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IN

PATHOLOGY

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INTRODUCTION

IN THE world of medicine pathology is everyone's business. Most, if not all, of the great advances in medical and surgical diagnosis and treatment and in the prevention of disease have been based on the results of pathological research and on new pathological understanding. The *Modern Trends* volumes already published bear witness to this fact, chapters or sections on the relevant pathology having appeared in most of these specialist volumes. This very circumstance has helped to resolve some of the problems that arose in choosing from an almost limitless range a small number of topics for inclusion in *Modern Trends in Pathology*, since it seemed that the emphasis should here be on new work that illuminates either pathology in general or major fields of pathology in which interest is widespread. Naturally, the final selection of topics is a personal one determined by a predilection for those with which the Editor has himself had some contact that has fired his interest.

The authors of the various chapters are each experts in the field of which they write. Their contributions are designed not so much for fellow initiates in their particular branch of knowledge as for readers whose interests are more general or mainly concentrated in other channels. It is an advantage of a book of this sort that its chapters can be written with greater freedom of style and with greater breadth of view than is possible in most conventional articles. The whole purpose of the book will have been achieved if it is found to give a clear, stimulating and up-to-date account of the matters upon which it touches, and if it serves as a guide and a source of reference for further studies.

Pathologists are losing the shackles that previously bound them to the rigid disciplines of morbid anatomy and descriptive morphology, and the excitement of adventuring in pathology today lies in the freedom with which use is made of information and techniques culled from biology, chemistry, immunology, physics and physiology. Nevertheless, the triumph of those pioneers of cellular anatomy and cellular pathology who flourished just a century ago is still manifest in the fact that the microscope remains the pathologist's primary tool and the cell the focus of his attention. All will agree with Sir Roy Cameron (p. 15) that the future of pathology lies in those investigations that "possess the merit of welding together structural and functional observations into a coherent story". But we are not yet at such a stage of technical development that disturbances of structure and of function can be equally detected and recorded in every process of disease. Sometimes, in the clinical or the experimental laboratory, impairments or disorders of function are the more accessible to the investigator; sometimes it is the structural changes that are the more accurately observable. The purely morphological and descriptive record of pathological lesions will always have its use in identifying, or distinguishing between, different disease processes; new diseases and new agents of disease arise, rare tumours and other lesions continue to be discovered, and, now that the less charted parts of the world are coming within medical surveyance, the nature of neoplastic and other lesions there appearing must be accurately established.

These and many other aspects of present-day pathology are revealed in the pages of this volume. If, for the reasons given in my first paragraph, the choice of subjects seems to be biased in favour of what in the undergraduate curriculum is called "general pathology", and in postgraduate practice is sometimes referred to as "academic pathology", it is worth-while reflecting on the foolishness of the attempt to separate sharply the various categories of pathological practice—clinical, academic, applied, general, special; these are artificial divisions occasioned mainly by the circumstances under which one's work is pursued. Behind every pathological investigation lies an essentially humanitarian impulse, yet at the same time there is always the motive of scientific inquiry. Every pathologist has a dual role to perform. His training makes him see in each case of disease, on the one hand, something immediately demanding appropriate action and, on the other hand, a small facet of life-processes perverted from the normal from which he may collect and analyse data bearing on a wider field than the individual case, leading, perhaps, to the final objective of all pathological research—the elucidation of the nature and causes of disease.

This volume, then, is addressed to pathologists of every category. It has been a great privilege to assemble and edit the material, and I am deeply grateful to all my collaborators for their care, thought and enthusiasm in preparing their contributions.

January, 1959 DOUGLAS H. COLLINS

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CHAPTER 1

THE INJURED CELL

SIR ROY CAMERON

WE ARE passing through a quiet revolution that promises profound changes in our outlook on matters pertaining to the cell. The invention of many new and intriguing techniques for novel attacks on the structure and working of the normal cell has eased the task of investigating what goes wrong when it is injured and has given fresh encouragement to the investigation of problems of general pathology.

