr. Macfarlane suggests, to differences in configuration amongst the lecithinases on the one hand and amongst the species-specific red corpuscles on the other. Such investigations are likely to throw light not only on immunological differences between bacterial species, but on the structure of the cell membrane.

The Cell Nucleus

It would scarcely seem possible that doubts should exist in our minds about the main functions of the nucleus. For more than 150 years contributions to nuclear knowledge have been unending, and we take it for granted that, as Mazia (1952) has so well put it, "the cell without a nucleus is a cell without a future." Yet what do we really know about the nature of that unique intracellular organ, save that within its confines go on great perturbations of cell energy from which come rifts in intranuclear and cellular structure which ensure the propagation of the species? Let me consider for a few moments the sum and substance of our knowledge about this mysterious body in the light of recent investigations.

Methods now exist whereby the nuclei of many cells may be isolated from their correlated cell structures and studied as isolated entities. Electron photomicrographs of isolated nuclear membranes have been obtained and present evidence that such membranes consist of two layers, an inner homogeneous film and an outer porous layer with pores of 400 Å diameter regularly arranged at 1,000 Å intervals (Callan *et al.*, 1949; Callan and Tomlin, 1950). Some at least of these membranes seem to be freely permeable to molecules of subcolloidal dimensions (Goldstein and Harding, 1950), so that it is possible that a wide variety of toxic compounds may be able to pass into the nuclear substance as well as being able to exert an influence on the membrane itself. In this way chromosomes, which are elastic structures enclosed in a limiting membrane, may become the targets of radiations and nuclear poisons, but no one can say at present what is the mode of action of such agents on the chromosome. Until we know more about chromosomal membranes and internal structure we can scarcely hope to follow the biochemical intricacies of chromosomal injury. Electron-microscope investigations show no structure that was not previously known, nor is there any evidence of orientation of smaller units forthcoming from x-ray diffraction studies.

Nuclear enzymes have been the subject of intensive investigation recently. Here, too, isolated nuclei have been employed, and with startling results, for it seems that no case has been encountered in which an enzyme is exclusively localized in the nucleus, or in which even the greater part of the cell's supply is concentrated in the nucleus. Even more unexpected is the discovery that enzymes peculiar to

energy-yielding processes are either absent or remarkably low in concentration. This applies in particular to the glycolytic system, succinic dehydrogenase, and cytochrome oxidase. On the other hand, there are a few systems—for example, alkaline phosphatase, desoxyribonuclease—which may be present in higher concentrations in the nucleus than in the cytoplasm. Probably the nucleus is concerned with the long-term continuous replacement of the cell activities, for turnover of metabolic intermediates and proteins has been demonstrated but not of cytoplasmic enzymes or enzyme groups (Mazia). Such replacements would require the transfer of some nuclear product to the cytoplasm; there is reason to believe that this is true of material rich in ribose-nucleic acid from certain chromosomes to the periphery of the nucleus and thence to the cytoplasm (Caspersson, 1947; Brachet, 1950). So far as its energy supply is concerned, the nucleus might be said to be parasitic on the cytoplasm (Mazia).

It is now possible to remove the nucleus successfully from the living cell or unicellular organism—for example, amoeba—and even to transplant a nucleus from an intact cell into one deprived of its nucleus (Comandon and de Fonbrune, 1939; Mazia, 1952; Lorch *et al.*, 1952; Lorch and Danielli, 1953). After enucleation, various cell activities go on uninterruptedly, while others continue for a time. Amongst the former are amoeboid movement—although movement may be uncoordinated—cytoplasmic streaming, co-ordinated ciliary movement, photosynthesis. Synthetic activities and secretory functions involved in the capture and digestion of food continue, but soon become ineffective. With some forms of life, the enucleated portion of the cell makes a start towards regeneration, but apparently it cannot carry this to completion (Haemmerling, 1934). The enucleated *Arbacia* egg is not abnormally permeable to water nor is enucleated *Amoeba proteus* or pieces of enucleated amoeba abnormally permeable to D₂O (Prescott and Mazia, 1954).

A cell that has been without its nucleus for three days recovers in all respects when it receives another nucleus, and can produce many offspring. This shows that the cell can persist unchanged in its normal function throughout a short period when deprived of its nucleus. After an interval it begins to run down and it eventually dies because synthesis and absorption give out.