In this short survey I shall try to find out how helpful these discoveries are proving in charting the seats and causes of disease at a level undreamed of by Morgagni and the pioneers of medical research. Let me say at once that I firmly believe in the need for resolving complex pathological processes into factors closely bound to the minute intracellular organs. There need be little hesitation in referring some, at least, of the cell functions to the organelles but the assumption may be too simple. Belief in co-ordination of functional units is inescapable and so far no cellular locus has been discovered for such directive activities. Nevertheless, a fresh point of view, even though it be restricted by obvious handicaps, deserves consideration if merely for the planning of future research.

THE CELL MEMBRANE

Up to the present the electron microscope has given us little evidence of a specialized cell membrane, though such a structure, 50–80 Å thick, is claimed for the lymphocytes of the mouse (Vogel, 1958), for renal epithelium and for the villous lining cells of the small intestine (Rhodin, 1958; Zetterquist, 1956). It is said to consist of two thick layers in apposition with a thinner lamina, and it gives rise to the intercellular bridges now accepted by all histologists.

Through such a membrane diffuse those materials concerned in the growth and survival of the cell. Diffusion is speeded up and facilitated when the substance is quickly utilized by the cell, as seems to be the case with amino acids that fuse with the cell proteins. In other cases, specific enzymes in the membrane mediate active transport, and this process is linked to cellular respiration. Lundegårdh (1948) holds the cytochrome system responsible for ion permeability in plant cells, and Conway (1953, 1955) has proposed a redox pump theory to explain similar facts in animals. Koch (1954) thinks that cholinesterase to some extent controls ionic penetration in various cells, while Gourlay (1952) links phosphate entry into red blood corpuscles with the formation of adenosine triphosphate (ATP) at the cell surface. Glycolysis would appear to play a part in the uptake of glucose (Prankerd and Altman, 1954; Prankerd, 1956), and certain areas of the membrane may be

selective in facilitating transport of the sugar (Le Fevre, 1954). The involved mechanism of water transport and its relation to hydropic degeneration have been discussed elsewhere (Cameron, 1956). Such observations should prepare us for the discovery of agents capable of modifying cell behaviour profoundly through their specific action on the membrane. Some such action may apply in the case of oleic acid, a compound that strongly depresses the oxygen uptake of certain tumour cells through lysis of the cell membrane (Bennett, Connon, and Schoenberg, 1951). The damage resembles that associated with haemolysis by sex-steroids and bile salts (Bennett and Connon, 1957).

Certain cells liberate chemical substances that are formed either at their surface or within the cell, and these exert a profound influence on related or distant cells. Some are very large molecules so that evidently the membrane can allow their passage, to and fro, as circumstances demand. Brachet (1957) assures us that ribonuclease with a molecular weight of 13,000, as well as protamines and histones, can penetrate cells so long as the respiratory mechanism is normal. Certain hormones no doubt penetrate the cell in this way, to exert their special actions on intracellular organelles. And this suggestion prepares us for the idea that bacterial toxins, growth hormones and the like, may pass unhindered through the membrane to reach the intracellular regions wherein they perform their apportioned task.

THE NUCLEUS

We have come to look upon the nucleus as a vast storehouse of desoxyribonucleic acids (DNA) which are closely associated with histones—protamines in the case of ripe spermatozoa—to form nucleohistones. These massive complexes constitute the morphological elements we know as chromatin. DNA, and probably histone, are remarkably constant in amount in the resting nucleus, showing, at most, small physiological variations. The nucleolus, however, is rich in ribonucleic acids (RNA), especially when the cell vigorously synthesizes proteins.

In striking contrast with these stores of nucleic acids is the dearth of enzymic protein. Isolated nuclei contain neither cytochrome oxidase nor succinic dehydrogenase which means that the essential respiratory enzymes are absent. Urease, D-amino acid oxidase and other oxidative enzymes are also missing (Brachet, 1957); but glycolytic enzymes are found in high concentrations, suggesting that glycolysis may be an important source of energy for the cell nucleus which thus depends predominantly on anaerobic metabolism (Stern and Timonen, 1954). Enzymes concerned with nucleotide metabolism are present in higher concentration in the nuclei than in the cytoplasm (Stern and his colleagues, 1952), but we are still very much in the dark about the incorporation of amino acid into nucleoproteins. The membrane is readily permeable to fairly large molecules, including enzymes such as ribonuclease, and the electron microscope gives evidence of a pore structure of the membrane, which no doubt facilitates ingress.

Thus it seems that the functions of the nucleus in the resting cell are still far from settled. However, the nucleus would appear to be largely concerned in the formation of protein by way of the nucleic-acid cycle. It may also help in the production of co-enzymes and exert an influence on the ergastoplasm, since the latter loses its basophilia and finely granular structure in amoebae robbed of their nuclei (Brachet, 1957).

Nuclear behaviour after injury is a little better understood. The changes known as karyorrhexis and pyknosis are now known to follow depolymerization of DNA and enzymic digestion of broken-down chromatin (Leuchtenberger, 1950). Intranuclear inclusions are also proving accessible to the electron microscope which shows that often they are invaginations of cytoplasmic particles into the nucleus, associated with nuclear oedema (Wessel, 1958). But the ability of certain cells to survive many days without a nucleus, their life terminating only because the machinery of existence runs down, compels us to expect fresh shocks in the future. Elsewhere I have urged the uncertainty of many of our time-honoured beliefs about nuclear changes leading to cell death (Cameron, 1956).

MITOCHONDRIA

Mitochondria display a galaxy of enzymes, including those of most importance in oxidative mechanisms. If provided with the appropriate soluble co-factors, ions, and substrates from the embracing hyaloplasm, they carry out oxidative phosphorylation, so that in one sense they are the power stations of the cell. The energy produced by substrate oxidation is stored in the phosphate bonds of ATP.

Mitochondria of liver cells

Electron microscope studies have concentrated attention on mitochondrial structure and its disturbance after cell injury. When liver cells are damaged by oxygen deficiency or by carbon tetrachloride their mitochondria shrink and lose their cristae within an interval of 2 hours, and about 3 hours later they become vacuolated or compressed by large vacuoles within the ergastoplasm (Oberling and Rouiller, 1956; Rouiller, 1957; Mölbert and Guerritore, 1957; Mölbert, 1958). Such changes are greatest between 12 and 24 hours after injury (Figs. 1–3), when cell respiration is diminished (Christie and Judah, 1953), and sooner or later they may lead to the formation of fatty droplets. Eventually the mitochondria end up as almost empty shells which are lost in the necrosis that overwhelms the cell. All such changes are preceded and accompanied by profound disturbances in the ergastoplasm (*see* p. 9). Clearly, the recognition of these changes is merely a prelude to the detailed investigation of organellar injury, and the next great advance will come when we learn to locate various functions in the mitochondrial framework.

Mitochondria of kidney cells

Renal mitochondria have been studied with some success under various conditions. Oliver's demonstration that the absorption of large molecules, especially egg albumin and haemoglobin, is closely associated with tubule-cell activity has been confirmed by Rhodin (1958) who has identified changes in the mitochondria as soon as 7 hours after administration of egg white to mice. The absorbed protein leads to clumping of mitochondria which become impregnated with electron-dense material that fills up their interior. Some of the lamellae may disappear and it is often difficult to find any that are normal. During the recovery phase, 30–60 hours after egg-white administration, the large globules decrease in number and normal mitochondria reappear. But quite a number remain vacuolated and some certainly

break up. At this stage, disturbance of water and sodium reabsorption is pronounced; it seems that the cell loses much of its energy for resorptive processes as mitochondrial function is disturbed. Miller and Sitte (1956) have come to similar conclusions, as also did Zollinger (1951) who used phase-contrast methods.

No less interesting is Rhodin's investigation of calculus formation in renal tubules. When rats are injected subcutaneously over some weeks with parathyroid extract large amounts of material that gives a positive reaction with the periodic acid Schiff (P.A.S.) technique appear in the proximal tubule cells, along with hyaline cylinders in the lumina. The latter react strongly with P.A.S. staining. Microradiography shows the presence of calcium concrements in nearby tubules at this stage. Simultaneously fine granules of great electron density form along the basal membrane of the tubule cells: they seem to be built up from ultramicroscopic calcium deposits. They clump together and appear to break through the basal membrane from the adjacent capillaries. As the granules become larger and denser they slowly fill most of the cell and obscure the mitochondria. They are invariably associated with P.A.S.-staining material which makes up the matrix for precipitation of a dense fibrillar material that is probably apatite. From the overflow of these masses into the lumen comes calculus formation.