I confess I am puzzled by the application of these discoveries to cellular pathology. I think that most of us have been brought up in the belief that nuclear damage, as displayed by the familiar disturbances of karyorrhexis, pyknosis, and karyolysis, is an early and serious sign of cell injury, from which there is seldom recovery. Now it seems that cells may survive for many days without a nucleus, presenting no obvious morphological disturbance and very little functional decline. Surely the time has come when we must reconsider the nuclear field and be prepared to discard much of what we have long held to be established.

Mitochondria

Among the most interesting discoveries in cytology that promise revolutionary advances are those which concern the mitochondria. Despite a long period of uncertainty about their very nature as entities, and the clash of rival views touching their functions, we now possess clear evidence that they are important cell organelles which can be isolated and studied apart from other cell constituents and used as tools for the investigation of toxic agents. It is the latter enterprise that I wish to embark upon, for I believe that here lies to hand a vast unexplored region of cellular pathology. But before doing so, let me mention what we have learned in recent years about the properties of normal mitochondria.

When living cells are subjected to centrifugal forces several hundred thousand times gravity they display a high resistance to disintegration, which suggests that cytoplasm or, at any rate, the cortical zone of cytoplasm consists of a framework capable of deformation but not disruption by the strong centrifugal forces (Beams, 1943). Nevertheless,

a certain amount of stratification of cell contents occurs under these conditions, and organelles and other cell contents become arranged in various layers, as might be expected if the milieu possessed a gel structure (Runnström, 1952). Since these displaced organelles return to their usual locations after centrifugation is over, it seems more than likely that a great deal of give and take must exist within the cyto-skeleton, a view that is supported by the cell's possession of movement, the complex phenomena of mitotic division, and the expansion and collapse of vacuoles. All these observations prepare us for the discovery that mitochondria are, indeed, entities in which are localized special functions.

The early studies on tissue culture cells, especially the use of cinephotography for the recording of structural change in living cells, taught us that mitochondria vary from granular to rod forms from moment to moment, seeming to anastomose and assuming bizarre patterns and even dividing when the cell divides (Lewis and Lewis, 1915; the Cinti film; Hughes and Fell, 1949). These apparently pointless facts received fresh significance when, with the invention of new methods for the segregation of organelles, mitochondria were isolated and their functional capacities laid bare to investigation.

Two such methods are in common use. Holter and his co-workers centrifuge cells or unicellular organisms at 1,000–2,000 g for 30–60 minutes and separate cell fragments containing concentrates of various cytoplasmic constituents by means of micro-needles (Holter, 1954). The method has the advantage of being less apt to produce artifacts than more violent centrifugation, but the amount of material so obtained is very small and such cytoplasmic samples are never pure. Although still in the early stages of improvement, this technique has already yielded valuable results which are complementary to those obtained from other studies.

The second method is much more ruthless, for the tissue is homogenized after freeze-drying, and then subjected to differential centrifugation whereby the particulates of the disrupted cells are isolated in practically pure form. Since there is a tendency for the granules to agglutinate during centrifugation isotonic sucrose is used for suspensions, and this proves satisfactory without disturbing the osmotic relationships between particulates and medium. Mitochondria are spun down by centrifugation for 20 minutes at 24,000 g; finer particles still, the so-called microsomes, are obtained from the supernatant after centrifugation for two hours at 41,000 g. These methods, developed largely by American and Belgian workers, allow of the quantitative fractionation of the whole cellular body; indeed, an essential safeguard of the method requires that the sum of each distinct component thus separated must account at the end, in mass or specific activity, for the total cell mass or the total specific activities exhibited originally by the unfractionated cell (Claude, 1954). In other words, a balance sheet must be struck which shows how much of a function is accounted for by each fraction in terms of the total activity of the cell under consideration.

In my department, we stick closely to the methods laid down by Claude, Lehninger, Hogeboom, and their collaborators, employing a machine capable of differential centrifugation up to 60,000 g. Photographs of isolated mitochondria in isotonic sucrose show them to be yeast-like bodies with little structure, but the recent electron-microscope studies of Pallade and Beams indicate a limiting membrane and a complex pattern of leaves and compartments not unlike that of a beehive. Mitochondria are profoundly influenced by changes in the salt concentration of the medium in which they are suspended, for they swell enormously and break up in hypotonic saline or distilled water in much the same way as do red corpuscles (Claude). Recent studies by Dr. W. G. Spector on electrolyte flux in mitochondria during the action of lytic and spherulating agents confirm the view that isolated mitochondria behave as osmometers.