A start has been made, too, with the investigation of other pathological changes in the kidney, such as the osmotic nephrosis produced in rabbits by peritoneal dialysis of a sodium-free glucose solution (Rouiller and Modjtabai, 1958), the Masugi nephritis of rats (Miller, Sitte and Bohle, 1958) and the glomerular changes in rabbits due to the Shwartzman phenomenon (Bohle, Sitte and Miller, 1958), but reports are still incomplete.

Mitochondria in cells of the lung

A fascinating study of the lung of the hibernating dormouse and the foetal rat by Schulz (1957) confirms the association of mitochondria with cell respiration. It has long been known that respiration is considerably decreased in the hibernating animal (Pembrey, 1901; Benedict and Lee, 1938). In the cytoplasm of the alveolar epithelium of the hibernating dormouse are found coalescing vacuoles filled with circular, concentric lamellae and bordered by strongly osmiophilic remnants of mitochondrial lamellae. Few normal mitochondria can be found in these cells. The raised alveolar carbon dioxide tension and the accompanying anoxia are associated with a tremendous alteration in the ultra-structure of mitochondria in the alveolar cells and may lead to the complete exhaustion of the normal mitochondrial supply. Schulz describes numerous finger-like infoldings of the alveolar cell membranes opposite the air and blood surfaces, associated with numerous tiny bubbles, and he suggests that these may represent devices for the maintenance of oxygen flow from the stagnant air passages to the capillaries.

Schulz also records the presence of oval bodies within the alveolar spaces of 21-day-old rat foetuses that have not breathed. He thinks that these bodies come from mitochondria or their precursors as the result of greatly raised production of carbon-dioxide in the lungs. Some, however, may be derived from mast cells. It is possible that they constitute part of the hyaline membrane that forms in premature births, although electron microscopic study of the membrane in infants suggests that it consists largely of a finely fibrillar matrix, which is apparently fibrin, with enmeshed cellular debris (van Breemen, Neustein and Bruns, 1957).