Chemical Composition

Chemical analyses of lysed mitochondria show them to be complex in their composition: 25% of their substance is lipid, especially phospholipid; a protein fraction which includes their important "soluble" enzymes and a dialysable fraction represented by ribose nucleic acid have been separated. It seems that one-third of the total nitrogen and one-fifth of the ribose nucleic acid of rat-liver cells repose in their mitochondria. And there is reason to believe that mitochondria in turn may be broken down to smaller particulates, about 0.1μ in diameter, which carry nearly all of the complement of ribose nucleic acid possessed by the parent elements. We are thus confronted with cell elements in the form of mitochondria which are built up according to a specialized pattern in which are concentrated lipids, rich in phosphorus, and ribose nucleic acids.

But it is the enzyme systems which point the way to the elucidation of mitochondrial function. You will recall a most unusual discovery that was made by Warburg over 40 years ago when he demonstrated that cellular oxidations may be bound to granules. It now seems certain that such respiring granules are, in very truth, mitochondria, for localized within isolated mitochondria have been discovered enzyme systems which enable molecular oxygen to be utilized. Cytochrome oxidase exists exclusively there; indeed, about 80% of the total cellular cytochrome oxidase activity can be traced to the mitochondrial fraction. Claude (1954) attributes 70% of succinic dehydrogenase activity and 50% cytochrome c to the mitochondria, and thinks that oxidative phosphorylation processes are restricted to them. The enzymes of the Krebs tricarboxylic acid cycle are also present. It has been shown that energy released in these oxidations is transferred to energy-rich phosphate bonds and these can be built up within the mitochondria and sent out to other regions of the cell. Co-enzymes such as di- and tri-phosphopyridine nucleotide and flavine-adenine nucleotide are important components present in bound form; they supplement the activity of the oxidative and phosphorylating processes. Small wonder is it, therefore, that Claude labels mitochondria as the main power plants of the cell, because they utilize molecular oxygen and distribute energy-yielding components to other organelles.

The studies of Krebs and his colleagues with radioactive tracers indicate, too, that a continual exchange goes on between the ions of the mitochondria and the suspending media. Thus the whole of the mitochondrial Na and K can readily be replaced by that of the environment. In some of these exchanges the expenditure of energy is concerned notably in the uptake of inorganic phosphate, as is the case, it will be remembered, with heart muscle or red cells; in other instances the exchange recalls that which occurs across a semi-permeable membrane in a Donnan system. Krebs suggests that mitochondria may be the basic units responsible for secretory and absorptive activity of cells. Secretion, therefore, is a process that takes place not only in glands but perhaps in all cells. Absorption, too, may well be a common feature of all cells. At the heart of these processes stand the mitochondria, which are thus not only the sites where most of the available energy is generated, but the mechanism whereby this energy is directed into channels for the transport of solutes and the maintenance of secretory and absorptive activities (Bartley *et al.*, 1954).

Researches into Mitochondrial Function

I have spent some time in viewing the advancing tide of investigation into mitochondrial function because I believe that it is in precisely this field that fresh discoveries may be anticipated that will be of vital importance for cellular pathology. In support of this claim I will describe briefly some of the indications that influence my belief.

For some time now my assistants have been interested in the effects produced by poisons on the liver. Recently Dr. J. D. Judah and Dr. G. Christie, of Melbourne, have completed an important study of the action of carbon

tetrachloride on liver cells which, to my mind, throws a great deal of light on these matters. Briefly the outcome of their investigation is the theory that this fat-soluble agent enters liver cells, attacks the mitochondria physically so that they lose their power of retaining their small stores of enzyme co-factors (pyridine nucleotides especially), and this results in the disorganization of the chain of enzymes constituting the tricarboxylic acid or Krebs cycle. In consequence, a loss of the integrated enzymatic activity necessary for the production of energy in the cell follows, as one would expect from our knowledge of mitochondrial function, despite the fact that individual enzymes may still retain activity. Most of this work has been done on isolated mitochondria, but Christie and Judah (1954) have shown that quite likely similar damage is induced within liver cells when a rat is exposed to a toxic dose of carbon tetrachloride. It will be remembered that within a few hours of such an exposure liver cells swell and develop clear vacuoles which may extend throughout the greater portion of the cytoplasm. These are filled with water and a little emulsified fat, and some pathologists have long believed that they arise from mitochondria.