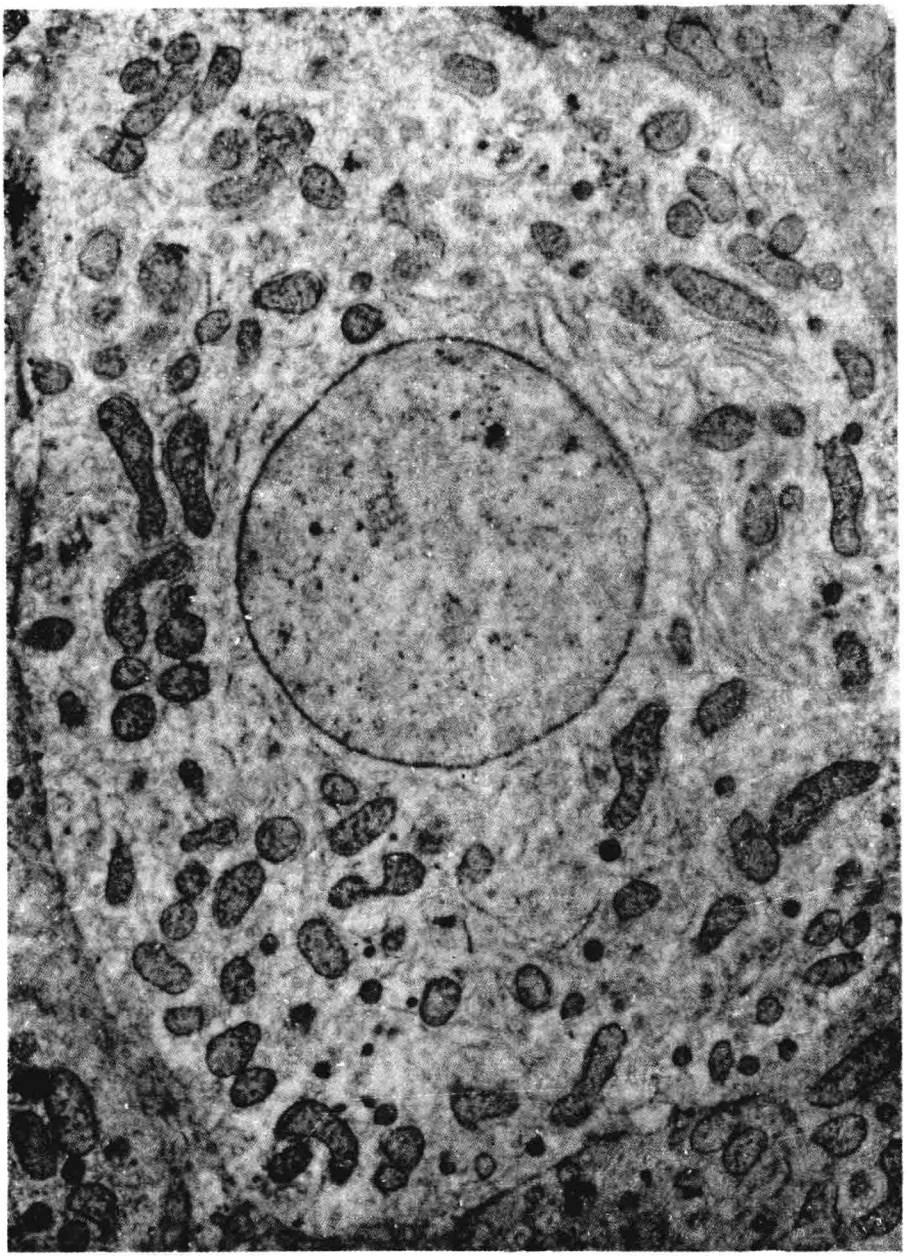

FIG. 1.—Electron micrograph of a normal rat liver cell, showing nucleus, mitochondria, and ergastoplasm, the latter appearing as wavy double lines between the mitochondria. Fixation in buffered osmium tetroxide. × 7,000.

(By courtesy of the Chester Beatty Institute, London)

Mitochondria in cells of the brain

Brain mitochondria are susceptible to the tranquillizer chlorpromazine (Abord 1955) which partially inhibits phosphorylation. Salicylic acid and related compounds uncouple oxidative phosphorylation in brain mitochondria of rats (Penniall, Kalnitsky, and Routh, 1956). Hartmann (1956) has shown that the mitochondria become vacuolated in the rat's brain after low doses of cortisone and swell up with high doses, but glial cells are unaffected. Aldridge and Cremer (1955) found that diethyl-tin-chloride specifically inhibits α-keto-acid oxidation in rat brain and liver mitochondria whereas triethyl-tin-sulphate uncouples oxidative phosphorylation with partial inhibition of DPN-linked oxidations. The mushroom poison, phalloidin, inhibits phosphorylation coupled to oxidation of ferrocytochrome *c* (Hess, 1956). Such "biochemical lesions" may well prove to be the key to many obscure diseases of the central nervous system.

FIG. 2.—Liver cell of albino rat, showing normal mitochondria and ergastoplasm and a portion of the nucleus at the left upper corner. ×32,900.

(By courtesy of Dr. E. Mölbert, Path. Instit., Freiburg-i.-Br.)