My assistants have actually separated the vacuoles from their parent cells by centrifugation and proved that they possess the characteristics of altered mitochondria. Christie and Judah found that mitochondrial respiration is diminished by carbon tetrachloride poisoning as the result of bottlenecks at certain phases of the tricarboxylic acid cycle as well as at some stages of breakdown of fats. Since these mechanisms lie at the very foundations of water, fat, protein, and carbohydrate metabolism, is it any wonder that cell function is grossly disturbed and that water and fat, and quite possibly products of protein breakdown, accumulate within the cell to give the well-known morphological changes associated with carbon tetrachloride damage? My assistants have also shown that choline delays the onset of the enzymatic changes, and this may be the basis of the prevention of fatty liver associated with chronic carbon tetrachloride poisoning.

Isolated mitochondria have also been used by Dr. W. G. Spector in investigations of the action of lytic and spherulating agents, and he has shown that under such conditions mitochondria lose much of their potassium and some of their sodium while retaining magnesium and calcium. Potassium loss is prevented by suspending isolated mitochondria at 37°C . in a medium which allows oxidation of tricarboxylic acids and the coupled phosphorylations to go on. This suggests that the retention of potassium by normal mitochondria against a gradient depends upon the associated metabolic activities, and Spector has demonstrated that it is oxidative phosphorylation which maintains the potassium concentration against an adverse gradient. This recalls similar phenomena in red corpuscles where potassium loss accompanies ageing or damage, but in that case, as Maizels (1949) has taught us, a glycolytic mechanism is concerned in preserving the potassium concentration. All such studies convince me of the importance of these organelles for the economy of the cell and indicate their sensitivity to injury of various kinds. We still have a long way to go before we can express cell response to abnormal conditions in terms of precise biochemical reactions, but I think you will agree that an encouraging start has been made. At the moment, Dr. Judah and Mr. Gallagher, of Sydney, are endeavouring to find out more about cell behaviour in the presence of certain dietary deficiencies, especially copper, and their results already promise a valuable harvest. We have planned other investigations in this field which range over many fascinating facets of pathology, but it would be premature to mention them.

"Lethal Synthesis"

I am tempted to include here a short reference to the brilliant studies of R. A. Peters and his school at Oxford which have led to the introduction into the theory of disease of the new concept of "lethal synthesis." As the outcome of his investigations of fluoroacetic acid (FCH_2COOH), a

compound which is the toxic principle of the South African poison plant known as gifblaar (*Dichapetalum cymosum*), Peters has shown that this substance induces a purely biochemical lesion in the cell. It does this through an enzymic block in the tricarboxylic cycle, the enzyme involved being aconitase. In this way the metabolism of both carbohydrate and fat within the cell is "jammed" at the tricarboxylic acid stage with the consequent increase of citrate inside the cell and the slowing or stoppage of metabolism. The astonishing thing is that fluoroacetate itself is not toxic to enzymes. The trouble arises through fluoroacetate being misguidedly synthesized by enzymic action, into a fluorotricarboxylic acid, probably fluorocitric acid, and this inhibits aconitase in minute amounts. Thus a compound apparently harmless in itself is joined with relatively innocuous cellular metabolites to bring about serious disturbances of cell function. Peters suggests that the latter takes place in two ways: (1) by the reduction of the energy supply by "jamming" of the tricarboxylic cycle, and (2) through the immobilization of Ca ions by the increasing citrate concentrations in the cell with altered cellular excitability, especially in the case of nerve cells.

Such a "lethal synthesis" contrasts strongly with the classical protective syntheses in which molecules foreign to the organism are converted to less toxic compounds. With fluoroacetate we are confronted with a substance that "can so deceive the normal metabolic enzymes concerned with getting energy into making a substance which will poison them, and only then because the enzymes are organized in the mitochondria" (Peters, 1952). I feel sure it will be agreed that Peters has given us a new lead in cell pathology from which may come many fascinating discoveries.

I now turn to the last of the cell components that I have chosen for consideration, the tiniest and least investigated of the organelles, the microsomes.

Microsomes

Cytologists have long been aware of the existence of minute cell components that lie just on the fringe of microscopic dimensions. In some cells these take the form of tiny granules which contribute to the underlying staining reactions of the cytoplasm, even imparting a distinct basophilic element to the more usual acidophilia. With the coming of differential centrifugation, elements of the cytoplasm that are submicroscopic in their dimensions have been separated from every active cell, and these, without doubt, correspond to the minute cell structures that were long sensed to exist. They have been named microsomes.