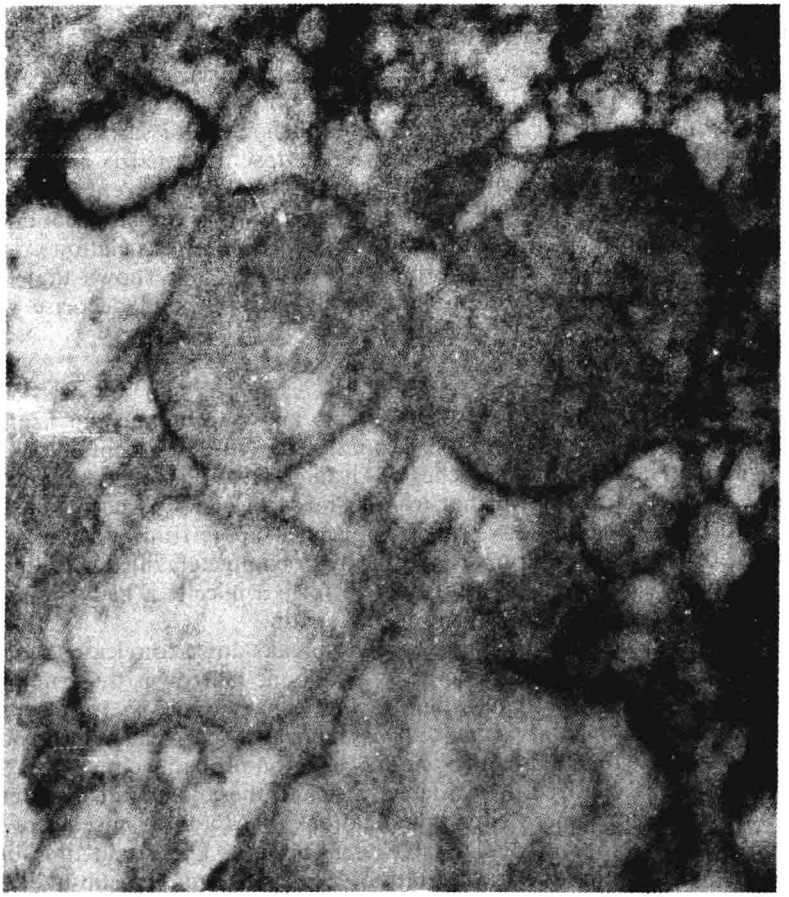

FIG. 3.—Liver cell of albino rat, 17 hours after exposure to carbon tetrachloride, showing degenerating and "optically empty" mitochondria with vacuolation of ergastoplasm. ×47,250.

(By courtesy of Dr. E. Mölbert, Path. Instit., Freiburg-i.-Br.)

Hormones and mitochondrial activity

There may also be an important link between the action of hormones and mitochondrial activity. The effect of cortisone on the mitochondria of the rat's neurones has already been mentioned. Lowe, MacKinney, and Sarkaria (1955) say that cortisone reduces the number and gives rise to swelling of liver mitochondria, but there is no decrease in the ability of such mitochondria to oxidize succinate or to carry out oxidative phosphorylation (Lowe and Lehninger, 1955). Hypophysectomy also depletes the mitochondrial population of the rat adrenal cortex (Lever, 1956). Restoration follows temporary lowering of the sodium:potassium ratio in the diet, or administration of ACTH. Growth hormone treatment of hypophysectomized rats leads to a threefold increase in the liver mitochondria (Reid, 1956; Stevens and Reid, 1956). Though the meaning of these discoveries is obscure, no one will deny their need for further investigation.

Mitochondria in cardiac muscle cells

The close association of mitochondria with injury is clearly shown in pathological conditions of heart muscle. Rats undergoing phosphorus poisoning display fat globules intimately related to heart muscle mitochondria or sarcoplasm reticulum (ergastoplasm). The mitochondria also swell up, lose their matrix and outer membranes, clump, and run together. Such changes are most severe 24 hours after phosphorus administration when fatty degeneration is most pronounced and ergastoplasmic damage is present (Poche, 1958). A similar association exists in the heart muscle of the hibernating dormouse. Poch'e has also shown that the myofibrils increase in number and size during hypertrophy and decrease when the heart atrophies.

Haemosiderin and ferritin

One final example from a field that seems to know no bounds must suffice. For a long time, pathologists have been puzzled by the iron-containing pigment haemosiderin. The brilliant researches of Granick (1949) have largely resolved the dilemma and it is now known that iron is stored in many tissue cells as ferritin. This consists of a colourless protein molecule—apoferritin, with a molecular weight of 500,000—with four holes in it partly or completely filled with up to 1,000 ferric hydroxide units, all linked together to form a micelle. The ferric hydroxide may make up 36 per cent dry weight of the ferritin.

When an animal is bled, ferritin in the liver breaks down to release the iron, and the protein is converted into amino acids. The bone marrow is thus supplied with iron and amino acids, both of which facilitate haemopoiesis. Iron supplied to tissues such as the alimentary canal or liver is quickly incorporated into apoferritin which may increase fourfold in a few hours. The iron probably circulates as ferrous bicarbonate and is oxidized as it attaches itself to the apoferritin. Richter (1957) has shown by electron-microscope investigations that the iron micelles of haemosiderin and ferritin contain similar molecular sub-units and are at times indistinguishable, but in haemosiderin the state of aggregation of the sub-units is quite variable. Both haemosiderin and ferritin aggregates are often situated within discrete organelles, especially during early haemosiderosis. These organelles appear to be derived from mitochondria. Thus haemosiderin and ferritin may be either synthesized or concentrated within mitochondria and later discharged into the cytoplasmic matrix. Perhaps a similar process is concerned in lead poisoning when the red corpuscles present iron granules, 50 Å diameter, composed of 20–50 iron atoms surrounded by an envelope of protein (Bessis and Breton-Gorius, 1957).