Microsomes range in size from 60 to 250 m μ and make up as much as 20% of the dry cell mass. They consist of delicate vesicles or slender strands which fragment easily, and in so doing they release part of their soluble constituents. They, too, are rich in lipids—as much as 40% of their mass is lipid, over two-thirds of which is phospholipid—and they contain 50 to 55% proteins (Claude). At least 50% of the total ribose nucleic acid of the cell is associated with the microsomes, and this no doubt is the reason for the basophilic tint of the cell ground substance. Microsomes must therefore be important centres for protein synthesis in the growing cell, especially for ribose nucleic acid production, and labelled amino-acids have been shown to be incorporated within them, probably taking part in forming peptide bonds within the protein molecules (Greenberg *et al.*, 1948). However, they are poorly off for enzymes except in the kidney, where certain phosphatases—glucose 6-phosphatase and alkaline phosphatase—appear to be closely associated with microsome fractions. Their osmotic behaviour suggests that they are enclosed by a semipermeable membrane. Since this is the extent of our knowledge at the moment, we can do no more than conjecture their function. My object in drawing attention to them is to urge the need for further investigation of what may prove a rich field of pathological discovery. I know of no information about microsomal reactions to injurious agents; I can only ask whether they, too, play a part in the cellular

degenerations, if they are linked closely to the mitochondria or the nucleus in co-operative function, and speculate that they may be concerned in the storage and production of hormones, antibodies, and other secretions.

Conclusion

My survey of these selected topics of cell behaviour has reached its end, and I am faced more than ever with feelings of insecurity and incompleteness. But can the position be otherwise when a field of investigation is making such rapid strides forward? Rather may we not find grounds for congratulation in the uncertainties and dissatisfactions of modern cell theory and of cell pathology in particular, provided we never cease to give up the attempt to eliminate or to rectify our illusions?

But this is not the way to end a lecture such as this, and I must return to my initial plan and attempt to convince you that some progress has actually been achieved. I set out with the purpose of trying to decide whether we really understand how a cell is injured by a poison, whether it be chemical, in the ordinary sense of the word, or toxic, the product of living matter. Let me say at once that I am sure of some success. In support of this optimism, allow me to draw a simplified sketch of how a poison such as carbon tetrachloride—or its close relative chloroform—brings about its harmful effects on one type of cell.

When carbon tetrachloride is brought to the liver in the blood plasma, partly in solution, partly attached in unexplained fashion to the blood lipids, its capacity to penetrate the cell is determined by its lipid and water solubility, its molecular size, and possibly other factors about which we know very little. It will thus be thrust along the pores of the surface sieve by way of the water spaces and in intimate contact with the lipid lining of the pores. Some molecules will no doubt come into contact with the membrane respiratory patches, and these will be affected in much the same fashion as the respiratory enzyme set-up of the mitochondria has been shown to be involved during *in vitro* experiments. Those molecules that succeed in penetrating the membrane now thread their way through the labyrinth of the cellular ground substance; they are directed towards various regions of the cell by obscure forces and according to unknown rules. Whether they loiter on the way or fall by the wayside into traps prepared for their destruction we do not know; indeed, the whole mysterious trek is one of pure conjecture and awaits the inspired investigator.

But sooner or later contact is made with the organelles and once again problems of surface membranes are faced, which are, we may guess, similar to those previously encountered. At any rate, things go wrong with the respiratory functions congregated in the mitochondria, and when a number of these are disturbed the overall effect on the cell is serious, for its machinery begins to run down. Imagine the confusion and chaos throughout the cell that ensues when all kinds of co-ordinated enzymic systems get out of hand. Little wonder is it that, sooner or later, cell structure begins to crumble as order fails, and that the innumerable interphases now fall into the ways of dead membranes. Deprived of energy supply, the mitochondrial dynamos run down, nuclear function falls into enigmatic confusion and no longer directs the future of the cell according to the primordial law. And so disintegration spreads, with cleavage and disruption of function from structure until the cell collapses and falls victim to its own forces of dismemberment.