Movement of mitochondria

One property of mitochondria, movement, has received little attention though it is well known to occur in tissue-culture cells. Frédéric and Chevremont (1952) and Frédéric (1954) have shown that moderate concentrations of detergents cause shortening of mitochondria and loss of movement while higher concentrations lead to their swelling and eventual rupture. Paralysis of mitochondrial movements with shortening is also seen after exposure to ethyl urethane and other metabolic inhibitors. Anaerobic conditions make the mitochondria elongate and break into small pieces. Dinitrophenol stimulates their activity for a short while; the mitotic inhibitor, trihydroxy-N-methylindol, shortens mitochondria and inhibits their

movement. Obviously all such disturbances require the expenditure of considerable energy but no precise information is available about the chemical processes that underlie such outbursts.

GROUND SUBSTANCE, ERGASTOPLASM, AND MICROSOMES

Electron-microscope photographs constantly pick out a very delicate structure in the ground substance of the normal cell which is thought to be a finely divided vacuolar system. In glandular cells of pancreas and liver it shows up as well-developed elongated trabeculae lying in the basophilic part of the cell. The trabeculae form enlarged cisternae separated by small granules, of 100–150 Å diameter, which are roughly proportional in number to the RNA content of the cell (Palade, 1953, 1955). Opinions vary as to its nature, and its very existence has been questioned for it is found only in tissue fixed in buffered osmium tetroxide.

Microsomes are also under dispute. Most likely they are fragments of ergastoplasm, for they appear in electron micrographs as vesicles containing many small granules at their surface. Brachet (1957) thinks the granules are formed of minute portions of nucleoprotein, containing 50 per cent RNA, surrounding a lipid membrane and holding together a number of hydrolytic enzymes. Their function is obscure. Microsomes seem to be very active in protein synthesis through incorporating amino acids into protein (Borsook and his colleagues, 1950; Borsook, 1956). The lipids are chiefly cholesterol and phospholipids (Hogeboom and Schneider, 1955). Microsomal enzymes number among their battery cholinesterase and vitamin-A esterase, various enzymes concerned in oxidative phosphorylation, a special type of cytochrome, and others that are concerned in the synthesis of taurocholic acid. In the liver, the specific glucose-6-phosphatase is concentrated in the microsomes, the more common acid phosphatase in the mitochondria.

Recent pathological investigations support the idea of an ergastoplasm, for changes in it have been demonstrated soon after the administration of carbon tetrachloride and well before the mitochondria are involved. Affected liver cells show dilatation of the ergastoplasmic cisternae with formation of microscopic vacuoles, disappearance of the granules, and reduction of supplies of ribonucleic acid. Vacuolation may progress to an irreversible hydropic degeneration or to the typical balloon-cell formation (Oberling and Rouiller, 1956; Rouiller, 1957). Mölbert and Guerritore (1957) and Mölbert (1958) also stress the part played by ergastoplasm in the production of vacuoles and hydropic degeneration. Mölbert says that hydrogen cyanide can induce vacuolation of mitochondria and ergastoplasm in liver and heart-muscle cells within a few minutes. Malonic-acid intoxication leads to the dissociation of ergastoplasmic lamellae, vacuolation and, eventually, to hydropic swelling. If the cell survives new lamellae appear especially in the neighbourhood of the mitochondria.

In an interesting investigation on anoxia, Mölbert and Guerritore (1957) kept guinea-pigs at atmospheric pressures equivalent to those at 12,000–13,000 metres above sea level. Electron micrographs of liver cells from these animals showed severe mitochondrial changes after 40–60 minutes' exposure, with vacuolation and dissolution of ergastoplasm and the development of large vacuoles in the cytoplasm. Similar changes, without vacuolation, have been noted in rats after extreme fasting (Gansler and Rouiller, 1956).