More complex must be the scheme that directs contact between bacterial toxins and the cell, even though the outcome—degeneration and destruction—is the same as with other varieties of injury. I have shown that certain toxins such as *Cl. welchii* α toxin act in the first instance through a lecithinase which disrupts key centres in the cell membrane; indeed, the highly important target areas may get the worst of it, with disorganization of cellular reactions

to stimuli of many kinds. Other toxins seem to block processes concerned in the transmission of co-ordinating stimuli between groups of cells. This appears to be the case with the tetanus and botulinum toxins, which interfere seriously with the acetylcholine mechanism (Ambache *et al.*, 1948a, 1948b; Burgen *et al.*, 1949). In other instances, especially with the endotoxins now being disentangled from Gram-negative bacteria, cellular metabolic functions seem to be the foci of attack, but there is an appalling lack of information about these matters. Clearly we are on the threshold of a vast new world of cellular research from which we may gain rich treasure if we explore it critically and with imagination. The reward will include, I feel sure, progress not only in fundamental knowledge about life but in applied pathology, carrying with it new ideas about the recognition and treatment of disease no less than the forestalling of sickness.

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According to a report of an informal working party on the treatment and disposal of sewage sludge (H.M.S.O., 1954, price 2s. 6d. net), the efficient treatment and disposal of sewage sludges, together with the production of useful and valuable by-products, constitute the most difficult and costly work undertaken at sewage works. The report discusses five methods of disposing of sewage sludge. These are in the order in which they should generally be given consideration: (1) Disposal in a liquid state, preferably after digestion, as a method of manuring grasslands. (2) Disposal in a liquid state, preferably after digestion, to manure agricultural land for general purposes. (3) Failing the possibility of final disposal in liquid form, efforts should be made to obtain an end-product in some dry state suitable, preferably after maturing and pulverization, to form slow-acting manure for agricultural land. (4) A still drier product than the above can be made, but this entails the use of expensive equipment. (5) Favourable conditions, usually to be found in rural areas and small market towns, may exist for the method of disposing of liquid sludge by composting with other waste materials, generally separated from household refuse,

POTASSIUM-LOSING NEPHRITIS PRESENTING AS A CASE OF PERIODIC PARALYSIS

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Recent workers (Platt, 1950; Nickel *et al.*, 1953) have emphasized a tendency to urinary loss of electrolyte in renal failure in addition to the more familiar excess retention of end products of nitrogen metabolism. In some patients this electrolyte loss dominates the clinical picture, and in the last 10 years cases of both sodium and potassium deficiency as a result of renal disease have been reported. The type characterized by excess sodium loss was first described by Thorn *et al.* (1944) under the name of "salt-losing nephritis." Such cases have many features like Addison's disease, in that weight loss, hypotension, cachexia, and low serum sodium are present, often associated with some pigmentation of the skin. They differ in that there is no favourable response to deoxycortone or to cortisone, but only to liberal supplements of sodium chloride.

Potassium loss often does not produce such obvious clinical effects, and there is little doubt that many cases of potassium deficiency due to excess urinary loss have passed unrecognized. Brown *et al.* (1944) first described cases of periodic attacks of hypokalaemic paralysis or weakness as a result of renal tubular acidosis. More recently, hypokalaemic paralysis has been described in the de-Toni-Fanconi syndrome, of both the adult and the infantile types (Milne *et al.*, 1952; Bickel *et al.*, 1953). In this paper we describe metabolic studies of a case of chronic pyelonephritis with severe urinary potassium loss.

Case Report

The patient was a married negress aged 41. She had lived in Nigeria until 1953, when, during a visit to Great Britain, her illness was first diagnosed and treated. She had been in good health until 1944, when she suddenly felt her legs becoming heavy and weak. There was complete paralysis of the legs and soon afterwards of the arms, lasting for two weeks. Since then she has suffered from periodic attacks of weakness of the limbs lasting from 2 to 20 days before relief occurs. There has never been any paralysis of the muscles of deglutition, respiration, or phonation, nor any diplopia. The attacks come on about every three months and tend to be precipitated by exposure to cold. She has always felt reasonably well between the attacks, but there is usually some slight residual muscular weakness. Her last attack started whilst on air passage to Great Britain. She was admitted to another hospital in this country with complete paralysis of the legs and weakness of the arms. A diagnosis of hypokalaemic paralysis of unusual type was made, and she was transferred to Hammersmith Hospital for further study.

On inquiry it was found that she had had three episodes of dysuria and frequency of micturition associated with pyrexia in 1945, 1946, and 1947. For the last three years there had been polyuria with nocturnal frequency of micturition. There was no family history of any similar urinary or paralytic symptoms.

The patient was co-operative and healthy-looking. On first admission to hospital she was unable to move her legs